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الملخص

تعتبر *Ammoides atlantica* (*A. atlantica*) و *Athamanta sicula* L. (*A. sicula* L.) من النباتات الطبية الهامة في الاستخدامات التقليدية للفلورا الجزائرية. تهدف الدراسة الحالية إلى التحليل الكيميائي وتقييم خارج الحي (في الزجاج) لمضادات الأكسدة ، مضادات الأسيتيل-كولينستراز ومضاد البوتيريل-كولينستراز، مضادات التيروزيناز ومضادات ألفا-أميلاز ومضادات ألفا-غليكوزيداز لمستخلصات *A. atlantica* و *A. sicula* L. المائية-الميثانولية (AACE و ASCE) وأجزائها الفرعية، الهكسان (AAHE و ASHE) ، الكلوروفورم (AAChE و ASCHE) ، أسيتات الإيثيل (AAEAE و ASEAE) ، البوتانول (AABE و ASBE) والمستخلصات المائية (AAWE و ASWE). علاوة على ذلك، تم تقييم النشاط المضاد للقرحة المعدية و التأثير على الحركية المعدية المعوية (إفراغ المعدة والعبور المعوي) في الحي لكل من المستخلصين AACE و ASCE. أظهر تحليل GC-MS أن الكارفاكرول، وحمض اللينوليك، حمض الأوليك (9-حمض أوكتاديسينويك (Z)) وحمض البالمتيك كانت المركبات الرئيسية في AAHE. في حين كان الكارفاكرول، حمض اللينوليك، الأبيول وحمض البالمتيك المكونات الرئيسية في ASHE. أظهر تحليل UHPLC-MS وجود إحدى أو العديد من ايزومر حمض الكافويلكوينيك و/أو مشتقاته كمكونات رئيسية في AACE و أجزائه الفرعية، في حين أن حمض مالونيل-3،5-ديكافويلكوينيك I، استراغالين حمض الفورميك، استراغالين، و / أو سينارين كانت المكونات الرئيسية في ASCE وأجزائه الفرعية. أظهرت المستخلصات AAEAE و ASEAE أعلى كمية من البوليفينول والفلافونويد والفلافونول والدباغ. أظهرت جميع المستخلصات أنشطة مضادة للأكسدة ومضادة للإنزيمات. كان للمستخلصين AAEAE و ASEAE أعلى نشاطا مع معظم الاختبارات. تسبب كل من AACE و ASCE في تأخير إفراغ المعدة بصورة معتمدة على الجرعة في الفئران و قد تعود هذه التأثيرات الى تدخل مسارات إنزيمات الأكسدة الحلقية في الحيوانات المعالجة بـمستخلصات AACE و ASCE و كذلك المسارات الكولينرجية في الفئران المعالجة بـمستخلص AACE. أدى كل من المستخلصين AACE و ASCE إلى تأخير العبور المعوي بصورة معتمدة على الجرعة. ينتج هذا التأثير عن تدخل مسارات أكسيد النيتريك و إنزيمات الأكسدة الحلقية عند الفئران المعالجة بمستخلص AACE أو ASCE والمسارات الكولينرجية عند الحيوانات المعالجة بمستخلص AACE. قدمت AACE و ASCE حماية معتمدة على الجرعة للغشاء المخاطي ضد القرحة المعدية في الجرذان. و قد تشمل آليات هذا التأثير على الأقل زيادة محتوى مخاط المعدة، المسارات الكولينرجية ، مسارات أكسيد النيتريك وتخفيض الضرر التأكسدي المحدث بالغشاء المخاطي للمعدة. تظهر هذه النتائج لأول مرة بعض المكونات و الأنشطة الجديدة لـ *A. atlantica* و *A.sicula* L. والتي يمكن استغلالها في مجال الصناعات الدوائية والغذائية ومستحضرات التجميل.

الكلمات المفتاحية: *A. atlantica*، *A. sicula* L.، مضادات الأكسدة، مضادات إنزيمية، إفراغ معدي، عبور معوي، القرحة المعدية.

Abstract

Ammoides atlantica (*A. atlantica*) and *Athamanta sicula* L. (*A. sicula* L.) are two important plants in traditional uses of the Algerian flora. The present study aimed to chemically analyze and to evaluate *in vitro* antioxidant**,** anti-acetylcholinesterase and anti-butyrylcholinesterase, anti-tyrosinase, anti-α-amylase and anti-α-glucosidase of *A. atlantica* and *A. sicula* L. crude (hydromethanolic) extracts (AACE and ASCE) and their sub-fractions; hexane (AAHE and ASHE), chloroform (AAChE and ASChE), ethyl acetate (AAEAE and ASEAE), butanol (AABE and ASBE) and water (AAWE and ASWE) extracts. Moreover, the gastro-protective and gastro-intestinal motility (gastric emptying and intestinal transit) effects of the AACE and ASCE were also evaluated *in vivo*. GC-MS analysis revealed that carvacrol, linoleic acid, oleic acid (9-Octadecenoic acid (Z)), palmitic acid were the major compounds in AAHE, whereas, carvacrol, linoleic acid, apiole, palmitic acid were the main constituents in ASHE. The UHPLC-MS analysis showed the presence of one or several of isomer of caffeoylquinic acid and/or its derivative as the main constituent in AACE and its sub-fractions, whereas, malonyl-3,5-dicaffeoylquinic acid I, astragalin formic acid adduct, astragalin and/ or cynarin were the main components in ASCE and its sub-fractions. AAEAE and ASEAE had the highest amount of polyphenols, flavonoids, flavonols and tannins. All extracts showed antioxidant and anti-enzymatic activities. The AAEAE and ASEAE fractions were the most active with the majority of tests. AACE and ASCE induced a dose dependent gastric emptying delay in mice. These effects may involve the cyclooxygenase in AACE and ASCE treated animals as well as cholinergic pathways in AACE treated mice. AACE and ASCE extracts dose dependently decreased the intestinal transit. The effect involves NO, cyclooxygenase pathways in AACE and ASCE treated mice and cholinergic system in AACE treated animals. AACE and ASCE offered a dose-dependent protection to the gastric mucosa against ethanol-induced ulceration in rats. This effect may involve at least in part gastric mucus content, cholinergic and nitric oxide pathways and a reduction in the oxidative damage of the mucosa. These results show for the first time new constituents and activities of *A. atlantica* and *A. sicula* L. that can be exploited in the field of pharmaceutical, food and cosmetic industries.

Keywords: *A. atlantica, A sicula* L., antioxidant activities, anti-enzymatic activities, anti-diabetic activity, gastric emptying, intestinal transit, gastric ulcer.

Résumé

Ammoides atlantica (*A. atlantica*) et *Athamanta sicula* L. (*A. sicula* L.) sont deux plantes importantes dans les usages traditionnels de la flore algérienne. La présente étude visait à analyser chimiquement et à évaluer *in vitro* les activités antioxydante, anti-acétylcholinestérase, anti-butyrylcholinestérase, anti-tyrosinase, anti-α-amylase et anti- α-glucosidase des extraits bruts (hydrométhanolique) de A. *atlantica* et *A. sicula* L. (AACE et ASCE) et leurs sous-fractions; extraits d'hexane (AAHE et ASHE), de chloroforme (AAChE et ASChE), d'acétate d'éthyle (AAEAE et ASEAE), de butanol (AABE et ASBE) et d'eau (AAWE et ASWE). De plus, les effets de la gastro-protection et de la motilité gastro-intestinale (vidange gastrique et transit intestinal) des extraits AACE et ASCE ont également été évalués *in vivo*. L'analyse GC-MS a révélé que le carvacrol, l'acide linoléique, l'acide oléique (acide 9-octadécénoïque (Z)), l'acide palmitique étaient les principaux composés de l'AAHE, tandis que le carvacrol, l'acide linoléique, l'apiole, l'acide palmitique étaient les principaux constituants de l'ASHE. L'analyse UHPLC-MS a montré la présence d'un ou plusieurs isomères de l'acide caféoylquinique et/ou de son dérivé comme constituants principaux de l'AACE et de ses sous-fractions, tandis que l'acide malonyl-3,5-dicaféoylquinique I, l'adduit d'acide formique d'astragaline, l'astragaline et/ou la cynarine étaient les principaux composants de l'ASCE et de ses sous-fractions. L'AAEAE et l'ASEAE avaient la plus grande quantité de polyphénols, de flavonoïdes, de flavonols et de tanins. Tous les extraits ont montré des activités antioxydantes et anti-enzymatiques. Les fractions AAEAE et ASEAE étaient les plus actives avec la majorité des tests. L'AACE et l'ASCE ont induit un retard, dose-dépendant, de la vidange gastrique chez la souris. Ces effets peuvent impliquer la cyclooxygénase chez les animaux traités par AACE et ASCE ainsi que les voies cholinergiques chez les souris traitées par AACE. Les extraits d'AACE et d'ASCE ont diminué de manière dose-dépendante le transit intestinal. L'effet implique les voies de NO et de la cyclooxygénase chez les souris traitées avec AACE et ASCE et le système cholinergique chez les animaux traités avec AACE. L'AACE et l'ASCE ont offert une protection dose-dépendante de la muqueuse contre l'ulcération induite par l'éthanol chez le rat. Cet effet peut impliquer au moins en partie la teneur en mucus gastrique, les voies cholinergiques et NO et une réduction des dommages oxydatifs de la muqueuse. Ces résultats montrent pour la première fois de nouveaux constituants et des activités d'*A. atlantica* et *A. sicula* L. qui peuvent être explorées dans le domaine des industries pharmaceutiques, alimentaires et cosmétiques.

Mots clés: *A. atlantica*, *A sicula* L., activités antioxydants, activités anti-enzymatiques, vidange gastrique, transit intestinal, ulcer gastrique.

List of abbreviations

Aβ: Amyloid beta

ABTS: 2.2´-Azino-bis-(3-ethylbenzenothiazoline-6-sulfonic acid)

Ach: Acetylcholine.

AChE: Acetylcholinesterase

AChEI: Acetylcholinesterase inhibitors

AD: Alzheimer's disease

AGEs: Advanced glycation end products

$AlCl_3$: Aluminium tri-chloride

AMPK: AMP-activated protein kinase

a-MSH: a-melanocyte stimulating hormone

APP: Amyloid β precursor protein

BChE: Butyrylcholinesterase

BE: Butanol extract

BH4: Tetrahydrobiopterin

BHT: Butyl hydroxyl toluene

CAT: Catalase

CBF: Cerebral blood flow

CCK: Cholecystokinin

CE: Crude extract

ChE: Chloroform extract

CGRP: Calcitonin gene related peptide

ChEI: Cholinesterase inhibitor

CMC: Carboxymethyl cellulose

CM: Circular muscle

CNS: Central nervous system

COX: Cyclooxygenase

CUPRAC: Cupric ion reducing antioxidant capacity

DB: Diabetes mellitus

DMNX: Dorsal motor nucleus of the vagus nerve

DMSO: Dimethyl sulfoxide

DOPA: 3,4-Dehydroxyphenylalanine

DPPH: 1, 1-Diphenyl-2-picryl-hydrazyl

DRG: Dorsal root ganglion

DTNB: 5, 5' -Dithio-bis (2-nitrobenzoic acid)

DW: Dried weight.

EAE: Ethyl acetate extract

EC: Enterochromaffin

EDTA: Ethylenediaminetetraacetic acid

EGCG: Epigallocatechin gallate

eNOS: Endothelial nitric oxide synthase

ENS: Enteric nervous system

EPSPs: Excitatory postsynaptic potential

GC-MS: Gas chromatography-mass spectrometry

GE: Gastric emptying

GI: Gastrointestinal

GLP-1: Glucagon-like peptide-1

GLUT-4: Glucose transporter type 4

GPx: Glutathione peroxidase

GR: GSH reductase

GSH: Glutathione

GSSG: Glutathione disulphide

HE: Hexane extract

HNE: 4-Hydroxy-2, 3-nonenal

HNO_2: Nitrous acid

H_2O_2: Hydrogen peroxide

HOCl: Hypochlorous acid

5-HT: 5-hydroxyryptamine or Serotonin

ICC: Interstitial cell of Cajal

ICC-MY: Myenteric interstitial cells of Cajal

IKI: Iodine/potassium iodide

IL: Interleukin

iNOS: Inducible nitric oxide synthase

LD_{50}: Lethal dose, 50%

LDL: Low-density lipoprotein

LM: Longitudinal muscle

L-NAME: L-NG-nitro arginine methyl ester

L-NMMA: L-NG-monomethyl-L-arginine

L-NNA: L-nitro-n-arginine

$LOO^{\bullet}$: Lipid peroxyl

LOOH: Lipid peroxide

LOX: Lipoxygenase

LPO: Lipid peroxidation

LTP: Long-term potentiation

MAM: Mitochondrial-associated endoplasmic reticulum membrane

MAO-B: Monoamine oxidase-B

MDA: Malondialdehyde

MeOH: Methanol

MMC: Migranting motor complexes

M: Muscarinic receptor

MP: Myenteric plexux

MPO: Myeloperoxidase

NADPH: Nicotinamide adenine dinucleotide phosphate

NANC: Non-adrenergic non-cholinergic

n-BuOH: n-Butanol

NFkB: Nuclear factor kB

NFTs: Neurofibrillary tangles

NG: Nodose ganglion;

NK: Neurokinin

NMDAR: N-methyl-D-aspartate receptors

nNOS: Neuronal nitric oxide

NO: Nitric oxide

$NO^{\bullet}$: Nitrogen free radical

$NO_2^{\bullet}$: Nitrogen dioxide

N_2O_3: Dinitrogen trioxide

NOS: Nitric oxide synthase

NPY: Neuropeptide Y

NSAIDs: Non-steroidal anti-inflammatory drugs

NTS: Nucleus tractus solitarius

$^1O_2$: Singlet oxygen

$O_2^{\bullet-}$: Superoxide radical

O_3: Ozone

$OH^{\bullet}$: Hydroxyl radical

$ONOO^-$: Peroxynitrite

PACAP: Pituitary adenylate cyclase-activating polypeptide

PAS: Peripheral anionic site

PD: Parkinson's disease

PGs: Prostaglandins

PKC: Protein kinase C

PPARs: Peroxisome proliferator-activated receptors

RAGE: Receptor for advanced glycation end products

RNS: Reactive nitrogen species

RO˙: Alkoxyl

ROO˙: Peroxyl

ROS: Reactive oxygen species

RT: Retention time

SMP: Submucosal plexus

SOD: Superoxide dismutase

SP: Substance P

TBA: Thiobarbituric acid

TCA: Trichloroacetic acid

TCP: α-Tocopherol

TFC: Total flavonoid content

TIC: Total ion chromatogram

TK: Tachynin

TNB: 2-Nitro-5-thiobenzoic acid

TNF: Tumor necrosis factor

TOM: Translocase of outer membrane

TPC: Total phenolics content

UHPLC-MS: Ultra-High performance liquid chromatography-mass spectrometry

UV: Ultraviolet

V: Vessel.

VIP: Vasoactive intestinal peptide

WE: Water extract

WHO: World Health Organization

XO: Xanthine oxidase

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Introduction

Oxidative stress is described as a condition in which cellular antioxidant defenses are inadequate to totally inactivate reactive oxygenspecies (ROS) and reactive nitrogen species (RNS) (Aquil et *al*., 2013). This oxidative stress is considered to be a major aetiological or pathogenic cause of many diseases including Alzheimer's disease, hyperpigmentation, diabetes, gastrointestinal (GI) disorders, cardivascular diseaseand cancer (Suzuki et *al*., 2011; Kim et *al*., 2012; Ahmad et *al*., 2017; Kanlayavattanakul and Lourith., 2018; Vona et *al*., 2021).

Before the development of synthetic drugs, herbswere the base for almost all medicinal therapies (Patti et *al*., 2019). It has been stated that the plant's chemical composition directly influences its biological activity (Lemos et *al*., 2016). The protective effects of many herbs and plant spicesis due to the antioxidative constituents present in their tissues. Antioxidantshave an increasinginterest due to their influence in food and pharmaceutical products againstpathological processes caused by oxidative stress (Gulcin, 2020). Research interest has increased significantly in discovering naturally occurring antioxidants to replace synthetic antioxidants in foods and medicinal materials, because of their side effects (Fernandes et *al*., 2015; Alisi et *al*., 2018). The antioxidant activity of various plant extracts is attributed mainly to the presence of phenolic compounds, particularly flavonoids (Sousa et *al*., 2015). Thus in the food supply industry, phenolics are often sought to substitute synthetic antioxidants (Fernandes et *al*., 2015). They are also one of the most important bioactive compounds in medicinal plants and many health benefits have been related to them, including cardiovascular disease, diabetes mellitus (DB), cancer, neurological disease prevention (Del Rio et *al*., 2013). The pharmacological potential of plants rich in phenolic compounds as antioxidants is enormous since these compounds act on the adsorption, neutralization, removal of free radicals and the decomposition of peroxides (Pacifico et *al*., 2015). Oxidative stress destroys multiple molecules and cellular structures that cause body malfunction. Increasing evidence points to the involvement of oxidative stress in the physiopathology of various chronic diseases that require prolonged periods of pharmacological treatment (García-Sánchez et *al*., 2020). As phenolics stimulate cellular defenses, they prevent oxidative damage and thus prevent the occurrence of chronic diseases (Oviedo-Solís et *al*., 2018), such as, Alzheimer's disease, Parkinson's disease, diabetes mellitus and others via the inhibition of the enzyme activities involved in these ailments. Indeed, many modern drugs have been reported to have side effects or low efficacy in dealing with these pathways (Adhikari et *al*., 2008; Orhan et *al*., 2015; Zengin et *al*., 2015; Shanmugam et *al*., 2016). The inhibition of enzymes is becoming a popular and effective tool in the discovery of new drugs in order to prevent or treat such diseases (Asghari et *al*., 2018).

In line with this, medicinal plants are a rich source of enzyme inhibitors that are used as drugs for the treatment of various physiological disorders (Rauf and Jehan, 2017).

The World Health Organization (WHO) acknowledged the use of herbal medicine and has considered it as an important part of the world health care system. Several studies on the biological effects of plants usually used as traditional remedies have been carried out to improve memory, prevent diabetic complications and protect the digestive tract (Loizzo et *al*., 2008; Liu et *al*., 2015; Basso et *al*., 2021).

The digestive system provides the body's nutritional needs (sugars, proteins, vitamins, water and electrolytes ...) by fulfilling several important functions. Any imbalance in these functions leads to various diseases. Among these disorders; gastriculcer, is one of the most common diseases affecting the digestive system resulting from the destruction of the mucous layer in the GI tract. This destruction is induced by many factors, including smoking, taking non steroidal anti-inflammatory drugs (NSAID) and alcohol consumption. It's an easy disease to contract and difficult to cure, it has serious repercussions on human health and can even lead to death. Its treatment is sometimes difficult, depending on the causing agent and the conditions of the ulcer. The treatment with modern medicine is expensive, whichis a handicap for the patient and is not free of side effects. In order to overcome these problems, people around the world are exploiting the benefits of natural products in dealing with these ailments. In Algeria, the use of natural products in the treatment and prevention of diseases is quite well established and is dating back to early ages. In recent decades, a good body of literature is accumulating mainly on the ethnobotany and pharmacology (Gharzouli et *al*., 2002; Amira et *al*., 2012; Boudjelal et *al*., 2013; Benchikh et *al*., 2016).

The Apiaceae (Umbelliferae) is a cosmopolitan family. In Algeria, this family is represented by about 130 species distributed on 55 genera (Quezel and Santa, 1963). *A. atlantica* belongs to the genus Ammoides, whereas *A. sicula*L. belongs to the genus Athamanta. Few studies have been devoted to these plants and their beneficial contribution to the health system. Thus, the main objectives of the present study were to put forward the scientific basis for the evaluation of these two plants in the management of some diseases by:

- Analyzing their phytochemical constituents

- Evaluation of the *in vitro* antioxidant properties using different methods.

- Assessment of their enzymes inhibitory effects against key enzymes involved in Alzheimer's, hyperpigmentation and diabetes mellitus diseases.

- Evaluation of the *in vivo* acute oral toxicity.

- Investigation of their GI motility effects and the mechanisms involved

- Assessment of their gastric mucosal protection against ethanol-induced ulceration and the elucidation of the mechanisms of action.

Literature review

1. Oxidative stress

1.1. Definition of oxidative stress

The delicate balance between the production and elimination of reactive determines the normal function of cells. However, when cells are unable to maintain redox homeostasis via the detoxification of these reactive species produced and/or repair the damage produced, oxidative stress or nitrosative stress prevails (Navarro-Yepes et *al*., 2014). Thus, oxidative stress is an imbalance between pro-oxidant and antioxidants in favor of oxidants (Navarro Yepes et *al*., 2014; Wu et *al*., 2015). Oxidative stress eventually leads to partial or total functional loss of physiological systems in the body. Currently, the incidence of free radical imbalance at the onset and during the evolution of many diseases (Inflammation, gastric disorders, neurodegenerative and cardiovascular diseases, diabetes, respiratory disorders, aging, ischaemia, tumor progression and carcinogenesis) has been demonstrated (Gönenç et *al*., 2013; Wu et *al*., 2015).

1.2. Oxygen species and reactive nitrogen species

In the literature, we often notice a symbolic point next to an abbreviation chemical such as ($OH^{\bullet}$), this point signifies a free radical (Scheibmeir et *al*., 2005). Free radicals are defined as atoms or molecules containing one or more unpaired electrons in the outer orbit. This unpaired electron(s) is unstable and usually gives a significant degree of reactivity to the free radical (Valko et *al*., 2007; Birben et *al*., 2012). This reactivity is very variable depending on the nature of the radical, its half-life and its place of production. Indeed, the more unstable the electron, the shorter the half-life of the radical, the less it diffuses, and the more it risks attacking neighboring molecules contained in cells, in particular lipids, proteins, and DNA (Halliwell and Gutteridge, 2015). ROS and RNS include radical and non-radical species. Oxygen-free radicals include superoxide ($O_2^{\bullet -}$), hydroxyl ($OH^{\bullet}$), peroxyl ($ROO^{\bullet}$), lipid peroxyl ($LOO^{\bullet}$), alkoxyl ($RO^{\bullet}$) radicals. Nitrogen free radicals include nitric oxide ($NO^{\bullet}$) and nitrogen dioxide ($NO_2^{\bullet}$). Oxygen and nitrogen free radicals can be readily converted to other non-radical reactive species which are harmful to health. Hydrogen peroxide (H_2O_2), ozone (O_3), singlet oxygen ($^1O_2$), hypochlorous acid (HOCl), nitrous acid (HNO_2), peroxynitrite ($ONOO^-$), dinitrogen trioxide (N_2O_3), lipid peroxide (LOOH) are not free radicals and generally named oxidants and can easily lead to free radical reactions in living organisms (Valko et *al*., 2007; Sen and Chakraborty, 2011; Nunes et *al*., 2012). Oxygen-derived radicals represent the most important

class of radical species generated in living systems due to the importance of aerobic metabolism (Valko et *al*., 2007). Indeed, the ROS are the majority but nitrogenous radicals, sulfur, phosphorus or carbon are also formed (Sekli-Belaidi, 2011).

1.3. Main sources of ROS and RNS

The production of these reactive species ROS and RNS in the body of animals and humans is continuous and a normal part of human physiology (Kumari and Kakkar, 2008). They are also produced under pathologic conditions (Montagnier, 2009). On other hand, free radicals can be produced from both endogenous and exogenous origins.

1.3.1. Endogenous sources

Major sources of ROS and RNS include, but are not limited to, NADPHoxidases, xanthine oxidase, NO synthases, mitochondrial respiratory chain enzymes (Figure01).

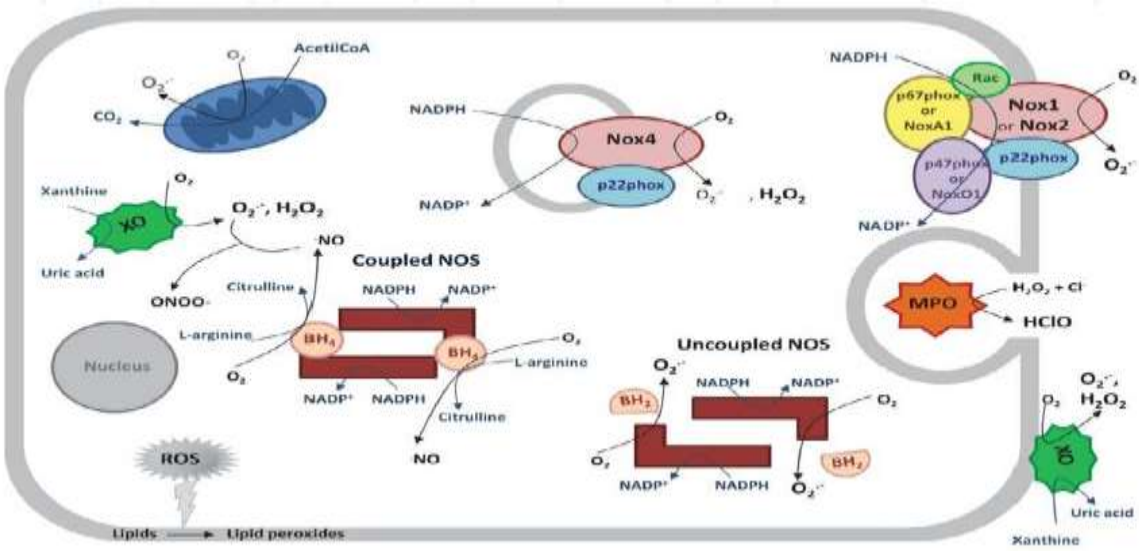

Figure 01: Endogenous sources of ROS and/or RNS generation (Sousa et *al*., 2012).

a) Mitochondrial respiratory chain enzymes

The mitochondrial respiratory chain is responsible for the production of 90% of ROS in the cell (Balaban et *al*., 2005). The production of $O_2^{\bullet-}$ results from the leakage of electrons during their transfer by the respiratory chain complexes (Zhang and Gutterman, 2007) (Figure 02).

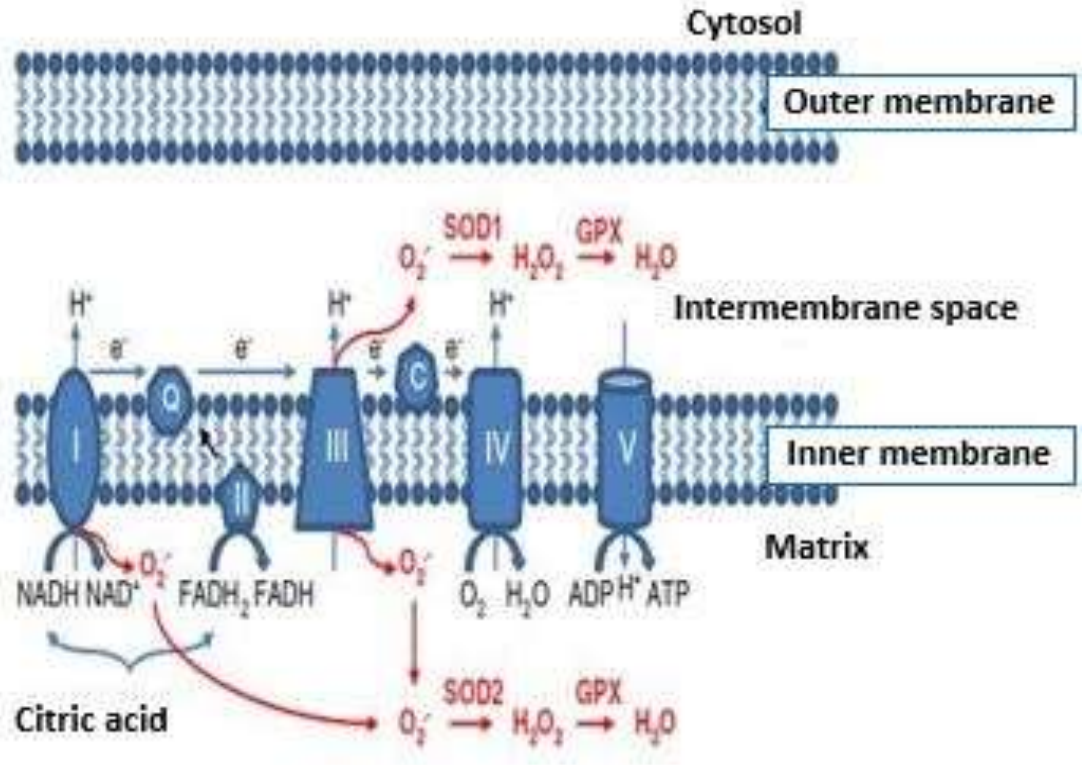

Figure 02: The production sites of ROS in the mitochondrial level (Li et *al*., 2013).

b) NADPH oxidases

As a result of the stimulation of phagocytes, including neutrophils, mononuclear and macronutrients, in the presence of foreign bodies like bacteria, NADPH oxidase produces high amounts of radical $O_2^{\bullet-}$ converts electrons from NADPH to molecular oxygen.

$$2O_2 + NADPH \longrightarrow 2O_2^{\bullet-} + NADP^+ + H^+$$

The resulting radical $O_2^{\bullet-}$, can be precucer of other compounds that have a greater ability to oxidize biological molecules (Quinn and Schepetkin, 2009). Fibroblasts and vascular smooth muscle cells contain this enzyme that uses NAD to produce NADPH (Griendling et *al*., 2000; Reyand Pagano, 2002).

c) Xanthine oxidase

Xanthine oxidase (XO) is an important source for producing the radical $O_2^{\bullet-}$ and H_2O_2, through oxidation of xanthine or hypoxanthine to uric acid during inflammatory diseases (Kelley et *al*., 2010). XO can convert nitrate and nitrite to nitrite and $NO^{\bullet}$, respectively. Also,

this enzyme can stimulate the reaction of $NO^{\bullet}$ and $O_2^{\bullet-}$ to generate $OONO^{\bullet-}$ (Godbar et *al*., 2000).

d) Nitric oxide synthase and cofactor tetrahydrobiopterin

Nitric oxide radical ($NO^{\bullet}$) produced in animal tissues by the enzyme nitric oxide synthase which is present in three forms; endothelial nitric oxide synthase (eNOS), neuronal nitric oxide (nNOS) and inducible nitric oxide synthase (iNOS). The first and second forms are regulated by the presence of calcium ions. These two enzymes catalyze L-arginine oxidation in the presence of molecular oxygen and tetrahydrobiopterin (BH4) and NADPH as cofactor to produce low quantities of $NO^{\bullet}$to perform certain physiological functions (Lavie, 2015). However, in conditions of limited bioavailability of BH4 or the substrate L-arginine, such as in condition of oxidative stress, these enzymes become unstable and reduce molecular oxygen to $O2^{\bullet-}$ instead of $NO^{\bullet}$ production (uncoupled NOS) (Li et *al*., 2013; Lavie, 2015). While iNOS independently of calcium is regulated by LDL and stimulated in the presence of inflammatory mediators such as cytokines and bacterial products, leading to the produce of NO with 100 times more than that produced by essential enzymes (Lavie, 2015).

e) Myeloperoxidase

Myeloperoxidase (MPO) is a heme protein secreted by activated neutrophiles and monocytes in inflammatory conditions and produces several oxidizing molecules that can affect lipids and proteins. MPO uses H_2O_2 to produce ROS such as HOCl, chloramines, tyrosyl radicals and nitrogen dioxides. Although MPO-derived ROS have a primary role in microbial killing, they also cause tissue damage in the heart, vessels, kidney and brain and appear to contribute to endothelial dysfunction (Sousa et *al*., 2012).

f) Cycloxygenase and lipoxygenase

During the metabolism of arachidonic acid, lipoxygenases (LOX) and cycloxygenases (COX 1 and 2) also generate ROS (iron metalloenzymes) (Ahsan and al., 2003; Lee et *al*., 2004; Valko et *al*., 2007). LOX and COX are another important source of ROS in the vascular walls (Droge, 2002). 5-lipoxygenase (5-LOX) is an important source of ROS production in lymphocytes (Bonizzi et *al*., 2000). 5-LOX catalyzes the oxidation of polyunsaturated fatty acids to give hydroperoxides toxic to the cell, as it is involved in the formation of H_2O_2 by T cells in response to interleukins-1β (Droge, 2002). Many specific enzymes such as 15-LOX,

12-LOX, 5-LOX, oxidize arachidonic acid to give compounds that alter the oxidation balance recalling causes the induction of signal transduction and gene expression pathways (Yamamoto, 1992). The enzyme cycloxygenase contributes to ROS production in cells induced by tumor necrosis factor (TNF) and interleukin-1 and bacterial lipid polysaccharides (Feng Xia et *al*., 1995).

1.3.2. Exogenous sources

a) Pollution: Different types of pollutants, like air pollutants (cigarette smoke, asbestos, benzene, carbon monoxide, chlorine, formaldehyde, ozone, and toluene), chemical solvents (cleaning products, glue, paints, paint thinners, perfumes, and pesticides), and water pollutants (chloroform and other trihalomethanes) are all potent generator of free radicals. Burning of organic matter during cooking, forest fires, and volcanic activities also can generate free radicals (Sen and Chakraborty, 2011; Gaston, 2016).

b) Radiation: UV radiations, medical and dental x-rays, gamma rays, and microwave radiation can lead to free radical generation (Birben et *al*., 2012; Rahal et *al*., et *al*., 2014).

c) Dietary factors: Additives, alcohol, foods that have been barbecued, fried, grilled, foods that have been browned or burned, hydrogenated vegetable oils, processed foods containing high levels of lipid peroxides can also produce free radicals (Sen and Chakraborty, 2011; Birben et *al*., 2012).

d) Toxins and drugs: Carbon tetrachloride, paraquat, benzopyrene, aniline dyes, toluene and drugs like adriamycin, bleomycin, mitomycin C, nitrofurantoin, and chlorpromazine increase free radical productions (Sen and Chakraborty, 2011; Rahal et *al*., 2014; Ye et *al*., 2014) .

1.4. Physiological roles of ROS and RNS

Most cells can produce ROS/RNS constitutively while others have inducible ROS/RNS release system. For example, defense against infectious agents by phagocytosis, killing of cancer cells by macrophages and cytotoxic lymphocytes, detoxification of xenobiotics by cytochrome P450, generation of ATP in mitochondria (energy production), cell growth, and the induction of mitogenic responses at low concentrations are some key beneficial activities of ROS and RNS (Sen and Chakraborty, 2011). ROS and RNS with low/moderate concentrations play important physiological roles, and they are used as regulating media for biological functions, where ROS play important role on different cellular signalling at low concentration

like activation of several cytokines and growth factor signalling, non-receptor tyrosine kinases activation, protein tyrosine phosphatases activation, release of calcium from intracellular stores, activation of nuclear transcription factors. Also, ROS exert vital actions such as gene transcription, and regulation of soluble guanylate cyclase activity in cells. NO produced by endothelial cells is essential for regulation of vascular tone and is key for neural plasticity, and mediator of the immune response by activated macrophages (Valko et *al*., 2007; Rahal et *al*., 2014). Moreover, previous studies also suggest that the ROS, such as superoxide, hydrogen peroxide may act as second messengers, but may be harmful as the accumulation of these free radicals are increased (Rahal et *al*., 2014). Their overproduction leads to molecular level damage such as lipid oxidation, including membrane cholesterol, free fatty acids, macromolecules in the extracellular fluid and collagen (Favier, 2003; Michel et *al*., 2004) thus the ROS at its high concentrations lead to important development of many diseases, such as diabetes, atherosclerosis, neurodegenerative diseases, and rheumatism (Dröge, 2002).

1.5. Biological targets and pathological consequences of oxidative stress

An excess of ROS often results in the appearance of cell and irreversible tissue damage. The most biological targets are lipids, proteinsand deoxyribonucleic acid (DNA)(Birben et *al*., 2012; Gaston, 2016).

1.5.1. Biological targets

a. Effects of oxidative stress on lipids

Peroxidation of lipids is initiated by the attack of a species, which can remove a hydrogen atom from a methylene group, resulting the formation of an unpaired electron on the carbon atom ($L^{\bullet}$). Carbon radical thus formed is stabilized by molecular rearrangement to produce a conjugated diene, which then can react with an oxygen molecule to form a lipid peroxyl radical ($LOO^{\bullet}$). These radicals can react with other lipid molecules to abstract hydrogen atoms further, so that lipid hydroperoxides (LOOH) form and at the same time propagate other lipid peroxidation further (Figure 03). Products of lipid peroxidation, such as malondialdehyde (MDA) and unsaturated aldehydes, are capable of inactivating many cellular proteins by forming protein cross- linkages (Birben et *al*., 2012; Kalyanaraman, 2013; Lavie, 2015). Lipid peroxidation can damage cell membranes by disrupting fluidity and permeability and thus changes the functions of many receptors and transmitters and the process of transmitting signals (Catala, 2006).

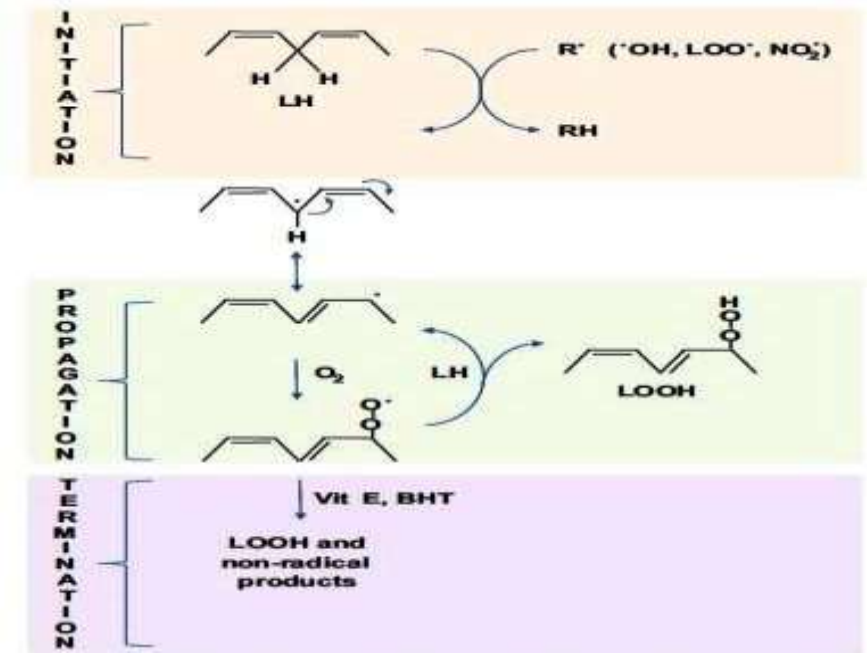

Figure 03: Lipid peroxidation; oxidation of unsaturated fatty acids to lipid hydroperoxide (Kalyanaraman, 2013).

b. Effects of oxidative stress on DNA

Reactive species (ROS and RNS) interfere with DNA and lead to oxidative damage. DNA is highly susceptible to damage by the free radicals such as $OH^{\bullet-}$ which can react with DNA by addition or loss of hydrogen atoms from the sugar moiety. In particular, the C4-C5 double bond of pyrimidine is very sensitive to attack by $OH^{\bullet-}$, which results generation of a spectrum of oxidative pyrimidine damage products, such as thymine glycol, uracil glycol, urea residue, 5- hydroxydeoxyuridine, 5-hydroxydeoxycytidine, hydantoin and others. Likewise, purines are susceptible to attack by $OH^{\bullet-}$ which leads to the generation of 8-hydroxydeoxyguanosine (8- OHdG), 8-hydroxydeoxyadenosine, formamidopyrimidines and other less characterized purine oxidative products (Sen and Chakraborty, 2011; Birben et *al*., 2012). Free radicals attack also causes the activation of the poly (ADP-ribose) synthetase enzyme which can lead to fragmentation of DNA and programmed cell death. This process depletes the cellular level of NAD^+ levels thereby disrupting electron transport chain function (Sen and Chakraborty, 2011; Birben et *al*., 2012; Lavie, 2015).

c. Effects of oxidative stress on proteins

ROS can cause fragmentation of the peptide chain, alteration of electrical charge of proteins, cross-linking of proteins and oxidation of specific amino acids and therefore lead to

increased susceptibility to proteolysis by specific proteases. Also, enzymes that have metals on or close to their active sites are especially more sensitive to metal catalyzed oxidation. Cysteine and methionine residues in proteins are particularly more susceptible to oxidation. For example, methionine can be oxidized to methionine sulfoxide and phenylalanine to O-tyrosine; sulfhydryl groups can be oxidized to form disulfide bonds; and carbonyl groups may be introduced into the side chains of proteins (Gutiérrez et *al*., 2014; Lavie, 2015).

1.5.2. Pathological consequences

Oxidative stress is involved in a large number of pathologies as a triggering factor or associated with complications (Favier, 2003). In fact, several studies, both epidemiological and clinical, confirm that oxidative stress is involved in the development of more than a hundred different human pathologies such as atherosclerosis and cancer as well as inflammatory diseases, diabetes, accelerated aging, asthma, hepatitis, dermatitis, cataracts, amyotrophic lateral sclerosis, acute pulmonary distress syndrome and pulmonary edema. It is a factor in the genesis of several diseases such as Alzheimer's disease, Parkinson's, GI infections, ulcers, rheumatism and cardiovascular diseases (Cohen et *al*., 2000; Packer and Weber, 2001; Atawodi, 2005; Favier, 2006; Valko et *al*., 2007; Roberts and Sindhuk, 2009; Alain et *al*., 2011). Most diseases induced by oxidative stress appear with age, because aging decreases antioxidant defenses and increases mitochondrial production of radicals accompanied by a decrease in the efficiency of repair systems and degradation of oxidized constituents (Favier, 2003).

1.6. Antioxidants

Antioxidants are compounds of endogenous or exogenous origin that serve to control the level of reactive species to neutralize them and minimize oxidative damage.

1.6.1. Endogenous antioxidants

1.6.1.1. Enzymatic antioxidants

The major enzymatic antioxidants are superoxide dismutase (SOD), catalase (CAT), GSH peroxidase (GPx) and GSH reductase (GR) (Figure 04) (Birben et *al*., 2012; Ye et *al*., 2014).

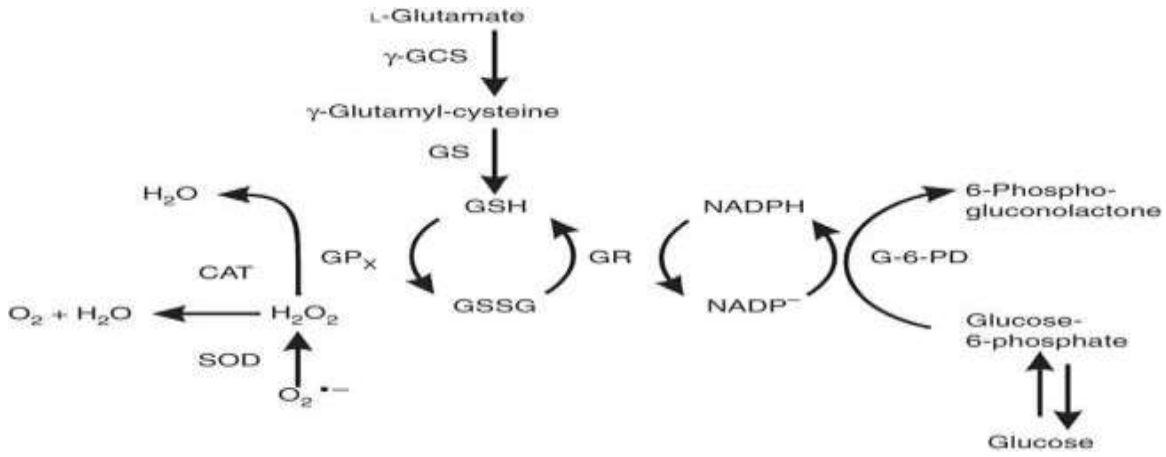

Figure 04: Methods of integration of enzymatic antioxidants (Weydert and Cullent, 2010).

a) Superoxide dismutase

These metalloproteins; Superoxide dismutase (SOD) act as the first-line defense system against ROS and catalyzes the dismutation of $O_2^{\bullet-}$ to H_2O_2 and O_2 (Sen and Chakraborty, 2011).

$$O_2^{\bullet-} + O_2^{\bullet-} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

The reaction mechanism is catalyzed by a metal located at the heart of the enzyme. SOD can exist in three metal containing isoforms which are found in different cellular compartments: SOD_1 (copper-zinc SOD) is in the cytoplasm, nucleus and plasma membrane; SOD_2 (manganese SOD) is mainly in mitochondria; and SOD_3 (Copper-zinc SOD) is unique in scavenging superoxide in the extracellular compartment and dismutating superoxides generated during the inflammation (Favier, 2006; Athiroh et *al*., 2014; Ye et *al*., 2014).

b) Catalase

The catalase (CAT) enzyme is present in most parts of the body, but it is present in high concentrations in the liver and its activity is high in the peroxisome. In the case of high H_2O_2 concentrations, this enzyme converts it into water and oxygen (Cemeli et *al*., 2009) according to the following reaction:

$$2H_2O_2 \xrightarrow{CAT} 2H_2O + O_2$$

Each CAT molecule can decompose millions of hydrogen peroxide molecules produced by SOD to water and oxygen in every second (Sen and Chakraborty, 2011).

c) Glutathione peroxidase

Glutathione peroxidase (GPx) is spread in human and animal tissues, while its high levels are in the kidneys and liver. GPxs found in several isoforms (encoded by different genes, which vary in cell location and substrate specificity) are found in plasma, cytosol, mitochondria and cell membrane. Itprotects the cell against oxidative injury caused by H_2O_2 and prevents the formation of hydroxyl radical from H_2O_2. GPx remove H_2O_2 by coupling its reduction to H_2O with oxidation of two molecules of GSH that are transformed into GSSG (Favier, 2003; Cemeli et *al*., 2009; Navarro-Yepes et *al*., 2014) according to the following two reactions:

$$H_2O_2 + 2GSH \longrightarrow 2H_2O + GSS$$

$$ROOH + 2GSH \longrightarrow ROH + H_2O + GSSG$$

d) Glutathione reductase (GR)

Glutathione reductase (GR) is an important cellular antioxidant necessary for the conversion of GSH. The oxidised glutathione is glutathione disulphide (GSSG) which reduced back to GSH by the presence of enzyme GR which uses NADPH as an electron donor.

$$GSSG + NADPH + H^+ \longrightarrow 2GSH + NADP^+$$

GSH-reductase is found in the cytosol and in the mitochondria, thus at the same level as GSH-peroxidase. The ratio of GSH/GSSG is an important general measure of oxidative stress of an organism, very high concentration of GSSG may damage many enzymes oxidatively (Sen and Chakraborty, 2011; Birben et *al*., 2012; Navarro-Yepes et *al*., 2014). The enzyme glucose-6-phosphate-dehydrogenase (G6PD) intervenes to convert $NADP^+$ to NADPH. Thus, there is a complementarity between the roles of antioxidant enzymes (Figure 04).

1.6.1.2. Non-enzymatic antioxidants

This class includes endogenous compounds of low molecular weight which can be either synthetic products (glutathione, histidine dipeptide) or resulting from cellular metabolism (uric acid). Proteins such as ferritin, ceruloplasmin and albumin in turn contribute to secondary

antioxidant defense by chelating transition metals to prevent hydroxyl radical formation (Martinez-Cayuela, 1995). Also, coenzyme Q10 has important anti-oxidant properties (Alam and Rahman, 2014).

a) Reduced glutathione

Reduced glutathione (GSH) is an antioxidant proteinthat is highly abundant in all cell compartments. GSH is also a cofactor for several detoxifying enzymes, such as GSH-Px and GSH transferase. Also, it is able to regenerate oxidized vitamins E and C. GSH protects cells against apoptosis by interacting with proapoptotic and antiapoptotic signaling pathways. It also regulates and activates several transcription factors, such as AP-1, NF-kB, and Sp-1. Also, GSH donates protons to membrane lipids and protects them from oxidant attacks (Birben et *al*., 2012; Sousa et *al*., 2012).

b) Uric acid

Uric acid is one of the best antioxidants in plasma, covering 35-60% of total antioxidant capacity (Johnson et *al*., 2009). It has the ability to quenche $HO^{\bullet -}$ and $ONOO^-$ and may prevent lipid peroxidation. It also acts as a chelator of iron in extracellular fluids (Sousa et *al*., 2012). Uric acid can be oxidized into different products, the predominant of which is allantoin which increases in the muscles under exertion (Hellsten et *al*., 2001), then it will be regenerated by vitamin C (Vasconcelos et *al*., 2007).

c) Coenzyme 10

Coenzyme 10 (Q10), which is also called ubiquinol 10, is a fat-soluble compound found in almost all cells. It is known for its important function in producing energy at the level of the inner membrane of the mitochondrion. It is an intermediate that has the ability to convert electrons from one enzyme compound to another (cytochrom reductase, and NADH deshydrogenase). It mainly resides in the reducted form (Ubiquinol: CoQ10H2) and it has important anti-oxidant properties for its ability to inhibit the direct superoxidation of lipids. It also contributes to the regeneration of vitamin E, in addition to it protects LDL against oxidation more than the protection provided by the vitamin E, β-carotene and lycopene (Alam and Rahman, 2014).

1.6.2. Exogenous antioxidants

Many phyto-nutrients have been identified as antioxidants. The most important are carotenoids, flavonoids, phenols, phytosterols and glucosinolates, vitamins and some metals such as Cu, Zn and Se. They have interesting antioxidant properties as scavengers / inhibitors of lipid radicals, superoxide anions, singlet oxygen or as a regulator of the antioxidant system (Berger, 2006; Dufour et *al*., 2007; Traber and Atkinson, 2007; Gobert et *al*., 2009).

1.6.2.1. Vitamins

a) Vitamin E

Vitamin E (tocopherols) is the main antioxidant in cell membranes, especially those of mitochondria (Traber and Atkinson, 2007). It is a lipid-soluble vitamin concentrated in the hydrophobic interior site of cell membrane and the principal defense against oxidant-induced membrane injury. Vitamin E donates electron to peroxyl radical, which is produced during lipid peroxidation. Vitamin E triggers apoptosis of cancer cells and inhibits free radical formations by converting $O2^{\bullet -}$ and $HO^{\bullet}$ to less reactive forms. α-Tocopherol is the most active form of vitamin E and the major membrane-bound antioxidant in cell, because it contains three methyl groups. It is produced only by plants and algae and is present in all parts of the plant (Kruk et *al*., 2008; Birben et *al*., 2012). Subsequently, the oxidized vitamin E can be reconverted by vitamin C but also by other compounds such as vitamin A, GSH and ubiquinol. Vitamin E can also activate SOD and catalases (Margaritis et *al*., 2003; Lyn Patrick, 2006). Vitamin E removes the $^1O_2$ radical, but its high concentrations and in the presence of the reduct minerals lead to its transformation into an oxidized precursor (Blokhina et *al*., 2003).

b) Vitamin C

Vitamin C (Ascorbic acid) is a water-soluble vitamin that provides intracellular and extracellular decoction-phase antioxidant capacity primarily by scavenging ROS and lipid hydroperoxides. It acts directly on ROS and indirectly through its action of regenerating vitamin E and GSH. Its plasma levels have been shown to decrease with age (Birben et *al*., 2012). At high doses, vitamin C can exert a pro-oxidant action via its ability to reduce the ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}), which participates in the Fenton and Haber-Weiss reactions (Sayre et *al*., 2005).

c) Vitamin A

Vitamin A acts on ROS by forming a vitamin A radical which can act as an antioxidant by reacting with another radical to form a non-radical, or will be regenerated into vitamin A (Clarkson and Thompson, 2000; Fisher-Wellman and Bloomer, 2009). In excess, vitamin A could act as pro-oxidants, and promotes the oxidation of DNA (Van Helden et *al*., 2009).

d) Vitamin B6

Vitamin B6 exerts an indirect antioxidant role by promoting the synthesis of cysteine from methionine and thus reinforces the production of GSH (Dalto and Matte, 2017).

1.6.2.2. Vegetable pigments

a) Chlorophylls

Chlorophyll and its derivatives are known to have antioxidant activity, being associated with reduced risks of diseases induced by free radicals, such as certain types of cancers (Carrapeiro et *al*., 2007; Barros et *al*., 2011).

b) Carotenoids

Carotenoids are liposoluble pigments derived from plants. They are among the most nature widespread pigments and have also received substantial attention because of both their provitamin and antioxidant roles (Carrapeiro et *al*., 2007; Barros et *al*., 2011). Carotenoids neutralize $OH^{\bullet-}$ avnd $ROO^{\bullet-}$ radicals, which makes them capable of inhibiting lipid peroxidation chains. In addition, carotenoids have a specific role of capturing $^1O_2$, which allows them to exercise protection against damage induced by the ultraviolet rays of sunlight (Gardès-Albert et *al*., 2003). Carotenoids show their antioxidant effects in low oxygen partial pressure but may have pro-oxidant effects at higher oxygen concentrations. Carotenoids also affect apoptosis of cells (Birben et *al*., 2012; Tanumihardjo, 2013).

- **Lycopene**

Lycopene is one of the most potent antioxidants among the dietary carotenoids and may help lowering the risk of chronic diseases including cancer and heart disease (Omoni and Aluko, 2005).

- **β-carotene**

More than 600 compounds of carotenoids have been identified and β-carotene is the most important form. Primarily, β-carotene has been found to react with peroxyl ($ROO^{\bullet}$), hydroxyl ($OH^{\bullet -}$), and superoxide ($O_2^{\bullet -}$) radicals. β-Carotene inhibits the oxidant-induced NF-kB activation and interleukin (IL)-6 and tumor necrosis factor α production (Birben et *al*., 2012; Tanumihardjo, 2013).

1.6.2.3. Polyphenols

a) Definition

Polyphenols are products of plant secondary metabolites used to defend against ultraviolet rays or foreign bodies. It includes more than 8000 identified natural compounds. Divided into several chemical classes which all have in their structure at least one aromatic ring with 6 carbon atoms, to which is directly linked at least one free hydroxyl group (OH) or engaged in another ether, ester or heteroside function. The classification of polyphenols is essentially based on the structure, the number of aromatic nuclei and the structural elements that bind these nuclei (Tapiero et *al*., 2002; Achat, 2013). They can be categorized as simple phenolic compounds, phenolic acids (derived from cinnamic and benzoic acids), coumarins, flavonoids, stilbenes, tannins and lignins (Fraga et *al*., 2010; Fadel et *al*., 2011). Flavonoids, tannins and phenolic acids are the most widely represented secondary metabolites (Khadiy et *al*., 2010) and they form the most important group (Beta et *al*., 2005).

b) The important classes of polyphenols

- **Phenolic acids**

They are simple phenolic molecules that represent the basic unit for building other phenolic compounds (Morton et *al*., 2000). They are found in medicinal plants (Psotova et *al*., 2003) and are divided into two main parts, namely the acids derived from hydroxybenzoic acid and the acids derived from hydroxycinnamic acid (Figure 05).

- ✓ **Acids derived from hydrobenzoic acid**

They had a structure consisting of C1-C6 as galic acid. They are the basic compounds for building hydrophilic tannins, both gallotanins and ellagitanins (Clifford and Scalbert, 2000).

- ✓ **Acids derived from hydroxycinnamic acid**

This is the most common section compared to the hydroxybenzoic acid derivatives and includes an acid p-coumaric acid, cafeic acid, ferulic acid, and sinapic acid, these acids are rarely present freely. The associated forms are derivatives of glucocorticoids or esters of quinic, shikimic, and tartaric acids. Cafeic acid combines with quinic acid to form a chlorogenic acid. Free and bound cafeic acid accounts for 75 to 100% of hydroxycinamic derivatives in most fruits. It is considered a ferulic acid one of the most common phenolic acids found in seeds (Manach et *al*., 2004).

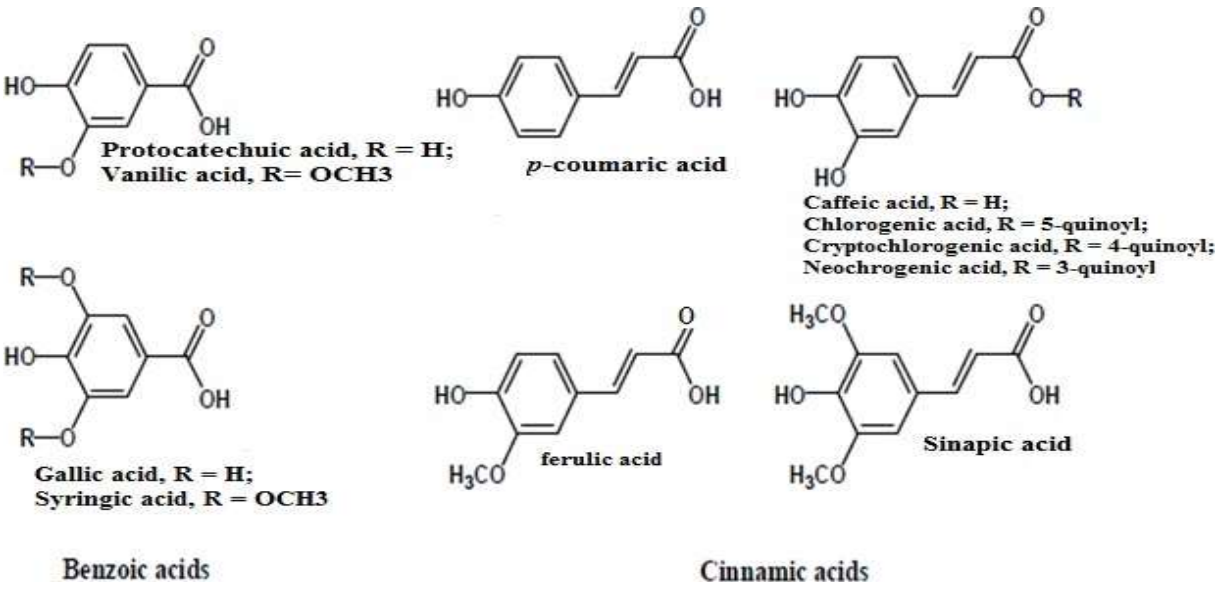

Figure 05: Different subgroups of phenolic acids (Tsao, 2010)

- **Flavonoids**

Flavonoids (the term is derived from the Latin word "flavus", meaning yellow) are ubiquitous plant secondary products that are best known as the characteristic red, blue, and purple anthocyanin pigments of plant tissues (Procházková et *al*., 2011). More than 5000 flavonoids have been identified (Akhlaghi and Bandy, 2009), each with a basic structure containing two benzene rings (A and B) with a pyrane ring in the middle (Ring C) (Figure 06) (Singh et *al*., 2014).

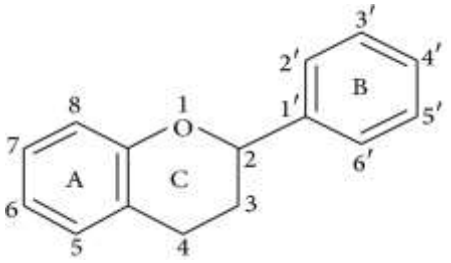

Figure 06: The basic structure of flavonoids (Kumar and Pandy, 2013).

Flavonoids can be divided into several subfamilies according to the degree of addition of the hydroxyl groups and the changes at the level of the C ring, being flavonols, flavanones, flavones, flavanes (essentially, flavan-3-ols), isoflavones, and anthocyanidins, the most relevant for human diets (Figure 07) (Wu et *al*., 2015). Most of the flavonoids are formed by the binding of ring B to carbon 2 of the ring C, however in some flavonoids such as isoflavonoids and neoflavonoids ring B is linked to carbon 3 and 4, respectively (Tsao et *al*., 2010) (Figure 07). Starting from a basic chemical structure, plant biosynthetic pathways introduce different hydroxyl group patterns, methyl groups, and sugars (Fraga et *al*., et *al*., 2010).

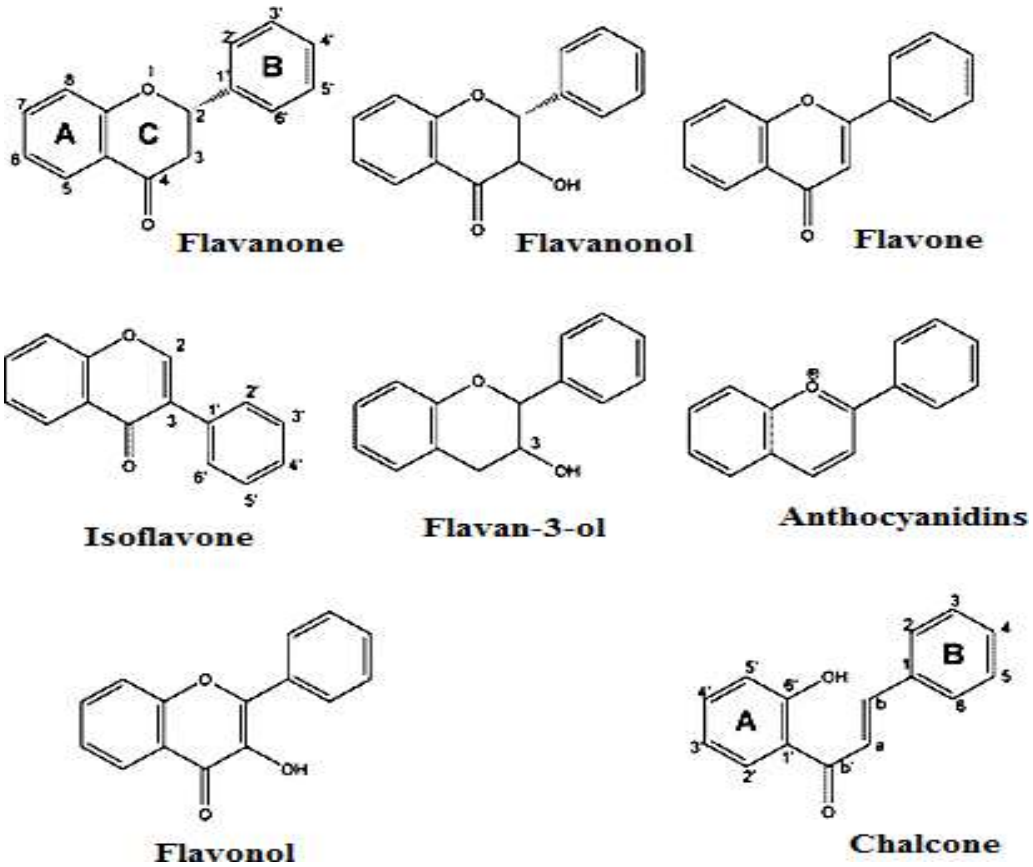

Figure 07: Different subgroups of flavonoids (Lago, 2014).

- **Tannins**

Tannins are complex compounds of polyphenols known as secondary metabolites with high molecular weights ranging between 500 and 3000 Daltons, which have the ability to form complexes with proteins, sugars, alkaloids, nucleic acids and minerals. Tannins are divided into two groups, which are the hydrolysable and condensed tannins (Figure08) (Frutos et *al*., 2004).

✓ **Hydrolysable tannins**

They a heterogeneous polyunsaturated unit formed as a result of the esterification of the hydroxylated groups of glucose with phenolic acids, whether it is gallic acid and called tannins in this case by gallotanins or ellagic acid and is called then ellagitanins. Hydrolysable tannins are easily degraded in acidic and basic medium and by some enzymes to release glucose and phenolic acids (Chung et *al*., 1998).

✓ **Condensed tannins**

The flavan-3-ols (catechin) and flavan-3, 4-dilos are the basic units in the formation of condensed tannins, and they are linked by carbon-carbon bonds, which makes them more resistant to hydration (He et *al*., 2008).

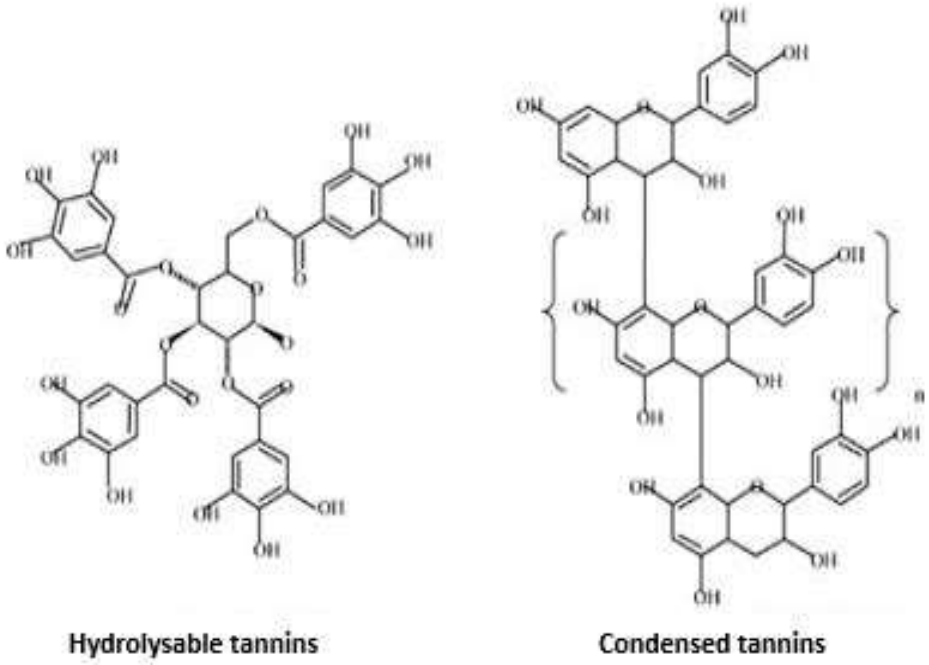

Figure 08: Tannins sections (Krause, 2005).

c) Antioxidant and other biological activities of polyphenols

It was noted that a diet rich in phenolic compounds plays an important role in human protection, as eating fruits, vegetables and grains known to be rich in phenolic compounds is linked to reduce the risk of many diseases such as cancer, cardiovascular disease, inflammation and many degenerative diseases (Duthie and Brown, 1994; Scalbert et *al*., 2005). The polyphenols are found to be powerful antioxidants and the antioxidant effect is related to the structure of the phenolic compounds (Rice-evans et *al*., 1996; Guo et *al*., 2009).

In addition to the known role of flavonoids in giving the color and aroma of the plant, these compounds work to control the growth and development of the plant by interfering in a complex way with plant growth hormones in addition to their primary role in protecting plants from bacterial and fungal infections. Flavonoids possess the ability to interfere with many biological activities, by inhibiting and reducing various enzymes such as; lipooxygenase, telomerase and cyclooxygenase and contribution to the cellular signal transduction pathways and the regulation of the cell cycle (D'archivio et *al*., 2007). These compounds also reduce inflammatory cytokines secreted by immune cells (Gao et *al*., 2001). Flavonoids stimulate programmed cell death and prevent cancer cell proliferation (Hirota et *al*., 2005). Thus, the flavonoids possess anti-inflammatory, antiviral, antibacterial, vasodilatory, anti-ischemic (Procházková et *al*., 2011), antilipoperoxidant, antitumoral, antiplateletand antiallergic activities. In addition, flavonoids exhibit strong antioxidant capacities through scavenging oxygen free radicals, promoting the expression of antioxidant enzymes in the antioxidant system or inhibiting the expression and activity of oxidase enzymes (Fraga et *al*., 2010; Fadel et *al*., 2011) (Figure 05). For these reasons, the biochemistry and medicinal aspects of flavonoids have received increasing attention recently (Wu et *al*., 2015).

d) The action mechanisms of polyphenols as antioxidant

* Direct scavenge of free radicals: Polyphenols are capable of reducing oxidizing free radicals ($R^{\bullet}$) such as superoxide, peroxyl radical, alkoxyl radical and $OH^{\bullet-}$ by hydrogen transfer (Delattre et *al*., 2005; Sandhar et *al*., 2011).

* Chelation of metal ions: Flavonoids are considered to be good chelators of these metal ions (Delattre et *al*., 2005; Verdan et *al*., 2011).

* Inhibition of lipid peroxidation: Polyphenols act against lipid peroxidation in two ways: by protecting target lipids against initiators of oxidation or by stabilizing the propagation phase. In the first case, antioxidants so-called preventives inhibit the formation of ROS or eliminate reactive species responsible for initiation of oxidation such as $O_2^{\bullet -}$, $^1O_2$ and OH. In the second case, antioxidants so-called chain breakers generally lose a hydrogen atom to favor of oxidation propagating radicals (LOO^-) to stop the spread of peroxidation (Delattre et *al*., 2005; Laguerre, 2007).

* Enzymatic inhibition: Flavonoids are able to inhibit a wide range of enzymes that generate $O_2^{\bullet -}$ and other ROS, such as xanthine oxidase, protein kinase C (PKC), cyclooxygenase, lipooxygenase, microsomal monooxygenase, and glutathione S-transferase (GST). Flavonoids having a catechol moiety on the B cycle inhibit mitochondrial succinoxidase and NADH oxidase (Pietta, 2000; Sandhar et *al*., 2011).

* Polyphenols can also act as co-factor of other antioxidants by regenerating vitamins (Zhou et *al*., 2005)**.**

* Polyphenols stimulate antioxidant enzymes, glutathione peroxidase, catalase, superoxide-dismutase (Du et *al*., 2007).

e) The relationship between chemical structure and antioxidant properties

The antioxidant activity is related to the structure of the phenolic compounds. The chemical structure of polyphenols that allows it to giving electrons or hydrogen atoms, makes it powerful antioxidants. Where, the addition of the hydroxyl group at the carbon 3 of the flavonol makes them potent antioxidants (Rice-evans et *al*., 1996; Guo et *al*., 2009). It has been found that flavonoids chelate the minerals in the presence of a group 3', 4'- o-diphenol at sites dihydroxy of B ring (Figure 09 a) and structure 4-keto with 3-hydroxyl of C ring (Figure 09 b) or 4-keto with 5-hydroxy of C and A ring (Figure 09 c) (Rice-evans et *al*., 1997).

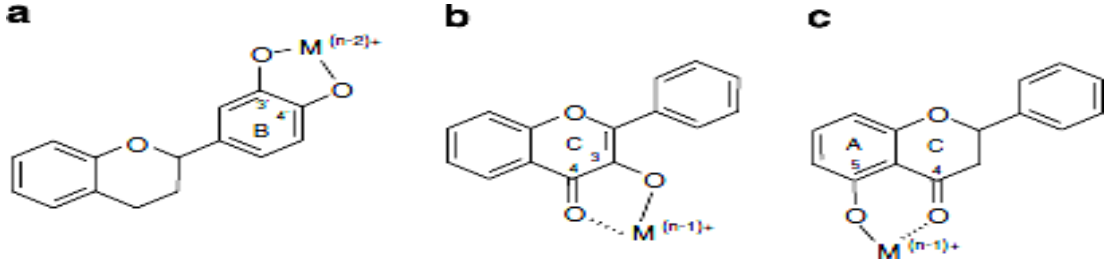

Figure 09: Iron chelates sites by flavonoids (Laguerre et *al*., 2007).

The scavenging capacity of the flavonoids is related to the C-ring structure and the type and number of groups attached to the B and C rings, where they give a free electron to be called semiquinone radical, and then quinone (Cotelle et *al*., 2001) (Figure 10). Most of the sites that affect scavenging are:

1) The structure ortho-dihydroxy in B ring, which has the best electron-giving properties, giving it greater stability and participates in electron transfer between the hydroxyl groups of the ring B (Balasundram, 2006).

2) The double bond between C2 and C3 is associated with the 4-oxo function in the C ring responsible for transferring electrons from ring B (Balasundram, 2006).

3) Combination of the hydroxyl groups in 3 and 5 with 4-oxo of the A and C rings for maximum activity. Thus, strong antioxidant activity requires the presence of 3-OH combined by the double bond (2-3) and the carbonyl function in the ring C (Rice-Evans et *al*., 1996).

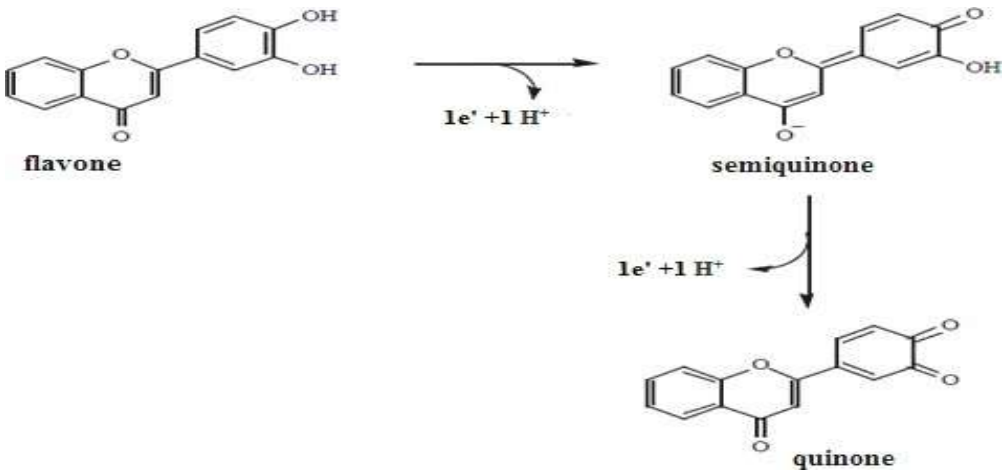

Figure 10: Mechanism of the free radical scavenging effect of flavonoids (Cotelle et *al*., 2001).

There is a relationship between the chemical structure of flavonoids and their XO inhibitory activity. Only flavonols and flavones have the ability to inhibit this enzyme, this indicates the importance of the double bond (3-2), and by comparing the activity of flavonols and flavone, it is confirmed that the absence of the OH group in carbon 3, slightly increases this inhibition ability, and it was found that the flavonoids associated with sugars have less activity than the compounds non-associated with sugars in the inhibition process (Cos et *al*., 1998).

All of the flavonoids quercetin, morin, rutin, dihydroquercetin (taxifolin), apigenin, catechin and hesperidin are known for their anti-viral activity against 11 types of viruses. This

activity is related to the presence of non-sugar flavonoids and flavonoids with hydroxyl group in site 3 (Tapas et *al*., 2008). It was also found that flavonols are more effective than flavones against *Herpes simplex* virus (Tim Cushnie and Andrew, 2005). The consumption of fruits and vegetables rich in flavonoids from a type of flavonol "quercetin" it protects against many types of cancer (lung, prostate, stomachand colon). Flavonoids affect as anti-cancer by inhibiting gene expression of P53 (kumar and Pandy, 2013).

f) The oxidant effect of polyphenols

Polyphenols are antioxidant, inflammatory and anticancer compounds, but high concentrations of them and under certain conditions, these compounds can affect in a negative way as catalysts of oxidation (Rucinska et *al*., 2007). Galati et *al*., (2002) note that excessive intake polyphenols can be converted by the enzyme peroxidase into the oxidized form (phenoxyl radical) which in some cases is sufficiently effective for oxidizing GSH and NADH associated with a large oxygen consumption and thus the formation of ROS. Also polyphenols containing catechol rings oxidize ascorbic acid by the intervention of semiquinone radical resulting from oxidation of flavonoids. Experiments also showed that incubating hepatocytes in the presence of polyphenols with multiple phenolic rings leads to partial oxidation of GSH to GSSG present in these cells while the phenolic compounds with catechol rings oxidize all GSH by converting it to conjugate GSH. Phenolic compounds with multiple rings act on the oxidation of oxyhemoglobin protein existing in human red blood cells, causing their degradation more and faster than the presence of phenolic compounds with one catechol ring, and thus phenolic compounds with multiple phenol rings are considered generally more inciting on oxidation than phenolic compounds with catechol single-ring. The enzymatic and chemical oxidation of flavonoids lead to the formation of semiquinones radicals which can be removed by GSH and thus the flavonoids are regenerated but with the production of a new type of radicals resulting from oxidation GSH (Thiyl radical). The latter interacts with another GSH molecule to form a disulfide radical which rapidly reduces molecular oxygen (O_2) and forms ($O_2^{\bullet -}$) radicals (Rietjens et *al*., 2002). In another study, it has been shown that flavonoids can induce oxidative stress at the DNA level by producing free radicals in the presence of copper and molecular oxygen. Itcan also catalyzes lipid peroxidation in the presence of minerals Al, Mg, Zn and Ca (Sakihama et *al*., 2002).

2. Alzheimer's, hyperpigmentation and diabetes mellitus diseases and their relation with oxidative stress

2.1. Alzheimer's disease

2.1.1. Definition

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that is clinically characterised by cognitive dysfunction and disturbances in behaviourand personality. With its late-onset and age dependency, AD affects over 10% of people over the age of 65 years, accounting for approximately 70% of dementia cases, with no cure or adequate treatments yet available (Cassidy et *al*., 2020). A study performed by Prince et *al*., (2013), estimated that a total of 47 million individuals worldwide suffered from dementia, with that statistic expected to rise to over 110 million people by 2050.

2.1.2. Factors

Alzheimer's disease is a complex multifactorial disorder resulting from genetic, epigenetic and environmental risk factors (Tanzi and Bertram, 2005).

2.1.3. Molecular mechanism of Alzheimer's disease pathophysiology

The molecular mechanism of AD pathophysiology emanates from the deposition of amyloid β (Aβ) plaques and the formation of hyperphosphorylated tau proteins (p-tau) causing neurofibrillary tangles (NFTs) (Bloom et *al*., 2014) which associated with significant presence of oxidative damage.

2.1.3.1. The role of Amyloid β in Alzheimer's disease

Amyloid β is a product of the sequential cleavage of amyloid β precursor protein (APP) by the β-secretase (Yonemura et *al*., 2016). APP is a transmembrane protein that plays a significant role in neuronal development and growth and anterograde axoplasmic transport trafficking (Musardo and Marcello, 2017). The Aβ peptides released by cleavage are free to undergo oligomerization and subsequent fibrillization to form the characteristic β amyloid plaques visible in the atrophied brain tissue of deceased AD patients (Kametani and Hasegawa, 2018). Aβ42 appears more neurotoxic than Aβ40 or Aβ38 as Aβ42 is more prone to oligomerization, potentiating its ability to exert cytotoxic effects (Dimitrov et *al*., 2013; Siegel

et *al*., 2017). Conversely, Panza et *al*., (2019) propose that elevated Aβ may be associated with the brain's response to injury and its attempt to mitigate or repair the insult, emphasising that AD-related neuronal death causes increased Aβ and not vice-versa.

2.1.3.2. The role of tau in Alzheimer's disease

Tau proteins are crucial to the physiological functioning of neurons, as they are responsible for microtubule polymerisation, microtubule stabilisation, facilitation of the transportation of organelles and enzymes along the cellular cytoskeleton, and promotion of neuronal growth (Cassidy et *al*., 2020). Tau may undergo numerous post-translational modifications, including phosphorylation by kinases such as JKN, AMPK (AMP-activated protein kinase) and GSK-3β, as tau proteins possess over 30 potential phosphorylation sites due to a high number of serine and threonine phosphate accepting residues (Chow et *al*., 2010; Sery et *al*., 2013). Hyperphosphorylation of tau proteins results in large amounts of aggregation forming tau oligomers and eventually paired helical filaments or straight filaments which constitute NFTs. NFT formation contributes to neuronal toxicity by decreasing tau affinity for microtubules (Evans et *al*., 2000). The mechanism of tau-mediated neurotoxicity occurs via two major mechanisms: toxic loss of function, in which physiological tau protein loses function causing microtubule destabilization (Andorfer et *al*., 2005) and toxic gain of function, in which highly phosphorylated tau display the toxic effects in neurons (Cassidy et *al*., 2020).

2.1.3.3. The role of oxidative stress in Alzheimer's disease pathogenesis and progression

Oxidative stress has been demonstrated to be a major contributor to the progression of Alzheimer's disease (Chen et *al*., 2012). For over twenty years, oxidative stress has been recognised and discussed as a major factor in AD pathogenesis, with several review articles highlighting the critical role that ROS play in AD pathology (Cheignon *et al.*, 2018; Cassidy et *al*., 2020). In the atrophied brains of patients with AD, there is often significant oxidative damage to neuronal tissue (Valko et *al*., 2005; Gella and Durany, 2009). Oxidative stress contributes to AD progression in 3 main ways: macromolecule peroxidation, Aβ metal ion redox potential and mitochondrial dysfunction, all of which have an effect on cell homeostasis, the generation of ROS and the up-regulation of Aβ and p-tau formation (Figure 11) (Cassidy et *al*., 2020).

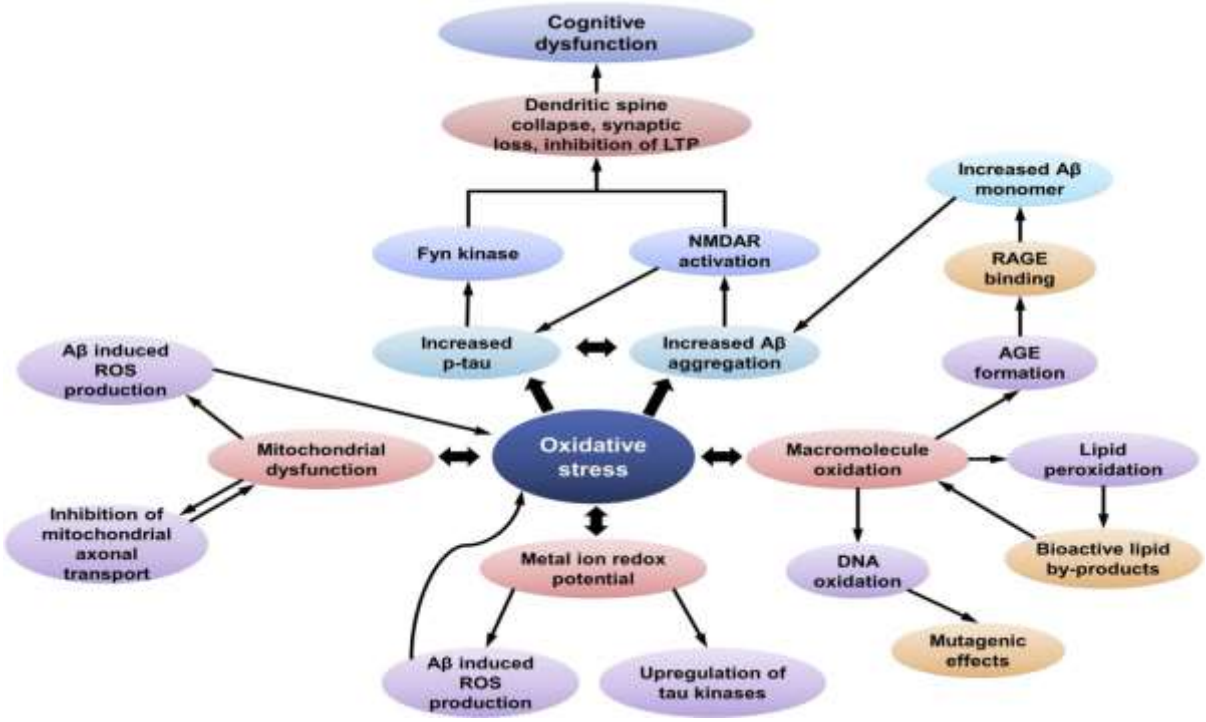

Figure 11: The interplay between oxidative stress and Alzheimer's Disease (AD) (Cassidy et *al*., 2020)

ROS; reactive oxygen species, p-tau; hyperphosphorylated tau protein, Aβ; amyloid beta, AGE; advanced glycated end products, RAGE; receptor for advanced glycated end products, NMDAR; N-methyl-D-aspartate receptors, LTP; long-term potentiation.

a) Macromolecule oxidation

Lipid peroxidation, interruption of DNA protein cross-links and oxidative cellular damage have been demonstrated to accelerate the process of ageing and numerous chronic diseases including diabetes, some cancers, and degenerative diseases including Parkinson's disease and AD (Lobo et *al*., 2010; Al-Abd et *al*., 2015). Peroxidation of the double bonds in polyunsaturated neuronal lipid products results in the generation of biochemically active lipid byproducts including 4-hydroxy-2, 3-nonenal (HNE), MDA and F2-isoprostanes (Bradley-Whitman and Lovell, 2015; Cassidy et *al*., 2020). These molecules are extremely reactive and are able to stimulate phosphorylation and dysfunction of tau, disruption of intracellular Ca^{2+} signalling pathway and induction of an apoptotic cascade (Cassidy et *al*., 2020). In addition, oxidative modification of lipids by ROS and generation of HNE were reported to be increased in neurons containing NFTs and in pyramidal hippocampal cells (Sayre Lawrence et *al*., 2002). Nucleotides present in nuclear and mitochondrial DNA show great vulnerability to oxidative

damage by ROS via hydroxylation, carbonylation and nitration (Cassidy et *al*., 2020). These epigenetic regulations lead to the alternation of transcription factor binding, interruption of crosslinking between DNA and protein, and the induction of mutagenic properties (Zawia et *al*., 2009; Santos et *al*., 2012). Oxidation of nucleotides produces biomarkers which can be used to determine the extent of oxidative damage, such as 8- hydroxydeoxyguanine (8−OHdG) which were found to colocalise with Aβ and p-tau formations in the temporal, parietal and frontal lobes (Cassidy et *al*., 2020). Finally, ROS has been demonstrated to accelerate the oxidation of glycated proteins, which are formed by the reaction of saccharides with proteins. These advanced glycation end products (AGEs) are potent neurotoxins and proinflammatory species, as they are able to bind to the receptor for advanced glycation end products (RAGE) to induce the release of inflammatory mediators such as nitrous oxide, interleukin-1 and tumour necrosis factor-α, further facilitating neuronal tissue damage (Wong et *al*., 2001; Gella and Durany, 2009). Furthermore, physiological tau proteins are capable of being glycated and transformed into AGEs, inhibiting the ability to bind to microtubules and contributing to the formation of higher order fibril generation. Similarly, glycation and AGE formation from Aβ monomers have been demonstrated to enhance the formation of oligomerization and higher order plaque formation by improving Aβ cross-linking (Cassidy et *al*., 2020).

b) Metal ion redox potential

Metal ions such as copper, zinc and iron are involved in biological functions of metalloproteins and in neuronal processes (Cheignon et *al*., 2018). Metal ion homeostasis is disrupted in neurodegenerative diseases, such as AD, with ion concentrations and distribution deviating from physiological levels. In AD brains, neuronal Cu^{2+} and Zn^{2+} levels can increase up to three times the physiological value of healthy control brain (Kozlowski et *al*., 2012). Cations Zn^{2+} and Cu^{2+} are able to bind to the hydrophilic N-terminal ends of Aβ peptides, where they are able to undergo continuous redox reactions, generating substantial amounts of ROS, creating a positive feedback loop of increased oxidation and increased ROS production (Barnham et *al*., 2003; Chen et *al*., 2012). Oxidised Aβ protein can accumulate in the synaptic cleft, where it reacts with the high concentration of Zn^{2+} ions to form a precipitate of toxic Aβ peptides unable to exit the synapse (Atwood et *al*., 2003). The binding of metal ions to Aβ monomers allows for easier aggregation into neurotoxic oligomers and eventually into plaques. In a similar fashion, metal ions are able to interact with tau proteins. It has been demonstrated that Zn^{2+} is able to bind tau, promoting its phosphorylation (Cristóvão et *al*., 2009). Likewise, Mo et *al*., (2009) found that even micromolar concentrations of Zn^{2+} ions induce and accelerate

tau fibrillization and paired helical filament formation through the upregulation of kinases that phosphorylate tau, such as GSK-3β, and inhibiting kinases responsible for the dephosphorylation of tau proteins, such as protein phosphatase 2A (Sun et *al*., 2012). Furthermore, Fe^{3+} can bind to p-tau monomers, inducing aggregation and oligomer formation (Bader et *al*., 2001). Accumulation of Fe^{3+} ions in NFTs allows them to undergo continuous Fenton redox reactions, generating significant amounts of ROS and causing further intracellular oxidative stress (Cassidy et *al*., 2020).

c) Mitochondrial dysfunction

Under physiological conditions, ROS can be derived from complexes 1 and 3 in the mitochondrial electron transport chain, where electrons can be directly donated to molecular oxygen to form a superoxide anion (Murphy Michael, 2009). To counter this, mitochondria possess a diverse antioxidant defence mechanism for catalysing ROS, including such enzymes as cytochrome C oxidase (Reddy, 2005). A study performed by Mutisya et *al*., (1994) reported a severe decrease in cytochrome C oxidase in the hippocampi of AD patients. In addition, Aβ oligomers that accumulate in the inner mitochondrial membrane continue to disrupt key electron transport chain complexes and subsequently increase the formation of ROS beyond the capacity of mitochondrial defence mechanisms (Picone et *al*., 2014; Hawking, 2016). Aβ is able to enter the mitochondria via two potential mechanisms, which include increased expression of proteins associated with the mitochondrial-associated endoplasmic reticulum membrane (MAM), and translocation via the translocase of the outer membrane (TOM) complex (Islam et *al*., 2019). The result of Aβ induced mitochondrial dysfunction is the generation of substantial amounts of ROS in the intracellular compartment, causing a vicious cycle that further facilitates AD pathology (Picone et *al*., 2014; Hawking, 2016). Mitochondrial axonal transport is impaired in AD brains due to hyperphosphorylated tau dissociating from microtubules. Physiologically, anterograde and retrograde mitochondrial trafficking occurs in neurons to transport functional and dysfunctional mitochondria via kinesin and dynein motor proteins (Sheng and Cai, 2012). However, accumulation of excess p-tau acts as obstacles, affecting anterograde and retrogradetransport of mitochondria, resulting in oxidative stress inside neurons (Sheng and Cai, 2012; Sery et *al*., 2013).

2.1.3.4. Amyloid β and p-tau upregulation and toxicity

Oxidative stress has been shown to upregulate both tau phosphorylation and Aβ production (Guglielmotto et *al*., 2009). Increased Aβ processing and peptide aggregation may perturb the lipidmicroenvironment surrounding nicotinic acetylcholine receptors resulting in modification of their function (Fabiani and Antollini, 2019). The result of increased Aβ and p-tau generation and aggregation into oligomers due to ROS production and oxidative stress is the toxic gain of function by the migration of species to dendritic spines, disrupting functional receptors on the postsynaptic membrane and the eventual activation of proteins causing the dendritic spine collapse and loss of synapse (Nisbet et *al*., 2015) .Aβ oligomers are able to act on extrasynaptic Nmethyl-D-aspartate receptors (NMDAR), causing Ca^{2+} influx into the neuron resulting in enhanced activation of tau protein kinases such as AMPK and GSK-3β (Parsons and Raymond., 2014). Increased tau phosphorylation also causes p-tau to bind to Fyn kinase, causing migration of p-tau to the dendritic neuronal spines (Nisbet et *al*., 2015). Furthermore, Aβ and Fyn-bound p-tau have synergistic effects, resulting in decreased surface NMDARs and eventual death of the dendritic spine and synaptic loss, resulting in inhibition of hippocampal long-term potentiation, thus affecting memory consolidation (Musardo and Marcello, 2017). Oxidative stress produces a positive feedback loop that increases ROS production, Aβ generation and tau phosphorylation leading to further oxidative stress. Ultimately, this pathophysiological cascade of neurodegeneration results in substantial neuronal damage (Gella and Durany, 2009). Therefore, it is evident that the interplay between oxidative stress, p-tau and Aβ is crucial to the clinical effects displayed by AD patients (Cassidy et *al*., 2020).

2.1.4. Current Alzheimer's disease treatment strategies and future prospects

The current treatment options for AD are limited in efficacy and scope, with these therapies mainly focussing on the treatment of mild to moderate AD symptoms. For instance, tacrine, approved for AD treatment in 1993, provided very mild improvements in cognitive function, but included adverse side effects (Tumiatti et *al*., 2010; Simunkova et *al*., 2019). Similarly, physostigmine, the first cholinesterase inhibitor (ChEI) for AD treatment, was abandoned due to its adverse effects (Orhan et *al*., 2009). As a result, more drugs, such as donepezil, rivastigmine, galantamine, and memantine, bapineuzumab, and solanezumab, were designed and approved for use after 1995 (Deardorff and Grossberg, 2016; Wang et *al*., 2016; Simunkova et *al*., 2019). Donepezil (5−10 mg/day), rivastigmine (1.5−6 mg/day) and galantamine are selective acetylcholinesterase (AChE) inhibitors, aiming to delay the

progression of AD symptoms by restoring cholinergic pathways in the forebrain and cortical areas (Herholz, 2008; Hawking, 2016). Memantine (5−20 mg/day) is an NMDA receptor antagonist that prevents excess levels of glutamate from binding, inhibiting excitotoxicity (Zhu et *al*., 2013). Bapineuzumab, and solanezumab are anti-Aβ monoclonal antibodies (Wang et *al*., 2016). However, these medications do not have the ability to fully alleviate AD pathology, acting only to slow symptom progression and possessing adverse side effects (e.g. nausea, hepatotoxicity, bradycardia, and diarrhoea), and with some patients exhibiting resistance to treatment entirely (Lleo et *al*., 2006). Although these compounds may have potential in combination therapies, current regimes possessing a single one of these drugs do not provide suficient eficacy for the management of AD (Folch et *al*., 2016). Therefore, there is a need to explore new sources of drugs that may possess protective effects against cognitive decline in AD that are eficacious, well-tolerated by patients and do not have adverse side effects (Folch et *al*., 2016). Due to the extensive damage resulting from oxidative stress and ROS production on the brains of patients suffering from AD, the suggestion that antioxidant administration may have potent neuroprotective effects against neurodegenerative disorders is topical (Chen et *al*., 2012; Alavi Naini, 2015; Hawking, 2016). There have been numerous studies examining the eficacy of severalantioxidant substances in various *in vivo* AD models, with some displaying encouraging results (Cassidy et *al*., 2020). Desferrioxamine B, a compound with potent Fe ion chelating capacity, led to a statistically signifcantreduction in cognitive decline in human patients, decreased Aβ levels and decreased metal ion overload in the brain (Cassidy et *al*., 2020). Similarly, Clausen et *al*., (2012) found that the administration of EUK-207, a synthetic superoxide dismutase mimetic with potent antioxidant properties, to a 3xTg-AD transgenic mouse model of AD resulted in a decrease in oxidative stress biomarkers, disease phenotypes and AD biomarker accumulation in the amygdala and hippocampus of mice with late-stage AD. Treatment with methylene blue, a phenothiazine that has electron donating and redox cycling properties, showed reduced HNE production and oxidative stress, promotion of mitochondrial biogenesis and decreased tau hyperphosphorylation in a P301S transgenic mouse model (Stack et *al*., 2012). Finally, Elipenahli et *al*., (2012) demonstrated that the administration of coenzyme Q10, a vital component of the electron transport chain with potent antioxidant characteristics, to a P301S tau mutated transgenic mouse model reduced oxidative stress, upregulated key complexes in the electron transport chain, and increased neuronal survival rates and cognitive behavior. These synthetic antioxidant compounds display promising outcomes as potential treatment for AD pathology. However, there has been an increased interest in the use of naturally derived substances in the management of AD due to the pleiotropic effects, superior

tolerability and wider availability of natural products; and in response to the lack of eficacy and serious side effects of current synthetic agents (Cacabelos, 2018).

2.1.4.1. Naturally derived drugs and therapeutics

There are thousands of potential plant-derived compounds that could possess potent physiological action to treat chronic incurable diseases. For instance, the use of plant extracts in Alzheimer's disease (AD) prevention and treatment could be the key to alleviating the symptoms of this devastating and currently untreatable neurodegenerative disorder. Medicinal plant extracts not only have the potential for multi-targeted effects, which are advantageous for complex disease, but can also complement existing drug treatments for greater synergistic outcomes (Czarnecka et *al*., 2018; Simunkova et *al*., 2019).Using a diet supplemented in antioxidant-rich α-tocopherol, a type of vitamin E, there are studies in a Tg2576 transgenic mouse model and one study in a T44 human tau isoform transgenic mouse model demonstrated improved cognitive function, a decrease in mortality and amelioration of higher order tau and Aβ aggregates in both animal models (Nakashima et *al*., 2004; Sung et *al*., ., 2004).The mechanism of α-tocopherol mediated neuroprotection is due to the prevention of Aβ-induced ROS production, reducing p-tau and Aβ aggregation and activation of the PP2A tau kinase (Abdel Moneim, 2015). The principal compound present in the rhizomes of turmeric (Curcuma longa), curcumin (a hydrophobic polyphenol), has been investigated *in vitro* and *in vivo* (Priyadarsini, 2014; Alavi Naini and Soussi-Yanicostas, 2015). The antioxidant properties of curcumin derive from its β-diketone structure and phenolic groups, which curcumin uses to scavenge free radicals (Gulcin, 2008). Park et *al*., (2008) demonstrated protective effects of curcumin in a PC12 cell line against Aβ induced toxicity through the inhibition of oxidative damage and tau hyperphosphorylation. Whereas, the *in vivo* study performed on a pharmacological AD mouse model showed that curcumin displayed a signifcant neuroprotective properties by scavenging okadaic acid-induced ROS production (Rajasekar et *al*., 2013). Moreover, curcumin administration to several Drosophila AD genotypes displayed a reduction in Aβ oligomerinduced neurotoxicity (May Brian et *al*., 2017). These studies show promising results for the use of *Curcuma longa* extracts as a treatment for AD *in vitro* and *in vivo*. The beneficial effects displayed by extracts are derived from phytochemicals, which are substances produced by metabolic pathways in plants (Randhir et *al*., 2004; Kim et *al*., 2010). Of the medically signifcant phytochemicals, polyphenolic compounds have been demonstrated to be the most eficient due to their ability to scavenge and inhibit ROS, donate hydrogen atoms

or electrons, chelate metal cations and scavenge electrophiles (Sharma et *al*., 2012). Therefore, extracts of plants containing high concentrations of phenolic compounds may have potential neuroprotective effects against cognitive decline in AD. There have been numerous studies conducted using naturally derived polyphenols that have shown diverse neuroprotective effects (Kim, 2010).

a) Polyphenolics as antioxidants in Alzheimer's disease

As antioxidants, polyphenolic compounds are able to protect tissue by quenching endogenous oxidants or inhibiting the production of highly ROS (Ratnam et *al*., 2006; Cirillo et *al*., 2016). Critically in the case of neuronal oxidative disorders, polyphenols and metabolic products have been shown *in vitro* to cross the blood-brain barrier and exert neuroprotective effects (Figueira et *al*., 2017; Johnson et *al*., 2019). In a tissue system perturbed by redox imbalance, such as that of the AD brain, there is the potential for antioxidant compounds to have a positive effect on disease progression and possibly disease onset (Soobrattee et *al*., 2005). For this reason, the review of Cassidy et *al*., (2020) focussed specifically on polyphenol compounds in relation to oxidative stress in AD. This suggested that naturally occurring compounds, such as plant polyphenols have potential neuroprotective effects against AD due to their diverse array of physiological actions, which includes potent antioxidant effects. Flavonoids are polyphenolic compounds that naturally occur in plants. Flavonoids may exhibit ROS scavenging and redox metal-chelating properties, and interact with signal transduction in neuron and glial cells (*via* PI3K/Akt, MAPK, PKC, tyrosine kinase pathways) (Spencer, 2010; Simunkova et *al*., 2019). The potential use of flavonoids in AD treatment may be limited to the early stages, however, hybrid containing flavonoids may improve cognitive function in the later stages (Sureda et *al*., 2017; de Andrade Teles et *al*., 2018). Examples of flavonoid type drugs include resveratrol, myricetin, morin, rutin, apigenin, and quercetin (Cassidy et *al*., 2020). Resveratrol, a stilbene compound extracted from plant sources, such as pine trees and cocoa bushes, has potent ROS scavenging capacity. In a study of its neuroprotective effects, oral administration of resveratrol (10 and 20 mg/kg over 25 days) to a colchicine-induced AD rat model reduced lipid peroxidation and improved cognitive function (Kumar et *al*., 2007). Myricetin, morin, rutin, apigenin, and quercetin (0.1–1 μM) all exhibit anti-amyloidogenic and Aβ fibril-destabilisation properties (Ono et *al*., 2003). It has been suggested that the higher number of hydroxyl groups on these flavonoids, the higher their potential for anti-Alzheimer's disease activity (Ono et *al*., 2003). Quercetin, present in apples, onions and berries, displays

anti-amyloidogenic effects via ROS scavenging, inhibition of Aβ oligomer formation, and decreased Aβ-stimulated apoptosis in primary rat neuronal cultures (Ansari et *al*., 2009). Rosmarinic acid is a polyphenol carboxylic acid present in herbs, such as rosemary and oregano, and possesses ROS scavenging capacity. In anAβ-stimulated mouse model, administration of rosmarinic acid (0.25, 1, 2, and 4 mg/kg day, i.p.) prevented Aβ-25-35 induced memory impairment (Alkam et *al*., 2007). The action of rosmarinic acid is thought to directly mitigate oxidative damage induced by Aβ, through quenching peroxynitrites and reducing ROS formation, caspase-3 activation, and tau hyperphosphorylation (Iuvone et *al*., 2006; Alkam et *al*., 2007). There is still a plethora of research being conducted on the potential protective effects of numerous other natural substances, providing exciting future prospects for potential AD therapy (Hassaan et *al*., 2014). However, further human clinical trials and research into the intracellular and molecular targets of bioactive polyphenols in the brain will be necessary to confirm the efficacy of these natural substances (Singh et *al*., 2008).

b) Acetylcholinesterase inhibitors as treatment of Alzheimer's disease

Acetylcholinesterase (AChE) is an acetylcholine hydrolase enzyme with esterase activity, localized in the synaptic gaps of the central and peripheral nervous system. This membrane-bound enzyme is projected into the synapse and terminates nervous impulses by catalysing the hydrolysis of the neurotransmitter acetylcholine, a neurotransmitter of cholinergic system, which amongst other roles is involved in memory formation (Roseiro et *al*., 2012). Loss of acetylcholine signalling in the brain correlates with the severity of AD, the most common form of dementia in elderly people. There are several AChE inhibitors that have been designed and clinically approved to inhibit the breakdown of acetylcholine in the brain, thus leading to increases in cholinergic neurotransmitter activity and for alleviating the symptoms of AD (Hashimoto et *al*., 2003; Salawu et *al*., 2011).AD is a complex biochemical process, for which there is no cure. The administration of cholinesterase inhibitors is based on the cholinergic hypothesis, briefly meaning that the loss of cholinergic function in the central nervous system is associated with the cognitive decline in AD (Mesulam, 2004). This loss in cholinergic function leads to a gradual loss of functional neurons in the brain of elderly people (Osborn and Saunderds, 2010). Many factors may be associated/correlated with this loss of cholinergic activity, including amyloid- peptide (A) formation and tau protein aggregation reviewed in (Suh and Checler, 2002), excessive transition metals (Hedge et *al*., 2011) and oxidative stress (Mohandas et *al*., 2009). Interestingly, AChE inhibitors have been observed to

affect the "amyloid cascade" (Racchi et *al*., 2005), in which insoluble A-peptide is deposited in the brain (Ohnishi and Takano, 2004). However, AChE may also be involved in the formation of A-peptides (Roseiro et *al*., 2012) accelerating its formation and it appears that the (peripheral anionic site) PAS of the enzyme is crucial for this activity (Talesa, 2001). Although surgical interventions have been used effectively to treat patients suffering from AD by improving cerebral blood flow (CBF) (Goldsmith, 2011), presently invasive surgical intervention is not an option in all patients and other mechanisms are also not wholly effective (Ji and Zhang, 2006). Thus there is still a requirement for additional drug treatments such as AChE inhibitors. Acetylcholinesterase inhibitors (AChEI) are used clinically to counteract various pathologies, including AD (Racchi et *al*., 2004). The active site of AChE containsPAS (Roseiro et *al*., 2012). Several studies have revealed that binding to the PAS sub-site could change the conformation of the active site, thus inhibiting enzyme activity (Akhtar et *al*., 2011). Treatment is known to improve symptoms by enhancing cholinergic functions and increasing the amount of acetylcholine present in cholinergic synapses. To be effective, inhibitors must reversibly bind to the active site of the enzyme as irreversible binding may lead to severe consequences, including death (Quinn, 2011). The use of reversible AChEI has attracted particular attention, in particular for the treatment of Alzheimer-type dementia (Racchi et *al*., 2004). However, synthetic AChEI such as physostigmine, tacrine and donepezil have been reported to have adverse effects, such as hepatotoxicity and GI complaints. Numerous plant extracts have been investigated for their potential to treat cognitive disorders and neurodegenerative diseases. Galantamine was the first compound isolated from a plant source that was found to possess powerful AChEI activity, and although it was not without its secondary effects it is in clinical use. As such, there is interest in the development of new AChEI and among these non-alkaloid compounds, including polyphenols have emerged as promising candidates (Vermerris et *al*., 2006).

- **Polyphenols as acetylcholinesterase inhibitors**

Polyphenols such as flavonoids have been investigated for their AChE inhibitory activity (Uriarte-Pueyo and Calvo, 2011; Roseiro et *al*., 2012), along with other phenolic compounds (Khan et *al*., 2009; Uriarte-Pueyo et *al*., 2010; Uriarte-Pueyo et *al*., 2011). A survey of medicinal plants and their extracts in order to evaluate their medicinal and therapeutic potential have revealed that there are many polyphenols that have a potential to act as AChEI (Roseiro et *al*., 2012) (Table 01). With regards to the AChE inhibition, the flavanone naringenin, a major

flavonoid present in citrus has been shown to exert AChE inhibitory activity *in vitro* and anti-amnesic activity *in vivo* (Heo et *al*., 2004). The flavonol quercetinhas not been tested for its inhibitory activity *in vivo* but also appears to influence cerebral blood flow and cholinergic dysfunction in brain (Tota et *al*., 2010). The authors suggested that consumption of a diet rich in quercetin may prevent dementia associated with vascular and neurodegenerative disorders, such as AD. However, whilst various polyphenols have been proposed to inhibit AChE activity (Table 01). Future work is required to establish whether these compounds reach the brain and directly affect AChE activity (Roseiro et *al*., 2012). Intervention with a water-soluble extract of propolis led to significant inhibition of hippocampal AChE activity along with cognitive improvements that may be mediated by apigenin, kaempferol and luteolin (Chen et *al*., 2008). In addition, a decoction extract of *Pseuderanthemum palatiferum*, a polyphenol-rich tea used predominantly in Thailand, has also been shown to inhibit AChE in the brain (hippocampus), serum and red blood cells in rats (Buncharoen et *al*., 2010). An extract of the more widely consumed green tea (0.5%; containing 60% flavanols) was effective in enhancing learning and memory in aged rats and also the selective inhibition of AChE (Kaur et *al*., 2008). These results are in agreement with other studies in scopolamine-treated mice, where tea polyphenols significantly reversed cognitive deficits and exhibited a dramatic inhibitory effect on AChE activity (Kim et *al*., 2004). The studies performed on the water extracts of olive pomace and carob kibbles, indicate that both extracts are rich in polyphenols and express significant AChE inhibitory action (Roseiro et *al*., 2012). Rosmarinic acid, the main phenolic compound found in *Plectranthus barbatus* decoction, (consumed as an herbal tea), was detected in the brain of rats, where AChE activity was decreased by 30% in comparison with control animals (Fale et *al*., 2011). These results indicate that rosmarinic acid may permeate the blood brain barrier and act as an AChEI in the brain (Roseiro et *al*., 2012). The diets rich in phenolic compounds might significantly influence memory and cognition, and that part of this action may be mediated by actions on AChE activity (Roseiro et *al*., 2012). Lastly, phytochemical-rich foods also contain compounds other than polyphenols, such as terpenes and alkaloids that may also be effective as AChEI either alone or in synergy with phenolics (Roseiro et *al*., 2012). Of course, the neuroprotective effects attributed to the polyphenols are not only mediated by their AChE inhibitory activity, but also by othermechanisms of action, namely, anti-inflammatory activity, protection against A-induced toxicity, Monoamine Oxidase-B (MAO-B) inhibiting potential, and interactions with brain cell signalling (Ji and Zhang, 2006). However, their ability to interact with and inhibit AChE may contribute to their reported physiological effects and

knowledge about this may add weight to the evidence regarding theirprophylactic/therapeutic potential to combat specific neurological disorders (Roseiro et *al*., 2012).

Table 01: Natural phenolic compounds as AChE inhibitors.

Compound	**Source**	**Reference**
Quercetin	Manyfruits, vegetables, beverages	(Jung and Park, 2007; Tota et *al*., 2010)
Curcumin	Tumeric	(Kennedy and Wightman, 2011)
Resveratrol	Red wine	(Kennedy and Wightman, 2011)
Naringenin	Citrus junus	(Heo et *al*., 2004)
Phlorotannins: 6,6-bieckol, diphlorethohydroxycarmalol	Marine brown algae (Phaeophyta)	(Singh et *al*., 2006)

- **Molecular mechanisms of acetylcholinesterase inhibition by the polyphenols**

There are only a few studies that define the molecular mechanisms of AChE inhibition by polyphenols (Roseiro et *al*., 2012). Furthermore, studies with choline derivatives of caffeic acid (Figure 12b) have revealed that the presence of a methyl group increases the inhibitory activity (Sebestik et *al*., 2011), as one of the methoxy groups orientates towards PAS sub-site, as is also the case with donepezil. Flavonoid orientation inside the enzyme gorge also looks similar to that presented by donepezil. Flavonoids have an aromatic ring (B-ring) (see figure 6), which contains OH groups that establish bonds with the PAS of the enzyme. Flavonoids appear not to induce modifications in the ternary structure of the enzyme, meaning that the inhibition process occurs due to the blockage of the entrance to the active site (Roseiro et *al*., 2012). The −OH group of the phenyl side (B-ring) shows hydrogen bonding with residues in the PAS sub-site. Such H-binding confers flavonoids with enhanced AChEI activity relative to other phenolic compounds like caffeic acid (Figure 12b) (Khan et *al*., 2009; Rcaffeicoseiro et *al*., 2012). In summary, it seems that phenolic compounds having structural motifs similar to caffeic

acid are capable of fitting into the gorge of the active site of AChE by positioning the aromatic ring into the PAS sub-site (Akhtar et *al*., 2011).

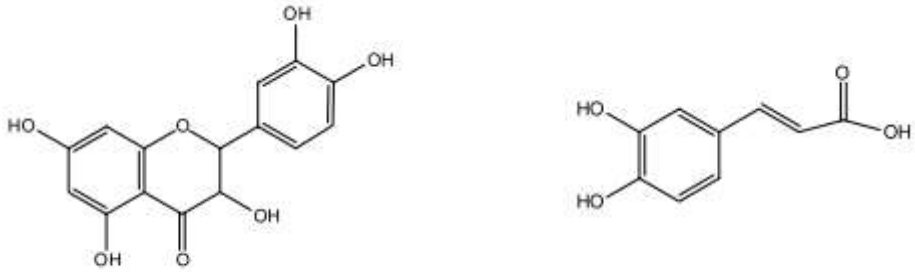

Figure 12: Structures of different phenolics compounds with AChE inhibition: a) flavonoid (quercetin), b) phenolic acid (caffeic acid) (Roseiro et *al*., 2012).

2.2. Hyperpigmentation

2.2.1. Definition

The hyperpigmentation (dark spots) on faces is a highly anxiety-producing symptom, especially for women from the aspect of beauty (Shigeta et *al*., 2004, Adhikari et *al*., 2008). It is a common skin disorder in the world regions where people have to be exposed to strong sunlight (Tengamnuay et *al*., 2006). Hyperpigmentation in the epidermis is the result of excessive melanin synthesis (Sugimoto et *al*., 2003). Epidermal and dermal hyperpigmentation can be dependent on the increased numbers of melanocytes and the activity of melanogenic enzymes. Moreover, ultraviolet (UV) light, chronic inflammation and rubbing of the skin as well as abnormal a-melanocyte stimulating hormone (a-MSH) release, are the triggering factors for these disorders (Im el al., 2002; Briganti et *al*., 2003). Melanin is the pigment responsible for the color of human skin and hair, secreted by melanocytes distributed in the basal layer of the epidermis (Nerya et *al*., 2003). Although melanin in human skin acts as a major defense mechanism against UV light from the sun, the production of abnormal pigmentation such as melasma, freckles, age spots, liver spots and other forms of melanin hyperpigmentation can be a serious aesthetic problem (Chang et *al*., 2005). Melanins, the end-products of complex multistep transformations of l-tyrosine (Adhikari et *al*., 2008). The synthesis of melanin starts with the conversion of l-tyrosine to l-3, 4-dihydroxyphenylalanine (L-DOPA). The subsequent oxidation of L-DOPA yields dopaquinone, which is the initial step in melanin synthesis. Tyrosinase is the key enzyme in the melanin biosynthesis and participates in the oxidation of

tyrosine to dopaquinone via L-DOPA (Ohguchi et *al*., 2003; Seo et *al*., 2003). Melanin biosynthesis can be inhibited by avoiding UV light exposure, by inhibiting melanocyte metabolism and proliferation, by inhibiting tyrosinase activity or by removing melanin by corneal ablation (Wang et *al*., 2006; Adhikari et *al*., 2008).

2.2.2. Hyperpigmentation factors

Hyperpigmentation of skin is caused by several factors. UV exposure, in addition to oxidative stress, elevates inflammatory mediators stimulating melanogenesis (Kanlayavattanakul and Lourith, 2018). Radical and inflammatory mediators are several factors that show accumulation during the hyperpigmentation of skin (Gillbro andOlsson, 2011; Kanlayavattanakul and Lourith, 2018).

2.2.3. Skin hyperpigmentation treatment

2.2.3.1. Natural treatment

Herbal-derived compounds for improving skin lightness are gaining interest as they are perceived to be milder, safer, and healthier than fully synthetic products (Kanlayavattanakul and Lourith, 2018).

a) Active ingredients for skin hyperpigmentation treatment

Skin-lightening agents, of which phenolics are the most widely used, can be employed in a formulation as either a single compound or a combination of actives. The application of herbal extracts containing several actives acting synergistically to improve the efficacy is also encountered in cosmetic formulations, with such actives proving highly desirable candidates (Kanlayavattanakul and Lourith, 2011; Lourith and Kanlayavattanakul et *al*., 2013). Commonly used agents are hydroquinone, vitamin C or ascorbic acid, arbutin, and kojic acid and derivatives thereof. Mulberry extracts are also used (Shin and Park, 2014; Ong and Maibach, 2017) in cosmetic formulations. Hydroquinone was one of the most commonly used agents for the treatment of skin hyperpigmentation. It inhibits the conversion of 3,4-dehydroxyphenylalanine (DOPA) to melanin by tyrosinase inhibition, and also RNA and DNA syntheses in melanocytic cells, and is responsible for melanosome degradation (Panich et *al*., 2011). A range of vitamins has been shown to exhibit skin-lightening effects, with the derivatives of these having enhanced stabilities and efficacies, and allowing for more facile dermal penetration. Melanin production is inhibited by vitamin C via the reduction of the ortho-

quinone, with its antioxidant activity triggering melanogenesis (Panich et *al*., 2011). Furthermore, vitamin C is able to reduce the oxidized form of melanin, resulting in skin brightening. Vitamin B3 in the amide form (niacinamide) is able to inhibit melanosome transfer from the melanocytes to the epidermal keratinocytes. Accordingly, increased skin lightness was observed in human subjects following application of 5% niacinamide over a 4-week period (Hakozaki et *al*., 2002). Arbutin (hydroquinone-O-β-D-glucopyranoside) and its phenolic derivatives (e.g. monobenzyl ether) are generally used as lightening agents in cosmetics (Kanlayavattanakul and Lourith, 2018). Kojic acid (5- hydroxy-2-(hydroxymethyl)-H-pyran-4-one) is a fungal metabolite, which acts to inhibit the catecholate activity of tyrosinase through copper chelation (Kanlayavattanakul and Lourith, 2018). Furthermore, it prevents the cascade conversion process of O-quinone to DL-DOPA, and subsequently to dopamine and its corresponding melanin (Kanlayavattanakul and Lourith, 2018). Also, several phenolics are emerging as potential actives for skin-lightness formulations (Kanlayavattanakul and Lourith, 2018). Gallic acid is non-cytotoxic to B16F10 melanoma cells at concentrations ranging from 12.5 to 100 μM, and exhibits significant IC_{50} values against melanin production and tyrosinase activity (18.3 and 24.8 μM, respectively) (Kanlayavattanakul and Lourith, 2018). Studies in zebrafish indicated that exposure to gallic acid resulted in decreased body pigmentation (Kumar et *al*., 2013). Topical application of gallic acid to UVB-hyperpigmented mice twice daily for four weeks resulted in skin-whitening effects comparable with those obtained for 50 μM kojic acid (Kanlayavattanakul and Lourith, 2018). Resorcinol derivatives are also gaining interest as lightening agents. 4-Butylresorcinol is a potent human tyrosinase inhibitor (Kolbe et *al*., 2013). Resveratrol has been shown to significantly decrease melanin production in B16F10 melanomas and be non-cytotoxic, even at 100 μM concentration. This phenolic suppresses the expression of α-MSH-induced tyrosinase. Its skin-lightening efficacy has been confirmed in animal models (guinea pigs) showing UVB-induced pigmentation, where topical application of 1% resveratrol solution (200 μL) for 2 weeks resulted in clear reductions in skin dullness (Lee et *al*., 2014). The lightening efficacy was confirmed by assessment of the reduction of the pigment index using the Dermalab combo system®, with these results being in accordance with histological studies highlighting the decreased melanin production on resveratrol treatment (Lee et *al*., 2014). Fatty acids are also promising actives for the treatment of pigmented skin. Oleic acid, linoleic acid, and linolenic acid were shown to decrease the levels of melanin synthesis and tyrosinase activity (Kanlayavattanakul and Lourith, 2018). Guinea pigs exhibiting UVB-induced hyperpigmentation were topically applied with oleic, linoleic, and linolenic acids daily for 3 weeks. A visible reduction in pigmentation was noticed after 2 weeks, with linoleic

acid treatment giving a lighter skin tone than linolenic acid (Kanlayavattanakul and Lourith, 2018).

b) Plants for skin hyperpigmentation treatment

Treating aesthetically displeasing skin disorders using naturally derived actives including herbal extracts is gaining interest amongst consumers due to their perception of safety (Kanlayavattanakul and Lourith, 2018). Tea (*Camellia sinensis*) extract, specifically of black tea, was investigated for its skin-brightening potential. Skin-lightening efficacy of black tea extracts was assessed in guinea pigs hyperpigmented by UVB irradiation. The results indicated that hydroquinone and black tea extracts were comparable (Choi and Kim, 2011). Catechins, especially (-)-epigallocatechin-3-gallate (EGCG), are the key actives present in tea extracts (Kim et *al*., 2004). Cellular melanin production was found to be significantly suppressed at low EGCG concentrations (1 μM), with activity being dose-dependent (Kim et *al*., 2004). Licorice (*Glycyrrhiza glabra*) is widely employed for the treatment of skin dullness, with its activity due to the presence of glabridin (Asl and Hosseinzadeh, 2008). Glabridin is a more potent tyrosinase inhibitor than kojic acid (Yamauchi, 2011), with additional inhibitory action in B16 melanoma cells and animal models (guinea pig) (Kanlayavattanakul and Lourith, 2018). In addition to glabridin, liquiritin isolated from licorice is also efficient at reducing dark spots on the skin, as examined in human volunteers (Amer and Metwalli, 2000). The extract from sea buckthorn (*Hippophae rhamnoides*) berries macerated in 70% methanol showed inhibitory effects against mushroom tyrosinase (Khan et *al*., 2013). Evening primrose (*Oenothera biennis*) saponified oil (12.5 μg/ml) significantly inhibited melanin production in B16F10 melanoma cells. A trial in three healthy men having UVB-induced hyperpigmentation on their forearms showed that visible reductions in skin pigmentation were observable after topical application of the oil twice daily for 2 months. Fatty acid components linoleic acid (65–75%), linolenic acid (7–10%), and oleic acid (9%) were deemed responsible for the skin-lightening effects (Koo et *al*., 2010). Extracts of rice (*Oryza sativa*), one of the world's important staples, have been widely used to treat skin disorders. Rice bran oil having a high content of essential fatty acids can be added to cosmetic remedies for hyperpigmentation. γ-Oryzanol, the key ingredient of rice bran oil, significantly inhibits cellular melanin production in B16F1 melanoma cells (Jun et *al*., 2012). In addition, rice phenolics such as p-coumaric acid, caffeic acid, ferulic acid, and gallic acid are of importance in skin treatments (Kanlayavattanakul et *al*., 2015; Shao and Bao, 2015). Rice panicles enriched with these phenolics inhibited mushroom tyrosinase and suppressed melanogenesis in B16F10 melanoma cells via tyrosinase inhibitory effects (Kanlayavattanakul

et *al*., 2016). Ginseng (*Panax ginseng*) is a traditionally used therapeutic in Asian culture and has been demonstrated to possess antimelanogenic effects in B16 melanoma cells through tyrosinase inhibition. Ginsenosides serve as the active constituents, having activities against α-MSH and cAMP. Another component, p-coumaric acid, shows anti-melanogenesis activity, with the lightening of UV-tanned human skin being noticeable following 8 weeks of topical application (0.1% ginsenoside F1) (Kim, 2015). Ethanolic extracts of thyme (*Thymus vulgaris*) rich in rosmarinic acid had skin-lightening potential by inhibition melanogenesis in hairless mice subjected to UVB irradiation (Sun et *al*., 2016).

c) Polyphenol as tyrosinase inhibitory

Several tyrosinase inhibitors were reported from both natural and synthetic sources, but only a few of them are marketed as skin-whitening agents, primarily because of various safety concerns (Petit and Pierard, 2003; Adhikari et *al*., 2008). A series of more complex phenolic compounds from a variety of sources, have been described as potent tyrosinase inhibitors. Among the first class, flavonoids, in particular chalcones, occupy a prominent role as natural inhibitors, followed by hydroxystilbenes (mainly resveratrol derivatives) (Panzella and Napolitano 2019). Several flavones and flavonols with a catechol moiety in their B-ring have been shown to be competitive inhibitors of mushroom tyrosinase. Due to their structural resemblance, these compounds can displace L-DOPA and their catechol group then binds with the copper ions in the catalytic domain of tyrosinase (Arroo et *al*., 2020). Experiments with murine B16/F10 melanoma cells have shown that treatment with polyhydroxy flavones results in reduced melanogenesis (Kumagai et *al*., 2011; Horibe et *al*., 2013; Arroo et *al*., 2020). Also, similar experiments with humans rather than murine melanocytes have shown that polymethoxy flavones inhibit the induction of melanogenesis (Kim et *al*., 2015; Arroo et *al*., 2020).

2.3. Diabetes mellitus

2.3.1. Definition

Diabetes mellitus (DM) is a chronic endocrine and metabolic disorder which is underlined by insulin deficiency or insulin insensitivity or both, and characterized by hyperglycemia and vascular complications (micro and macro) (Ighodaro, 2018). Chronic hyperglycemia results in mortality and morbidity due to microvascular complications such as neuropathy, nephropathy, retinopathy, cataracts, and even stroke damaging blood vessels, peripheral nerves, and nephrons (Kitada et *al*., 2010; Alam et *al*., 2019). DM has grown into a

major health risk worldwide. It has been observed to increase with time. The chronic disease has hurt more than 171 million persons globally in 2000, and the frequency is estimated to grow gradually to 366 million by 2030 (Zhu, 2013; Alam et *al*., 2019).

2.3.2. Oxidative stress in diabetes mellitus

The role of oxidative stress in the occurrence and development of DM is both critical and pivotal. Oxidative stress has been widely linked with the incidence of DM. Several studies have shown that oxidative stress is a key element in the development and progression of diabetes and its associated complications. In line with this view, Brownlee had earlier proposed oxidative stress as a major participant in the pathophysiology of diabetes and its complications. Oxidative stress has been shown to compromise the two major mechanisms failing during diabetes which are insulin secretion and insulin action (Ighodaro, 2018). The role of oxidative stress in diabetes does not only promote the onset of diabetes but also exacerbates the disease condition and its associated complications. Experimental evidence implicates the role of ROS in impaired beta-cell function caused by autoimmune reactions, cytokines and inflammatory proteins in type 1 diabetes (Prattichizzo et *al*., 2018). Also, hyperglycemia has been noted to promote oxidative stress through free radical generation and suppression of the antioxidant defense systems (Giacco et *al*., 2010). In chronic hyperglycaemic conditions, production of ROS is perpetuated and hence, the antioxidant enzymes and non-enzymatic antioxidants are severely suppressed in various tissues, which further exacerbate oxidative stress (Maritim et *al*., 2003; Rains et *al*., 2011; Asmat et *al*., 2016).

2.3.3. Treatment of diabetes mellitus

2.3.3.1. Antidiabetic drugs

Diabetes mellitus disease can be prevented by regulating the blood sugar level with various types of medicines, acquiring to different exercise or yoga therapy or diet plan (Narayan et *al*., 2012; Verma et *al*., 2018). Currently, available therapies of DM are insulin treatment for type 1 DM and other oral hypoglycemic drugs such as sulphonylureas, thiazolidinediones, peptide analogs for treatment of type 2 DM (Reddy et *al*., 2000; Bordoloi et *al*., 2014; Verma et *al*., 2018).

The three most common anti-diabetic drugs that act mainly by inhibiting carbohydrate digestion and absorption are:

*Acarbose; this inhibitor was the first α-glucosidase inhibitor commercially available for diabetes treatment which inhibits the activities of α-amylase, sucrase and maltase (Liang et *al*., 2014; Singh et *al*., 2014; Agarwal and Gupta, 2016).

*Miglitol; strongly inhibits sucrose, glucoamylase and isomaltase activities (Van de Laar et *al*., 2005; Van de Laar, 2008; Agarwal and Gupta, 2016).

*Voglibose; it is of bacterial origin which inhibits the activities of α-glucosidase, sucrose, isomaltase and maltase (Agarwal and Gupta, 2016).

a) α- Amylase inhibitory as treatment of diabetes mellitus

There are many digestive enzymes in humans and among them, the most important one is pancreatic α-amylase, that act as catalysis in the reaction which involves the hydrolysis of the α-1,4 glycosidic linkages of the starch, amylopectin, amylose, glycogen, and numerous maltodextrins and is responsible for starch digestion. The other important enzyme is α-glucosidase or maltase which catalyzes the final step of the digestive process of carbohydrates mainly starch by acting upon 1,4-alpha bonds and producing glucose as the final product (Tundis et *al*., 2010; Agarwal and Gupta, 2016). The large molecules like starch cannot cross the blood brain barrier as glucose has to reach the brain thus; to overcome this problem alpha-amylase cleaves the large starch molecules into smaller fragments of sugars in order to cross the blood brain barrier. If there will be excess conversion of starch to sugars, it will increase the sugar level in blood, then the role of insulin will come into action by ordering cells to metabolize the excess sugar moieties and store as energy sources i. e. glycogen. This cycle is endlessly happening in a healthy person. But in some cases, due to excess activity of amylase enzyme and insulin deficiency or resistance to insulin, level of blood glucose arises which might result in hyperglycaemia. To control hyperglycaemia several studies on inhibition of amylase enzyme activity is being studied (Agarwal and Gupta, 2016).

b) α-Glucosidase inhibitory as treatment of diabetes mellitus

The α-glucosidase is an important enzyme found in human body. α-Glucosidase is found in the brush border of intestinal mucosa where they catalyze the end product of digestion into carbohydrates. It is involved in postprandial hyperglycemia. Inhibition of this enzyme in intestine results in delayed carbohydrates digestion. It also results in lowering of glucose level after meal. Consequently, the mechanism of α-glucosidase inhibition signifies the

pharmacological optimization of the nutritional principle of late carbohydrate absorption. Acarbose when given orally inhibited the enzymes α-glucosidase in the brush border of the small intestines and reduces the rate of digestion of complex carbohydrates. So the inhibitors of α-glucosidase work in a similar fashion and retard the glycation of proteins and therefore reduced the glycated hemoglobin and glycation end products in collagen. The result is the improvement of biochemical parameters and act against the neuropathy and diabetic nephropathy. Therefore, treatment of diabetic animals with α-glucosidase inhibitors improves the metabolic state and prevents the onset of diabetic complications (Alam et *al*., 2018).

2.3.3.2. Herbal treatment of diabetes mellitus

Not only medicinal plants are effective in DM, but many of them also possess a variety of effects on other disease states, including the complications of DM. The medicinal plants have exerting hypoglycemic effects, decrease accompanying complications such as nephropathy, neuropathy, retinopathy, hypertension, and/or hyperlipidemia among individuals with DM. Such plants may be appropriate alternatives or adjuncts to available antidiabetic medications.Studies on the antidiabetic mechanisms of medicinal plants have shown that most of them produce hypoglycemic activity by stimulating insulin secretion, augmenting peroxisome proliferator-activated receptors (PPARs), inhibiting α-amylase or α-glucosidase, glucagon-like peptide-1 (GLP-1) secretion, advanced glycation end product (AGE) formation, free radical scavenging plus antioxidant activity (against ROS/RNS), up-regulating or elevating translocation of glucose transporter type 4 (GLUT-4), and preventing development of insulin resistance (Nazarian-Samani, 2018).

a) The role of natural antioxidants in the management of diabetes and its complications

Production of ROS and disturbed capacity of antioxidant defense in diabetic subjects have been reported. It has been suggested that enhanced production of free radicals and oxidative stress is central event to the development of diabetic complications. This suggestion has been supported by demonstration of increased levels of indicators of oxidative stress in diabetic individuals suffering from complications. Therefore, it seems reasonable that antioxidants can play an important role in the improvement of diabetes. Use of antioxidants reduces oxidative stress and alleviates diabetic complications (Rahimi et *al*., 2005). The most common antioxidant deficiencies reported in diabetes are lower levels of ascorbate, glutathione and superoxide dismutase (Jialal et *al*., 2002). Therefore, it seems that plants particularly those

with high levels and strong antioxidant compounds have an important role in improvement of disorders involving oxidative stress such as DM. There are many investigations that have studied the effects of these plants and their antioxidant ingredients on diabetes and its complications and achieved good results (Rahimi et *al*., 2005). Rajasekaran et *al*., (2005) showed that *Aloe vera* had positive effects on diabetes by their antioxidant properties, leading to increase the levels of GSH, SOD, CAT, GSH-Px, and GST in liver and kidney, and decrease lipid peroxidation level. Ryu et *al*., (2005) showed that *Panax ginseng* had positive effects on Type 2 diabetic in rats by scavenging free radical's mechanism.

b) Enzyme inhibitors from natural products

There are many enzymes involved in diabetic pathways, and finding an appropriate enzyme inhibitor can be useful to identify new sources of antidiabetic drugs. Such inhibitors synergistically with natural products may play a crucial role in the development of a drug for diabetes. Many studies have taken place to study natural sources from plants on different mechanism pathways involved in diabetes such as inhibition of intestinal α-glucosidase, inhibition of amylase, protection against oxidative, by lowering plasma glucose levels, by synthesis and release of insulin from pancreatic β cells, inhibition of postprandial hyperglycemia, delaying absorption of glucose, increase utilization of glucose via GLUT4 (Alam et *al*., 2018). *Allium sativum* is commonly known as "Garlic" that belongs to onion family Amaryllidaceae. It showed a dose-dependent inhibitory effect against α-glucosidase and aldose reductase with IC_{50} greater than 500 mg/ml (Wu et *al*., 2015). Methanolic extract of the shade-dried bark of cinnamon reduces postprandial intestinal glucose absorption by inhibiting α-glucosidase with IC_{50} of 676 μg/ml while stimulating glucose uptake in the cells via GLUT4 and reduces hyperglycemic response by 42.5% (Shihabudeen et *al*., 2011). Lyophilized aloe gel from *Aloe barbedensis* contains aloe-emodin-8-O-glycoside, which enhances glucose transport by modulating the proximal and distal markers involved in glucose uptake and its transformation into glycogen while modulating the expression level of GLUT4 and decreasing the activity of hexokinases (Alam et *al*., 2018). It also inhibited the protein tyrosine phosphatase in a dose-dependent manner (Anand et *al*., 2010). Luteolin isolated from *Aloe vera*Tourn showed inhibition of procaine pancreatic α-amylase with an IC_{50} of 50–500 μg/ml. Quercetin from red pepper showed an IC_{50} of 1.59 μg/ml against α-glucosidase (Alam et *al*., 2018). Curcumin pyrazole derivatives from *Curcuma longa* inhibited the α-amylase with an IC_{50} ranging from 37.35 ± 0.15 to 53.87 ± 0.57 μmol/l (Puneeth and Sharada, 2015). Ethanolic

extract of *Curcuma longa* inhibited the α-glucosidase with an IC_{50} of 28.4 μg/ml (Hasimun and Lisnasari, 2016).

c) Polyphenol as α-amylase and α-glucosidase inhibitory

The polyphenol-rich functional foods have been proposed to be unique supplementary and nutraceutical treatments for DM. Inhibition of α-amylase and α-glucosidase enzymes using natural products (especially polyphenols) is a novel oral policy to regulate carbohydrate metabolism and hyperglycemia (Tadera et *al*., 2006; Nyambe-Silavwe et *al*., 2015). In previous study of Rasouli et *al*., (2017), the structural analysis revealed that caffeic acid (hydroxycinnamic acid), curcumin (other polyphenols), cyaniding (anthocyanidins), daidzein (isoflavones), epicatechin (flavan3-ols), eridyctiol (flavanones), ferulic acid (hydroxycinnamic acid), hesperetin (flavanones), narenginin (flavanones), pinoresinol (lignans), quercetin (flavonols), resveratrol (stilbenes) and syringic acid (hydroxybenzoic acid), show high binding affinities for interaction with the α-glucosidase active site. This type of binding mode into the active site can decrease or fully inhibit the α-glucosidase enzyme activity. It concludes that these compounds have great potential for inhibiting the α-glucosidase enzyme and can be considered as natural inhibitors for the glucosidase enzyme (Rasouli et *al*., 2017). In previous study of Rasouli et *al*., (2017), the screening showed that catechin, hesperetin, kaempferol, silibinin and pelargonidin, have great potential to interact with an α-amylase enzyme active site. The docked pose of the α-amylase enzyme with the ligands catechin (flavan-3-ols), hesperetin (flavanones), kaempferol (flavonols), silibinin (flavonolignans) and pelargonidin (anthocyanidins) is clearly speculated for the binding position of the ligands with the active site (Rasouli et *al*., 2017). The results of study performed by Rasouli et *al*., (2017), speculate thatthese compounds are potent α-amylase inhibitors. Therefore, the primary structure of polyphenols can change the inhibitory effect versus the α-amylase and α-glucosidase enzymes. Thus the consumption of polyphenol-rich functional foods (by considering the best dose of each compound and assessing their possible side effects) in diabetic patients useful for regulating carbohydrate metabolism and related disorders. The chemical flavonoids are based upon a fifteen-carbon skeleton consisting of two benzene rings (A and B) linked via a heterocyclic pyrane ring (C). They can be divided into a variety of classes. The various classes of flavonoids differ in the level of oxidation and pattern of substitution of the C ring, while individual compounds within a class differ in the pattern of substitution of the A and B rings. Due to the occurrence of a specific pattern of some functional groups in the various rings, some

polyphenols may be more susceptible inhibitors for the specific enzymes. Researchers reported that the degree of hydroxylation, has an important role in inhibiting digestive enzymes (especially α-amylase) (Xiao et *al*., 2013). Based on docking study, the OH groups present in position 3 (ring C), 7 (ring A), 4′ and 5′ (ring B) are apparently particularly important for the α-amylase and α-glucosidase inhibitory effects of the studied compounds (Rasouli et *al*., 2017). Specific polyphenols bind directly to the active sites and there is the possibility that this will inhibit their biological activities through occluding the active sites or locking of the catalytic cycle (Sales et *al*., 2012). In previous study of Rasouli et *al*., *(*2017), the docking results, indicate that among the polyphenol families only specific polyphenols are possible potent inhibitors of the α-amylase and α-glucosidase enzymes, while many other polyphenols (e.g. glycosylated phenols) are very weak inhibitors or are not inhibitors.

3. Digestive system

The digestive system consists of the alimentary tract or canal and the associated organs such as teeth, salivary glands, the liver and the pancreas. The alimentary tract is a hollow tube that extends from the mouth to the anus. It is divided into the mouth, pharynx, esophagus, stomach, duodenum, jejunum, ileum, colon, rectum, and anus. The duodenum, jejunum and ileum form the small intestine, whereas the colon is sometimes called the large intestine. In some parts of the tube, a large number of glands in its wall empty their secretions in the lumen. Moreover, the associated glandular glands, such as the salivary glands, the pancreas and the liver also empty their secretions in the lumen (Rhoades and Pflanzer, 1989). Both the stomach and the intestine are called the GI tract. The principal function of the GI tract is to ensure the proper digestion and absorption of nutrients by passing through the mucous membrane to the blood or lymph, and the expulsion of undigested residue and unwanted waste. This complex process requires the coordinated propulsion of endoluminal content along the length of the GI tract, which in turn relies on the activity of specialized cells, including smooth muscle cells, and a hierarchy of intrinsic and extrinsic neurons to regulate the motor programs (Knowles et *al*., 2013). GI tract also serves as a barrier from the directly contiguous external and internal environment. GI tract is also one of the largest endocrine organs and plays a critical role in the regulation of satiety and weight control (Urbanska et *al*., 2016).

3.1. Histology of the digestive tract

The structure of the digestive or alimentary tract varies from one region to another, but there are similarities in the four main layers that constitute its wall. These are from the inside to the outside, the mucosa, the submucosa, the muscularis externa, and the serosa that covers the wall of the tract except the esophagus and the distal rectum (Figure 13) (Ganong, 2010).

a) The mucosa is composed of the epithelium, the lamina propria, and the muscularis mucosae. The epithelium is a single layer of specialized cells that line the lumen; the most abundant are cells called absorptive enterocytes, enteroendocrine cells and mucinsecreting cells. Besides this, in the gastric epithelium specialized cells produce protons, pepsins and bicarbonate. The nature of the epithelium changes from one part to another in the tract. Beneath the epithelium, the lamina propria is connective tissue rich in glands and contains nerve fibers, capillaries, and lymph vessels and nodules. The muscularis mucosa is a thin layer of smooth muscle (Daniel et *al*., 2001; Furness, 2012).

b) The submucosa is a connective tissue that contains larger nerve trunks, blood vessels, and lymph vessels, together with the first plexus of the enteric nervous system (ENS); the submucosal plexus (Meissner plexus) which controls secretions from the digestive tract, and it is present from the stomach to the rectum (Daniel et *al*., 2001; Furness, 2012).

c) The muscularis externa consists of two muscular layers; the inner circular layer and the outer longitudinal layer. Between the two layers lies the second plexus of the ENS; the myenteric plexus (Auerbach's plexus) which controls motility in the digestive tract, and it extends along the entire length of the GI tract (Daniel et *al*., 2001; Furness, 2012).

d)The serosa or adventitia is the last or the outermost layer. It consists of a layer of squamous mesothelial cells and it is a part of the mesentery (Rhoades and Pflanzer, 1989; Daniel et *al*., 2001; Furness, 2012).

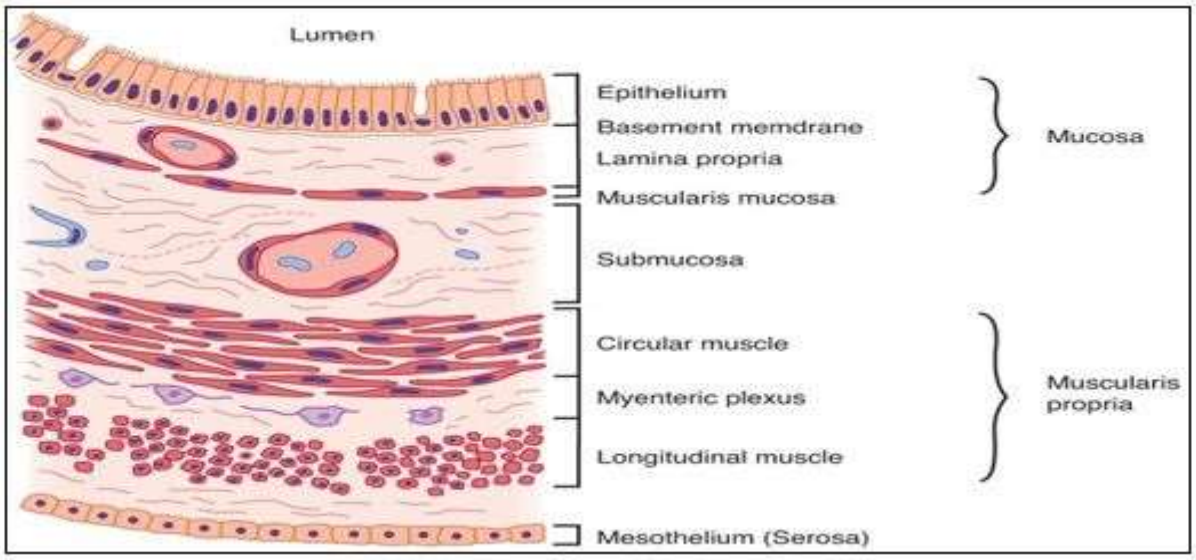

Figure 13: Digestive tract wall histology (Ganong, 2010).

3.2. Innervation of the digestive tract

The motility of the digestive tract is dependent on the central nervous system (CNS) and local neuro-hormonal mechanisms.

3.2.1. Autonomous extrinsic innervation of the digestive tract

Although ENS can function independently from CNS, the latter has an important role in coordinating the various functions of ENS (Goyal and Hirano, 1996).

3.2.1.1. Extrinsic motor innervation (efferent)

The extrinsic motor innervation of the digestive tract is twofold; parasympathetic cholinergic activity which generally causes stimulation of smooth muscles, and sympathetic noradrenergic activity which inhibits these muscles (Holzer et *al*., 2001), with the exception of sphincters which stimulates contraction (Janig, 2009). Neurons of the sympathetic and parasympathetic systems influence smooth muscles indirectly by acting on neurons in the myenteric plexus (Hansen, 2003a).The CNS exerts its role in the control of GI functions through parasympathetic pathways originating from the dorsal motor nucleus of the vagus nerve (DMNX) and sacral marrow, and sympathetic pathways originating from the thoracolumbar spinal cord (Janig, 2009).The parasympathetic pathways are composed of the vagus nerve which controls motor and secretomotor functions of the upper GI tract and the pelvic nerve which innervates the distal colon and rectum (Goyal and Hirano, 1996). Sympathetic

innervation takes place via the splanchnic nerves (Figure 14) (Van Oudenhove et *al*., 2004). Extrinsic efferent parasympathetic neurons (cholinergic) are preganglionic fibers acting on cholinergic neurons in the ENS. In the myenteric plexus, they control motor activity; in the submucosal plexus they regulate the secretory activity of glandular cells and visceral circulation (Konturek et *al*., 2004). The sympathetic fibers that enter the digestive tract are adrenergic, postganglionic, the cell bodies of which are in the pre-vertebral ganglia. They have at least four targets in the GI tract: secretomotor neurons containing the vasoactive intestinal polypeptide (VIP), presynaptic cholinergic nerve endings, submucosal blood vessels and GI sphincters. The cell bodies of adrenergic neurons are absent at the enteric plexus (Goyal and Hirano, 1996).

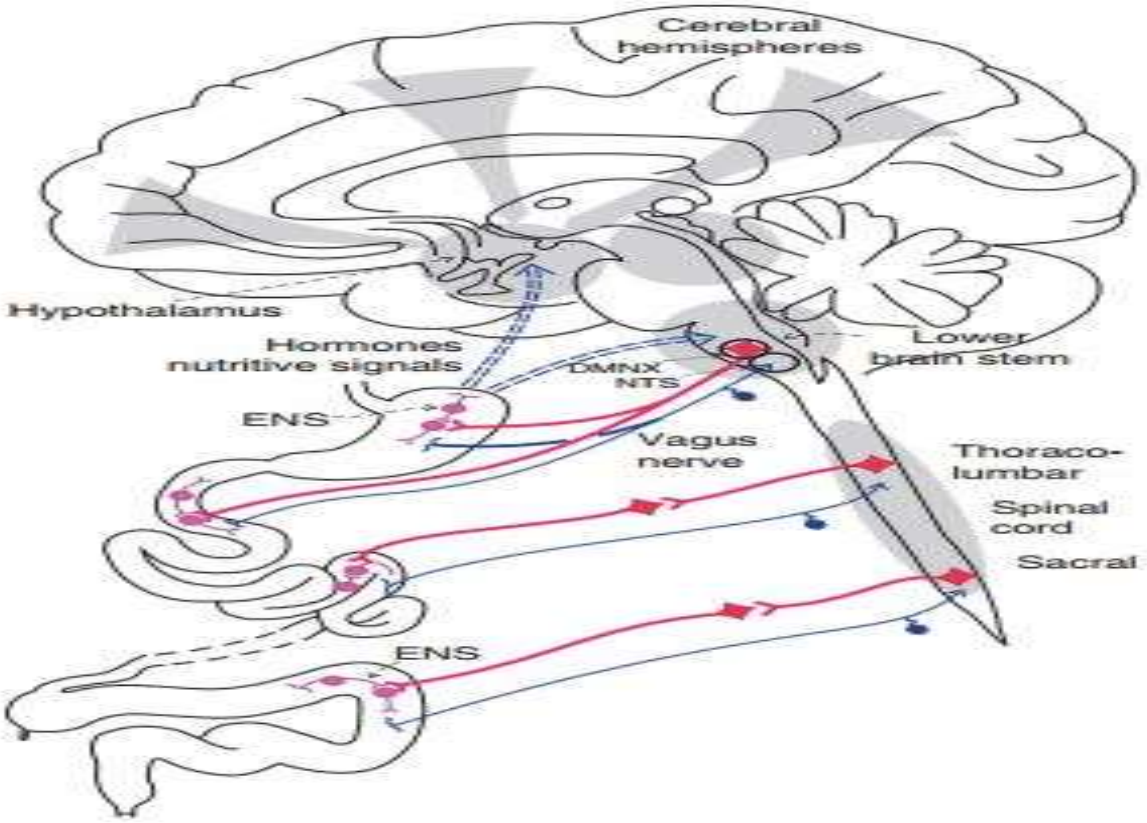

Figure 14: Afferent and efferent pathways connecting GI tract (Janig, 2009).

DMNX; dorsal motor nucleus of the vagus nerve, NTS; nucleus tractus solitarius, ENS; enteric nervous system.

3.2.1.2. Extrinsic sensory innervation (afferent)

Afferent fibers carry information from the digestive tract (visceral and chemical sensitivity) to the brain (Grundy et *al*., 2006). There are two groups of primary extrinsic afferent neurons that innervate the digestive tract: vagal afferents and spinal afferents (Van Oudenhove et *al*., 2004). Vagal afferent fibers have their cell bodies in the nodous ganglia while those of

the spinal afferent fibers are located in the dorsal root ganglia (Grundy et *al*., 2006). The vagus nerve and splanchnic nerve are involved in extrinsic innervation of the stomach and proximal intestine, while the pelvic nerve innervates the distal intestine (Hansen, 2003a). The endings of vagal and spinal sensory neurons extend to muscles, mucosa, serosa, and enteric ganglia (Grundy, 2006). This enabled them to respond to chemical and mechanical alterations within the digestive tract (Holzer et *al*., 2001). Vagal afferent fibers are generally involved in the transmission of physiological signals, while spinal afferent fibers carry pathophysiological signals (Grundy, 2006) (Figure 15).

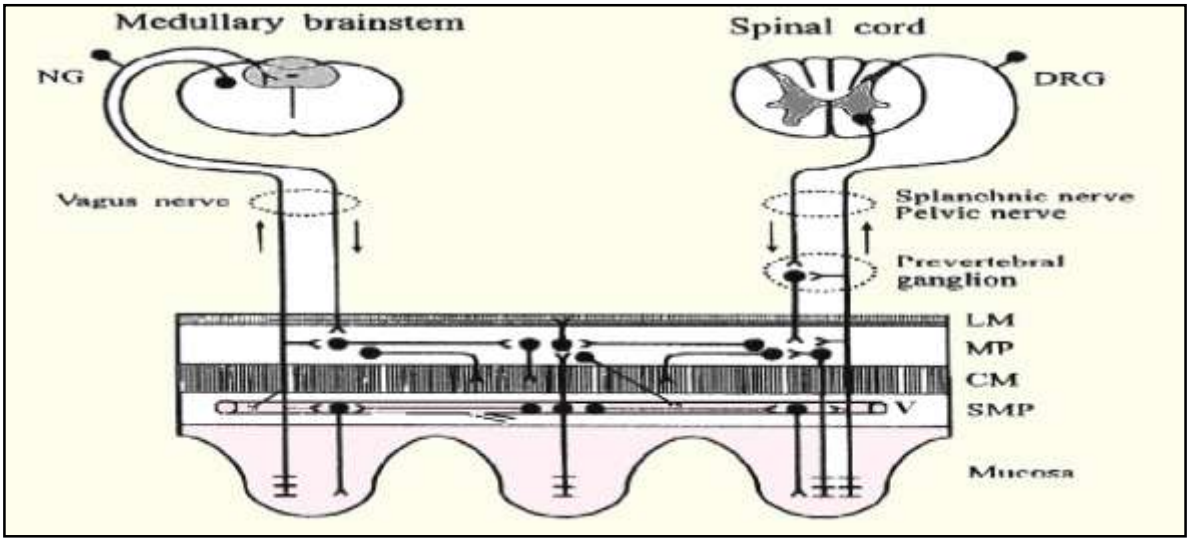

Figure 15: Schematic diagram showing the multiple innervation of the GI tract (Holzer et *al*., 2001).

LM: longitudinal muscle; CM: circular muscle; MP: myenteric plexux; SMP: submucosal plexus; DRG: dorsal root ganglion; NG: nodose ganglion; V: vessel.

3.2.2. The enteric nervous system

The enteric nervous system (ENS) is the autonomous part of the nervous system that controls digestive functions, both for motor activity (peristalsis and vomiting) and for secretions and vascularization (Costa et *al*., 2000). It functions more or less independently of the CNS (Van Oudenhove et *al*., 2004). In the ENS, the cell bodies of neurons are grouped together in small ganglia which are connected by a network of axonal projections thus forming two large plexuses, called the myenteric plexus (or Auerbach's plexus), which lies between the muscles longitudinal and circular muscles, and the submucosal plexus (or Meissner's plexus), located in the submucosa (Figure 15). The first controls motor skills and the second controls secretions

(Goyal and Hirano, 1996). The ENS of the stomach differs from other regions of the digestive tract, of which it is formed only of a single ganglion plexus, since the submucosal plexus is very scarce or completely absent (Schemann et *al*., 2008). The intrinsic innervation of the stomach is therefore located mainly in the myenteric plexus. The density and size of myenteric nodes increase from the fundus towards the anterior (Brookes and Costa, 2002).

3.2.2.1. Type of neurons in the enteric nervous system

Within the enteric nervous system, neurons are classified according to their functions, morphologies, their electrical properties and their neurochemical properties (Furness, 2006).

a) Functional classification

Enteric neurons are classified according to their functions into sensory, motor and interneurons neurons (Furness, 2006).

- **Sensory neurons**

Sensory neurons (intrinsic afferent neurons) are Dogiel II type neurons located in the myenteric and submucosal plexuses. These neurons contain specialized receptors that sense changes in the environment (tension, contraction, chemical stimuli), which are then transmitted along sensory nerve fibers to other parts of the nervous system (Costa et *al*., 2000).

- **Motor neurons**

They are excitatory and inhibitory neurons that innervate longitudinal, circular smooth muscle and muscularis mucosa along the digestive tract (Furness, 2006). Circular muscle excitatory motor neurons are of the Dogiel I morphological type. They project onto the muscle, where they form a dense arrangement of nerve endings in the myenteric muscle plexus (Costa et *al*., 2000). They use acetylcholine and tachykinins as neurotransmitters (Brookes and Costa, 2006). The inhibitory motor neurons of circular muscles are also of Dogiel type I. They project onto the circular muscle where their axons are intimately associated with those of the excitatory motor neurons in the myenteric plexus. They use multiple neurotransmitters including NO, VIP, and ATP (Hozler et *al*., 2001). Both types of neurons act directly on smooth muscle or indirectly through interstitial cell of Cajal(ICC) (Costa et *al*., 2000). Axons of motor neurons from longitudinal muscle project onto muscle, their cell bodies are usually located in the myenteric plexus. In the guinea pig small intestine, almost all motor neurons of longitudinal muscles (over

97%) are cholinergic, and therefore excitatory. This suggests that the longitudinal muscle layer receives relatively little direct inhibitory innervation (Brookes and Costa, 2006).

- **Interneurons**

Interneurons are interposed between primary afferent neurons and motor or secretomotor neurons. Interneurons involved in motor reflexes are directed orally or anal and are referred to as ascending or descending, respectively. They form polysynaptic pathways along the digestive tract. Several subgroups of interneurons have been defined on the basis of their neurotransmitter content (Goyal and Hirano, 1996). One class of ascending interneurons and three classes of descending interneurons have been identified (Brookes and Costa, 2006). Ascending interneurons are primarily cholinergic, while descending interneurons have a complex of neurotransmitters, including acetylcholine, NO, VIP, serotonin (5-HT), and somatostatin (Hansen, 2003b). Morphological classification of enteric neurons was established by Dogiel. He described three types of neurons with his name Dogiel I, II, and III. It is based on the morphology of nerve cells (cell shape and size, number of dendrites and axons) (Furness, 2006).

b) Electrophysiological and neurochemical properties

Enteric neurons express a combination of different neurotransmitters (Hansen, 2003b); Acetylcholine, tachykinins, NO, ATP, VIP, neuropeptide Y (NPY) and 5-HT are the main neurotransmitters of ENS (Hozler et *al*., 2001). Excitatory transmission in the ENS can be divided into two categories: rapid transmission by nicotinic acetylcholine receptors, P2X purinoceptors and 5-HT3 receptors, and slow transmission via muscarinic acetylcholine receptors and tachykinin NK1 and NK3 receptors (Wood, 2006). Two types of neurons have been identified according to their electrophysiological character: S neurons (synaptic) and AH neurons (afterhyperpolarization) (Furness, 2006). Type S neurons have the morphology of Dogiel I type (Hansen, 2003b). They receive rapid excitations and are interneurons and motor neurons (Furness, 2000). AH-type neurons are most often of the Dogiel II morphological type (Wood, 2006). They are intrinsic sensory neurons and receive slow excitations (Furness, 2000).

3.2.1.2. Neurotransmitters of the enteric nervous system

In addition to the classical neurotransmitters acetylcholine and adrenaline/noradrenaline, other neurotransmitters involved in the nerve regulation of GI

motility have been identified. These non-adrenergic, non-cholinergic (NANC) neurotransmitters can be peptides, amines (serotonin, dopamine), amino acids (GABA, glutamate), and gases (NO and CO) (Olsson and Holmgren, 2001).

a) Acetylcholine

Acetylcholine is the primary excitatory neurotransmitter of the ENS (Furness, 2006). The two types of receptors, M2 and M3, are the predominant muscarinic receptors in GI muscle (Lecci et *al*., 2002). The activation of M2 receptors, coupled to the G protein, is linked to an inhibition of the activity of adenyl cyclase, which reduces the inhibitory effect of this enzyme on muscle contraction, and the opening of channels non-selective cationic that cause muscle depolarization and entry of Ca^{2+}. Activation of M3 receptors in turn activates phospholipase C, and causes the release of calcium from intracellular stores and activation of PKC (Furness, 2006). Nicotinic receptors are of the ionotropic type: they are present in myenteric neurons. They cause rapid depolarization of the membrane (rapid excitatory postsynaptic potential, EPSPs), which facilitates rapid communication between neurons (Brookes and Costa 2002).

b) Tachykinins

Pharmacological blockade of cholinergic transmission does not completely suppress excitatory transmission; residual transmission is observed and blocked by tachykinin receptor antagonists (Kunze and Furness, 1999). Indeed, pharmacological and immunohistochemical studies have shown that tachykinins are excitatory neurotransmitters and they are co-transmitters with acetylcholine (Furness, 2006). In the ENS, the NK1, NK2 and NK3 receptors are preferentially activated by substance P (SP), neurokinin A (NKA) and neurokinin B (NKB), respectively (Galligan, 2002).

c) Serotonin

The 5-HT synthesized, stored and released by enterochromaffin cells (EC) and enteric neurons, participates in excitatory transmission through 5-HT3 and 5-HT4 receptors located on cholinergic neurons (Briejer et *al*., 1995; Holzer et *al*., 2001).

d) Noradrenaline

It is the main neurotransmitter of postganglionic fibers of the sympathetic nervous system (De ponti et *al*., 1996). Adrenergic receptors are of three types α1, α2 and β (Brookes

and Costa, 2002). In the stomach, stimulation of α – adrenergic receptors produce excitatory and inhibitory effects, depending on the portion of the stomach, the degree of existing mechanical activity, the concentration of the agonists used and the species (De Ponti et *al*., 1996). Several studies have shown that stimulation of α-adrenergic receptors generally produces muscle relaxation by direct action on postsynaptic α-adrenergic receptors located on smooth muscles and by indirect action on the pre-synaptic α2-adrenergic receptors located on the nerve endings of enteric cholinergic neurons (Kelly et *al*., 1990; MacDonald et *al*., ., 1990; Verplanken et *al*., 1984). Activation of presynaptic α2-adrenergic receptors inhibits the release of acetylcholine in ENS (Tack and Wood, 1992). Relaxation of the proximal stomach by direct stimulation or by nociceptive stimuli of gastric sympathetic nerves is hampered by specific blockade of adrenergic neurons (guanethidine or bretylium) (Mc Intyre and Thompson, 1992). Activation of α2-adrenergic receptors inhibits gastric motility, GI transit, and colon motility in rats (Asai et *al*., 1997; Umezawa et *al*., 2003).

e) Nitrogen oxide

NO is a NANC neurotransmitter of the central, peripheral and enteric nervous systems (Goyal and Hirano, 1996). It is produced by NOS (nitrogen oxide synthase) localized in enteric neurons and NO diffuses through the smooth muscle cell membrane to activate soluble guanylate cyclase in the cytoplasm, causing the cell to relax (Furness, 2006). Inhibition of NOS by L-nitro-n-arginine (L-NNA) delays gastric emptying in mice (De Winter, 2002) and small intestine and colon transit in dogs (Chiba et *al*., 2002). In humans, pharmacological NOS inhibitors increase the frequency of gastric contractions, decrease gastric emptying time, and decrease fundus volume before and after meals (Tack et *al*., 2002).

f) Vasopressin intestinal peptide and pituitary adenylate cyclase-activating polypeptide

Vasopressin intestinal peptide (VIP) and pituitary adenylate cyclase-activating polypeptide (PACAP) have been proposed as mediators of inhibitory neurotransmission (Ekblad and Sundler, 1997). Stimulation of VIP receptors activates adenyl cyclase, leading to the synthesis of cAMP and activation of protein kinase A (Lecci et *al*., 2002). VIP can also stimulate the release of NO from nerve fibers and smooth muscle cells (Mashimo et *al*., 1996). PACAP, found in enteric neurons, is also involved in the relaxation of intestinal muscle (Furness, 2006).

g) Adenosine triphosphate

Adenosine triphosphate (ATP) is a purine; thus, transmission involving ATP is called purinergic. ATP acts on P2Y receptors coupled to G protein (metabotropes) and ionotropic P2X receptors. ATP acts on smooth muscles as an inhibitory neurotransmitter through P2Y receptors, as it is released as an excitatory neurotransmitter and acts via P2Y and P2X receptors (Bertrand, 2003). It is now well established that inhibitory neurons use neurotransmitters, including ATP, NO, and peptides of the VIP family (Furness, 2006).

3.2.3. Interstitial cell of Cajal

The smooth muscles of the GI tract show spontaneous electrical and mechanical activity, which persists in the absence of any stimulation. This activity results from a distinct group of cells, known as Interstitial cell of Cajal (ICC), which are located between muscle cells, or between neural plexuses and muscle layers. Three functional groups of ICC are present in the GI tract (Hirst and Ward, 2003). A group of ICCis present in the myenteric region (ICC-MY), between the circular and longitudinal muscle layers. ICCs of the second group are dispersed among smooth muscle cells (intramuscular ICC or ICC-IM), and ICC-DMP, which constitutes the third group, are located inside the deep muscle plexus in the small intestine (Ward and Sanders, 2006). In humans and animals, there are two classes of ICC in the stomach, intramuscular ICC and ICC myenteric, and they are more numerous in the body and antrum than in the fundus (Huizinga, 2001; Radenković et *al*., 2005). ICCs are electrically coupled to each other and to neighboring muscle cells via gap junctions (Sanders et *al*., 2006). Each depolarization produced by a group of ICC will passively depolarize neighboring muscle cells. If the depolarization is large enough to activate the L-type calcium channels in smooth muscle, a contraction will occur. Conversely, if a group of ICC produces hyperpolarization, neighboring muscle cells will be hyperpolarized, making them less susceptible to contraction (Hirst and Edwards, 2006).

3.3. Gastrointestinal circulation

The circulation of the stomach, small and large intestines, pancreas and liver are called the splanchnic circulation. The heart pumps blood to these organs through arteries that branch from the abdominal aorta. The blood that leaves the stomach, intestines, and pancreas goes next to the liver by way of the portal vein. The significance of this vascular organization is that many absorbed digestion products are subject to metabolic processing by the liver before being distributed to the cells of the body. The splanchnic circulation receives almost one fourth of the

cardiac output. Its resting flow rate is about 1400 ml per minute, which is greater than that of any of the other major peripheral circulations. Flow of blood through the splanchnic circulation increases when a meal is eaten, this increase facilitates the removal of the digestion products from the digestive tract. In addition, extra oxygen and other nutrients are supplied to meet the enhanced demands for energy imposed by the increased muscular, secretory and absortive activities that occur after a meal (Rhoades and Pflanzer, 1989).

3.4. The stomach

Anatomically, the stomach is divided into five regions: cardia, fundus, corpus (body), antrum and pylorus; functionally, there are two: proximal and distal (Figure 16 A). The proximal region (cardia, fundus and proximal one-third of the corpus) acts as a reservoir; the distal region (distal two-thirds of the corpus, antrum and pylorus), which is more muscular, mixes the stomach contents before delivering them to the duodenum (McDonald and MacFarlane, 2018). The gastric mucosa contains many deep glands. In the pyloric and cardiac regions, the glands secrete mucus. In the body of the stomach, including the fundus, the glands contain parietal cells, which secrete hydrochloric acid and intrinsic factor, and chief (zymogen, peptic) cells, which secrete pepsinogens (Figure 16 B). These sectretions mix with mucus secreted by the cells in the necks of the glands. Mucus is also secreted along with HCO3- by mucus cells on the surface of the epithelium between glands (Ganong, 2010). The stomach has a very rich blood and lymphatic supply. Its parasympathetic nerve supply comes from the vagi and its sympathetic supply from the celiac plexus. Stomach contains three muscle layers: outer longitudinal muscle, intermediate oblique muscle layer, while the myenteric plexus is located in between the circular and longitudinal muscle layer. Central and peripheral stimuli of gastric acid secretion are mediated through activation of histaminergic, gastrinergic, and cholinergic pathways coupled to intracellular second messenger systems that determine the trafficking and activity of H^+/K^+-ATPase, the proton pump of the parietal cell. The major stimulants of acid secretion are histamine, gastrin, and acetylcholine, whereas, the main inhibitor is somatostatin (Ganong, 2010).

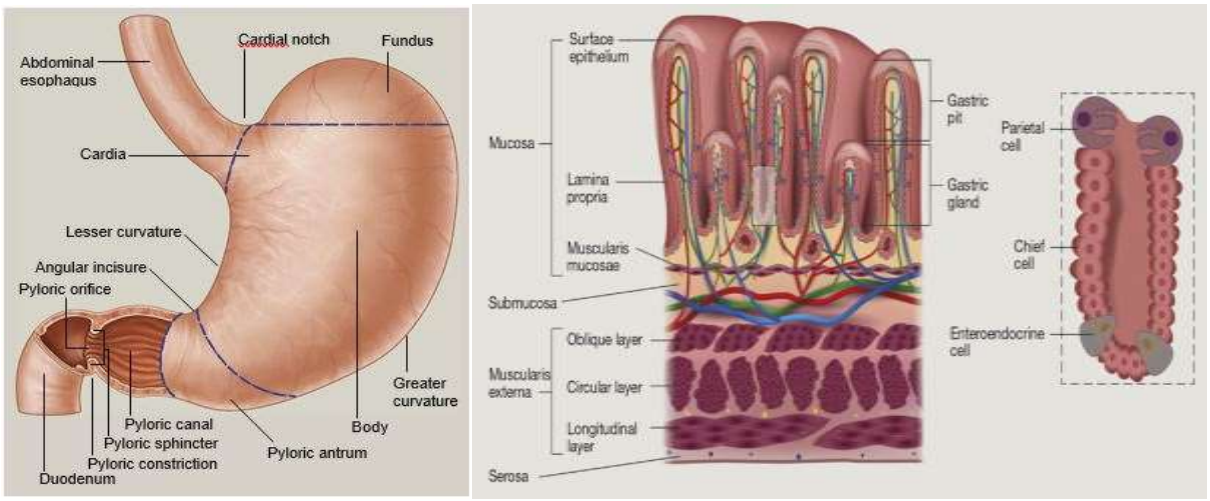

Figure 16: Parts anatomically and functionally of stomach (McDonald and MacFarlane, 2018).

3.5. The intestines

The small intestine is the part of the gastrointestinal (GI) tract between the stomach and the large intestine. In the small bowel, food is mixed with bile, pancreatic juice and the secretions of cells lining the small intestine. These include mucus, which lubricates and protects the epithelium, and various digestive enzymes, which break down disaccharides and peptides. Digestion, which started in the mouth and stomach, is completed and the products absorbed along with most of the water in the small bowel. The small bowel receives approximately 9 litres of water per day (with 2 litres from the diet and 7 litres from secretions) and approximately 1-2 litres pass into the colon. The small intestine has three distinct regions: the duodenum, jejunum, and ileum. ileum is rich in lymphatic tissue. The small bowel is covered in finger-like projections or villi, containing a lymphatic vessel, an (end) artery, a vein and a capillary network. The villi, in turn, are covered with microvilli, making up the 'brush border'. The mucosa is incredibly folded and it has been estimated that the total area available for absorption in the small bowel is 200 m^2. These villi and microvilli increase the surface area available for the absorption of nutrients. The primary function of the small intestine is the absorption of nutrients and minerals from food. The main function of the colon or large intestine is absorption of water, Na+and other minerals (converting 1-2 litres of iso-osmotic chyme into 200-250 ml of semi-solid faeces). By removal of about 90% of the fluid, it converts the 1000-2000 ml of isotonic chyme that enters it each day from the ileum to about 200-250 ml of semisolid feces. There are no villi on the mucosa. The colonic glands are short inward projections of the mucosa that secrete mucus. Many mucous glands secrete mucus into the hollow lumen of the large

intestine to lubricate its surface and protect it from rough food particles (Ganong, 2010; McGuiness, 2010; McDonald and MacFarlane, 2018).

3.6. Gastrointestinal motility

3.6.1. Gastric motility

In the stomach, motor functions include receiving food, mixing its contents, kneading and stirring solid food, and regulating the evacuation of its contents to the duodenum (Brookes and Costa, 2002). The motility of fasting is very different from that observed during feeding. During fasting, the proximal GI tract is animated by well-organized contractions constituting "migranting motor complexes" (MMC) (Camilleri, 2006), in which three successive phases are distinguished. Phase I is a period of total rest (absence of contraction), it is followed by a variable period of irregular contractile activity (irregular contractions, of variable amplitude), called phase II. Phase III then consists of a short period (five to ten minutes) of intense, frequent (three to four per minute in the stomach) and regular contractions which migrate peristaltically distally, with a speed of one to two centimeters per minute. This phase (III) is still followed by phase I. The role of the MMC is to periodically empty the stomach and intestine of its contents in order to eliminate non-digestible food residues, thus avoiding endoluminal microbial proliferation (Tack, 2007). After eating a meal, the CMM disappears, the fundus and upper gastric body function as a reservoir for stomach contents. Adaptive relaxation gives the stomach the capacity to expand to store a complete meal without changing muscle tension. This is followed by a tonic contraction propelling the contents to the stomach (Hellström et *al*., 2006). On an empty stomach, the smooth muscle cells in this region generate significant tonic contractions (Tack, 2007). Inhibition of these contractions induces gastric relaxation known as receiver when swallowing and adaptive when filling the stomach (Schemann et *al*., 2008). The inhibitory neurons responsible for gastric relaxation are not adrenergic; they are intrinsic inhibitory motor neurons that use inhibitory neurotransmitters like NO, ATP, and VIP (Furness, 2006). When food enters the stomach, the organ relaxes to accommodate this increase in volume. This receptive relaxation allows storage of the meal without a concomitant increase in intragastric pressure. This relaxation of the gastric musculature is triggered by the movement of the pharynx and esophagus and is mediated via a vagal reflex and the release of NO (Ganong, 2010). In the two functional regions, proximal and distal, with existence of sphincters at each end, the cardia and the pylorus (Hellström et *al*., 2006). The proximal stomach (fundus and body) acts as a reservoir, as it exerts pressure on the gastric contents, allowing them to migrate

to the antral region (Brookes and Costa, 2002). The distal part of the stomach (the antrum) functions as a peristaltic pump involved in mixing and crushing gastric contents, allowing their passage into the duodenum through the pylorus (Schemann et *al*., 2008). Muscle cells in this region generate phasic contractions that allow this part of the stomach to perform its peristaltic role (Chang et *al*., 2003). Peristaltic waves propagate distally from their origin in the central body to the pylorus. These contractions facilitate the crushing and mixing of food with gastric juice (Tack, 2007). The substances contained in the antrum are both propelled forward and pushed back to the corpus of the stomach to obtaining a good mixture (Hellström et *al*., 2006).

3.6.1.1. Factors affecting gastric emptying

The mechanisms involved in emptying, or evacuation, of the stomach depends the chemical and physical composition of the food ingested (nature, solids, semi-solids, nutritious liquids, and non-nutritive liquids, volume, osmolarity and composition) (Hellström et *al*., 2006). The gastric emptying of liquids is faster than that of solids, their evacuation is of the exponential type with a half-emptying time of the order of 20 minutes (Camilleri, 2006). Isotonic fluids empty faster than those that are hypertonic or hypotonic (the duodenum seems to have osmoreceptors) (McDonald and MacFarlane, 2018). Ingested food may remain unmixed for up to 1 h after eating (Ganong, 2010). The process of eliminating digestible solids is two-phase (Fig. 4). During the first phase, which has the latency time (20 to 40 min), the solids are redistributed and broken down into small particles (1 to 2 mm) which can then pass through the pylorus during the linear phase of emptying (Horowitz et *al*., 2004). Emptying of solids takes approximately 3 to 4 hours, sometimes longer, especially if the meal is fatty or contains large particles (Camilleri, 2006). The rate of gastric emptying is proportional to the volume of chyme (with smaller particles emptying faster than large ones). Normally, chyme passing into the duodenum contains particles less than 2 mm in diameter; 90% of them are less than 0.25 mm (McDonald and MacFarlane, 2018). A decrease in duodenal pH (pancreatic secretions are alkaline) slows gastric emptying and inhibits gastric motility (McDonald and MacFarlane, 2018). Lipids also slow gastric emptying: products of fat digestion (glycerides and fatty acids) arriving in the duodenum stimulate the secretion of cholecystokinin (CCK) from endocrine cells in the duodenal epithelium, which closes the pyloric sphincter (McDonald and MacFarlane, 2018). If too much chyme with high osmolality passes into the duodenum, then there is a shift of extracellular fluid into the lumen. This can cause hypovolaemia, resulting in the ‘dumping syndrome’ sometimes observed after gastrectomy (McDonald and MacFarlane, 2018).

3.6.1.2. Gastric emptying mechanism

The main factor in the regulation of gastric emptying of food is inhibitory feedback, triggered by receptors distributed along the small intestine (Horowitz et *al*., 2004). These intestinal receptors respond to pH, osmolarity and distension, as well as the content of food (Horowitz et *al*., 2004). Nitrogen oxide, an important inhibitory neurotransmitter in the intestine, plays an essential role in the feedback control of gastric emptying (Kuo et *al*., 2007). The presence of food in the small intestine is associated with relaxation of the gastric fundus, suppression of antral contractions, and stimulation of tonic and phasic pyloric contractions (Kuo et *al*., 2007). When food enters the intestine, a number of hormones are released which can influence the speed of this process (Hellström et *al*., *2006*; Strader and Woods, 2005). Indeed, cholecystokinins inhibit gastric emptying and food intake by activating CCK-1 receptors (Dockray, 2006; Strader and Woods, 2005). A mechanism involving stimulation of CCK receptors in afferent vagal fibers and activation of a vagal efferent cholinergic pathway has been proposed as a mode of action of CCK. The important role of CCK in inhibiting gastric emptying is shown by exogenous administration of CCK, where the inhibition persists as long as the infusion of CCK is present (Moran and Kinzig, 2004). Gastrin is secreted by endocrine G cells of the antropyloric part under the influence of mucous membrane contact with food, especially protein (Dockray, 2006). It promotes fundic relaxation and decreases intragastric pressure inducing a slowing down of gastric emptying (Hunt and Ramsbottom, 1967). Motilin is secreted by endocrine cells in the upper jejunum mucosa. Motilin and its agonist, erythromycin, stimulate intestinal contractile activity and regulate inter-digestive motility by triggering phase III of CMM and increase gastric motor activity (Bruley Des Varannes et *al*., 1995). Motilin increases the rate of gastric emptying of fluids in dogs. However, in healthy volunteers, it inhibits gastric emptying of liquid meals (Ruppin et *al*., 1975). Food intake stimulates the secretion of glucagon-like peptide-1 (GLP-1) by L cells of the digestive tract (Wynne et *al*., 2005). GLP-1 causes a reduction in food intake by slowing gastric emptying in man, giving a feeling of satiety (Delgado-Aros et *al*., 2002). The YY peptide released by the entero-endocrine L cells of the intestine exerts an inhibitory action on gastric emptying and intestinal transit (Wren and Bloom, 2007; Englander and Greeley, 2006). In addition, Ghrelin, is a polypeptide secreted mainly by the stomach and to a lesser extent, by various organs (Wren and Bloom, 2007). It stimulates motility, increases food intake and gastric acid secretion (Camilleri, 2006).

3.6.2. Intestinal motility

The small intestine is moved by movements that allow the absorption and mixing of the chyme with the pancreatic juice. There are two main types of movements in the small intestine: segmentation contractions and peristaltic waves (Gwynne & Bornstein, 2007). Segmentation consists of a succession of stationary contractions without longitudinal displacement. These movements permit the movement of the chyme to and fro. They ensure the mixing of chyme and digestive juices and bring the contents of the intestine into contact with the mucous membrane to promote absorption (Gwynne & Bornstein, 2007; Ganong, 2010). Peristalsis is a reflex response of the smooth muscles in the digestive tract that is triggered when the wall is distended by substances in the lumen. Local distension of the intestine causes a circular contraction behind the stimulus (ascending excitatory reflex) and a zone of relaxation in front (descending inhibitory reflex). Sequential activation of these two reflexes promotes peristalsis, which is responsible for the coordinated propulsion of intestinal contents (Tonini et *al*., 1996).

3.6.2.1. Peristaltic reflex mechanism

Peptides involved in the endocrine and enteric nervous system as well as in the central nervous system exert concerted action on GI motility (Fujimiya and Inui, 2000). Although, both segmentation contractions and peristaltic waves can take place in the absence of extrinsic innervation, but require an intact myenteric plexus (Ganong, 2010). The enteric nervous system mediates these reflexes (peristalsis and segmentation), It controls intestinal peristalsis and involves sensory neurons and interneurons as well as ascending excitatory and descending inhibitors motor neurons (Brookes et *al*., 1992; Grider and Jin, 1994; Waterman et *al*., ., 1994).

Stretching the circular muscle and stimulating the mucosa activates different sensory pathways:

Stimulation of the mucosa activates intrinsic sensory neurons, whose cell bodies are in the intestinal wall and nerve endings in the mucosa, while, muscle stretch activates extrinsic sensory neurons with cell bodies located in the dorsal root ganglia and axonal projections to enteric neurons (Grider and Jin, 1994).

The increased intraluminal pressure caused by the bolus, stimulates 5-hydroxytryptamine (5-HT) or Serotonin release from the EC (enterocromaffin) epithelial endocrine cells in response to stimulation of the intestinal mucosa. 5-HT acts on the 5-HT1P

and/or 5-HT4 receptors on the CGRP containing primary sensory neurons lacated in the submucosa plexus. This sensory neuron is coupled to 5-HT containing interneurones in the myenteric plexus and then the neurons are coupled to ascending excitatory motor neurons and descending inhibitory motor neurons (Fujimiya and Inui, 2000).

Mechanical and chemical stimuli which induce peptide release from the epithelial endocrine cells are the earliest step in the initiation of peristaltic activities. Gut peptides exert hormonal effects, but peptide-containing stimulatory (Ach, substance P, tachynin) and inhibitory (VIP, PACAP, ATP, NO) neurons are also involved in the induction of ascending contraction and descending relaxation, respectively (Fujimiya and Inui, 2000).

The contraction of the circular muscle involves the excitatory neurotransmitters acetylcholine and tachykinins (substance P and neurokinin A) (Holzer et *al*., 1998; Fujimiya and Inui, 2000).

The relaxation of the circular muscle is ensured by the inhibitory neurotransmitters, VIP, PACAP and by nitric oxide (Grider et *al*., 1994).

Activation of inhibitory motor neurons to circular muscle elicits a hyperpolarization and relaxation of smooth muscle (Bornstein et *al*., 2004).

The inhibitory neurotransmitters participate in peristalsis by promoting the inhibitory descending reflex, which is aimed to facilitate propulsion of the chyme towards the anus (Bornstein et *al*., 2004).

There is a unique relationship between the inhibitory transmitters in that NO regulates the release of VIP and PACAP from the myenteric neurons and VIP and PACAP in turn enhance the production of NO in smooth muscle (Bornstein et *al*., 2004) (Figure 17).

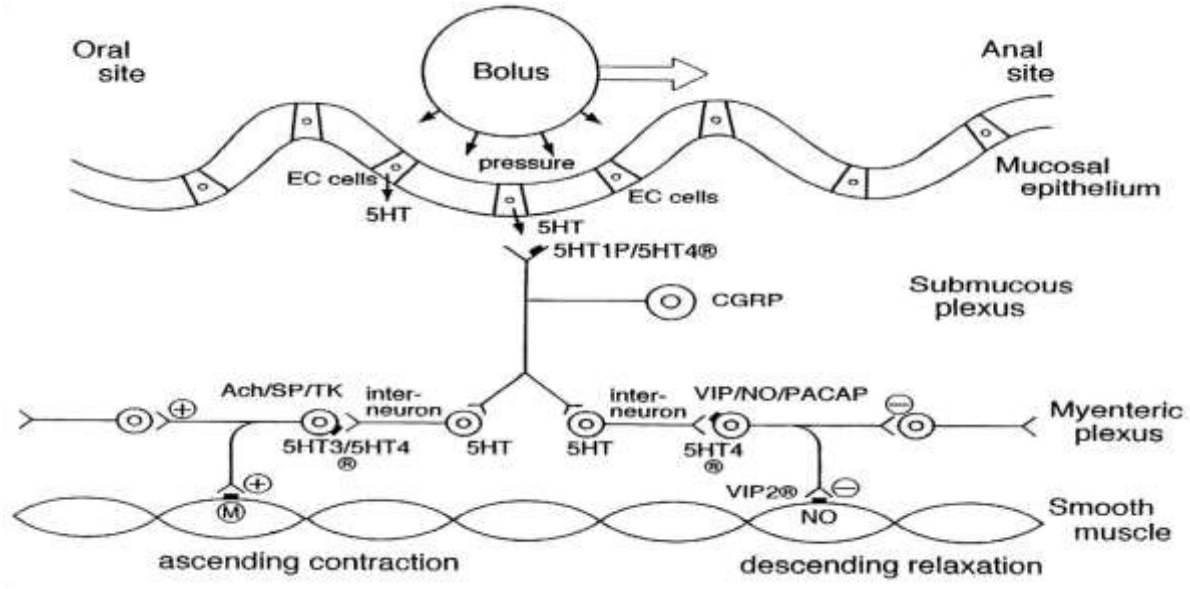

Figure 17: Mechanism of peristaltic reflex of the rat intestine (Fujimiya and Inui, 2000).

VIP: vasopressin intestinal peptide; NO: nitric oxide; PACAP: pituitary adenyl cyclase-activating peptide; CGRP: calcitonin gene-related peptide; TK: tachynin; SP: substance P; 5-HT: 5-hydroxytryptan; EC: enterochromaffin cells; Ach: acetylcholine.

3.6.3. Polyphenols effect on gastrointestinal motility

Polyphenols show relaxing effects on the contractile activity of various smooth muscles, such as vascular smooth muscles (Ajay et *al*., 2003; Mendes et *al*., 2003; Herrera et *al*., 1996), muscles of the bladder (Dambros et *al*., 2005) and the muscles of the uterus (Revuelta et *al*., 1997). Jeon et *al*., (2007) reported that the flavone induces dose-dependent relaxation of vascular smooth muscles. Epigallocatechin-3-gallate (EGCG) (catechins; a polyphenol) has also been shown to relax the aortic rings in rats after a transient increase in blood pressure. This relaxation is due in part to the inhibition of phosphodesterase and therefore the increase in the level of cAMP and cGMP, which can decrease the influx of calcium and the release of calcium from the intracellular stores of rat aortic cells. (Alvarez et *al*., 2006). In the GI tract, polyphenols show an inhibitory action against the contractions of smooth muscle cells. Indeed, Cappasso *et* et *al*., (1991) reported that 13 flavonoids have an inhibitory effect on the ileum contractions induced by PGE2, LTD4, acetylcholine and BaCl2. For contractions induced by PGE2, the inhibitory effect is observed for apigenin, quércetin, kaempferol and chrysin. Apigenin, quercetin and kaempferol are also active inhibitors of the contractions induced by LTD4. The authors have proposed that the effect of these flavonoids involves calcium. Ceregrzyn and Kuwahara (2003) reported that epigallocatechin gallate (EGCG) induces a dose-dependent

decrease in spontaneous activity in mouse jejunum. EGCG decreases the frequency and extent of contractions. A guanylate cyclase-dependent mechanism may be partially involved in the action of EGCG. In the stomach it is shown that apigenin and quercetin (1 µM – 100 µM) induce relaxation of the gastric smooth muscles of mice in a concentration-dependent manner, the action of apigenin is greater potent than quercetin. In a calcium-free medium or in the presence of nifedipine (voltage-gated L-type calcium channel blocker), the muscle relaxant effect of apigenin and quercetin is significantly reduced, suggesting that this effect is mainly due to their ability to inhibit the entry of calcium via voltage-gated calcium channels (Rotondo et *al*., 2009). Polyphenols also act on the intestinal transit thus exerting antidiarrheal effects. Di Carlo et *al*., (1993) showed that intraperitoneal administration of some flavonoids (apigenin, flavone, kaempferol, morine, myricetin, naringin and rutin; 12.5-50 mg / kg) reduced intestinal transit by 28-69% in mice. Other flavonoids (naringenin, silibinin, silymarin and taxifolin, 100-200 mg / kg) reduce intestinal transit by 23-41%, while hesperitin, catechin and phloridzin (more than 200 mg / kg) n 'have no effect. Flavonoids (quercetin, apigenin, naringenin and genistein) exert an inhibitory action on intestinal peristalsis. The action of naringenin and genistein is more potent than that of quercetin and apigenin. In fact, naringenin and genistein at a concentration of 10 µM significantly increase the threshold of peristaltic pressure; while apigenin and quercetin require a higher concentration (100 µM) (Gharzouli and Holzer, 2004).

3.7. Peptic ulcer

Peptic ulcer disease continues to be a source of significant morbidity and mortality worldwide. Approximately two-thirds of patients found to have peptic ulcer disease are asymptomatic. In symptomatic patients, the most common presenting symptom of peptic ulcer disease is epigastric pain, which may be associated with dyspepsia, bloating, abdominal fullness, nausea, or early satiety (Kavitt et *al*., 2019). The term peptic ulcer refers to acid peptic injury of the digestive tract, resulting in mucosal break reaching the submucosa. Peptic ulcers are usually located in the stomach or proximal duodenum, but they can also be found in the esophagus or Meckel's diverticulum (Lanas and Chan, 2017). Gastric and duodenal ulcers has been a major threat to the world's population over the past two centuries, with high morbidity and substantial mortality (Malfertheiner et *al*., 2009). Gastric ulcer is predominantly characterized by damage to the gastric mucosa in the stomach lining, resulting in abdominal pain, possible bleeding, chest pain, fatigue, vomiting, weight loss and other GI symptoms (Awaad et *al*., 2013; Son et *al*., 2015).

3.7.1. Gastric ulcer

Gastric ulcer is a common disorder of the digestive system (Bi et *al*., 2014).

3.7.1.1. Aetiology of gastric ulcer

The multifactorial etiology of gastric ulcer includes bacterial infection, excessivealcohol intake, emotional stress, free radicals, the use of steroidal and non-steroidal anti-inflammatory drugs (NSAIDs), and nutritional deficiencies that disrupt the gastric mucosal barrier and make it vulnerable to normal gastric secretions. Ulcerogenic risk factors, such as excessive alcohol consumption and use of NSAID drugs, cause dispersal of the protective mucus gel and the phospholipid bilayer, resulting in acid back diffusion and mucosal injury secretions (Son et *al*., 2015).

3.7.1.2. Pathophysiology of gastric ulcer

The pathophysiology of ulcer is due mainly to an imbalance between aggressive factors (acid, pepsin, H. pylori, NSAIDs and local mucosal defensive factors (mucus, blood flow, endogenous prostaglandins (PGs) secretion, nitric oxide (NO), antioxidant and bicarbonate, etc). The integrity of gastro-duodenal mucosa is maintained through a homeostatic balance between these aggressive and defensive factors (Sumbul et *al*., 2010; Kansara and Sakhreliya, 2013). The gastric and duodenal mucosa is covered by mucus and bicarbonate in order to protect against gastric acid. Mucus and bicarbonate are secreted by gastric epithelium and by Brunner's glands in the duodenum. The epithelium also has a role in acid protection. The apical cell membranes and the tight junctional complexes between the surface cells limit the penetration of hydrogen ion into the mucosa. In addition, mucosal blood flow transports nutrients and oxygen and bicarbonate to the surface to neutralise acid (Allen and Flemstrom, 2005).

3.7.1.3. Models of gastric ulcer

There are several models that are used to evaluate antiulcer drugs. However, the choice of a suitable model has proven to be difficult as each model has significant advantages as well as disadvantages. The choice of a particular model is sometimes influenced by local resources, the objectives of the study, the hypothesis being tested, or research questions being answered by the researcher. The choice of model may also depend on the relevance to the type of peptic ulcer disease under investigation (Adinortey et *al*., 2013; Thabrew and Arawwawala, 2016). Peptic ulcers can be induced by physiological, pharmacological or surgical manipulations in

several animal species. However, most experiments in peptic ulcer studies are carried out in rodents. Several models are used experimentally for testing or evaluating antipeptic ulcer activity of drugs/agents and the main of these are:

a. Ethanol-induced gastric ulcers

b. NSAIDs- (indomethacin, aspirin, and ibuprofen) induced gastric ulcers.

c. Water-immersion stress or cold-water-restraint or cold-restraint stress.

d. Acetic acid-induced gastric ulcers.

e. Histamine-induced gastric ulcers.

f. Reserpine-induced gastric ulcers.

g. Serotonin-induced gastric ulcers.

h. Pylorus-ligated-induced peptic ulcers (Adinortey et *al*., 2013; Thabrew and Arawwawala, 2016).

a. Ethanol-induced gastric ulcer model and its relationship to oxidative stress

Alcohol consumption may result in several diseases including gastric ulcer. Because of its damaging effect, ethanol has been explored as a model in gastric ulcer. It is one of the must widely used models in testing gastroprotective agents. The mechanisms of ethanol-inducing ulcer are diverse. It may directly affect the gastric mucosa by reducing bicarbonate and mucus secretions and exposing it to the damaging effect of acid and pepsin or by acting on neurohormonal factors that stimulate acid secretion (Bode and Bode, 1997). Ethanol may also decrease blood flow (Adinortey et *al*., 2013). One crucial mechanism through which ethanol provokes gastric ulceration is via increased oxidative stress and reducing the antioxidant system (Hirokawa et *al*., 1998; Samonina et *al*., 2004). ROS are responsible for oxidation of tissues leading to lipid peroxidation and tissue damage. They are also responsible for oxidation of bases in cellular DNA making them mutagenic, cytotoxic and cross-linking agents, which in turn causes uncontrolled expression of certain genes causing increased multiplication of cells leading to cancer. Antioxidants seemed to have protective role in gastric ulcers. Stress causes both sympathetic (causes direct arteriolar vasoconstriction) and parasympathetic (induces an

increased motility and muscular contraction) stimulation of stomach leading to local hypoxia and near or actual "ischemia". The ischemic condition caused an increase in the level of H_2O_2 (by the action of SOD), which, in conjugation with O_2 generates OH via the methyl catalyzed Haber-Weiss reaction. Hydroxyl radicals thus generated oxidizes important cellular constituents such as structural and functional proteins membrane lipids and depletes glutathione. Lipid peroxidation causes loss of membrane fluidity, impaired ion transport and membrane integrity and finally loss of cellular functions (Suzuki et *al*., 2012). In addition, ethanol causes a massive intracellular accumulation of calcium leading to cell death (Wong et *al*., 1991). Moreover, ethanol relaxed the pyloric sphincter and increased gastric emptying which may result gastric damage (Dinoso et *al*., 1972). The ethanol-induced ulcer model looks much more appropriate for evaluating cytoprotection and/or antioxydant agents and the general pathways of ethanol-inducing gastric ulcer couldbe summarized in Figure 18.

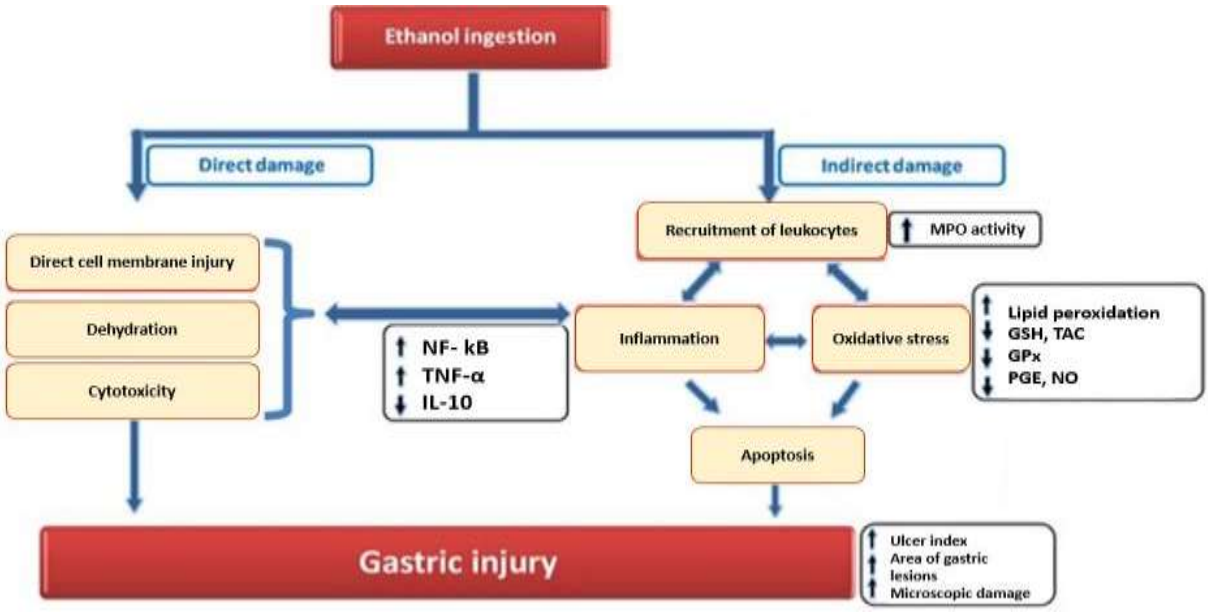

Figure 18: Main pathways of ethanol-inducing gastric ulcer (Arab et *al*., 2015).

3.7.2. Treatment

Peptic ulcer disease is a serious GI disorder that requires a well-targeted therapeutic strategy. For over a century, peptic ulcer disease has been one of the leading causes of GI surgery, with high morbidity and mortality rates. Several classes of pharmacological agents have proved to be effective in the management of acid peptic disorders. These groups include antacids (aluminum hydroxide, magnesium trisilicate), acid suppressive agents (Antisecretory drugs) which include proton pump H^+/K^+-ATPase inhibitors (omeprazole, lansoprazole),

histamine H2 receptor antagonist (cimetidine, ranitidine) and anticholinergic (pirenzepine), cytoprotective agents (sucralfate and prostaglandin analogs (misoprostol), antimicrobials for eradication of H. pylori (amoxicillin, clarithromycin) and Triple therapy (one week triple therapy consisting of a proton pump inhibitor such as Omeprazole and the antibiotics Clarithromycin and Amoxicillin) (Waller et *al*., 2005). Although these drugs are effective, they produce many adverse effects (headache, diarrhoea, abdominal pain and nausea), thus limiting their use. In recent years, there has been a growing interest in alternative therapies, especially those from plants due to their perceived relative lower side effects (Adinortey et *al*., 2013; Sriniva et *al*., 2013; Al-Snafi, 2016).

3.7.3. Herbal therapy

Numerous studies have demonstrated that herbal medicines can effectively treat gastric ulcer in humans and various animal models via divergent mechanisms. Previous studies updated the efficacy and safety of herbal medicines in treating gastric ulcer, and the mechanisms of their action in humans and animal models. Studies have demonstrated that the efficacy of herbal medicines is comparable or superior to that of drugs such as omeprazole or cimetidine in humans and animal models, and herbal medicines display fewer adverse effects. The mechanisms by which herbal medicines benefit gastric ulcer include stimulation of mucous cell proliferation, anti-oxidation, and inhibition of gastric acid secretion and H^+/K^+-ATPase activity. Some herbal medicines also exhibit antimicrobial properties. Utilization of herbal medicines could be a valuable alternative to treat gastric ulcer in humans effectively, with few adverse effects (Bi et *al*., 2014). These plants include:

Teucrium polium reduced the scarring of gastric ulcer. It induced mucosal healing, and the reduction of proliferation, mucosal hyper-plasia, migration ofinflammatory cells (Khazaei., 2018). Ngobidi et *al*., (2018) suggest that *camellia sinensis* (green tea) possess antiulcer activity, which could be due to its cytoprotective actions or strengthening of gastric mucosa, with the enhancement of mucosal defense. Glycyrrhizinic acid, a major component of *Glycyrrhiza glabra*, has antiulcer effect by raising the local concentration of prostaglandins that promote mucous secretion and cell proliferation in the stomach (Lohar et *al*., 2020). The anti-ulcer activity from *Aloe vera* (L.) Burm.f. is reported in Indomethacin induced ulcer model. The mechanism involved in production of antiulcer activity of the plant is due to its antioxidant, anti-inflammatory, mucus secreting, cytoprotective or healing activities (Borra et *al*., 2011; Srinivas et *al*., 2013). *Matricaria chamomilla* L.has a protective effect against ethanol-induced

gastric mucosal lesions, and this effect, at least in part, depends upon the reduction in lipid peroxidation and augmentation in antioxidant activity (Cemek et *al*., 2010). The Phenolics are one of the most important bioactive compounds in medicinal plants

3.7.3.1. Anti-inflammatory activity of polyphenols

Under the action of cyclo-oxygenase and lipo-oxygenase, arachidonic acid is converted into prostaglandins and leukotrienes, respectively, thus inducing inflammatory phenomena. The molecular mechanisms involved in the anti-inflammatory activity of polyphenols include inhibition of inflammation-related enzymes such as cyclooxygenase and lipooxygenase, as they can act on other molecular levels (Yoon and Baek, 2005). Landolfi et *al*., (1984) showed that flavone, chrysin, apigenin, and phloretin decrease cyclo-oxygenase activity and inhibit platelet aggregation. Quercetin, in particular, inhibits the activity of cyclo-oxygenase and lipo-oxygenase, thereby decreasing the formation of inflammatory metabolites (Kim et *al*., 1998). The phenolic compounds in olive oil inhibit the generation of leukotrienes B4 by acting on 5-lipo-oxygenase (De la Puerta et *al*., 1999). Apigenin and quercetin (0.5–50 µM) are good inhibitors of NO production by a concentration-dependent effect. On the other hand, quercetin, galangin, apigenin, and naringenin cause a remarkable decrease in the release of prostaglandins 2 and the expression of cyclooxygenase-2 in a concentration-dependent manner (Raso et *al*., 2001).

4. Selected Apiaceae plants

The Apiaceae (Umbelliferae) is a cosmopolitan family. In Algeria, this family comprises about 55 genera and 130 species (Laouer et *al*., 2014). The Apiaceae are important sources of bioactive compounds and volatile oils (Laouer et *al*., 2008). It is noteworthy that Apiaceae species contain compounds with several and different biological activities, such as antibacterial, anticancer, hepatoprotective, vasorelaxant, and cyclooxygenase inhibition (Okuyama et *al*., 1991; Pae et *al*., 2002). The richness of the Apiaceae family in secondary metabolites and their important biological properties, led us to investigate the phytochemical and biological activities of two plants of this family. The first of the genus Ammoides; *Ammoides atlantica* (Coss. & Dirieu) H. Wolff (*A. atlantica*), and the second belongs to the genus Athamanta, *Athamanta sicula* L. (*A. sicula* L.).

4.1. *Ammoides atlantica*

4.1.1. Botanical description

A. atlantica is a biennial or perennial plant with thick stem fitted with a rosette of basal leaves and slightly branched. Leaves umbels have 3-6 rays and fruit 2-2.5 mm. It is found in mountains above 1000 m (Ababsa et *al*., 2013), (Figure.19).

Figure 19: *Ammoides atlantica* (https://www.gbif.org/occurrence/1424815902).

4.1.2. Geographical distribution

The plant *A. atlantica* is endemic to Algeria (Quezel and Santa., 1962).

4.1.3. Botany systetematics of the plant

The taxonomy of *A. atlantica* is as follows:

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Apiales

Family: Apiacea Lindl

Genus: Ammoides Adans.

Species: *Ammoides atlantica* (Coss. & Dirieu) H. Wolff

4.1.4. Traditional uses

In Algerian folk medicine, *A. atlantica*, is widely used in infusions to treat headache, fever and diarrhea. It has also important digestive properties and is used in some recipes (Laouer et *al*., 2008). Sometimes, it is also used in compresses, alone or soaked in alcohol or vinegar and mixed with henna, to treat children affected by mental debility (Bellakhdar, 1997).

4.1.5. Chemical composition

The plant contains many biologically active compounds. Twenty compounds were characterized by GC-MS analysis, representing 97.9 % of the essential oil of fresh aerial part of *A. atlantica* collected from Jijel. The main components were safranal (17.9 %), endo-borneol (17.6 %), chrysanthenone (15.5 %), filifolone (12.1 %) and camphor (11.8 %) as main components (Boudiar et *al*., 2011). However, in essential oil from aerial parts of *A. atlantica*, collected in Megriss Mountain near Sétif, the main components were thymol (53.2%), γ-terpinene (19.4%) and p-cymene (10.6%) (Laouer et *al*., 2008). Phytochemical profiling of hydro-alcoholic extract of *A. atlantica* aerial parts were investigated using RPUHPLC-ESI-QTOF-MS. A total of 66 chemical compounds were detected, among these, 45 compounds were identified: hydroxycinnamic acid and derivatives (26), hydroxybenzoic acids (4), flavones (11), flavonols (3), and a lignan (Benteldjoune et *al*., 2021).

4.1.6. Pharmacological properties

The antioxidant activity of hydro-alcoholic extract of *A. atlantica* aerial parts was studied. The obtained results suggest that the antioxidant activity of the hydroalcoholic extract was comparable to common antioxidant additives (Benteldjoune et *al*., 2021). The *in vitro* antibacterial activity of the essential oil of *A. atlantica* was evaluated against several strains of bacteria, and this activity was very strong against most of the tested microorganisms, especially *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Enterococcus faecalis* ATCC 29212 (Laouer et *al*., 2008).

4.2. *Athamanta sicula* L.

4.2.1. Botanical description

The genus *Athamanta*. (Apiaceae) consists of about nine species, which are distributed mainly in Southeastern Europe (Camarda et *al*., 2008). In Algeria, there is only one species *A. sicula* L. (Syn. *Tinguarra sicula L*). The later is an annual perennial herb, growing to a height of 30 to 100 cm (Tutin, 1968; Mormile et *al*., 2002). 20-60 cm branched stems, leaves umbels with 10-20 rays, the flowers are white, oblong seeds, the very hispid fruit, 4-6 times longer than wide (Bouratoua., 2018). It occurs in calcareous vertical cliffs at altitudes of 100–1500 m (Tutin, 1968; Mormile et *al*., 2002).

Figure 20: *Athamanta sicula* L **(**Jijel Region in North-Eastern Algeria).

4.2.2. Geographical distribution

A. sicula L., endemic to South Italy, particularly in Sicily (Tutin, 1968; Mormile et *al*., 2002) and to the Maghreb countries in Algeria and Morocco (Quezel and Santa, 1963; Labed et *al*., 2012, El Ouadi, 2015; Redouan et *al*., 2020).

4.2.3. Plant systetematics

The taxonomy of *A. sicula* L. is as follows:

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Apiales

Family: Apiacea

Genus: Athamanta L.

Species : *Athamanta sicula L.*

4.2.4. Traditional uses

A. sicula L. is known in Sicilian popular medicine with the name of "spaccapietre" (rock splitters) because fresh roots infusions are indicated as diuretics and to treat urinary tract diseases; more specifically it is used to dissolve kidney stones (Baroni, 1977; Mormile et *al*., 2002). In Morroco the plant is used to treat leprosy (Redouan et *al*., 2020).

4.2.5. Chemical composition

The essential oils obtained separately from the leaves and the fruits of *A. sicula* L. collected in Sicilywere studied by capillary GC/MS. The main component in the leaf oil was myristicin (97.6%), while β-pinene (2.8%), β-phellandrene (5.8%), δ-3-carene (22.6%), terpinolene (20.5%) and myristicin (41.38) were the main components of the fruit oil (Camarda et *al*., 2003). The analysis of the chemical composition of essential oils from different parts of *A. sicula* L. in the plant material originating from Palermo contained apiol as the main constituent (Camarda et *al*., 2008). In another previous investigation of the essential oil composition of *A. sicula* L. collected in the Madonie area of central Sicily, it was found that myristicin was the main component (Camarda and Di Stefano., 2003). The observed differences in composition in the analyzed essential oils of *A. sicula* L., collected in the Palermo area could be explained by different habitat conditions or from the occurrence of various plant chemotypes (Camarda et *al*., 2003). In algeria essential oil of *A. sicula* L collected at Bekira- (Constantine,

Eastern Algerian) the main compound was myristicin (Ouadi, 2015), werheas, the main constituent of essential oil *A. sicula* L. collected at Didouche Mourad (Constantine - North-Eastern Algeria) was: germacrene B (Labed et *al*., 2012).

4.2.6. Pharmacological properties

Acetone extracts of leaves, flowers, and stems of *A. sicula* L. (collected in the flowering stage in Monte Pellegrino near Palermo, Italy, were investigated *in vitro* for antibacterial and cytotoxic activities. Antimicrobial activity was carried out against bacterial and fungal strains and antiproliferative activity against a group of human cancer cell lines (K-562, NCI-H460, and MCF-7). All acetone extracts, apiol and myristicin resulted inactive as antimicrobial agents at the maximum tested concentration of 200 µg/ml, but they induced signifcant antiproliferative activity on the tested cancer cell lines (Di Stefano et *al*., 2011). The essential oil of the aerial parts of *A. sicula* L. collected at Didouche Mourad, Constantine was tested against a wide range of bacteria, but the growth of Escherichia coli and Klebsiella pneumonia were the only strains inhibited by the essential oil of *A. sicula* (Labed et *al*., 2012).

Materials and methods

1. Materials

1.1. Plant materials

The aerial part from *A. atlantica* and *A. sicula* L. were harvested during the flowering season (June), from Setif and Jijel Regions in North-Eastern Algeria, respectively. Their scientific identification was performed by Professor. Hocine LAOUER, Department of Plant Biology and Ecology, University Setif 1, Algeria. A two vouchersnumbers 201 AA 16/6/17 SET/SA/HL and 202 AS 15/6/17 JIJ/SA/HL for *A. atlantica* and *A. sicula* L., respectively, were deposed at the laboratory of Phytotherapy Applied to Chronic Diseases. The samples were shade-dried in a dark, dry place and at room temperature for 30 days, and then crushed, using an electrical grinder.

1.2. Animals

Swiss albino mice weighing between 25 and 30 g and albino Wistar rats weighing 150–200 g were used in this study. Animals were purchased from Pasteur Institute, Algiers. They were initially housed in cages during 7 days in normal laboratory conditions and had free access to water and food ad libitum for a week. Before commencing the experiment, the animals were placed individually in cages with wide-mesh wire bottoms and were deprived of food for 18–20 h, but were supplied with water until 60 min before the start of the experiment.

2. Methods

2.1. Extraction procedure

100g of *A. atlantica* or *A. sicula* L. powder was mixed with 1 L of boiling distilled water (100 °C) and after 20 minutes, it was removed from the heat. The mixture was filtered using Wattman filter paper n°1 and then dried at 45 °C to obtain *A. atlantica* or *A. sicula* L. decoction extracts which were stored at 20°C until furtheranalysis (Ferreira et *al*., 2006).

A. atlantica and *A. sicula* L. methanolic extracts were done according to a method of Markham (1982). 100g of *A. atlantica* or *A. sicula* L. powder was macerated in 1 liter of methanolfor three days at room temperature. This procedure is repeated three times, using fresh solvents. The mixture was filtered usingWattman filter paper n°1 and then dried at 40 °C to obtain methanolic extract which was stored at 20°C until furtheranalysis.

A. atlantica and *A. sicula* L. hydromethanolic extracts were prepared according to the method described by Markham (1982). The plant powder (100 g) was macerated in 1liter methanol 80% (v/v) for three days at room temperature. This procedure is repeated three times, using fresh solvents. Solutions obtained after each maceration step were filtered using Whatman paper. The filtered solvent was concentrated by a rotary evaporator at 40°C, to give a syrupy residue, and then half of the size from this latter was dried at 40°C to obtain *A. atlantica* crude (hydromethanolic) extract (AACE) and *A. sicula* L. crude (hydromethanolic) extract (ASCE). The residue was diluted with distilled water and was then partitioned by successive extractions using water-immiscible solvents with increasing polarity; hexane, chloroform, ethyl acetate and butanol, respectively, for each plant. Each fraction was evaporated using a rotary evaporator in a water bath at 40°C, then dried at 40°C to give hexane (AAHE), chloroform (AAChE), ethyl acetate (AAEAE), butanol (AABE) and water (AAWE) extracts of *A. atlantica* plant, and hexane (ASHE), chloroform (ASChE), ethyl acetate (ASEAE), butanol (ASBE) and the remaining water extracts (ASWE) of *A. sicula* L. plant. The extraction yields were calculated and the extracts were then stored at -20°C until they are used.

2.2. Quantitative determination of phytochemicals

2.2.1. Total phenolics content

Total phenolics content (TPC) was estimated using the Folin–Ciocalteu method of Singleton and Rossi (1965) with slight modification. In brief, a 20 µl (1mg/ml) of sample (extract or standard) were mixed with 100 µl of Folin–Ciocalteu reagent (diluted ten times), rested at room temperature for 5 min and then 75 µl of sodium carbonate solution (75g/l) was added. The final mixture was incubated for 120 min in darkness at room temperature. The absorbance was read in a microplate reader at 765 nm. Gallic acid has been used as a standard drug for the calibration curve. The results were expressed in milligram equivalents gallic acid per gram of dried weight of plant extract (mg GAE/g DW)

2.2.2. Total flavonoids content

Total flavonoids content (TFC) was evaluated using the method of Topçu et *al*., (2007). A volume of 50 µl sample (1 mg/ml) was added to 10 µl of 10% aluminum nitrate, 10 µl of 1 M potassium acetate and 130 µl of methanol. After 40 min of incubation at room temperature, the absorbance was read at 415 nm in a microplate reader. Quercetin was used as a standard.

TFC is expressed in milligram equivalents quercetin per gram of dried weight of plant extract (mg QE/g DW).

2.2.3. Total flavonol content

The content of flavonol has been determined by utilizing the Kumaran and Karunakaran method (2007). A volume of 50 µl of the sample was mixed with 50 µl of $AlCl_3$ prepared in methanol (2%) and 150µl sodium acetate solution prepared in water (5 percent). The mixture was incubated for 2.5 h and the absorbance was read at 440 nm. The concentrations of flavonol were calculated using quercetin as a standard. The results were expressed in milligram quercetin equivalents per gram of dried weight of plant extract (mg QE/g DW).

2.2.4. Total tannins content

Total tannins content was evaluated utilizing the ability of the sample to precipitate hemoglobin from fresh bovine blood. A volume of hemolyzed bovine blood (absorbance=1.6) was mixed with the same volume of the sample. The mixture was incubated for 20 min, then centrifuged at room temperature at 4,000 rpm. The absorption of the resulting supernatant was measured at 576 nm (Gharzouli et *al*., 1999). Using a tannic acid calibration curve, the results were presented as milligram equivalent tannic acid per gram of dry plant extract weight (mgTAE/g DW).

2.2.5. Total chlorophylls and carotenoids content

Total chlorophylls and carotenoids in the samples were determined spectrophotometrically based on Lichtenthaler and Wellburn method (1983). A test solution of 10 mg of plant extract and 10 ml of distilled water were prepared. The absorbance (A) was read at 450, 645, and 663 nm. The concentrations (mg/100 g DW) of chlorophylls and carotenoids were then calculated using the equations:

$$\text{Chlorophyll a} = 12.7\ A_{663} - 2.69\ A_{645}$$

$$\text{Chlorophyll b} = 22.9\ A_{645} - 4.68\ A_{663}$$

$$\text{Total Chlorophylls} = 20.2\ A_{645} - 8.02\ A_{663}$$

$$\text{Total Carotenoids} = 4.07A_{450} - [(0.0435\times \text{Chlorophyll a}) + (0.367\times \text{Chlorophyll b})]$$

Where, A = Absorbance at the respective wavelength.

2.2.6. Total β-carotene and lycopenecontent

Using Barros et *al*., (2008) method, β-carotene and lycopene were evaluated. 100 mg of extract were added to 10 ml of acetone-hexane (4:6). The mixture was vigorously shaken for 1 min and then filtered through Whatman filter paper. At 453, 505 and 663 nm, the absorbance was read. The β-carotene and lycopene contents were determined using the following equations:

Lycopene (mg / 100 ml) = $-0.0458\ A_{663} + 0.372\ A_{505} - 0.0806\ A_{453}$.

β-Carotene (mg / 100 ml) = $0.216\ A_{663} - 0.304\ A_{505} + 0.452\ A_{453}$.

2.3. Phytochemical analysis

2.3.1. Ultra-High performance liquid chromatography-mass spectrometry analysis

For the Ultra-High performance liquid chromatography-mass spectrometry (UHPLC-MS) analysis, 50 mg of each extract were dissolved in 5 ml of methanol or water and the resulting solutions were filtered through a 0.2 µm Nylon membrane (Whatman). Three independent analyses were carried out for reproducibility. This technique was performed using a Thermo Scientific Ultimate 3000RSLC (Dionex) equipped with a Dionex UltiMate 3000 RS diode array detector and coupled to a mass spectrometer. The column used was a Thermo scientific hypersil gold column (1000 mm × 20 mm) with a part size of 1.9 µm and its temperature was maintained at 30 °C. The mobile phase was composed of (A) acetonitrile and (B) 0.1% formic acid (v/v), both degassed and filtered before use. The flow rate was 0.2 ml/min. The solvent gradient started with 5% of solvent B over 14 min followed by 40% of solvent B for 2 min, 100% over 7 min and finally 5% over 10 min. The injection volume was 2 µl. UV–vis spectral data were gathered in a range of 250 to 500 nm and the chromatographic profiles were documented at 253 nm. The mass spectrometer used was an LTQ XL linear ion trap 2D equipped with an orthogonal electrospray ion source (ESI). The equipment was operated in negative-ion mode ($[M^-H^-]$) with electrospray ionization source of 5.00 kV and ESI capillarity temperature of 275 °C. The full scan covered a mass range of 50–2000 m/z. Collision-induced dissociation MS/MS and MSn experiments were simultaneously acquired for precursor ions. The compounds identification was done using a local database (Faustino et *al*.,2018).

2.3.2. Gas chromatography-mass spectrometry analysis

Gas chromatography-mass spectrometry (GC-MS) analysis of each silylated hexane extract was performed using a GC–MS QP2010 Ultra Shimadzu equipped with a DB-5- J & W capillary column (30 m x 0.25 mm i.d. and a film thickness of 0.25 µm). Samples were injected with a split ratio 1:50 and helium as carrier gas with a flux of 1.13 ml/min. The temperature of the column was maintained at 130 °C for 1 min and then increased, first at 6 °C/min. until 190 °C, followed by 1.2 °C/min. until 205 °C and lastly 3 °C/min. until achieved 300 °C, which was maintained for 5 min. Injector temperature was at 320 °C and the transfer-line temperature was at 200 °C. The mass spectrometer was operated in the electronic impact (EI) mode with energy of 70 eV, and data were collected at a rate of 1 scan/s over a range of m/z 50–1000. The performed chromatography lasted a total of 60.2 min and the standards were analysed separately by GC–MS under the same chromatographic conditions.The photo components of the extracts from the different species were identified based on direct comparison with the library entries of mass spectra database (NIST14 Mass spectral and WILEY Registry TM of Mass Spectra Data) and by comparing the retention times and mass spectra data of the standard compounds injected in the same chromatographic conditions. The compounds identification was done using a local database (Faustino et *al*.,2017).

2.4. *In vitro* pharmacological effects

2.4.1. Antioxidant activities

2.4.1.1. DPPH free radical-scavenging assay

Free radical scavenging potential against DPPH (1, 1-diphenyl-2-picryl-hydrazyl) radical was determined spectrophotometrically according to the method of Blois (1958). A volume of 40 µl of the sample (extracts or standard) at different concentrations was mixed with 160 µl of 0.1 mM DPPH (in methanol). After incubation at room temperature for 30 min in dark, and using a microplate reader, the absorbance was read at 517 nm. Butyl hydroxyl toluene (BHT) was used as standard. Free radical scavenging potential was determined as inhibition percent by the following equation:

$$\text{Inhibition (\%)} = [(A_{control} - A_{sample})/A_{control}] \times 100.$$

Where $A_{control}$ represents the absorbance of the reaction mixture in the absence of sample, and A_{sample} represents the absorbance of the reaction mixture in the presence of extract or standard.

2.4.1.2. ABTS radical scavenging assay

The spectrophotometric analysis of $ABTS^{+}$ (2.2´-Azino-bis-(3-ethylbenzenothiazoline-6-sulfonic acid) radical scavenging activity was determined according to the method described by Re et *al*.,(1999). The $ABTS^{\bullet+}$was prepared by mixing 7 mM ABTS (in water) and 2.45 mM potassium persulfate, then the mixture was kept in darkness at ambient temperature for 16 h before use. The dilution of the obtained solution was done to give an absorbance of 0.700 to 0.75 at 734 nm. Then, to 40 µl of the sample (extracts orBHT as standard) at various concentrations was added to 160 µl of $ABTS^{\bullet+}$ solution. After the incubation period at room temperature for 10 min, the absorbance of the tested samples was measured at 734 nm by a microplate reader. The scavenging potential of $ABTS^{\bullet+}$ was determined by the following equation:

$$\text{Scavenging effect (\%)} = [(A_{control} - A_{sample})/A_{control}] \times 100.$$

2.4.1.3. Reducing power assay

The reducing antioxidant power was examined with small changes according to Oyaizu (1986). Briefly, 10 µl of the sample at various concentrations, 40 µl of 0.2 M phosphate buffer (pH=6.6) and 50 µl of potassium ferricyanide (1%) were mixed. Then the incubation process was performed for 20 min at a temperature of 50°C. After that, 50 µl of 10% trichloroacetic acid (TCA) were added, and the mixture was centrifuged for 10 min at 1000 rpm. A volume of 40 µl of the upper layer of solution, 10 µl of 0.1% ferric chloride and 40 µl of distilled water were mixed. The absorbance was read spectrophotometrically using a microplate reader at 700 nm. Higher absorbance shows a greater reducing power. The results were presented as $A_{0.5}$ (µg/ml) corresponding to the concentration indicating 50% absorbance intensity. The reducing potential of the various extracts was compared with that of α-tocopherol (TCP) as standard.

2.4.1.4. Cupric reducing antioxidant capacity assay

Cupric ion reducing antioxidant capacity (CUPRAC) was determined according to the modified method described by Apak et *al*., (2004). A volume of 50µl of $CuCl_2$ (10 mM), 50 µl of neocuproine (7.5 mM) and 60 µl of NH_4Ac buffer solutions (1 M, pH 7.0) were mixed. A

volume of 40 µl of the sample at various concentrations was then added to the mixture. After 60 min, the absorbance at 450 nm was recorded using a microplate reader. The results were presented as $A_{0.5}$ (µg/ml), like in the reducing power assay, and were compared with BHT as standard.

2.4.1.5. Phenanthroline assay

Phenanthroline activity was determined according to the method described by Szydlowska-Czerniaka et *al*., (2008). A volume of 10 µl of various concentrations of the extract, 30 µl of 0.5% phenanthroline (in methanol), 50 µl 0.2% $FeCl_3$ and 110 µl of methanol were mixed. The obtained mixture was incubated for 20 min at 30°C, and then the absorbance was read at 510 nm. The results were given as $A_{0.5}$ (µg/ml). BHT was used as a drug reference.

2.4.1.6. Metal chelating assay

The chelation of metal ions was evaluated using a previously reported method by Decker and Welch (1990). A volume of 40 µl of different concentrations of extracts or ethylenediaminetetraacetic acid (EDTA) as standard were added to 40 µl of methanol and 40 µl of $FeCl_2$ (0.2 mM). Then, 80 µl of ferrozine solution (0.5 mM) were then added. The obtained samples were incubated for 10 min at ambient temperature after vigorous stirring. The absorbance was measured spectrophotometrically by a microplate reader at 593 nm. This equation was used to calculate the Fe^{2+} chelating effect:

$$Fe^{2+} \text{ chelating effect } (\%) = [(A_{control} - A_{sample})/A_{control}] \times 100.$$

2.4.1.7. β-Carotene-linoleic acid bleaching assay

The β-carotene bleaching capacity was evaluated using the method described by Marco (1968). A stock solution of β-carotene/linoleic acid was initially prepared by mixing a solution of β-carotene (0.5 mg) in chloroform (1 ml) with linoleic acid (25 µl) and Tween 40 emulsifier (200 mg). The mixture was evaporated under a vacuum to remove chloroform. The above mixture was added to 100 ml of water saturated with oxygen, and then the mixture was shaken. A volume of 40 µl of the sample at different concentrations (extracts or BHT) was mixed with 160 µl of the above mixture. Immediately, A_0 (the absorbance at zero time) was recorded utilizing a microplate reader at 470 nm. The plate was incubated at 50°C for 2 h, and then, the absorbance (At) of the mixture was measured again at 470 nm. The following equation was used to calculate the bleaching rate of β-carotene:

$R = \ln (A_0/ A_t)/t$.

Where A_0 and A_t are absorbance at time zero and t = 120 min respectively. ln is the natural logarithm. The antioxidant activity was calculated in terms of percentage inhibition relative to the control using the equation:

Antioxidant activity (%)= $[(R_{control}-R_{sample})/R_{contol}] \times 100$.

2.4.2. Enzymatic inhibitory assay

2.4.2.1. Acetylcholinesterase and butyrylcholinesterase inhibition assay

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory capacities were measured according to the modified method described by Ellman et *al*., (1961). A volume of 10 µl of the sample at different concentrations, 150 µl of 100 mM sodium phosphate buffer (pH 8.0), and 20 µl AChE ($5.32 \times 10-3$ U) or BChE ($6.85 \times 10-3$ U) solution were mixed, then incubated for 15 min at 25°C. After that, 10 µl of 0.5 Mm DTNB (Dithiobisnitrobenzoic acid) reagent and 10 µl of 0.71 mM acetylthiocholine iodide or 10 µl of 0.2 mM butyrylthiocholine chloride were added. Galantamine was used as a standard. The absorbance was recorded at 412 nm against a blank (phosphate buffer, pH 8, and ethanol). Inhibition percentage of AChE or BChE enzymes in the presence of the sample was determined relative to the control which contains enzyme without sample, using the following equation:

AChE or BChE inhibition activity (%) = $[(A_{control}-A_{sample})/A_{control} \times 100]$.

2.4.2.2. Tyrosinase inhibition assay

Tyrosinase inhibitory activity was measured according to the method of Deveci et *al*., (2018). To 10 µl of different concentrations of sample, 150 µl of sodium phosphate buffer (100 mM) with pH=6.8, and 20 µl tyrosinase enzyme (from mushroom) solution in buffer were mixed and incubated at 37°C for 10 min, then 20 µl of L-DOPA (5 mM) were added. After 10 min of incubation at 37°C, the absorbance was measured at 475 nm using a 96-well microplate reader. Kojic acid was utilized as a standard inhibitor. The tyrosinase inhibition ability was evaluated relative to the control using the following equation:

Tyrosinase inhibition (%)=$[(A_{control}-A_{sample})/A_{control} \times 100]$.

2.4.2.3. α-Amylase inhibition assay

α-Amylase inhibitory ability was performed using IKI (iodine / potassium iodide) method developed by Zengin et *al*.,(2015), with some modifications. In brief, 25 µl of the sample (Extracts or acarbose as standard) were mixed with 50 µl of α-amylase solution (1U) and incubated at 37°C for 10 min. Then 50 µl of starch (0.1%) was added. After incubation for 10min at 37°C, 25 µl of HCl (1M) and 100µl IKI were added. The blank contained a sample without enzyme. The control contained all the reaction solutions except the sample. The absorbance was determined at 630 nm utilizing a microplate reader. The α-amylase inhibition potential was evaluated against a blank, in terms of inhibition percentage relative to the control.

α-Amylase inhibition (%)=$[(A_{control}-A_{sample})/A_{control}\times 100]$.

2.4.2.4. α-Glucosidase inhibition assay

The α-glucosidase inhibitor effect was evaluated according to the method described by Lordan et *al*., (2013). A volume of 50 µl of sample and 50 µl of p-nitrophenyl-a-D-glucopyranoside solution (5 mM) (prepared in phosphate buffer (100 mM) with pH (6.9), were mixed and incubated for 10 min at 37°C. Then, 100 µl of α-glucosidase (0.1 U/ml) were added. The absorbance was recorded against a blank of the sample using a microplate reader at 405 nm after 0 and 30 min set to 37°C, respectively. Acarbose was included as a standard. The activity of α-glucosidase inhibition was calculated as follows:

α-Glucosidase inhibition activity (%)= $[(A_{control}-A_{sample})/A_{control}\times 100]$.

2.5. *In vivo* biological activities

2.5.1. Acute oral toxicity

Acute oral toxicity of *A. atlantica* and *A. sicula* L. extracts was tested using mice according to guideline 423 (2001) proposed by the Organisation for Economic Cooperation and Development (OECD, 2008). The extracts were orally administered at 2 single oral doses (2000 and 5000 mg/kg). The animals were not fed for three hours following administration. The mortality, gross behavioral and clinical signs (unusual aggressiveness, restlessness, dullness, sedation, somnolence, twitch, tremor, agitation, catatonia, paralysis, convulsion, writhing, prostration and unusual locomotion, etc) were observed at short intervals for 24 h. If the animals survived, all of the experimental animals were maintained under close observation for 14 days,

and the number of mice that died within the study period was noted. After 14 days, mice were sacrificed and all the organs were removed for gross pathological examination. The LD_{50} was predicted to be above of tested dose (2000 mg/kg or 5000 mg/kg) if three or more mice survived.

2.5.2. Preliminary selection of tested doses for biological activities *in vivo*

Different doses of hydromethanolic extracts from both Apiaceae plants were tested in mice and rats, to choose the effective dose in biological activities. The tested extracts showed a remarkable effect starting of the 100 mg/kg dose, and a good effect at 400 mg/kg dose.

2.5.3. Gastric emptying and small intestine transit in mice

The test meal consisted of 0.1% phenol red (non-nutrient meal, a non-absorbable and easily detectable marker) dissolved in 1.5% carboxymethyl cellulose (CMC) was used in this study. Gastric emptying was measured according to the method described by Amira et *al*., (2005). After 18-20 h of fasting, mice were orally pretreated with AACE and ASCE (100, 200 and 400 mg/kg) and CMC 1.5% as negative control. Atropine (1 mg/kg) was used as a standard. After one hour of the treatment, each animal received 0.2 ml of the test meal and was sacrificed 20 min later. Under a laparotomy, the stomach and the small intestine were excised. The stomach was homogenized with its contents in 0.1 N NaOH. The homogenate was allowed to settle for 1 h at room temperature and an amount of the supernatant were added to trichloroacetic acid (33%) to precipitate proteins. After centrifugation (1600 g for 30 min), NaOH (2 N) was added to the supernatant. The mixture was homogenized and its absorbance (A) was read at 560 nm. The gastric emptying (GE) rate in the 20-min period was calculated according to the following formula:

$$GE\ (\%) = (A_{standard}\text{-}A_{test}/A_{standard}) \times 100.$$

Immediately after the excision of the stomach, the whole small intestine was grossly freed from its mesenteric attachments and its length measured using a ruler. The intestine was opened at the level of the front of the test meal, which was then exactly localized by a drop of 0.1 N NaOH. The rate of intestinal transit was expressed as the ratio between the distance travelled by the test meal and the total length of small intestine.After determining the most effective doses of the extract on the rate of gastric emptying and intestinal transit, another series of experiments was applied using the chosen dose (400 mg/kg) under pretreatment of animals with L-nitro-n-arginine (L-NNA), (1.36×10^{-1}mmol/kg), atropine (3.45×10^{-3}mmol/kg) or

indomethacine (5.58×10^{-2}mmol/kg) to assess possible interference of the extract with neurotransmitters that control GI motility. Atropine, indomethacine and L-NNA were injected intraperitoneally.

2.5.4. Ethanol-induced gastric ulceration in rats

2.5.4.1. Gastroprotective effect evaluation

The method followed is thatdescribed by Gharzouli et *al*., (2002). It consists in checking the protective action of AACE and ASCE at 100, 200 and 400 mg/kg against the ulcer caused in animals by administration of pure ethanol. A total of nine batches of eight rats were set up for the test, one batch for each extract dose (100, 200 and 400 mg/kg) for each plant, one batch for the normal control, one batch for the negative control (CMC 1.5 %) and one batch for ranitidine (40 mg/kg) which is used as a standard (positive control). The rats fasted for 24 hours with free access to the glycosylated water, they are placed separately in individual cages before experimentation. An hour after removing the water, and at time T=0, the rats received intragastrically 1 ml/200 g of sample (crude extract at different dose or ranitidine). The control group received only CMC (1.5%). One hour later, each rat received 0.5 ml/200 g of pure ethanol intragastrically. Half an hour after administration of the ethanol, the rats were sacrificed. The stomach of each rat was removed, opened with great curvature using a chisel, washed with physiological solution and then spread well and fixed on a tablet to better observe the ulcers formed. The stomachs were photographed for better vision. The total area of the lesions and the total area of the stomach were measured using Image J 1.52o software (Wayne Rasband, National Institutes of Health, USA). The percent of the ulceration was calculated for each group treated according to this formula:

% Ulceration = [UAs/ UAc] ×100.

Where: UAc: ulcer area of the control. UAs: ulcer area of the Sample.

Histological analyzes of the glandular gastric mucosa were performed to determine the severity of the ulcers. Mucus in gastric layer was determined, also, antioxidant activity was determined using following biochemical parameters: estimation of lipid peroxidation (MDA), reduced glutathione (GSH), catalase (CAT), Superoxide dismutase (SOD) and total proteins.

After determining the most protective doses of the AACE and ASCE against the ulcer, another series of experiments was applied using the chosen dose (400 mg/kg) with arginine

(1.72mmol/kg), L-NNA (1.36×10^{-1}mmol/kg) or atropine (3.45×10^{-3}mmol/kg) to assess possible interference of the extract with neurotransmitters that control Gastroprotective effect. Atropine and L-NNA were injected intraperitoneally and Arginine was administered intragastrically. % ulceration, histological analyzes of the glandular gastric mucosa, mucus in gastric layer and antioxidant activities were determined.

2.5.4.2. Histopathological preparations

The histological sections were made at the pathology anatomy laboratory (CHU of Sétif). A portion from glandular part of stomach from each experimental group was fixed in formalin (10%). They were cut into small pieces. These samples are dehydrated by passage through three successive ethanol baths of 30 min (70, 90 and 100°C). Then they are thinned in two baths of 20 minutes of toluene and included in the paraffin (two baths of 2 hours each). The operation is automated using an automaton (TISSUE-TEK). The final inclusion is then carried out in metal molds. The paraffin blocks obtained are then cut with a microtome and the 5 µm thick sections are spread on slides with a 2% gelatin gel and then dried in an oven set at a temperature of 35-42°C., rehydrated and dried, stained with hematoxylin-eosin. The tissue sections were examined by a pathologist using microscopic analysis. The slides were later photographed.

2.5.3.3. Determination of mucus in gastric layer

Adherent mucus measurement was carried out in rats according to the alcian blue dye method described by Corne *etal*. (1974), this cationic dye binds to glycoproteins and soluble mucopolysaccharides into insoluble complexes without penetrating mucosal cells. Each glandular portion of the stomach was immediately immersed in 0.1% alcian blue solution. At the end of the 2 h-period, the unbound dye was removed by two successive washings, first for of 15 min. and then for 45 min with 0.25 M sucrose. The mucus-bound dye was then eluted by immersion of the tissue in 0.5 M magnesium chloride and shaken for 2 h. Four milliliters of the blue extract were then briefly shaken with diethyl ether in order to dissolve possible particles, which could interfere with the spectrophotometric determination. The absorbance of the aqueous phase was then read at 605 nm (Shimadzu UV/Vis 1601 Spectrophotometer, Japan). The amount of alcian blue extracted per gram of wet glandular tissue was then calculated by linear regression with a calibration curve obtained from standard serial dilutions of different concentrations of the dye, and results were expressed as µg of alcian blue/g of tissue.

2.5.4.4. Evaluation of *in vivo* antioxidant activity of gastric homogenate

a. Preparation of homogenate

The glandular portion from each stomach was cut, weighed and homogenized in 50 mM Tris HCl buffer (pH 7.4) using dounce homogenizer in ice-cold condition to obtain 10 % (w/v) homogenate. Then the homogenate was centrifuged at 4000 g at - 4 °C for 15 min and the supernatant was collected and stored at -20 °C for the following biochemical parameters estimation: lipid peroxidation (MDA), reduced glutathione (GSH), catalase (CAT), Superoxide dismutase (SOD) and total proteins.

b. Estimation of gastric total proteins content

Gastric total proteins were determined by the method of Gornall et *al*., (1949) using the Biuret kit total protein reagent (potassium iodide, potassium sodium tartarate, copper sulphate and sodium hydroxide). Proteins give a blue-violet color with copper sulphate in alkaline medium. In brief, 1 ml of Biuret reagent was mixed with 25 µl of the tissue homogenate or standard (bovine serum albumin), then the mixture was incubated at room temperature for 10 min. The absorbance was then read at 540 nm. Total protein amount wascalculated according to the following formula:

Total protein (mg/ml) = $(A_{sample}/A_{standard}) \times n$.

Where n is standard concentration.

c. Estimation of catalase activity

Catalase (CAT) activity was measured by the method of Clairborne (1985) with slight modification. The principle of this assay is based on the hydrogen peroxide breakdown in the presence of catalase according to the following reaction:

$$2H_2O_2 \xrightarrow{CAT} 2H_2O + O_2$$

A solution of 19 mM H_2O_2 (2.9 ml) in 50 mM phosphate buffer pH 7.4 was put into a quartz cuvette, 50 µl of tissue homogenate was added. The rate of decomposition of H_2O_2 in presence of CAT was monitored spectrophotometrically at 240 nm immediately and at every 15 seconds for 1 min; the enzymatic activity was expressed as nmole H_2O_2/min/mg.

d. Assessment of reduced glutathione

Reduced glutathione (GSH) was measured by the method of Ellman (1959). The assay is based on the oxidation of GSH by 5, 5' -dithio-bis (2-nitrobenzoic acid) (DTNB) (Ellman's reagent). DTNB and GSH react to generate 2-nitro-5-thiobenzoic acid (TNB) which has an intense yellow color and maximum absorbance at 412 nm. For this assay, 50 µl of the tissue homogenate was diluted in 10 ml of phosphate buffer (0.1 M, pH 8). To 3 ml of the mixure of dilution, 20 µl of DTNB (0.01 M) were added and after 5 min of incubation, the yellow color developed was read at 412 nm. The concentration of GSH was calculated using the molecular absorption coefficient (ε TNB: 13.6 $.10^3$ $M^{-1}.cm^{-1}$), (Razinger et *al*.,2008). The results were expressed as mmol/mg of tissue.

e. Determination of superoxide dismutase activity

The method used to determine superoxide dismutase (SOD) activity was based on the capacity of SOD to inhibit pyrogallol autoxidation, according to Marklund and Marklund (1974) and Gao et *al*., (1998). Supernatant aliquots (5 µl) were added to 1 ml of buffer solution (50 mM Tris HCl, pH 8.2) and 10 µl pyrogallol (20 mM). An increase in absorbance was measured at 420 nm every 30 s for 1 min against a blank.

Inhibition % = $(A_{control}-A_{sample}/A_{control}) \times 100$.

The amount of enzyme that produces 50% inhibition of pyrogallol autoxidation, relative to the control, was defined as one unit of SOD activity.

f. Lipid peroxidation estimation

Stomach tissue lipid peroxidation (LPO) was assessed by measuring malondialdehyde (MDA) formation following the method of Ohkawa et *al*., (1979). The principle of this method consists of the reaction of MDA with thiobarbituric acid (TBA) in acid conditions and a higher temperature (100°C) to form a pink MDA-(TBA)2 complex. Briefly, 0.5 ml of TCA (20 % w/v) was added to 0.5 ml of tissue homogenate, then 1 ml of TBA (0.67 % w/v) was added. The mixture was incubated at 100 °C for 15 min, cooled immediately in ice and mixed with 4 ml of n-butanol and centrifuged at 3000 rpm for 15 min. The absorbance of the clear pink supernatant was measured spectrophotometrically at 532 nm against a blank. The concentration of MDA was calculated using the molecular absorption coefficient (ε MDA-TBA: 156 $mM^{-1}.cm^{-1}$),

(Razinger et *al*., 2008). The results were expressed as nmole of MDA/ g of tissue (nmole MDA/g of gastric tissue).

2.6. Statistical analysis

Statistical analysis was performed using GraphPad Prism7.00 software. *In vitro* results were expressed as mean ± standard deviation (SD). The *in vivo* pharmacological results were presented as mean ± standard error of mean (SEM). In all cases, the values were compared by using oneway analysis of variance (ANOVA) followed by Tukey's multiple comparison test. The P-values less than 0.05 were considered statistically significant.

Results

I. Comparative study between decoction, methanolic and hydromethanolic extracts

1. Extraction yields

The weight percentage (**%)** of the obtained dried extract (g) with respect to the initial amount of the dried powder (g) is defined as yield (g/g DW).

The percentage of extraction yield of hydromethanolic (22.1±0.63%) was similar to methanolic extract (20.38±0.41%; P>0.05) and lower than decoction extract (24.32±1.01%; P≤0.05) from *A. atlantica.* In *A. sicula*, the highestpercentage of extraction yield was found in hydromethanolic extract (26.97±0.92%) than decoction (P≤0.01) and methanolic (P≤0.001) extracts (Table 02).

Table 02. Percentage of extraction yields obtained from the decoction, methanolic and hydromethanolic extractsof*A.atlantica* and *A. sicula.*

Extraction yields % (g/g DW)	*A.atlantica*	*A. sicula*
Decoction extract	24.32 ± 1.01^{B}	23.18 ± 0.15^{C}
Methanolic extract	20.38 ± 0.41^{A}	21.19 ± 0.82^{D}
Hydromethanolic extract	22.1±0.63	26.97±0.92

[a]ns; no significant difference (P>0.05), [B]*; P≤0.05, C**; P≤0.01, [D]***; P≤0.001 *v*shydromethanolic extract.

2. Phytochemicals screening

The highest TPC was found in *A. atlantica* hydromethanolic extract (238.31±11.68 mg GAEq /g DW, P≤0.0001) followed by *A. atlantica* decoction and methanolic extracts, respectively. Also, *A. atlantica* hydromethanolic extract had the highest TFC (97.81±6.98 mg GAEq /g DW) compared to decoction (P≤0.001) and methanolic extracts (P≤0.0001). In *A. sicula,* hydromethanolic extract had a greater TPC (149.58±0.77mg GAEq /g DW) than decoction and methanolic extracts (P≤0.0001). Whereas, the TFC of hydromethanolic extract (40.48±3.97 mg QEq /g DW) was similar to methanolic extract (43.12±2.29 mg QEq /g DW; P>0.05) and higher than decoction (17.91±1.81mg QEq /g DW; P≤0.001) extracts (Table 03).

Table 03. Total phenolics and flavonoids contents in decoction, methanolic and hydromethanolic extracts from *A. atlantica* and *A. sicula.*

Extracts	TPC (mg GAEq / g DW)		TFC (mg QEq / g DW)	
	A.atlantica	*A. sicula*	*A.atlantica*	*A. sicula*
Decoction extract	141.74±0.44^{E}	58.01±1.18^{E}	61.87±6.7^{D}	17.91±1.81^{D}
Methanolic extract	85.56±4.71^{E}	100.37±8.03^{E}	40.55±4.09^{E}	43.12±2.29^{A}
Hydromethanolic extract	238.31±11.68	149.58±0.77	97.81±6.98	40.48±3.97

[a]ns; no significant difference (P>0.05), ^{D}***; P≤0.001, ^{E}**** ; P≤0.0001*v*shydromethanolic extract.

3. Antioxidant activities

The highest DPPH radical scavenging activity was found in *A. atlantica* hydromethanolic extract compared to *A. atlantica* methanolic and decoction extracts (P≤0.0001). *A. atlantica* hydromethanolic extract had a similar radical scavenging activity (no significant differences; P>0.05) compared to that of BHT (IC_{50}=22.32±1.19 µg/ml) as standard**.** *A. sicula* hydromethanolic extract had the highest DPPH radical scavenging potential compared to *A. sicula* methanolic (P≤0.0001) and decoction (P≤0.001) extracts.

In other hand, *A. atlantica* hydromethanolic extract had stongest ABTS radical scavenging activity (IC_{50} =9.17±0.81 µg/ml) than *A. atlantica* methanolic and decoction extracts (P≤0.0001). Whereas, *A. sicula* hydromethanolic extract (IC_{50} =31.14 ± 1.78 µg/ml) was less active than *A. sicula* decoction extract (IC_{50} = 18.29±0.75 µg/ml; P≤0.01) and no significantly different compared to ABTS scavenging effect of *A. sicula* methanolic extract (33.19±5.54; P>0.05).

There is no significant difference between metal-chelating activities of *A. atlantica* hydromethanolic and decoction extracts (P>0.05). The activity of these two extracts were higher than *A. atlantica* methanolic extract (P≤0.0001). Also, *A. sicula* showed that hydromethanolic extract had a best metal chelating activity (IC_{50} =29.24±2.63 µg/ml), with no significant difference compared to decoction extract (IC_{50}=30.39±2.44 µg/ml, P>0.05), and very significant difference (P≤0.0001) compared to methanolic extract.

Also, *A. sicula* hydromethanolic extract had the highest β-carotene bleaching activity compared to *A. sicula* methanolic (P≤0.01) and decoction (P≤0.0001) extracts. Whereas, the β-carotene bleaching activity of *A. atlantica* hydromethanolic extract was lower compared to *A.*

atlantica decoction extract (P≤0.0001) and higher compared to *A. atlantica* methanolic extract (P≤0.01).

Hydromethanolic extract from *A. atlantica* and *A. sicula* plants exhibited a best reducing power thanmethanolic and decoction extracts (P≤0.01 and P≤0.0001, respectively).

In phenanthroline assay, the antioxidant activity of *A. atlantica* hydromethanolic extract ($A_{0.5}$=57.33±1.57µg/ml) was similar to that in *A. atlantica* decoction extract ($A_{0.5}$= 74.09±6.22µg/ml, P>0.05), and highest than *A. atlantica* methanolic extract ($A_{0.5}$= 196.03±11.49 µg/ml, P≤0.0001). *A. sicula* hydromethanolic extract was more active ($A_{0.5}$= 167.2 ± 20 µg/m) than *A. sicula* methanolic and decoction extract (P≤0.01 and P≤0.0001, respectively).

Cupric reducing antioxidant capacity from *A. atlantica* was higher in decoction extract, followed by hydromethanolic and methanolic extracts, with no significant difference between decoction, hydromethanolic and BHT effects (P>0.05). Cupric reducing antioxidant capacity from *A. sicula* decreased in order of decoction, methanolic and hydromethanolic extracts, (Table 04).

Table 04. DPPH and ABTS radicals scavenging, reducing power, metal chelating and β-carotene bleaching activities of decoction, methanolic and hydromethanolic extracts from *A. atlantica* and *A. sicula.*

	Extracts		Decoction	Methanolic	Hydromethanolic	BHT	EDTA	α-Tocopherol
IC_{50} (µg/mL)	DPPH	*A. atlantica*	204.22±12 $^{e\,E}$	107.48±5.90 $^{e\,E}$	21.26±0.08 ^{a}	22.32±1.19	NT	NT
		A. sicula	59.17±0.59 $^{e\,D}$	79.55±2.55 $^{e\,E}$	51.54±0.58 ^{e}			
	ABTS	*A. atlantica*	20.01±0.28 $^{e\,E}$	32.87±1.33 $^{e\,E}$	9.17±0.81 ^{e}	1.29±0.30	NT	NT
		A. sicula	18.29±0.75 $^{d\,C}$	33.19±5.54 $^{e\,A}$	31.14 ± 1.78 ^{e}			
	Metal chelating	*A. atlantica*	36.57±4.73 $^{c\,A}$	222.06±11.02 $^{e\,E}$	45.88±2.16 ^{d}	NT	12.11±0.32	NT
		A. sicula	30.39±2.44 $^{d\,A}$	147.76±3.86 $^{e\,E}$	29.24±2.63 ^{d}			
	β-carotene bleaching	*A. atlantica*	112.45±6.66 $^{e\,E}$	296.17±10.32 $^{e\,C}$	251.08±12.49 ^{e}	1.05±0.01	NT	NT
		A. sicula	310.32±14.23 $^{e\,E}$	256.48±10.54 $^{e\,C}$	212.14±15.36 ^{e}			
$A_{0.5}$ (µg/mL)	Reducing power	*A. atlantica*	665.83±26.66 $^{e\,E}$	541.79±61.58 $^{e\,E}$	182.81±6.27 ^{c}	NT	NT	34.93±2.38
		A. sicula	621.67±68.25 $^{e\,E}$	386.77±25.91 $^{e\,C}$	204.69 ± 8.3 ^{c}			
	Phenanthroline method	*A. atlantica*	74.09±6.22 $^{e\,A}$	196.03±11.49 $^{e\,E}$	57.33±1.57 ^{d}	12.14±0.79	NT	NT
		A. sicula	376.31±21.67 $^{e\,E}$	238.02±3.75 $^{e\,C}$	167.2 ± 20 ^{e}			
	CUPRAC	*A. atlantica*	8.58±0.13 $^{a\,A}$	59.29±3.43 $^{e\,E}$	11.91±0.29 ^{a}	9.62±0.87	NT	NT
		A. sicula	7.62±0.24 $^{a\,E}$	32.69±1.59 $^{e\,C}$	38.6±1.94 ^{e}			

^{a}ns; no significant difference (P>0.05), ^{c}**; P≤0.01, ^{d}***; P≤0.001 and ^{e}****; P≤0.0001*vs* BHTor EDTA as standards. ^{A}ns; no significant difference (P>0.05), ^{C}**; P≤0.01, ^{D}***; P≤0.001, ^{E}****; P≤0.0001*vs* hydromethanolic extract.

II. Selected hydromethanolic extracts

1. Extraction yields

The percentage of extraction yields obtained from AACE and ASCE and their sub-fractionsshowed that the AACE had the highest extraction yield followed by AAWE, AABE, AAEAE, AAHE, and AAChE, respectively, for *A. atlantica* plant, and ASCE had the highest extraction yield followed by the ASWE, ASBE, ASEAE, ASHE and ASChE, respectively, for *A. sicula* plant (Table 05).

Table 05. Percentage of extraction yields obtained from the AACE and ASCE and their sub-fractions.

Extract yields % (g/g DW)						
Extracts	**CE**	**HE**	**ChE**	**EAE**	**BE**	**WE**
A.atlantica	22.1±0.6	0.96±0.02	0.35±0.01	1.49±0.2	2.93±0.03	11.49±0.4
A. sicula	26.97±0.9	1.77±0.07	0.4±0.05	2.34±0.18	4.1±0.04	17.71±0.6

CE: crude extract, HE: hexane extract, ChE: chloroform extract, EAE: ethyl acetate extract, BE: butanol extract, WE: water Extract, Extract yields % (g/g DW): dried extract (g) / 100 g of the initial amount of the dried plant powder, DW: dried weight.

2. Phytochemical screening

Total phenolic, flavonoid, flavonol, tannins, carotenoid, chlorophyll, lycopene and β-carotene contents in AACE and ASCE and their sub-fractions are presented in Table 06. These results demonstrate that the greatest total of polyphenols, flavonoids, flavonols and tannins were found in AAEAE (529.49±9.01 mg GAEq/g DW, 241.16±5.17 mg QEq/g DW, 70.76± 3.25 mgQEq/g DW and 296.18±8.74 mgTAEq/g DW, respectively) and ASEAE (382.92±4.01 mg GAEq/g DW, 109.25±2.14 mg QEq/g DW, 61.11±3.25 mgQEq/g DW and 194.54±6.19 mgTAEq/g DW, respectively) for *A. atlantica*and *A. sicula* L. extracts, respectively.

TPC and tannins contents from *A. atlantica*were decreased in the order of AAEA> AAChE> AABE> AACE > AAWE> AAHE. Whereas, TFC and flavonols contents from *A. atlantica*were decreased in the order of AAEA> AAChE> AACE> AABE > AAWE> AAHE. The total chlorophyll, carotenoid, lycopene and β-carotenecontents from *A. atlantica* were highest for AAChE and AAHE.

TPC and TFC from *A. sicula* L. extracts were decreased in the order of ASEA>ASChE>ASBE>ASCE>ASWE>ASHE. Flavonols and tannins content from *A. sicula*

L. were decreased as following: ASEA>ASChE> ASBE>ASCE>ASHE>ASWE and ASEA>ASChE> ASCE> ASBE > ASWE> ASHE, respectively. Whereas, the highest total pigments content (chlorophylls, carotenoids, lycopene and β-carotene) from *A. sicula* L. were found in non-polar extracts; ASHE and ASChE. In comparison between the two Apiaceae, a majority extracts showed that the highest phenolics content was found in *A. atlantica* extracts, whereas, the highest pigments content was found in *A. sicula* L. extracts.

Table 06. Total phenolics, flavonoids, flavonols, tannins, chlorophyll, carotenoids, lycopene, β-carotene contents in *A. atlantica* and *A. sicula* L. extracts.

Extracts		**CE**	**HE**	**ChE**	**EAE**	**BE**	**WE**
TPC (mg GAEq/g DW)	*A. atlantica*	238.31±11.68	30.54±2.76	280.96±6.24	529.49±9.01	280.25±5	91.45±3.5
	A. sicula	149.58±0.77	67.82 ± 3.89	274.78 ± 2.1	382.92±4.01	258.01±3.54	70.07±4.88
TFC (mg QEq/g DW)	*A. atlantica*	97.81±6.98	15.55±0.63	126.22±4.11	241.16±5.17	62.36±3.13	17.29±1.25
	A. sicula	40.48±3.97	6.94±0.36	83.09±1.53	109.25±2.14	52.15±2.82	9.37±0.17
Total flavonols content (mgQEq/g DW)	*A. atlantica*	32.15±0.76	6.47±0.24	48.19±2.13	70.76±3.25	17.38±1.16	10.02±0.21
	A. sicula	17.37±0.76	4.26±0.24	37.59±2.13	61.11±3.25	21.12±1.97	4.01±0.21
Total tannins content (mgTAEq/g DW)	*A. atlantica*	102.38±8.46	12.36±1.19	142.78±9.46	296.18±8.74	121.46±7.32	57.89±3.48
	A. sicula	98.16±7.12	27.82 ± 2.14	134.15±6.12	194.54±6.19	92.47±5.45	51.07±2.17
Total chlorophylls content (mg /g DW)	*A. atlantica*	2.97±0.185	4.36± 0.488	5.78± 0.365	0.78±0.049	0.43± 0.041	0.41±0.036
	A. sicula	1.79±0.04	12.08 ± 0.04	15.20±0.09	2.09±0.03	1.97±0.05	1.11±0.01
Total carotenoids content(mg/g DW)	*A. atlantica*	0.91±0.087	2.15±0.195	3.74±0.264	0.29±0.021	0.21± 0.002	0.04±0.003
	A. sicula	0.96±0.08	5.24±0.09	2.09±0.07	2.05±0.02	0.80± 0.05	0.62±0.02
Total β-carotene content(mg/g DW)	*A. atlantica*	0.21±0.018	0.22±0.023	0.27±0.02	0.12±0.015	0.14±0.09	0.06±0.008
	A. sicula	0.19±0.014	0.28±0.024	0.27±0.021	0.12±0.011	0.13±0.006	0.03±0.002
Total lycopene content (mg/g DW)	*A. atlantica*	0.34±0.03	0.38±0.027	0.35±0.032	0.16±0.015	0.21±0.02	0.10±0.009
	A. sicula	0.33±0.016	0.36±0.029	0.35±0.031	0.34±0.039	0.14±0.010	0.24±0.019

CE: crude extract, HE: hexane extract, ChE: chloroform extract, EAE: ethyl acetate extract, BE: butanol extract, WE: water Extract.

3. Phytochemical analysis

3.1 UHPLC-MS analysis

From the UHPLC/DAD/ESI-MS^n chromatogram, there are several peaks identification detected (Figure 21), and out of these 06, 11, 07, 06, 11, 09, 07, 09, 03 and 07 compounds were characterized from AACE, ASCE, AAChE, ASChE, AAEAE, ASEAE, AABE, ASBE, AAWE and ASWE respectively (Table 4). The main constituents of the AACE were 5-O-caffeoylquinic acid (m/z 353a, Rt: 7.92), 4-O-caffeoylquinic acid (m/z: 353, Rt: 8.41), luteolin 6-C-glucoside (m/z: 447, Rt: 12.02) and cynarine (m/z: 515, Rt: 13.50), respectively. The main components of the AAChE were Luteolin 6-C- glucoside (m/z: 447d, Rt :12.02) and Feruloylquinic acid derivative II (m/z: 431, Rt :14.07). The major constituents in the AAEAE are dicaffeoylquinic acid derivative (m/z: 515c, Rt: 12.88), 5-O-caffeoylquinic acid (m/z: 353a, Rt: 7.92) and then 3,4-O-dicaffeoylquinic acid (m/z: 515, Rt: 12.72). On other hand, the main components of the AABE were 5-O-Caffeoylquinic acid (m/z: 353a, Rt :7.92) and 4-O-caffeoylquinic acid (m/z: 353, Rt: 8.41). 5-O-caffeoylquinic acid (m/z: 353, Rt: 7.97) was the main compound in the AAWE. The main constituents ; in the ASCE were malonyl-3,5-dicaffeoylquinic acid I (m/z : 601, Rt : 13.72), astragalin formic acid adduct (m/z : 492, Rt : 12.09), astragalin (m/z : 447, Rt : 13.33) and then cynarine (m/z : 515, Rt : 12.86); in the ASChE were kaempferol (m/z : 285, Rt : 15.74), astragalin derivative (m/z : 492, Rt : 15.06) and feruloylquinic acid derivative (m/z : 431^c, Rt :14.05); in the ASEAE were astragalin formic acid adduct (m/z : 492, Rt : 12.09) and then cynarine (m/z :515 , Rt : 12.86); in ASBE were malonyl-3,5-dicaffeoylquinic acid I (m/z : 601, Rt : 13.11) and then astragalin (m/z : 447, Rt : 13.33); and in the ASWE was malonyl-3,5-dicaffeoylquinic acid I (m/z : 601, Rt : 13.11).

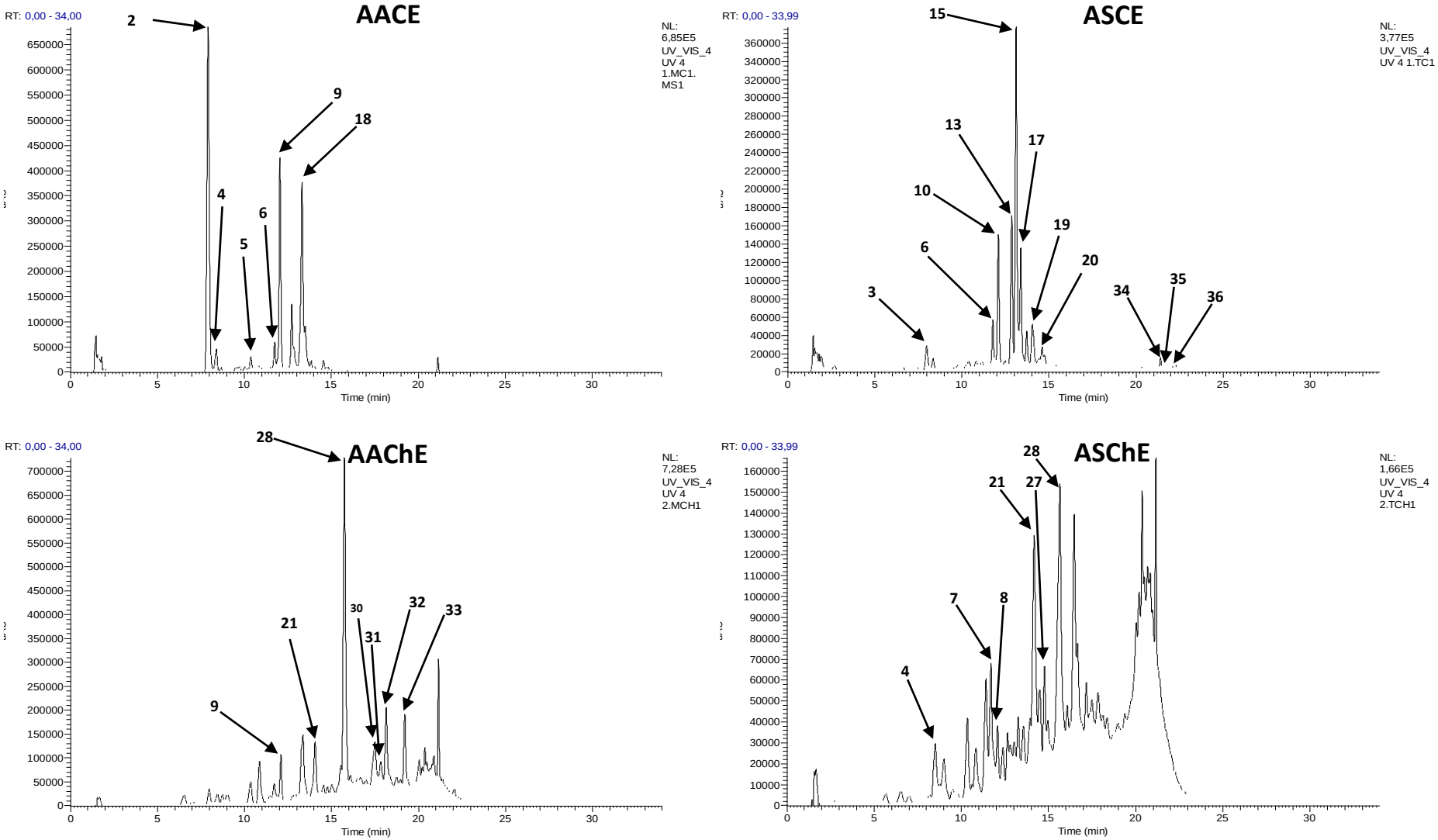

Figure 21 (continued)

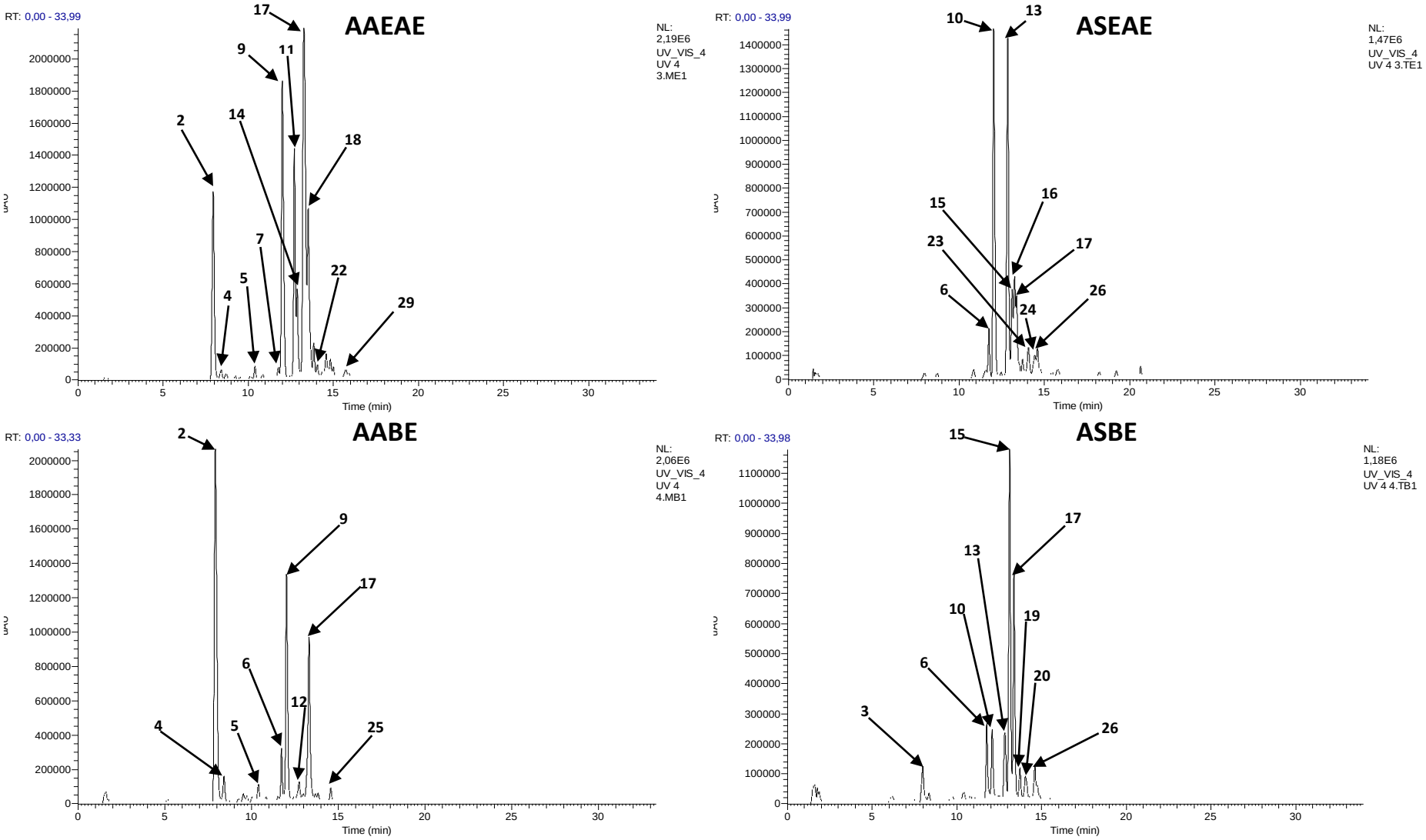

Figure 21 (continued)

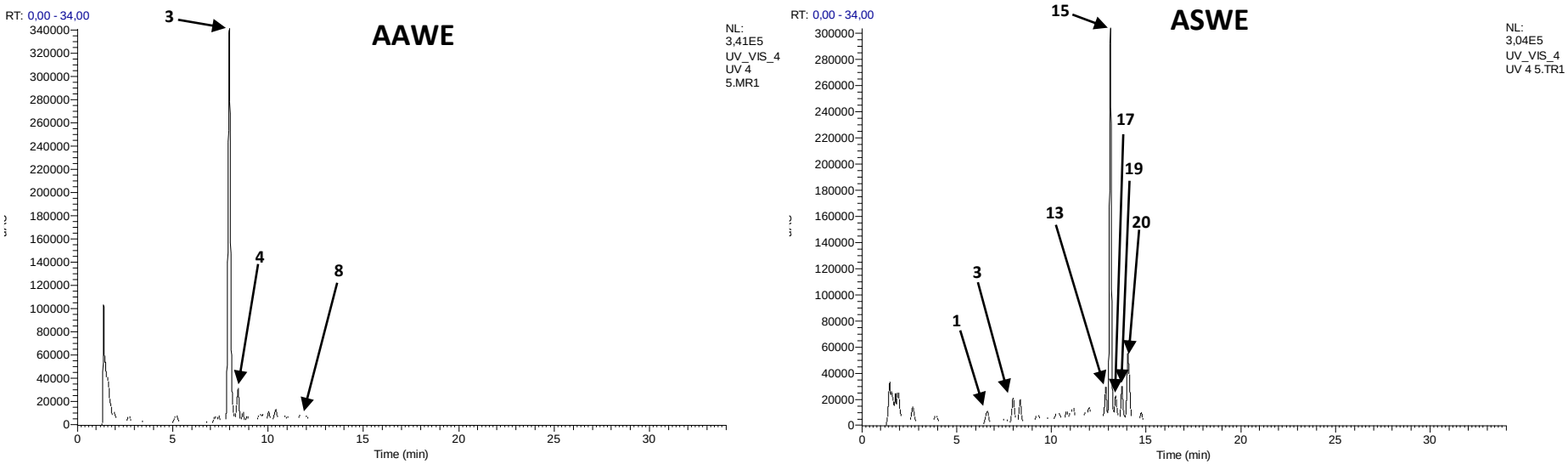

Figure 21: UHPLC chromatogram of *A. atlantica* and *A. sicula* L. hydromethanolic extracts and their sub-fractions recorded at 305 nm.

AACE; *A. atlantica* crude (hydromethanolic) extract, ASCE; *A. sicula* L. crude (hydromethanolic) extract, AAChE; *A. atlantica*chloroform extract, ASChE; *A. sicula* L. chloroform extract, AAEAE; *A. atlantica* ethyl acetate extract, ASEAE; *A. sicula* L. ethyl acetate extract, AABE; *A. atlantica* butanol extract, ASBE; *A. sicula* L. butanol extract, AAWE; *A. atlantica* water extract, ASWE; *A. sicula* L. water extract.

Table 07: Identification of UHPLC/DAD/ESI-MS^n data, and quantification of the most relevant compounds from the *A. atlantica* and *A. sicula* L. hydromethanolic extracts and their sub-fractions (Retention time (Rt), wavelength of maximum absorption in the UV-Vis region (λmax), pseudomolecular and MS^n fragment ions, quantification (mean ± SD) and identification of the phenolic compounds).

N	Rt (min)	λmax (nm)	Molecular ion [M-H]$^-$ (m/z)	MS^2 (m/z)	Quantification (mg)*										Compounds
					AACE	ASCE	AAChE	ASChE	AAEAE	ASEAE	AABE	ASBE	AAWE	ASWE	
1	6.61	206, 218, 327	439	359, 179,135	-	-	-	-	-	-	-	-	-	nq	Sulphated rosmarinic acid
2	7.92	220, 240, 293, 324	353[a]	191,179,135	0.079 ± 0.001	-	-	-	0.123 ± 0.009	-	0.205 ± 0.018	-	-	-	5-*O*-Caffeoylquinic acid
3	7.97	195, 219, 299, 325	353	308,191,179, 135		0.045 ± 0.002	-	-	-	-	-	0.032 ± 0.002	0.528 ± 0.007	0.031 ± 0.002	5-*O*-Caffeoylquinic acid
4	8.41	220, 239, 299, 324	353	335,191,179, 173, 135	0.028 ± 0.001	-	-	nd	0.052 ± 0.002	-	0.134 ± 0.001	-	0.046 ± 0.001	-	4-*O*-Caffeoylquinic acid
5	10.40	230, 239, 325	367[b]	193,191,173, 127, 85	0.002 ± 0.000	-	-	-	0.008 ± 0.001	-	0.010 ± 0.001	-	-	-	5-*O*-Feruloylquinic acid
6	11.76	204, 224, 265, 364	593	575,447,327, 285	0.002 ± 0.000	0.079 ± 0.001	-	-	-	0.034 ± 0.001	0.004 ± 0.001	0.057 ± 0.003	-	-	Kaempferol-3-*O*-(6-*p*-coumaroyl)-hexoside
7	11.77	203, 222, 266, 368	593	477,447,327, 285	-	-	-	nd.	nq	-	-	-	-	-	Kaempferol derivative
8	11.96	245, 323	401	293, 269, 161	-	-	-	nd	-	-	-	-	nq	-	Benzyl alcohol derivative
9	12.02	207, 220, 265, 350	447[d]	429, 327, 285	0.012 ± 0.001	-	0.012 ± 0.001	-	0.084 ± 0.005	-	0.068 ± 0.002	-		-	Luteolin 6-*C*-glucoside
10	12.09	204, 254, 265, 345	492	447, 429, 369, 327, 285	-	0.285 ± 0.003	-	-	-	0.193 ± 0.011	-	0.046 ± 0.002	-	-	Astragalin formic acid adduct
11	12.72	202, 244, 324	515	353, 335, 299, 255, 173	-	-	-	-	0.120 ± 0.010	-	-	-	-	-	3,4-*O*-Dicaffeoylquinic acid
12	12.73	202, 267, 344	789	447, 285, 136	-	-	-	-	-	-	0.003 ± 0.001	-	-	-	Luteolin derivative
13	12.86	193,220, 242, 329	515	497,471,353,335, 255,203, 191	-	0.229 ± 0.004	-	-	-	0.166 ± 0.005	-	0.043 ± 0.001	-	0.031 ± 0.001	Cynarin
14	12.88	219, 240, 327	515[e]	353, 335, 191	-	-	-	-	0.154 ± 0.006	-	-	-	-	-	Dicaffeoylquinic acid derivative
15	13.11	193, 219, 242, 323	601	583,557,515, 439, 395, 377	-	0.509 ± 0.011	-	-	-	0.052 ± 0.001	-	0.422 ± 0.005	-	0.371 ± 0.012	Malonyl-3,5-dicaffeoylquinic acid I
16	13.26	205, 297, 326	431	193, 191, 161	-	-	-	-	-	0.046 ± 0.002	-	-	-	-	Feruloylquinic acid derivative I
17	13.33	206, 248, 265, 339	447	429, 403, 327, 309, 285, 269	-	0.263 ± 0.002	-	-	0.091 ± 0.007	0.065 ± 0.001	0.042 ± 0.002	0.335 ± 0.010	-	0.034 ± 0.001	Astragalin
18	13.50	222, 240, 328	515	353, 335, 299, 255, 203, 191	0.016 ± 0.001	-	-	-	0.117 ± 0.016	-	-	-	-	-	Cynarin
19	13.72	243, 329	601	557, 515, 439, 395, 377, 233	-	0.043 ± 0.003	-	-	-	-	-	0.023 ± 0.001	-	0.032 ± 0.001	Malonyl-3,5-dicaffeoylquinic acid II
20	14.04	220, 271, 328	687	525, 329	-	0.019 ± 0.003	-	-	-	-	-	0.033 ± 0.004	-	0.065 ± 0.002	Tricin derivative
21	14.05	203,220, 295, 323	431[c]	193, 191, 173, 161, 85	-	-	0.011 ± 0.001	nd	-	-	-	-	-	-	Feruloylquinic acid derivative

Table 07 (Continued)

22	14.06	220, 246, 304	431[d]	385, 223	-	-	-	-	0.005 ± 0.001	-	-	-	-	-	Sinapic acid glucoside
23	14.07	198, 294, 328	431	193,191,173, 161	-	-	-	-	-	0.017 ± 0.003	-	-	-	-	Feruloylquinic acid derivative II
24	14.45	207, 247, 317	529	367, 193, 179	-	-	-	-	-	0.017 ± 0.002	-	-	-	-	Feruloylcaffeoylquinic acid
25	14.57	205, 268, 340	447	429, 357, 327 , 285	-	-	-	-	-	-	nq	-	-	-	Luteolin 8-*C*-glucoside
26	14.60	198, 227, 267, 333	517	355, 337, 295, 193	-	-	-	-	-	0.015 ± 0.001	-	0.074 ± 0.003	-	-	Ferulic acid derivative
27	15.06	209, 268, 347	492	343, 327, 285	-	-	-	nd	-	-	-	-	-	-	Astragalin derivative
28	15.74	200, 266, 300, 364	285	267, 257, 241, 217, 177, 149	-	-	0.072 ± 0.002	nd.	-	-	-	-	-	-	Kaempferol
29	15.74	222, 246, 301	285	241, 186	-	-	-	-	0.006 ± 0.001	-	-	-	-	-	Luteolin
30	17.36	203, 231, 278	809	647, 629, 471, 427	-	-	0.008 ± 0.001	-	-	-	-	-	-	-	Azukisaponin III
31	17.49	200, 264, 334	269	251, 239, 225, 201, 151	-	-	0.007 ± 0.001	-	-	-	-	-	-	-	Genistein
32	18.14	204, 228, 266, 314	329	312, 299, 293, 231, 196, 171	-	-	0.013 ± 0.001	-	-	-	-	-	-	-	Cirsiliol
33	19.21	195, 230, 266, 314	329	311, 293, 229, 211, 182, 171	-	-	0.006 ± 0.001	-	-	-	-	-	-	-	Jaceosidin
34	21.40	242, 280, 324	721	677, 559, 497, 353, 179, 161	-	0.022 ± 0.001	-	-	-	-	-	-	-	-	3,5,9-TCAOA
35	21.66	240, 311, 375	339	325, 193, 163, 149	-	0.017 ± 0.002	-	-	-	-	-	-	-	-	*p*-Coumaroylferulic acid
36	22.01	234, 288, 375	341	327, 195, 163, 151	-	0.014 ± 0.001	-	-	-	-	-	-	-	-	*p*-Coumaroylhydroferulic acid

AACE; *A. atlantica* crude (hydromethanolic) extract, ASCE;*A. sicula* L. crude (hydromethanolic) extract, AAChE;*A. atlantica*chloroform extract, ASChE;*A. sicula* L. chloroform extract, AAEAE;*A. atlantica*ethyl acetate extract, ASEAE;*A. sicula* L. ethyl acetate extract, AABE;*A. atlantica*butanol extract, ASBE; *A. sicula* L. butanol extract, AAWE;*A. atlantica*water extract, ASWE; *A. sicula* L. water extract.[a]m/z 398 $[M-H+HCO_2]^-$; [b]m/z 412 $[M-H+HCO_2]^-$; [c]m/z 476 $[M-H+HCO_2]^-$; [d]m/z 492 $[M-H+HCO_2]^-$; [e]m/z 560 $[M-H+HCO_2]^-$; [f]m/z 638 $[M-H+HCO_2]^-$; *This value is in mg of compound / sample in the vessel, using the three replicates for the error calculation ; nq; not quantified ; nd ; not determined. N: Compoundnumber.

3.2. GC-MS analysis

The *A. atlantica* and *A. sicula* L. hexane extracts were analyzed by GC–MS (Figure 22), leading to the identification of 24 and 30 compounds from AAHE and ASHE, respectively. The carvacrol (39.24%, Rt: 18.300), linoleic acid (14.09%, Rt :42.937), oleic acid (9-octadecenoic acid (Z)) (10.85%, Rt: 43.054) and palmitic acid (10.02%, Rt: 39.237) were identified as the major compounds in AAHE. While, carvacrol (15.15%, Rt : 18.300), linoleic acid (11.06%, Rt : 42.937), apiole (10.41%, Rt :29.62), linolenic acid (10.12%, Rt :43.071) and palmitic acid(8.03%, Rt : 39.237) were the major components from ASHE (Table 8).

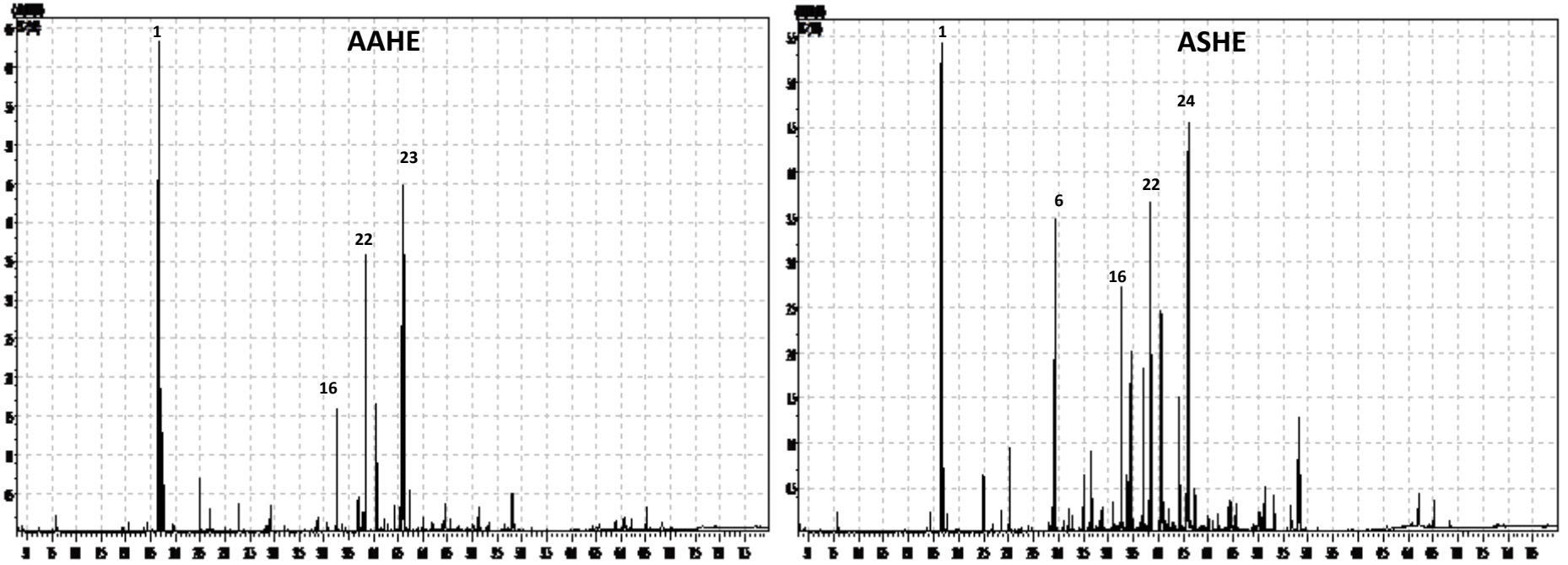

Figure 22: Total ion chromatogram (TIC) of *A. atlantica* and *A. sicula* L. hexane extracts.

AAHE; A. *atlantica* hexane extract, ASHE; A. *sicula* L. hexane extract.

Table 08: Identified compounds of the hexane extract from *A. atlantica* and *A. sicula* L.

Compound number	Rt. (min.)	Compound	Area%	
			AAHE	ASHE
1	18.300	Carvacrol	39,24	15.15
2	18.570	Thymol	3,78	1.11
3	18.868	3-Methyl-4-isopropylphenol	1,22	tr
4	25,090	Myristicin	-	1.60
5	26.363	4-*t*-Butylcatechol	0,77	-
6	29.62	Apiole	0,68	10.41
7	32,519	Saussurea lactone derivative	-	1.12
8	33,311	Saussurea lactone derivative	-	1.49
9	34.355	Myristic acid	0,43	-
10	35,494	7,10,13-Hexadecatrienoic acid, methyl ester	-	0.54
11	36.310	Palmitic acid, methyl ester	3,66	5.06
12	36,881	Pentadecanoic acid,	-	1.36
13	37,251	Gazaniolide	-	5.27
14	38,468	Unknown	-	3.26
15	38,960	3-Octenoic acid	-	0.59
16	39.237	Palmitic Acid	10,02	8.03
17	40.265	Linoleic acid, methyl ester	4,15	5.11
18	40.386	Linolenic acid, methyl ester	2,69	4.97
19	41.051	Stearic acid, methyl ester	0,33	Tr
20	42.096	Phytol	0,78	2.65
21	42.688	Mannonic acid, lactone	0,70	0.67

Table 05 (continued)

22	42.937	Linoleic acid	14,09	11.06
23	43.054	Oleic acid (9-Octadecenoic acid (Z))	10,85	-
24	43,071	Linolenic acid	-	10.12
25	43.189	9-Octadecenoic acid, (E)	0,32	-
26	43.645	Stearic acid	1,09	0.87
27	47.745	Arachidic acid	0,37	1.06
28	50.616	1-Monopalmitin	0,73	-
29	50,625	1-Monolinolenin	-	0.85
30	51,648	Behenic acid	-	0.42
31	53,268	2-Monolinolein	-	0.53
32	54.009	1-Monolinolein	1,49	3.19
33	54.182	1-Monolinolenin	0,46	1.48
34	65.313	Campesterol	0,58	-
35	66.048	Stigmasterol	0,46	1.18
36	67.590	β-Sitosterol	1,12	0.87

AAHE; A. *atlantica* hexane extract, ASHE; A. *sicula* L. hexane extract, tr; traces.

4. *In vitro* pharmacological effects

4.1. Antioxidant activities

4.1.1. DPPH radical scavenging activity

The results of the DPPH radical scavenging activity from *A. atlantica* and *A. sicula* L. extracts are presented in figure 23. For *A. atlantica*, AAEAE exhibited the greatest activity with an IC_{50} value of 13.59±0.24 µg/ml, followed by AACE [IC_{50}=21.26±0.08 µg/ml], AAChE [IC_{50}=27.49±0.24 µg/ml], AABE [IC_{50}=31.53±2.60 µg/ml], AAWE [IC_{50}=75.70±0.70 µg/ml] and AAHE [IC_{50}=93.64±6.06 µg/ml]. For*A. sicula* L., ASEAE possesses the highest DPPH effect with an IC_{50} value of 24.80 ± 0.33 µg/ml followed by ASChE [IC_{50}=29.46 ±0.56 µg/ml], ASBE [IC_{50}=32.77±0.47 µg/ml], ASCE [IC_{50}=51.54±0.58 µg/ml], ASWE [IC_{50}=108.99±4.87 µg/ml] and ASHE [IC_{50}=195.07±12.23 µg/ml]. Crude extract from *A. atlantica* and chloroform, ethyl acetate and butanol extracts from both *A. atlantica* and *A. sicula* L. had a similar radical scavenging activity (no significant differences; P>0.05) compared to that of BHT [IC_{50}=22.32±1.19 µg/ml] as standard. Crude, hexane and water extracts from *A. atlantica* had a best radical scavenging potential than that in *A. sicula* L. (P≤0.0001).

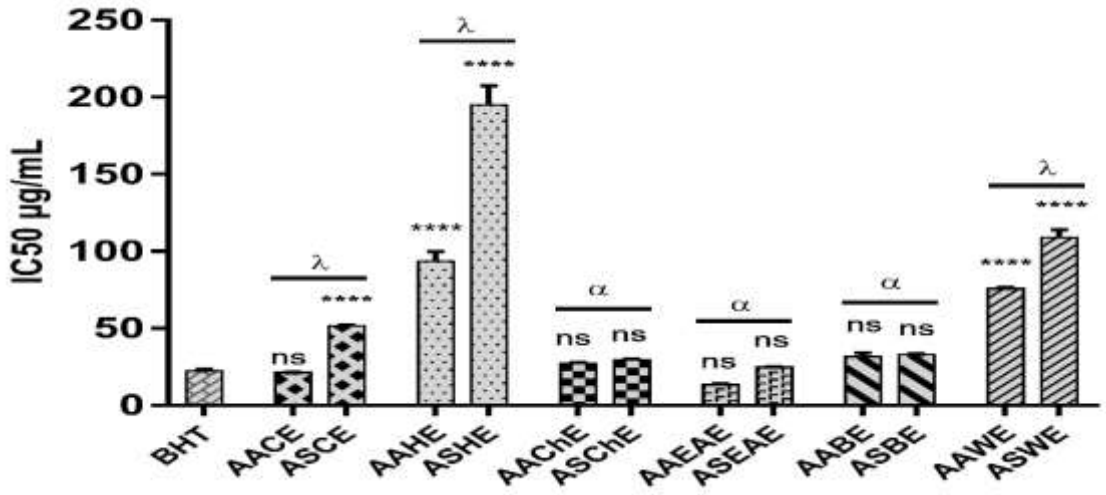

Figure 23: DPPH free radical scavenging potential of *A. atlantica* and *A. sicula* L. extracts.

ASCE; *A. sicula* crude extract, AACE;*A. atlantica* crude extract,ASHE; *A. sicula* hexane extract, AAHE;*A. atlantica* hexane extract, ASChE; *A. sicula* chloroform extract, AAChE;*A. atlantica* chloroform extract,ASEAE; *A. sicula* ethyl acetate extract, AAEAE;*A. atlantica* ethyl acetate extract,ASBE; *A. sicula* butanol extract, AABE; *A. atlantica* butanol extract,ASWE; *A. sicula* water extract, AAWE; *A. atlantica* water extract.BHT was used as reference antioxidant, ns;no significant difference (P>0.05),****; P≤0.0001*vs* BHT.$^{\alpha}$ns; no significant difference (P≥0.05), $^{\lambda}$****; P≤0.0001in comparison between the effects of *A. atlantica* and *A. sicula* L. extracts.

4.1.2. ABTS radical scavenging activity

The ABTS free radical scavenging abilities of *A. atlantica* and *A. sicula* L. extracts are shown in figure 24, compared to the BHT [IC_{50}=1.29±0.30 µg/ml] used as standard drug. The results showed that the *A. atlantica* extracts exhibited a good ability to scavenge the ABTS radical in the following decreasing order: AAEA [IC_{50}=7.09±0.22 µg/ml], AAChE [IC_{50}=7.2±0.2 µg/ml], AACE [IC_{50}=9.17±0.81 µg/ml], AABE [IC_{50}=12.78±0.61 µg/ml], AAWE [IC_{50}=32.92±0.66 µg/ml] and AAHE [IC_{50}=50±7.60 µg/ml]. *A. sicula* L. extracts exhibited a good ability to scavenge the ABTS radical in the following decreasing order of ASEAE [IC_{50}=6.98±0.50 µg/ml], ASChE [IC_{50}=8.79±0.50 µg/ml], ASBE [IC_{50}=17.58±1.60 µg/ml], ASCE [IC_{50}=31.14±1.78 µg/ml], ASWE [IC_{50}=45.43±0.27 µg/ml] and then ASHE [IC_{50}=66.8±8.20 µg/ml]. There were no significant differences (P>0.05) when comparing thefree radical scavenging from*A. atlantica* crude extract, *A. atlantica* and *A. sicula* L. chloroform and ethyl acetate extracts to BHT. ABTS free radical scavenging activity of crude, hexane and water extracts was highest in *A. atlantica* compared to *A. sicula* L. (P≤0.01 to P≤0.0001).

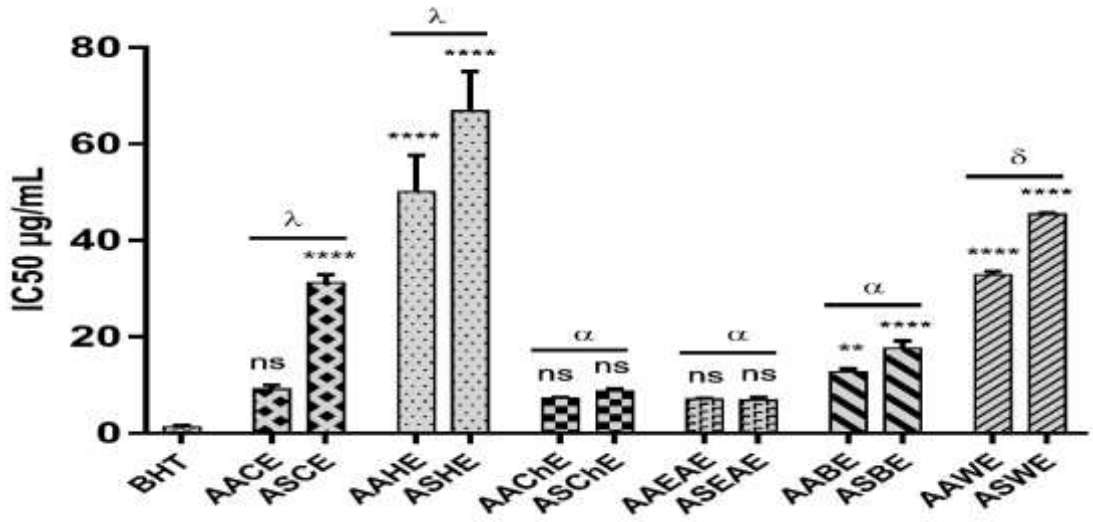

Figure 24: ABTS free radical scavenging activity of *A. atlantica* and *A. sicula* L. extracts.

ASCE; *A. sicula* crude extract, AACE; *A. atlantica* crude extract, ASHE; *A. sicula* hexane extract, AAHE;*A. atlantica* hexane extract, ASChE; *A. sicula* chloroform extract, AAChE; *A. atlantica* chloroform extract, ASEAE; *A. sicula* ethyl acetate extract, AAEAE;*A. atlantica* ethyl acetate extract, ASBE; *A. sicula* butanol extract, AABE; *A. atlantica* butanol extract, ASWE; *A. sicula* water extract, AAWE; *A. atlantica* water extract.BHT was used as standard antioxidant, ns;no significant difference (P>0.05), **; P≤0.01, ****; P≤0.0001*vs* BHT.$^{\alpha}$ns; no significant difference (P≥0.05), $^{\delta}$**; P≤0.01, $^{\lambda}$****; P≤0.0001in comparison between the effects of *A. atlantica* and *A. sicula* L. extracts.

4.1.3. Reducing power activity

The results represented in figure 25, demonstrated that all *A. atlantica* extracts exhibited a high reducing power which decreased in the following order of AAEAE [$A_{0.5}$=73.3±2.7 µg/ml], AABE [$A_{0.5}$=129.34±4.9 µg/ml], AACE [$A_{0.5}$=182.81±6.27 µg/ml], AAChE [$A_{0.5}$=283.48±0.69 µg/ml], AAWE [$A_{0.5}$=384.54±0.5 µg/ml] and AAHE [$A_{0.5}$=643.12±68.75 µg/ml]. The reducing power of *A. sicula* L. extracts was in the following order of ASEAE [$A_{0.5}$=90.65±2.07 µg/ml], ASBE [$A_{0.5}$=144.51±4.18 µg/ml], ASChE [$A_{0.5}$=151.56±2.4 µg/ml], ASCE [$A_{0.5}$=204.69±8.31 µg/ml], ASHE [$A_{0.5}$=208.6±12.74 µg/ml] and ASWE [$A_{0.5}$=380.43±54µg/ml], respectively. The reducing capacity of AAEAE and ASEAE revealed strong activity as to that of TCP; α-tocopherol [$A_{0.5}$=34.93±2.38 µg/ml] which is well known as a strong reducer (no significant difference; P>0.05). There is no significant difference in comparison between a majority of both plant extracts, except hexane and chloroform extracts showed that *A. sicula* L. was more active than *A. atlantica* (P≤0.001 to P≤0.0001).

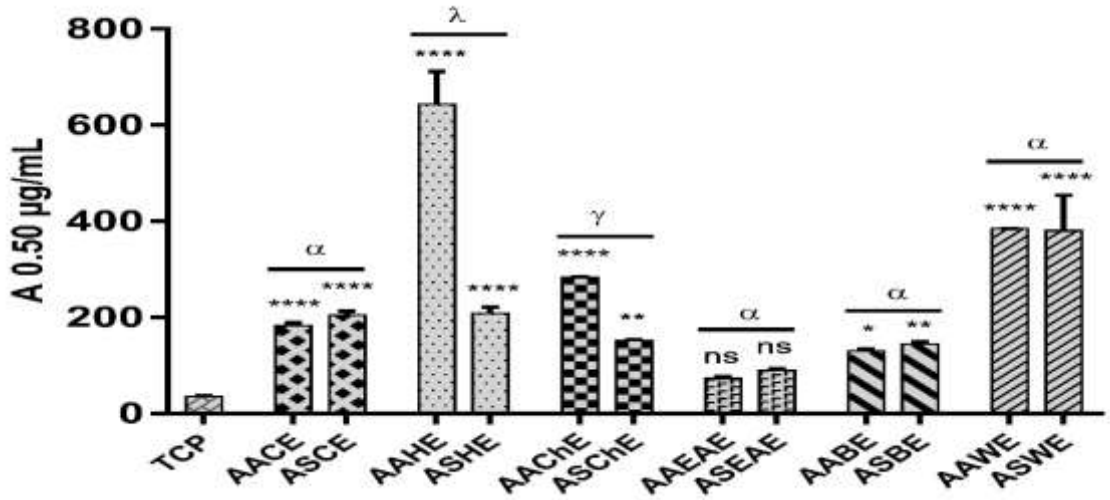

Figure 25: Reducing power of *A. atlantica* and *A. sicula* L. extracts.

ASCE; *A. sicula* crude extract, AACE; *A. atlantica* crude extract, ASHE; *A. sicula* hexane extract, AAHE;*A. atlantica* hexane extract, ASChE; *A. sicula* chloroform extract, AAChE; *A. atlantica* chloroform extract, ASEAE; *A. sicula* ethyl acetate extract, AAEAE;*A. atlantica* ethyl acetate extract, ASBE; *A. sicula* butanol extract, AABE; *A. atlantica* butanol extract, ASWE; *A. sicula* water extract, AAWE; *A. atlantica* water extract.TCP;α-tocopherol was used as positive control, ns;no significant difference (P>0.05), *; P≤0.05, **; P≤0.01, ****; P≤0.0001*vs* TCP.$^{\alpha}$ns; no significant difference (P≥0.05), $^{\gamma}$***; P≤0.001, $^{\lambda}$****; P≤0.0001in comparison between the effects of *A. atlantica* and *A. sicula* L. extracts.

4.1.4. Cupric ion reducing antioxidant capacity

In this assay, the *A. atlantica* and *A. sicula* L. extracts showed a promising result (Figure 26). AAEAE from *A. atlantica* displayed a good activity [$A_{0.5}$=7.68±0.55 µg/ml], followed by AACE [$A_{0.5}$=11.91±0.29 µg/ml], AABE [$A_{0.5}$=15.9±0.38 µg/ml], AAChE [$A_{0.5}$=15.93±0.07 µg/ml], AAHE [$A_{0.5}$=28.38±0.19 µg/ml] and AAWE [$A_{0.5}$=45.85±2.06 µg/ml]. AAEAE and AACE were the most active among the remaining *A. atlantica* extracts, with a similar activity to that of synthetic antioxidant BHT [$A_{0.5}$=9.62±0.87 µg/ml]; (no significant difference; P>0.05). ASHE showed the better activity among the investigated *A. sicula* L. extracts [$A_{0.5}$=4.48±0.18 µg/ml], followed by ASEAE [$A_{0.5}$=8.15±0.55 µg/ml], ASChE [$A_{0.5}$=15.13±0.95 µg/ml], ASBE [$A_{0.5}$=16.37±0.52 µg/ml], ASCE [$A_{0.5}$=38.6±1.94 µg/ml] and then ASWE [$A_{0.5}$=71.58±5.98 µg/ml]. ASHE, ASChE and ASEAE extracts from *A. sicula* L. exhibited a similar cupric ion reducing activity compared to that of the BHT (no significant differences; P>0.05). For crude and water extracts, *A. atlantica* was the most active (P≤0.0001), whereas, for hexane extract, *A. sicula* L. exhibited a best cupric ion reducing power than *A. atlantica* (P≤0.0001).

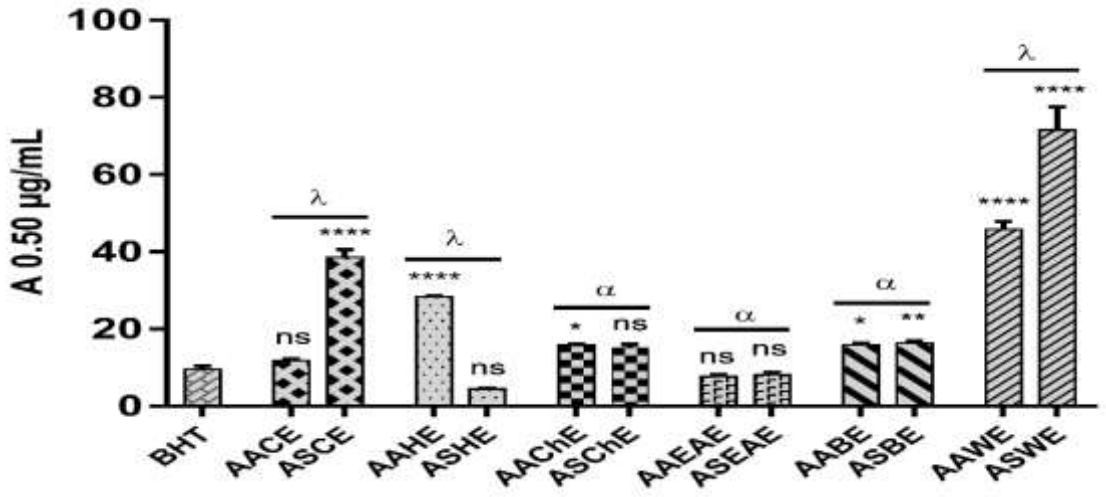

Figure 26: Cupric reducing antioxidant capacity of *A. atlantica* and *A. sicula* L. extracts.

ASCE; *A. sicula* crude extract, AACE;*A. atlantica* crude extract, ASHE; *A. sicula* hexane extract, AAHE;*A. atlantica* hexane extract, ASChE; *A. sicula*chloroform extract, AAChE; *A. atlantica* chloroform extract, ASEAE; *A. sicula* ethyl acetate extract, AAEAE; *A. atlantica* ethyl acetate extract, ASBE; *A. sicula* butanol extract, AABE; *A. atlantica* butanol extract, ASWE; *A. sicula* water extract, AAWE; *A. atlantica* water extract.BHT was used as standard, ns;no significant difference (P>0.05), *; P≤0.05, **; P≤0.01, ****; P≤0.0001*vs* BHT.$^{\alpha}$ns; no significant difference (P≥0.05), $^{\lambda}$****; P≤0.0001in comparison between the effects of *A. atlantica* and *A. sicula* L. extracts.

4.1.5. Phenanthroline activity

The antioxidant activity measured using the phenanthroline assay was also investigated as shown in figure 27. The order of high effect from *A. atlantica* extracts was; AAEA [$A_{0.5}$=17.95±0.31 µg/ml], AAChE [$A_{0.5}$=31.82±0.83 µg/ml], AACE [$A_{0.5}$=57.33±1.57 µg/ml], AABE [$A_{0.5}$=80.2±4.41 µg/ml], AAWE [$A_{0.5}$=403.49±45 µg/ml] and AAHE [$A_{0.5}$=591±65.60 µg/ml] at the low end. The antioxidant capacities of AAEAE, AAChE, AACE and AABE were superior to that of the other plant extracts, also, its $A_{0.5}$ showed no significant difference (P>0.05) compared to that of the BHT [$A_{0.5}$=12.14±0.79 µg/ml] as drug standard.Also, the results revealed that the order of the antioxidant activity of the *A. sicula* L. extracts was as following; ASEAE [$A_{0.5}$=35.32±0.09 µg/ml], ASChE [$A_{0.5}$=59.61±2.65 µg/ml], ASHE [$A_{0.5}$=65.33±2.65 µg/ml], ASBE [$A_{0.5}$=144.87±3.44 µg/ml], ASCE [$A_{0.5}$=167.2±20 µg/ml] and ASWE [$A_{0.5}$=305.42±40µg/ml] at the low end. Moreover, when comparing the $A_{0.5}$ of ASHE, ASChE and ASEAE, with BHT, there were no significant differences (P>0.05). AACE was more active than ASCE (P≤0.001), whereas, AAHE and AAWE were less active than ASHE and AAWE (P≤0.01to P≤0.0001).

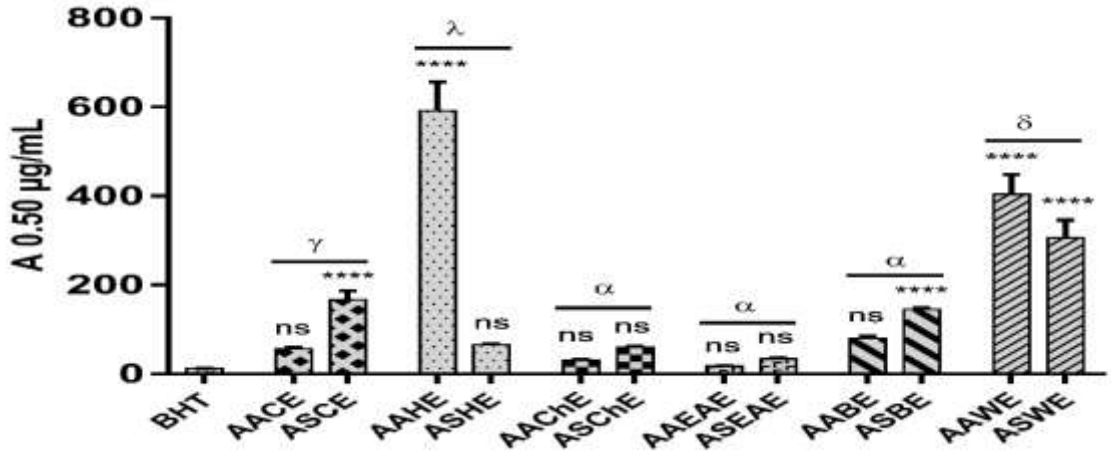

Figure 27: Antioxidant activity of *A. atlantica* and *A. sicula* L. extractsmeasured by phenanthroline method.

ASCE; *A. sicula* crude extract, AACE;*A. atlantica* crude extract, ASHE; *A. sicula* hexane extract, AAHE;*A. atlantica* hexane extract, ASChE; *A. sicula*chloroform extract, AAChE;*A. atlantica* chloroform extract, ASEAE; *A. sicula* ethyl acetate extract, AAEAE;*A. atlantica* ethyl acetate extract, ASBE; *A. sicula* butanol extract, AABE; *A. atlantica* butanol extract, ASWE; *A. sicula* water extract, AAWE; *A. atlantica* water extract.BHT was used as reference, ns;no significant difference (P>0.05), ****; P≤0.0001*vs* BHT.$^{\alpha}$ns; no significant difference (P≥0.05), $^{\delta}$**; P≤0.01, $^{\gamma}$***; P≤0.001, $^{\lambda}$****; P≤0.0001in comparison between the effects of *A. atlantica* and *A. sicula* L. extracts.

4.1.6. Metal chelating activity

The metal chelating effect in*A. atlantica*and *A. sicula* L. crude extract and their sub-fractionsare presented in figure 28. with a comparison to that of EDTA [IC_{50}=12.11±0.32 µg/ml] as a standard control. The scavenging and chelating activities of*A. atlantica* extracts decreased in the order of AABE [IC_{50}=4.82±0.31 µg/ml], AAChE [IC_{50}=24.83±3.24 µg/ml], AACE [IC_{50}=45.88±2.16 µg/ml], AAWE [IC_{50}=74.04±1.61 µg/ml], AAEAE [IC_{50}=215.55±0.83 µg/ml] and AAHE [IC_{50}=275.46±16.39 µg/ml]. AABE and AAChE extracts were found to be as strong as the reference drug, EDTA [IC_{50}=12.11±0.32 µg/ml] (no significant difference; P>0.05). The highest metal chelating activity of *A. sicula* L. extracts was found in ASCE [IC_{50}=29.24±2.63 µg/ml], followed by ASWE [IC_{50}=30.66±0.51µg/ml], ASEAE [IC_{50}=200.99±10.05 µg/ml], ASChE [IC_{50}=268.49±5.30 µg/ml], ASHE [IC_{50}=271.83±11.51 µg/ml], and ASBE [IC_{50}=280±6.90 µg/ml]. ASCE and ASWE effects were no significant compared to EDTA (P>0.05). The chloroform and butanol extracts showed that *A. atlantica* was more active than *A. sicula* L. (P≤0.0001), whereas, the *A. sicula* L. water extract was more active than *A. atlantica* water extract (P≤0.0001).

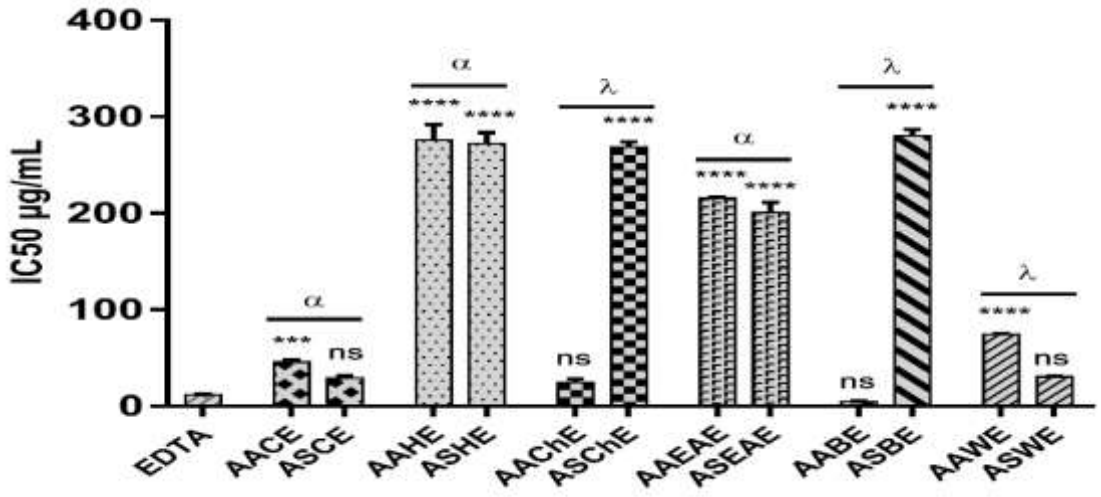

Figure 28: Metal chelating activity of *A. atlantica* and *A. sicula* L. extracts.

ASCE; *A. sicula* crude extract, AACE;*A. atlantica* crude extract, ASHE; *A. sicula* hexane extract, AAHE;*A. atlantica* hexane extract, ASChE; *A. sicula*chloroform extract, AAChE;*A. atlantica* chloroform extract, ASEAE; *A. sicula* ethyl acetate extract, AAEAE;*A. atlantica* ethyl acetate extract, ASBE; *A. sicula* butanol extract, AABE; *A. atlantica* butanol extract, ASWE; *A. sicula* water extract, AAWE; *A. atlantica* water extract.EDTA was used as standard antioxidant, ns;no significant difference (P>0.05), ***; P≤0.001, ****; P≤0.0001*vs* EDTA.$^{\alpha}$ns; no significant difference (P≥0.05), $^{\lambda}$****; P≤0.0001in comparison between the effects of *A. atlantica* and *A. sicula* L. extracts.

4.1.7. β-Carotene bleaching activity

The total antioxidant activity of *A. atlantica* and *A. sicula* L. extracts by inhibition of lipid peroxidation in the ß-carotene–linoleic acid system is shown in figure 29. The oxidation of β-carotene was effectively inhibited by *A. atlantica* extracts in the decreasing order of AAHE [IC_{50}=9.88±0.55 µg/ml], AAChE [IC_{50}=60.95±0.58 µg/ml], AAEAE [IC_{50}=190±14.14 mg/ml], AABE [IC_{50}=201.19±22.43 µg/ml], AAWE [IC_{50}=222.03±11.50 µg/ml] and then AACE [IC_{50}=251.08±12.49 µg/ml]. However, AAHE had no significant antioxidant effect compared to the BHT [IC_{50}=1.05±0.01 µg/ml] as an antioxidant standard (P>0.05). *A. sicula* L. extracts inhibited the peroxidation of linoleic acid in the following order: ASChE [IC_{50}=21.35±0.93 µg/ml], ASHE [IC_{50}=21.69±1.94 µg/ml], ASEAE [IC_{50}=191.13±6.65 µg/ml], ASCE [IC_{50}=212.14±15.36 µg/ml], ASWE [IC_{50}=257.44±16.13 µg/ml], ASBE [IC_{50}=266.97±7.12 µg/ml]. However, ASHE and ASChE had a similar antioxidant capacity compared to BHT (P>0.05). The butanol and water extracts showed that *A. atlantica* had a best activity than *A. sicula* L., (P≤0.05 to P≤0.0001). The crude and chloroform extracts were more active from *A. sicula* L. than *A. atlantica* (P≤0.05 to P≤0.01).

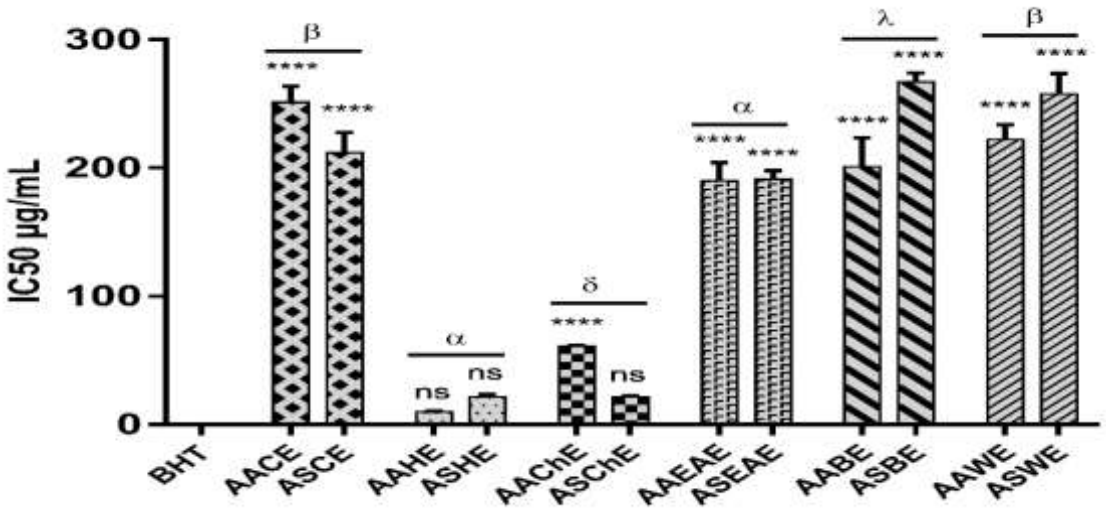

Figure 29: β-Carotene bleaching activity of *A. atlantica* and *A. sicula* L. extracts.

ASCE; *A. sicula* crude extract, AACE;*A. atlantica* crude extract, ASHE; *A. sicula* hexane extract, AAHE;*A. atlantica* hexane extract, ASChE; *A. sicula*chloroform extract, AAChE;*A. atlantica* chloroform extract, ASEAE; *A. sicula* ethyl acetate extract, AAEAE;*A. atlantica* ethyl acetate extract, ASBE; *A. sicula* butanol extract, AABE; *A. atlantica* butanol extract, ASWE; *A. sicula* water extract, AAWE; *A. atlantica* water extract.BHT was used as reference antioxidant, ns;no significant difference (P>0.05), ****; P≤0.0001*vs* BHT.$^{\alpha}$ns; no significant difference (P≥0.05), $^{\beta*}$P≤0.05, $^{\delta}$**; P≤0.01, $^{\lambda}$****; P≤0.0001in comparison between the effects of *A. atlantica* and *A. sicula* L. extracts.

4.2. Enzymatic inhibition activities

4.2.1. AChE inhibition activity

Results for inhibition of AChE by *A. atlantica* and *A. sicula* L. extracts are presented in figure 30. The best inhibitory activity from *A. atlantica* extracts was obtained with AAEAE which displayed an IC_{50} value of 15.33±0.57 µg/ml, followed by AABE [IC_{50}=18.68±1.72 µg/ml], AAChE [IC_{50}=20.38±1.32 µg/ml], AACE [IC_{50}=68.22±6.13 µg/ml], AAWE [IC_{50}=133.33±5.35 µg/ml] and AAHE [IC_{50}=433.36±25.06 µg/ml]. AAChE, AAEAE and AABE had no significant effect compared to that of the standard, galantamine [IC_{50}=6.27±1.15 µg/ml], a well-known anti-Alzheimer's medicine (P>0.05). All the tested samples from *A. sicula* L. were able to inhibit AChE activity. ASEAE showed better activity with an IC_{50} value of 16.79±1.45 µg/ml, followed by ASChE [IC_{50}=22.58±1.03 µg/ml], ASBE [IC_{50}=84.95±6.73 µg/ml], ASCE [IC_{50}=138.17±3.26 µg/ml], ASWE [IC_{50}=252.31±16.31 µg/ml] and ASHE [IC_{50}=620.34±19.42 µg/ml], consecutively. The AChE activity of ASChE and ASEAE revealed as strong activity as that of galantamine. In comparison between both Apiaceae, *A. atlantica* extracts showedthe highest AChE inhibition activity than *A. sicula* L. extracts (P≤0.0001), except the chloroform and ethyl acetate extracts, showed no significant effects.

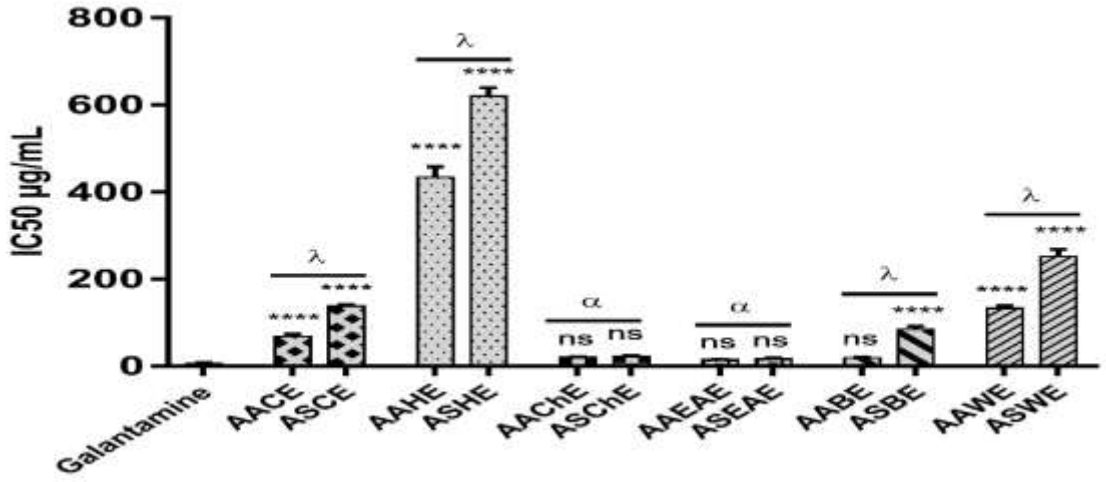

Figure 30: AChE inhibitory activity of *A. atlantica* and *A. sicula* L. extracts.

ASCE; *A. sicula* crude extract, AACE;*A. atlantica* crude extract, ASHE; *A. sicula* hexane extract, AAHE;*A. atlantica* hexane extract, ASChE; *A. sicula*chloroform extract, AAChE;*A. atlantica* chloroform extract, ASEAE; *A. sicula* ethyl acetate extract, AAEAE;*A. atlantica* ethyl acetate extract, ASBE; *A. sicula* butanol extract, AABE; *A. atlantica* butanol extract, ASWE; *A. sicula* water extract, AAWE; *A. atlantica* water extract.Galantamine was used as reference drug, ns;no significant difference (P>0.05), ****; P≤0.0001 vs galantamine.$^{\alpha}$ns; no significant difference (P≥0.05), $^{\lambda}$****; P≤0.0001in comparison between the effects of *A. atlantica* and *A. sicula* L. extracts.

4.2.2. BChE inhibition activity

BChE inhibitory effects by *A. atlantica* and *A. sicula* L. extracts were investigated with a comparison to that of galantamine [IC_{50}=34.75±1.99 µg/ml] as a control standard (Figure 31). AAChE extract from *A. atlantica* displayed a good activity with an IC_{50} value of 22.20±0.82 µg/ml, followed by AAEAE [IC_{50}=26.89±2.63 µg/ml], AABE [IC_{50}=34±2.6 µg/ml], AACE [IC_{50}=62.33±4.10 µg/ml], AAHE [IC_{50}=167.89±2.58 µg/ml] and AAWE [IC_{50}=184.19±19.8 µg/ml]. The AAChE, AAEAE and AABE extracts exhibited a similar BChE inhibition activity of galantamine [IC_{50}=34.75±1.99 µg/ml], (P>0.05). ASEAE indicated the highest activity among the investigated *A. sicula* L. extracts [IC_{50} = 72.48±4.10 µg/ml], followed by ASBE [IC_{50}=72.93±7.93 µg/ml], ASChE [IC_{50}=151.99±12.97 µg/ml], ASWE [IC_{50}=312.32±22.07 µg/ml], ASCE [IC_{50}=360.5±8.52 µg/ml] and ASHE [IC_{50}=615.45±10.94 µg/ml], consecutively. All *A. atlantica* extracts had a best BChE inhibition activity than *A. sicula* L. extracts (P≤0.01 to P≤0.0001).

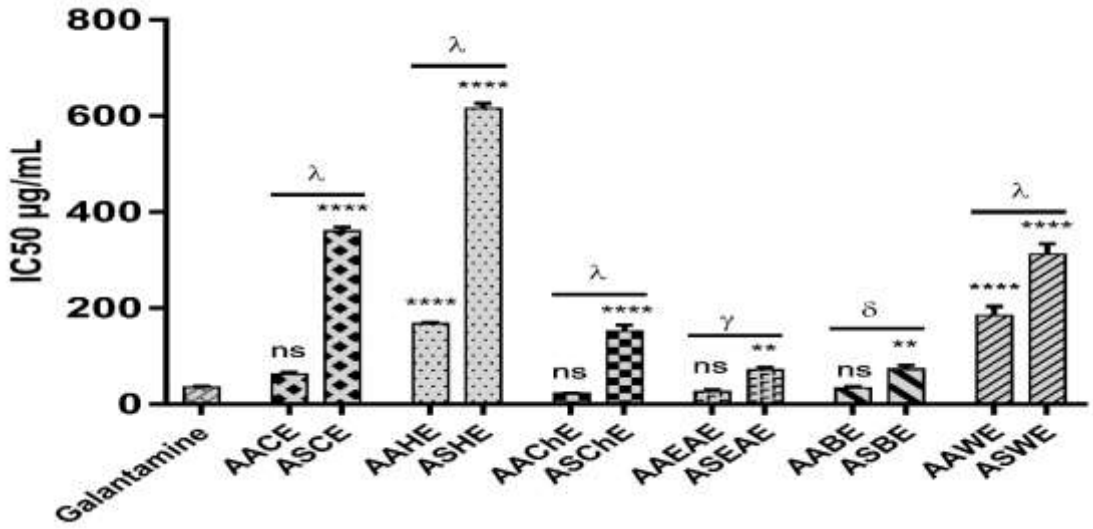

Figure 31: BChE inhibitory activity of *A. atlantica* and *A. sicula* L. extracts.

ASCE; *A. sicula* crude extract, AACE;*A. atlantica* crude extract, ASHE; *A. sicula* hexane extract, AAHE;*A. atlantica* hexane extract, ASChE; *A. sicula*chloroform extract, AAChE;*A. atlantica* chloroform extract, ASEAE; *A. sicula* ethyl acetate extract, AAEAE;*A. atlantica* ethyl acetate extract, ASBE; *A. sicula* butanol extract, AABE; *A. atlantica* butanol extract, ASWE; *A. sicula* water extract, AAWE; *A. atlantica* water extract.Galantamine was used as reference drug, ns;no significant difference (P>0.05), **; P≤0.01, ****; P≤0.0001*vs* galantamine.$^{\delta}$**; P≤0.01, $^{\gamma}$***; P≤0.001, $^{\lambda}$****; P≤0.0001in comparison between the effects of *A. atlantica* and *A. sicula* L. extracts.

4.2.3. Tyrosinase inhibition activity

The inhibitory effects of*A. atlantica* and *A. sicula* L. extracts on tyrosinase activity are presented infigure 32. *A. atlantica* extracts inhibited tyrosinase activity in following order AAEAE [IC_{50}=11.28±0.12 µg/ml], AAChE [IC_{50}=21.03±0.97 µg/ml], AABE [IC_{50}=39.54±0.47µg/ml], AAWE [IC_{50}=68.18±2.62µg/ml], AACE [IC_{50}=76.43±2.97µg/ml] and AAHE [IC_{50}=147.25±5.8µg/ml] respectively. With better tyrosinase inhibitory activity by AAEAE extract than to that of Kojic acid [IC_{50}=25,23±0,78µg/ml] a well-known tyrosinase inhibitor and whitening agent (P<0.05). Whereas, the AAChE extract had a similar tyrosinase inhibition activity compared to Kojic acid (P>0.05). *A. sicula* L. extracts showed an increased inhibition effect in the order of ASEAE [IC_{50}=30.58±2.73 µg/ml], ASChE [IC_{50}=51.19±1.33 µg/ml], ASBE [IC_{50}=78.52±1.5 µg/ml], ASCE [IC_{50}=159.22±1.96 µg/ml], ASWE [IC_{50}=306.10±6.97 µg/ml] and then ASHE [IC_{50}=373.42±9.81 µg/ml]. As well as, ASEAE had a similar effect of Kojic acid (P>0.05). All *A. atlantica* extracts exhibited a highest tyrosinase inhibitory potential than *A. sicula* L. extracts (P≤0.001 to P≤0.0001).

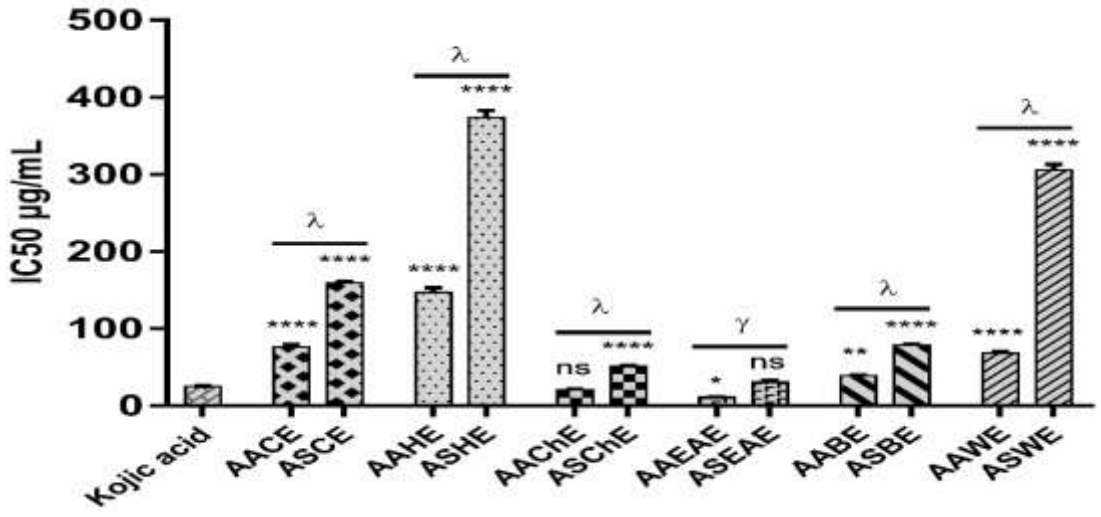

Figure 32: Tyrosinase inhibitory activityof *A. atlantica* and *A. sicula* L. extracts.

ASCE; *A. sicula* crude extract, AACE;*A. atlantica* crude extract, ASHE; *A. sicula* hexane extract, AAHE;*A. atlantica* hexane extract, ASChE; *A. sicula*chloroform extract, AAChE; *A. atlantica* chloroform extract, ASEAE; *A. sicula* ethyl acetate extract, AAEAE;*A. atlantica* ethyl acetate extract, ASBE; *A. sicula* butanol extract, AABE; *A. atlantica* butanol extract, ASWE; *A. sicula* water extract, AAWE; *A. atlantica* water extract.Kojic acid was used as reference, ns;no significant difference (P>0.05), *; P≤0.05, **; P≤0.01, ****; P≤0.0001 vs kojic acid.$^{\gamma}$***; P≤0.001, $^{\lambda}$****; P≤0.0001in comparison between the effects of *A. atlantica* and *A. sicula* L. extracts.

4.2.4. α-Amylase inhibition activity

Figure 33 reveals the α-amylase inhibition activity of *A. atlantica* and*A. sicula* L. extracts. The best α-amylase inhibition activity from *A. atlantica* was obtained in AAEAE with an IC_{50} value of 215.79±1.69 µg/ml, followed by AAChE [IC_{50}=362.4±11.44 µg/ml], AABE [IC_{50}=696.73±2.29 µg/ml], AACE [IC_{50}=1405.61±15.07 µg/ml], AAWE [IC_{50}=2169.12±65.81 µg/ml] and AAHE [IC_{50}=2249.65±233.73 µg/ml]. The investigation shows that the ASEAE from *A. sicula* L. had a strong α-amylase inhibition activity [IC_{50}=143.53±9.67 µg/ml] followed by ASBE [IC_{50}=800.92±6.22 µg/ml], ASChE [IC_{50}=1039.67±18.71 µg/ml], ASCE [IC_{50}=2533.89±34.29 µg/ml], ASHE [IC_{50}=2987.19±257.84 µg/ml] and ASWE [IC_{50}=3991.25±22.46 µg/ml], consecutively. The observed α-amylase inhibitory activity of all *A. atlantica* and *A. sicula* L. extracts was more potent than that of acarbose [IC_{50}=3650.93±10.7 µg/ml] as standard, a well-known α-amylase inhibitor, except for ASWE, which was lower active than to standard (P≤0.01). *A. atlantica* extracts exhibited a better -amylase inhibition activity than *A. sicula* L. extracts (P≤0.0001), except ethyl acetate and butanol extracts which were showed no significant effects in comparison between both plants.

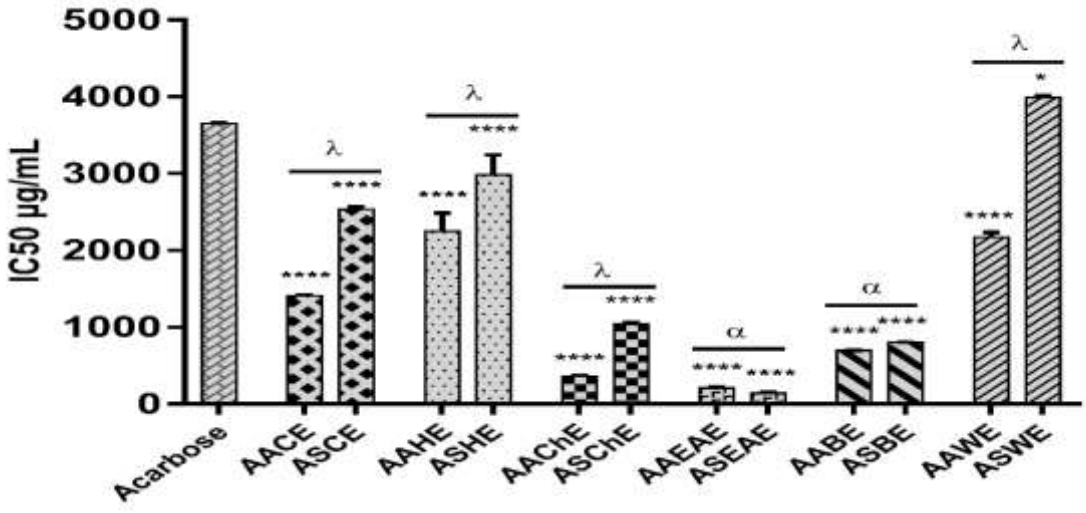

Figure 33: α-Amylase inhibitory activity of *A. atlantica* and *A. sicula* L. extracts.

ASCE; *A. sicula* crude extract, AACE; *A. atlantica* crude extract, ASHE; *A. sicula* hexane extract, AAHE; *A. atlantica* hexane extract, ASChE; *A. sicula*chloroform extract, AAChE; *A. atlantica* chloroform extract, ASEAE; *A. sicula* ethyl acetate extract, AAEAE; *A. atlantica* ethyl acetate extract, ASBE; *A. sicula* butanol extract, AABE; *A. atlantica* butanol extract, ASWE; *A. sicula* water extract, AAWE; *A. atlantica* water extract. Acarbose was used as reference drug. *; P≤0.05, ****; P≤0.0001*vs* acarbose. $^{\alpha}$ns; no significant difference (P≥0.05), $^{\lambda}$****; P≤0.0001in comparison between the effects of *A. atlantica* and *A. sicula* L. extracts.

4.2.5. α-Glucosidase inhibition activity

The α-glucosidase inhibitor effects of *A. atlantica* and*A. sicula* L. extracts are presented in figure 34. The investigation showed that the AAEAE from *A. atlantica* had a strong α-glucosidase inhibition effect with an IC_{50} value of 35.73±0.56 µg/ml, followed by AABE [IC_{50}=82.99±0.27 µg/ml], AAChE [IC_{50}=90.61±0.47 µg/ml], AACE [IC_{50}=155.67±2.82 µg/ml], AAWE [IC_{50}=440.85±3.71 µg/ml] and AAHE [IC_{50}=974.78±10.14 µg/ml]. The greatest α-glucosidase inhibition activity from*A. sicula* L. was obtained in ASEAE with an IC_{50} value of 66.13±1.24 µg/ml, followed by ASChE [IC_{50}=135.01±2.99 µg/ml], ASBE [IC_{50}=164.45±1.07µg/ml], ASCE [IC_{50}=421.14±15.82 µg/ml], ASHE [IC_{50}=466.22±20.71 µg/ml], and ASWE [IC_{50}=938.72±38.87 µg/ml] consecutively. *A. atlantica* crude extract and *A. atlantica* and *A. sicula* L. chloroform, ethyl acetate and butanol extracts had the highest α-glucosidase inhibition effect, and this biological activity was better than to that of the acarbose as a standard [IC_{50}=275.43±1.59 µg/ml] (P≤0.0001). A majority of extracts were more active from *A. atlantica* than *A. sicula* L.

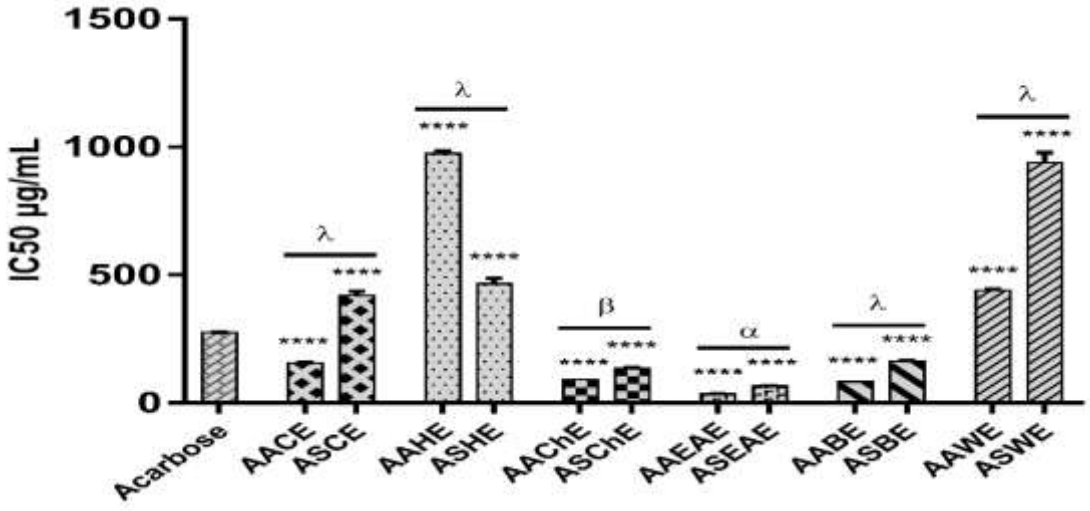

Figure 34: α-Glucosidase inhibitory activity of *A. atlantica* and *A. sicula* L. extracts.

ASCE; *A. sicula* crude extract, AACE; *A. atlantica* crude extract, ASHE; *A. sicula* hexane extract, AAHE; *A. atlantica* hexane extract, ASChE; *A. sicula*chloroform extract, AAChE; *A. atlantica* chloroform extract, ASEAE; *A. sicula* ethyl acetate extract, AAEAE; *A. atlantica* ethyl acetate extract, ASBE; *A. sicula* butanol extract, AABE; *A. atlantica* butanol extract, ASWE; A. *sicula* water extract, AAWE; *A. atlantica* water extract. Acarbose was used as standard drug. ****; P≤0.0001*vs* acarbose. $^{\alpha}$ns; no significant difference (P≥0.05), $^{\beta*}$P≤0.05, $^{\lambda}$****; P≤0.0001in comparison between the effects of *A. atlantica* and *A. sicula* L. extracts.

4.3. Correlation analysis

To understand the relationship between phytochemicals and bioactivity, correlation analysis was carried out and it is presented in tables 09 and 10. The correlation table showed that a good correlation between antioxidant activities as determined by DPPH, ABTS, phenanthroline, CUPRAC, or reducing power assays and phenolic contents; total polyphenol, flavonoid, flavonol or tannin contents. Also, a close correlation was found between phenolic contents and enzymatic inhibitory activities as determined by AChE, BChE, tyrosinase, α-amylase or α- glucosidase inhibition methods.

Table 09. Pearson's correlation coefficient between *in vitro* study variablesof *A. atlantica* extracts [Phytochemicalcontents (TPC, TFC, Flavonols, tannins, chlorophylls, carotenoids, lycopene and β-carotene content), and the sample concentration providing 50% of antioxidant (DPPH, ABTS, phenanthroline, CUPRAC, reducing power, metal chelating and β-carotene assays) and enzymatic (AChE, BChE, tyrosinase, α-amylase and α-glucosidase inhibition assays) activities ($A_{0.5}$ and IC_{50})].

	TPC	TFC	Flavonol	Tanin	Chlorophyll	Carotenoids	β-carotene	lycopene	DPPH	ABTS	Reducing power	CUPRAC	Phenanthroline	Metalchelating	β-carotenebleaching	ACHE	BCHE	Tyrosinase inhibition	α-Amylase inhibition	α-Glucosidase inhibition
TPC	1	0.9528	0.9138	0.9848	-0.2636	-0.1772	-0.0859	-0.2839	-0.8763	-0.8224	-0.8615	-0.7734	-0.8362	-0.0649	0.6919	-0.7452	-0.8302	-0.8323	-0.8985	-0.8424
TFC		1	0.9846	0.9671	-0.06831	-0.011	0.0455	-0.1447	-0.7981	-0.7309	-0.7075	-0.7558	-0.7386	0.1196	0.5904	-0.6001	-0.7440	-0.7254	-0.8242	-0.7430
Flavonols			1	0.9327	0.0524	0.1196	0.1321	-0.0864	-0.7938	-0.7501	-0.6661	-0.7210	-0.7501	0.0564	0.6066	-0.6186	-0.7451	-0.7489	-0.8298	-0.7366
Tannins				1	-0.2788	-0.1827	-0.1558	-0.3552	-0.7968	-0.7453	-0.7931	-0.6812	-0.7531	0.0386	0.5769	-0.6889	-0.7372	-0.8181	-0.8497	-0.7445
Chlorophylls					1	0.9640	0.9315	0.8824	0.1054	0.0932	0.4582	-0.1603	0.0885	0.0854	0.0124	0.3194	-0.0771	0.2679	0.0006	0.0177
Carotenoids						1	0.8800	0.7673	0.0634	0.0237	0.3936	-0.1457	0.0195	0.0119	0.0348	0.2033	-0.1597	0.0948	-0.1409	-0.0446
β-carotene							1	0.9503	-0.1329	-0.1134	0.2239	-0.4477	-0.1365	-0.227	0.2696	0.1670	-0.3359	0.1718	-0.1897	-0.2520
Lycopene								1	0.0656	0.1136	0.3947	-0.3312	0.0856	0.1155	0.0890	0.4020	-0.1189	0.4495	0.0680	-0.0490
DPPH									1	0.9815	0.9287	0.8347	0.9882	0.4411	-0.9519	0.8753	0.9318	0.8155	0.8765	0.9723
ABTS										1	0.9212	0.7366	0.9972	0.5752	-0.9597	0.9377	0.9104	0.8719	0.8689	0.9513
Reducing power											1	0.6606	0.9239	0.4843	-0.8556	0.9253	0.8094	0.8508	0.7916	0.8782
CUPRAC												1	0.7766	0.0602	-0.7820	0.4851	0.8972	0.4748	0.7946	0.8859
Phenanthroline													1	0.5520	-0.9651	0.9217	0.9357	0.8602	0.886	0.9713
Metalchelating														1	-0.6358	0.6918	0.3930	0.4864	0.2919	0.4486
β-carotenebleaching															1	-0.8503	-0.9045	-0.7146	-0.7812	-0.9492
ACHE																1	0.7672	0.9363	0.7812	0.8239
BCHE																	1	0.7678	0.9519	0.9872
Tyrosinase inhibition																		1	0.8748	0.7823
α-Amylase inhibition																			1	0.9214
α-Glucosidaseinhibition																				1

Table 10. Pearson's correlation coefficient between *in vitro* study variables of *A. sicula* L. extracts [Phytochemical contents (TPC, TFC, Flavonols, tannins, chlorophylls, carotenoids, lycopene and β-carotene content), and the sample concentration providing 50% of antioxidant (DPPH, ABTS, phenanthroline, CUPRAC, reducing power, metal chelating and β-carotene assays) and enzymatic (AChE, BChE, tyrosinase, α-amylase and α-glucosidase inhibition assays) activities ($A_{0.5}$ and IC_{50})].

	TPC	TFC	Flavonol	Tanin	Chlorophyll	Carotenoids	β-carotene	lycopene	DPPH	ABTS	Reducing power	CUPRAC	Phenanthroline	Metalchelating	β-carotenebleaching	ACHE	BCHE	Tyrosinase inhibition	α-Amylase inhibition	α-Glucosidase inhibition
TPC	1	0.9755	0.9544	0.9430	-0.0574	-0.2299	0.0063	-0.0256	-0.8083	-0.9142	-0.8204	-0.5217	-0.5980	0.4126	0.0639	-0.7833	-0.8468	-0.9313	-0.9606	-0.8539
TFC		1	0.9835	0.9797	0.0324	-0.2004	0.0564	0.1620	-0.8022	-0.9122	-0.7873	-0.4823	-0.6192	0.3270	-0.0446	-0.7910	-0.7864	-0.9190	-0.9082	-0.8220
Flavonols			1	0.9809	-0.0026	-0.1208	0.0018	0.2381	-0.7144	-0.8394	-0.7628	-0.4853	-0.6345	0.2944	-0.0490	-0.7108	-0.7214	-0.8426	-0.8689	-0.7736
Tannins				1	-0.1159	-0.2867	-0.0836	0.1875	-0.8139	-0.8939	-0.7060	-0.3547	-0.5159	0.1459	0.0755	-0.8126	-0.7725	-0.8903	-0.8353	-0.7256
Chlorophylls					1	0.6742	0.8253	0.5291	0.2933	0.1226	-0.2048	-0.4995	-0.5563	0.5965	-0.9634	0.2950	0.3069	0.1001	-0.0576	-0.2495
Carotenoids						1	0.6726	0.5674	0.7081	0.5377	-0.1968	-0.6200	-0.5983	0.5310	-0.8002	0.7421	0.6506	0.4653	0.0886	-0.1132
β-carotene							1	0.4533	0.2020	0.0981	-0.4862	-0.7159	-0.7037	0.6153	-0.8375	0.2836	0.3437	-0.0116	-0.2118	-0.4846
Lycopene								1	0.2482	0.1456	-0.1365	-0.2500	-0.4789	-0.0646	-0.7035	0.2258	0.4310	0.1342	0.0767	-0.0759
DPPH									1	0.9653	0.5091	0.0546	0.1380	0.0045	-0.3792	0.9890	0.9131	0.9516	0.7254	0.5916
ABTS										1	0.6279	0.2312	0.3287	-0.1929	-0.20004	0.9593	0.9351	0.9858	0.8400	0.7108
Reducing power											1	0.8743	0.8800	-0.6512	0.2504	0.4166	0.4680	0.7325	0.9239	0.9854
CUPRAC												1	0.9531	-0.8530	0.5610	-0.0396	0.0873	0.3498	0.6966	0.8366
Phenanthroline													1	-0.7390	0.6454	0.0679	0.1185	0.4255	0.7125	0.8458
Metalchelating														1	-0.5361	0.0663	-0.1789	-0.2644	-0.5980	-0.6777
β-carotenebleaching															1	-0.3867	-0.4217	-0.1629	0.0400	0.2527
ACHE																1	0.9217	0.9215	0.6669	0.5073
BCHE																	1	0.8967	0.7618	0.5580
Tyrosinase inhibition																		1	0.8949	0.8017
α-Amylase inhibition																			1	0.9528
α-Glucosidaseinhibition																				1

5. *In vivo* biological activities

5.1. Acute oral toxicity

In acute toxicity test, no visible signs of toxicity (behavioral, neurological or physical changes) or mortality was observed at two test doses (2000 mg/kg and 5000 mg/kg) from *A. atlantica* and *A. sicula* L. hydromethanolic extracts within the 14 days of observation. It is concluded that the minimum lethal dose of the plant extracts tested in our study is over 5000 mg/kg.

5.2. Gastric emptying and small intestine transit in mice

5.2.1. Effect of AACE and ASCE on gastric emptying in mice

Both two AACE and ASCE extracts exerted dose dependent decrease in activity of the emptied quantity of the test meal compared to the vehicle (CMC) as negative control (81.06±8.14%). The results are shown in figure 35. The tested doses (100, 200 and 400 mg/kg) from AACE (52.40±6.96%, 45.21±6.04% and 42.48±4.52%, respectively; $P \leq 0.0001$) and ASCE (62.69±7.45%, 52.15±7.40% and 46.42±5.44%, respectively; $P \leq 0.001$ to $P \leq 0.0001$) dose-dependently reduced the gastric emptying. The effects of AACE (200 and 400mg/kg) and ASCE (400 mg/kg) are significantly different compared to negative control. All doses from the two plants showed no significant difference in gastric emptying compared to the atropine as standard (53.15±6.07%; $P > 0.05$). The tested doses did not show any significant difference between AACE and ASCE effects ($P > 0.05$).

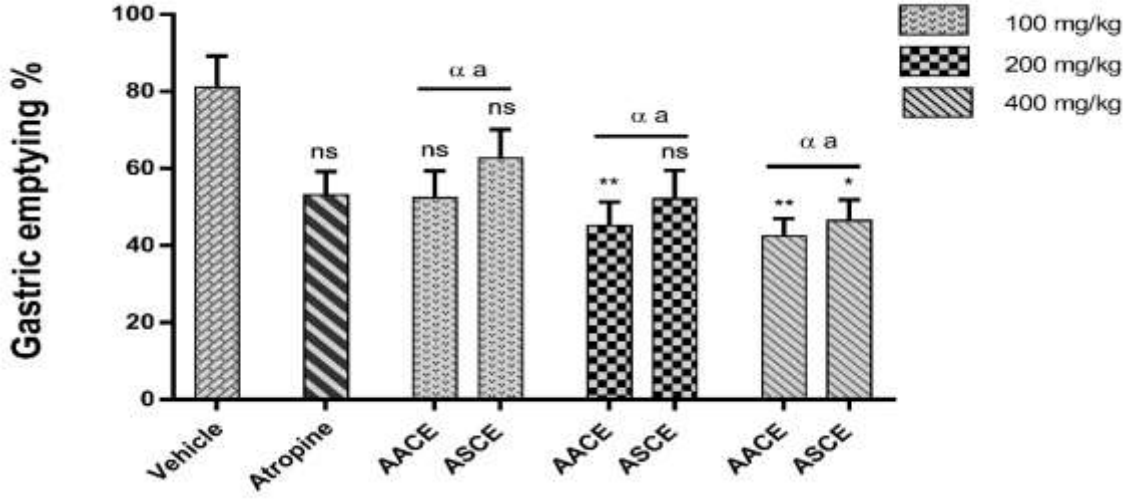

Figure 35: Effects of AACE and ASCE on gastric emptying in mice.

AACE; *A. atlantica* crude extract, ASCE; *A. sicula* crude extract. Bars represent means±SEM (n=12). ns; no significant difference, *; P≤0.05 and **; P≤0.01*vs* vehicle asnegative control.[a]ns; no significant difference (P>0.05) *vs* atropine as positive control. [α]ns; no significant difference (P>0.05) in comparison between the effects of AACE and ASCE at different doses (100, 200, 400 mg/kg).

5.2.1.1. Mechanisms of the AACE effect on gastric emptying

In series of experiments, the extract is administered (400 mg/kg) in the presence of different pharmacological substances; L-nitro-n-arginine (L-NNA), (1.36×10^{-1}mmol/kg), atropine (3.45×10^{-3}mmol/kg) or indomethacine (5.58×10^{-2}mmol/kg), to determine the possible pathway (s) involved in the effect of extract on gastric emptying. The injection of L-NNA alone; NOS inhibitor, very highly decreased the rate of gastric emptying to 24.13±1.97%compared to the vehicle (P≤0.0001). Under the inhibition of NOS, oral administrationof the AACE extract had a significant decreasein rate of gastric emptying (18.36±1.94%; P≤0.01) compared to the treatment with AACE alone. Blockade of ACh receptors withatropine strongly significantly reduces the rate of gastric emptying (52.14±1.00%; P≤ 0.001) compared to the vehicle. Administration of the AACE, in mice pretreated with atropine, did not show any decrease in rate of gastric emptying (31.14±1.02%; P>0.05) compared to the effect observed with AACE alone. The cyclo-oxygenase inhibitor, hence prostaglandins production inhibitor, indomethacin had significantly reduced gastric emptying (49.57±4.87%, P≤ 0.0001) compared to the vehicle. AACE effect wasno altered under the influence of indomethacin compared to the effect of, AACE alone (27.15±1.92%, P>0.05) (Figure 36).

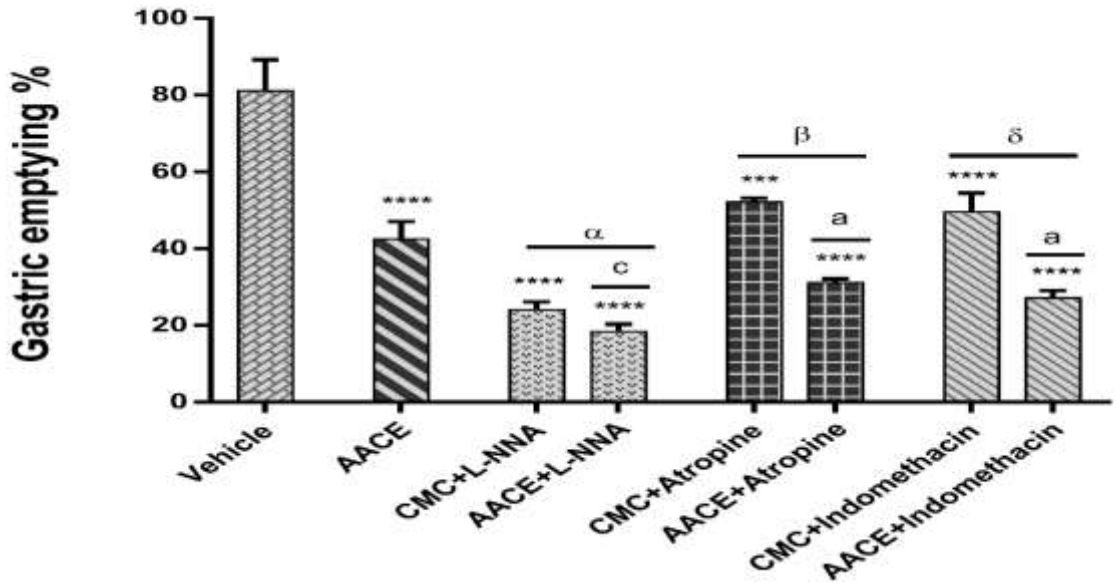

Figure 36: Effect of AACE in absence and in presence of L-NNA, atropine orindomethacin on gastric emptying.

AACE; *A. atlantica* crude extract. Bars represent means±SEM (n=12). ***; P≤0.001, ****; P≤0.0001 *vs*vehicle as negative control.[a]ns; no significant difference (P>0.05), [c]**; P≤0.01in comparison to both effects of ASCE in absence and in presence of L-NNA, atropine or indomethacin. [α]ns; no significant difference (P>0.05) and[β]*; P≤0.05, [δ]**;P≤0.01in comparison between both effectsof AACE (400mg/kg) in absence and in presence of L-NNA (1.36×10^{-1}mmol/kg), atropine (3.45×10^{-3}mmol/kg), or indomethacin (5.58×10^{-2}mmol/kg).

5.2.1.2. Mechanisms of the ASCE effect on gastric emptying

Under the inhibition of NOS, oral administration of the ASCE extract had significant effect on gastric emptying delay (24.89±2.36%; P≤0.05) compared to the animals pretreated only with L-NNA. A significant difference between the two treatments of ASCE in the absence and in the presence of atropine (22.36±3.17%; P≤0.05) was observed. The ASCE extract, administered to mice pretreated with indomethacin, had no significant effect on gastric emptying compared to the effect observed with ASCEalone (51.27±4.98%; P>0.05) (Figure 37).

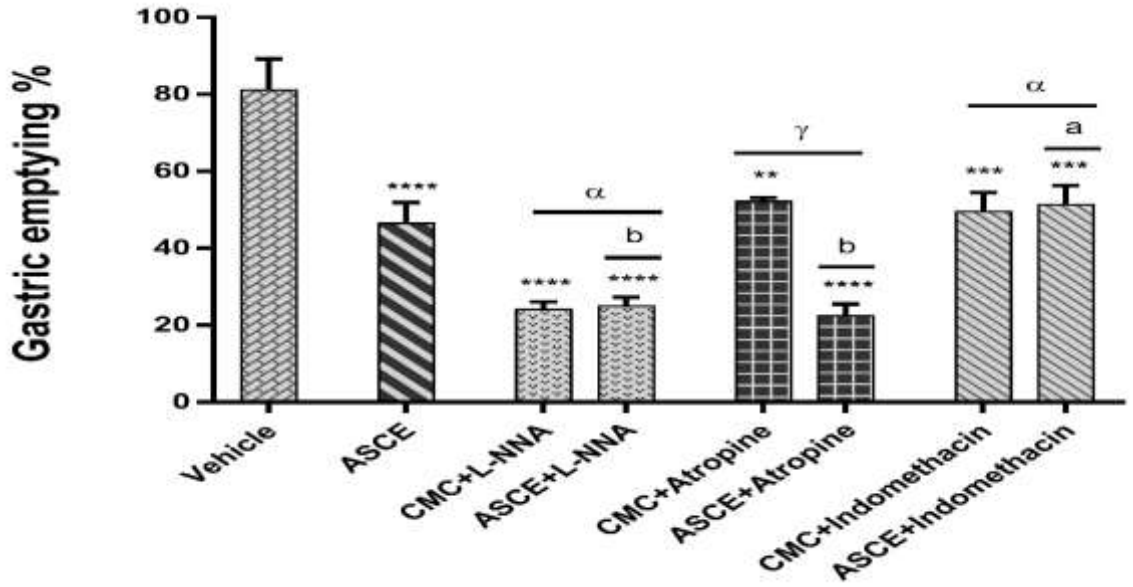

Figure 37: Effect of ASCE in absence and in presence of L-NNA, atropine or indomethacin on gastric emptying.

ASCE; *A. sicula* crude extract. Bars represent means±SEM (n=12). **; P≤0.01, ***; P≤0.001, ****; P≤0.0001*vs* vehicle as negative control. [a]ns; no significant difference (P>0.05), [b]*; P≤0.05in comparison to both effects of ASCE in absence and in presence of L-NNA, atropine or indomethacin. [α]ns; no significant difference (P>0.05) , [δ]**; P≤0.01 in comparison betweenboth effects of L-NNA (1.36×10^{-1}mmol/kg), atropine (3.45×10^{-3}mmol/kg), or indomethacin (5.58×10^{-2}mmol/kg) in the absence and in the presence of ASCE (400 mg/kg).

5.2.2. Effect of AACE and ASCE on intestinal transit in mice

The effects of AACE and ASCE on intestinal transit are shown in figure 38. Both two tested extracts decreased the transit of phenol red through the small intestine compared to the vehicle (65.17±3.69%). AACE and ASCE at the lowest dose (100 mg/kg) did not show any effect (54.18±2.14% and 56.45±3.09%; P>0.05, respectively), compared to the vehicle. The doses (200 and 400 mg/kg) from AACE showed a dose dependant reductionin intestinal transit (52.13±1.98% and 42.53±3.54%; P≤0.05 and P≤0.0001, respectively). Also, the doses 200 and 400 mg/kg of ASCE decreased the propulsive movement and transit of red phenol through the small intestine (50.89±2.14% and 43.26±1.18%; P≤0.05 and P≤0.0001), respectively. AACE and ASCE at 200 and 400 mg/kg showed no significant difference effects compared to atropine as positive control (40.45±4.01%; P>0.05). The intestinal transit effect of the tested doses was not significantly different in comparison between AACE and ASCE (P>0.05).

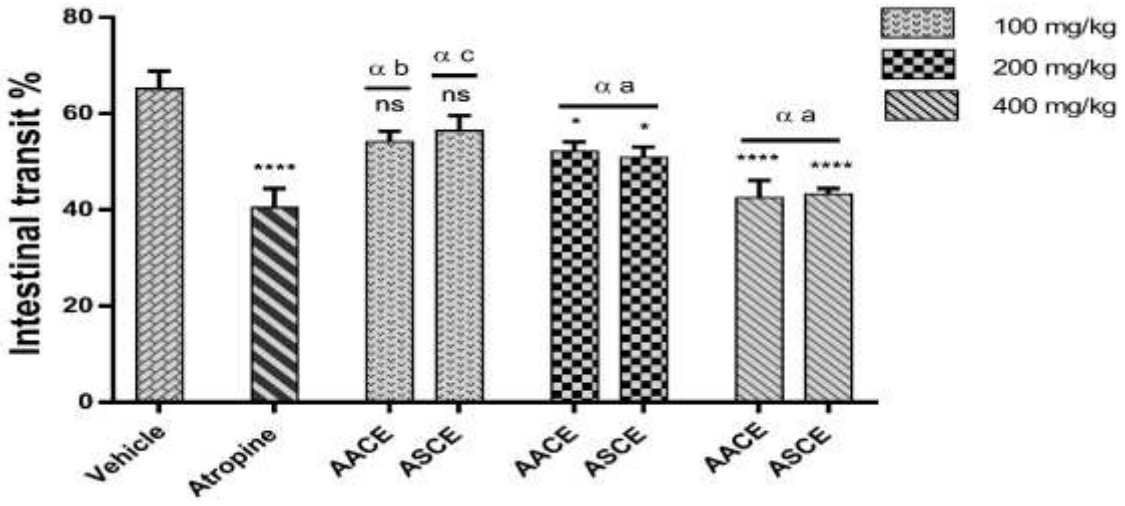

Figure 38: Effects of AACE and ASCE on intestinal transit in mice.

AACE; *A. atlantica* crude extract, ASCE; *A. sicula* L. crude extract. Bars represent means±SEM(n=12). ns; no significant difference (P>0.05), *; P≤0.05, ****; P≤0.0001*vs*vehicle as negative control.[a]ns; no significant difference (P>0.05), [b]*; P≤0.05, [c]**; P≤0.01 *vs* atropine as positive control. [α]ns; no significant difference (P>0.05) in a comparison between the effects of AACE and ASCE at different doses (100, 200, 400 mg/kg).

5.2.2.1. Mechanisms of theAACE effect on intestinal transit

Different pharmacological substances (atropine, L-NNA or indomethacin) were used to determine of AACE effect mechanism on intestinal transit (Figure 39). L-NNA had significantly decreased gastric emptying (49.07±1.62%; P≤0.01) compared to vehicle. However, the effect of AACE in the presence of L-NNA did not show any effect (53.67±1.64%; P>0.05) compared to the treatment with AACE in absence of L-NNA. The atropine reduced the rate of intestinal transit to 41.84±4.49% (P≤0.0001) compared to the vehicle. The effect of both AACE and atropine had no significant difference (40.12±3.79%; P>0.05) compared to the effect of AACE alone. The indomethacin significantly reduced gastric emptying compared to the vehicle (49.17±1.27; P≤0.01). No significant difference in a comparison between AACE effects in the absence and in the presence of indomethacin was observed (34.08±1.13; P>0.05).

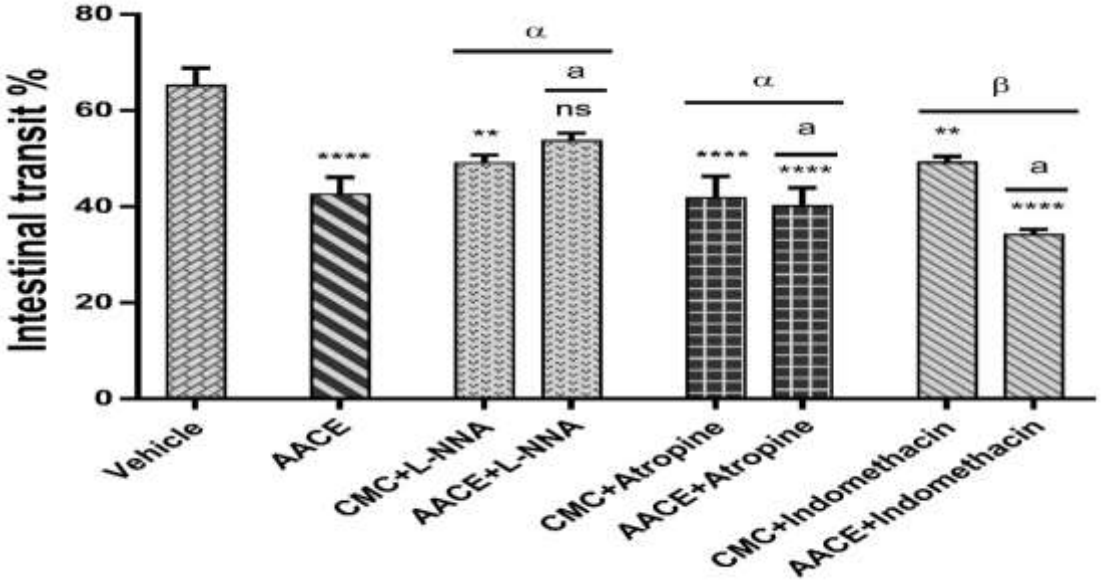

Figure 39: Effect of AACE in absence and in presence of L-NNA, atropine or indomethacin on intestinal transit.

AACE; *A. atlantica* crude extract. Bars represent means±SEM (n=12). ns; no significant difference (P>0.05), **; P≤0.01, ****; P≤0.0001 *vs* vehicle as negative control. [a]ns; no significant difference (P>0.05) in comparison to both effects of AACE in absence and in presence of L-NNA, atropine or indomethacin. [α]ns; no significant difference (P>0.05) , [β]*P≤0.05 in comparison between both effects of L-NNA (1.36×10^{-1}mmol/kg), atropine (3.45×10^{-3}mmol/kg), or indomethacin (5.58×10^{-2}mmol/kg) in the absence and in the presence of AACE (400mg/kg).

5.2.2.2. Mechanisms of the ASCE effect on intestinal transit

The mechanismseffect of ASCE on intestinal transit is shown in figure 40. No significant difference between the intestinal transit in the presence of ASCE alone and in the presence of ASCE with L-NNA extract was recorded (47.19±3.46; P>0.05). There is a significant difference between the ASCE effects in the absence and inthe presence of atropine (26.13±2.42%; P≤0.01). No significant difference was recorded between ASCE effects in the absence and in the presence of indomethacin (35.05±2.45%; P>0.05).

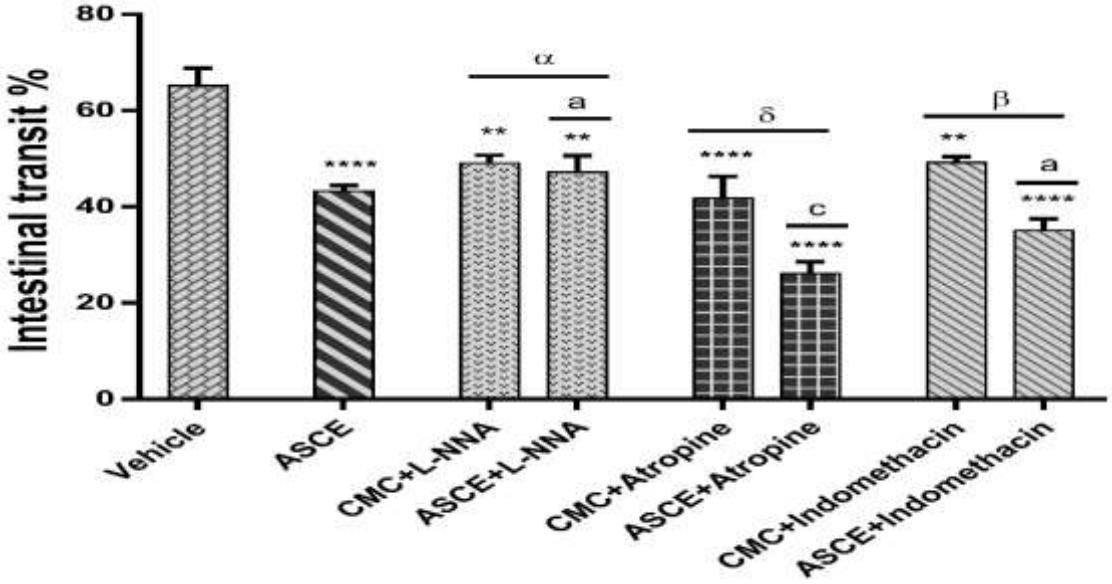

Figure 40: Effect of ASCE in absence and in presence of L-NNA, atropine or indomethacin on intestinal transit.

ASCE ; *A. sicula* L. crude extract. Bars represent means±SEM (n=12). **; P≤0.001, ****; P≤0.0001 *vs*vehicle as negative control. [a]ns; no significant difference (P>0.05), [c]*; P≤0.01 in comparison between both effects of ASCE in absence and in presence of L-NNA, atropine or indomethacin. [α]ns; no significant difference (P>0.05) , [β]*P≤0.05, [δ]**; P≤0.01 in comparison between both effects of L-NNA (1.36×10^{-1}mmol/kg), atropine (3.45×10^{-3}mmol/kg), or indomethacin (5.58×10^{-2}mmol/kg) in the absence and in the presence of ASCE (400mg/kg).

5.3. Ethanol-induced gastric ulceration in rats

5.3.1. Gastroprotective effect of AACE and ASCE

Intragastric administration of absolute ethanol to the untreated group of rats (the vehicle (1.5% CMC) as negative control) produced large bandlike hemorrhagic erosions in the glandular stomach (34.23±3.69%). Pretreatment with AACE at the tested doses (100, 200 and 400 mg/kg) offered different degrees of protection to the mucosa against all such damages caused by ethanol (20.94±2.20%, 18.32±1.55% and 1.16±0.19%, respectively; P≤0.001 to P≤0.0001). Compared with the 1.5% CMC vehicle-treated animals, all tested doses of ASCE extracts (100, 200 and 400 mg/kg) dose-dependently reduced significantly the ulcer gastric (23.78±2.45%, 21.17±2.16% and 5.13±0.21%, respectively; P ≤0.01 to P≤0.0001). AACE at the dose of 200 mg showed a similar effectas the positive control; ranitidine (10.00±0.46%; P≥0.05). The highest dose of AACE exhibited a similar form of the stomach to that of normal control (0.00±0.00%; P>0.05), and a better reduction of ulcer gastric than the positive control (P≤0.05). Whereas, the highest dose of ASCE exhibited a similar effect (P≥0.05) as normal and

positive control. The lowest tested doses (100 and 200 mg/kg), did not show any significant difference (P>0.05) in comparison between AACE and ASCE. But in 400 mg/kg dose, the protection against ulcer was best in AACE than ASCE (P≤0.05) (Figure 41).

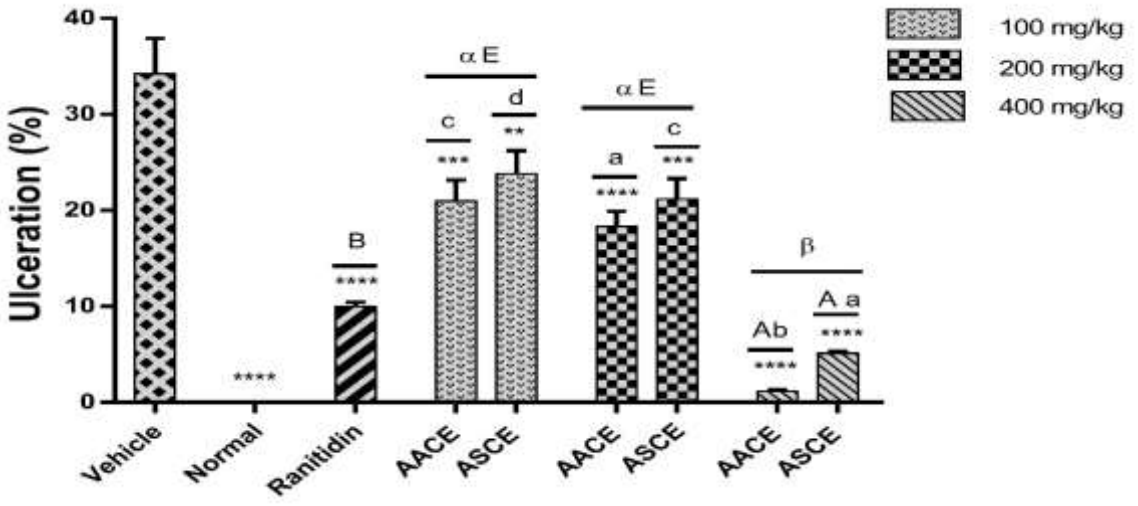

Figure 41: Effects of AACE and ASCE on gastric ulcer in rats.

AACE; *A. atlantica* crude extract, ASCE; *A. sicula* L. crude extract. Bars represent means±SEM (n=8). **; P≤0.01, ***; P≤0.001, ****; P≤0.0001 vehicle as negative control. ^{Ans}; no significant difference (P>0.05), ^{B}*; P≤0.05, ^{E}****; P≤0.0001 *vs* normal control. ^{a}ns; no significant difference (P>0.05), ^{b*}; P≤0.05, ^{c**}; P≤0.01, ^{d}***; P≤0.001 *vs* ranitidineas positive control. $^{\alpha}$ns; no significant difference (P>0.05), $^{\beta*}$;P≤0.05in comparison between the effects of AACE and ASCE at different doses (100, 200, 400 mg/kg).

5.3.1.1. Mechanisms of the AACE gastroprotective effect

In series of experiments, the extract is administered (400 mg/kg) in the presence of different pharmacological substances; L-nitro-n-arginine (L-NNA), (1.36×10^{-1}mmol/kg), atropine (3.45×10^{-3}mmol/kg) or indomethacine (5.58×10^{-2}mmol/kg), to determine the possible pathway (s) involved in the gastroprotective effect of extract. The arginine had significantly reduced the gastric ulcer to 1.43±0.18% (P≤0.0001) compared to the vehicle (34.23±3.69%). The AACE in the presence of arginine showed no significant difference effect (0.61±0.09%; P>0.05) compared to AACE alone. On other hand, L-NNA had a very highly significantly increased gastric ulcer than to the vehicle (50.69±1.43%; P≤0.0001). AACE in the presence of L-NNA, had significant increase on gastric ulcer compared to AACE alone (48.15±1.53%; P≤0.0001). The atropine had highly provided a protection to the mucosa against damages caused by ethanol (12.09±0.25%; P≤0.0001) compared to CMC vehicle treated animals. No

significant difference (0.82±0.07%, P>0.05) between both effects of atropine in the presence and in the absence of the extract was recorded (Figure 42).

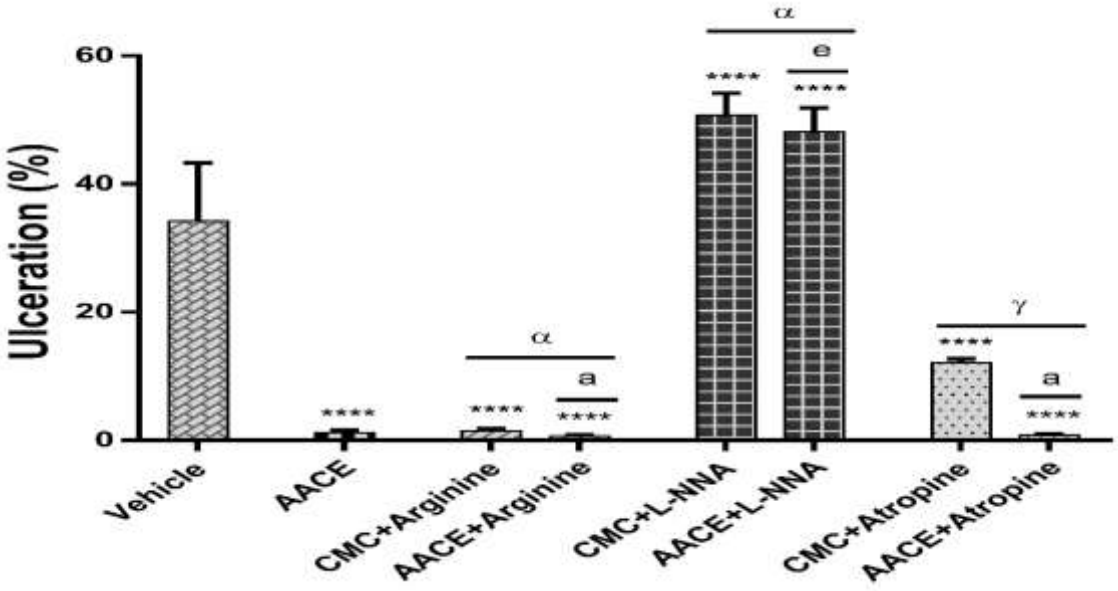

Figure 42: Effect of AACE in absence and in presence of arginine, L-NNA or atropine ongastric ulcerin ethanol-induced gastric mucosal lesions in rats

AACE; *A. atlantica* crude extract. Bars represent means±SEM (n=8). ****; P≤0.0001 *vs*vehicle as negative control.[a]ns; no significant difference (P>0.05), [e]***; P≤0.0001in comparison between both effects of AACE in absence and in presence of arginine, L-NNA or atropine. [α]ns; no significant difference (P>0.05), [γ]***; P≤0.001 in comparison between both effects of arginine, L-NNAor atropine in the absence and in the presence of AACE (400mg/kg).

5.3.1.2. Mechanisms of the ASCE gastroprotective effect

The mechanisms of ASCE gastroprotective effect is shown in figure 43. Pre-treatment with ASCE in presence of arginine offered very highly significant gastric protection against ulcer caused by ethanol (0.83±0.12%; P≤0.0001) compared to the vehicle (34.23±3.69%), with no significant difference between the two gastroprotective effects of ASCE in absence and in presence of arginine (P>0.05). A high significant difference was observed between the rate of ulceration in the presence of ASCE alone or with L-NNA (53.36±3.56; P≤0.0001). On another hand, ASCE under effect of atropine had provided significantly gastric protection against ulcer caused by ethanol (2.16±0.27%; P≤0.0001). No significant difference (P≤0.05) between both effects of ASCE in presence and in absence of atropine was recorded.

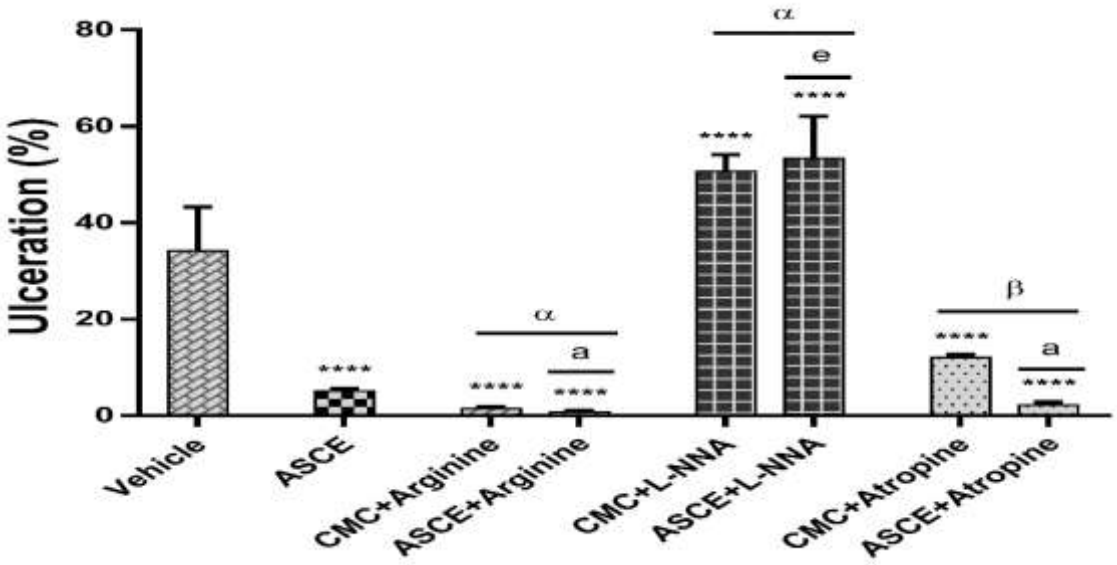

Figure 43: Effect of ASCE in absence and in presence of arginine, L-NNA or atropine on gastric ulcer in ethanol-induced gastric mucosal lesions in rats

ASCE ; *A. sicula* L. crude extract. Bars represent means±SEM (n=8). ****; P≤0.0001 *vs*vehicle as negative control. [a]ns; no significant difference (P>0.05), [e]***; P≤0.0001in comparison between both effects of ASCE in absence and in presence of arginine, L-NNA or atropine. [α]ns; no significant difference (P>0.05), [β*]; P≤0.05 in comparison between both effects of arginine (1.72 mmol/kg), L-NNA (1.36×10^{-1}mmol/kg) or atropine (3.45×10^{-3}mmol/kg) in the absence and in the presence of ASCE (400mg/kg).

5.3.2. Macroscopic and histopathological examination of AACE and ASCE effect on ethanol-induced gastric mucosa damage in rats

a) Macroscopic examination

The assay revealed an effect of ethanol on gastric tissues in the absence and in the presence of ASCE or AACE extracts at different doses, and the results are shown in figure 44. No injuries to the gastric mucosa are seen in macroscopic appearance of the intact stomach from a normal group (A). Absolute ethanol produced extensive visible hemorrhagic necrosis of gastric mucosa in the vehicle as negative control animals (ulcer control group) (Figure 44 B). The positive control group (ranitidine 40 mg/kg) showed milder injuries to the gastric mucosa (Figure 44 C) compared to the ulcer negative control rats. However, oral administration of AACE (Figure 44 D) and ASCE (Figure 44 E) effectively reversed the ethanol-induced gastric injury in a dose-dependent manner, with significant reduction of the gastric ulcer area. The protective properties of the ASCE extract at its highest dosage (400 mg/kg) appeared

similar to the positive group (Figure 44 E3), while the highest dose (400 mg/kg) from AACE showed a best protective activity than to the positive group (Figure 44 D3).

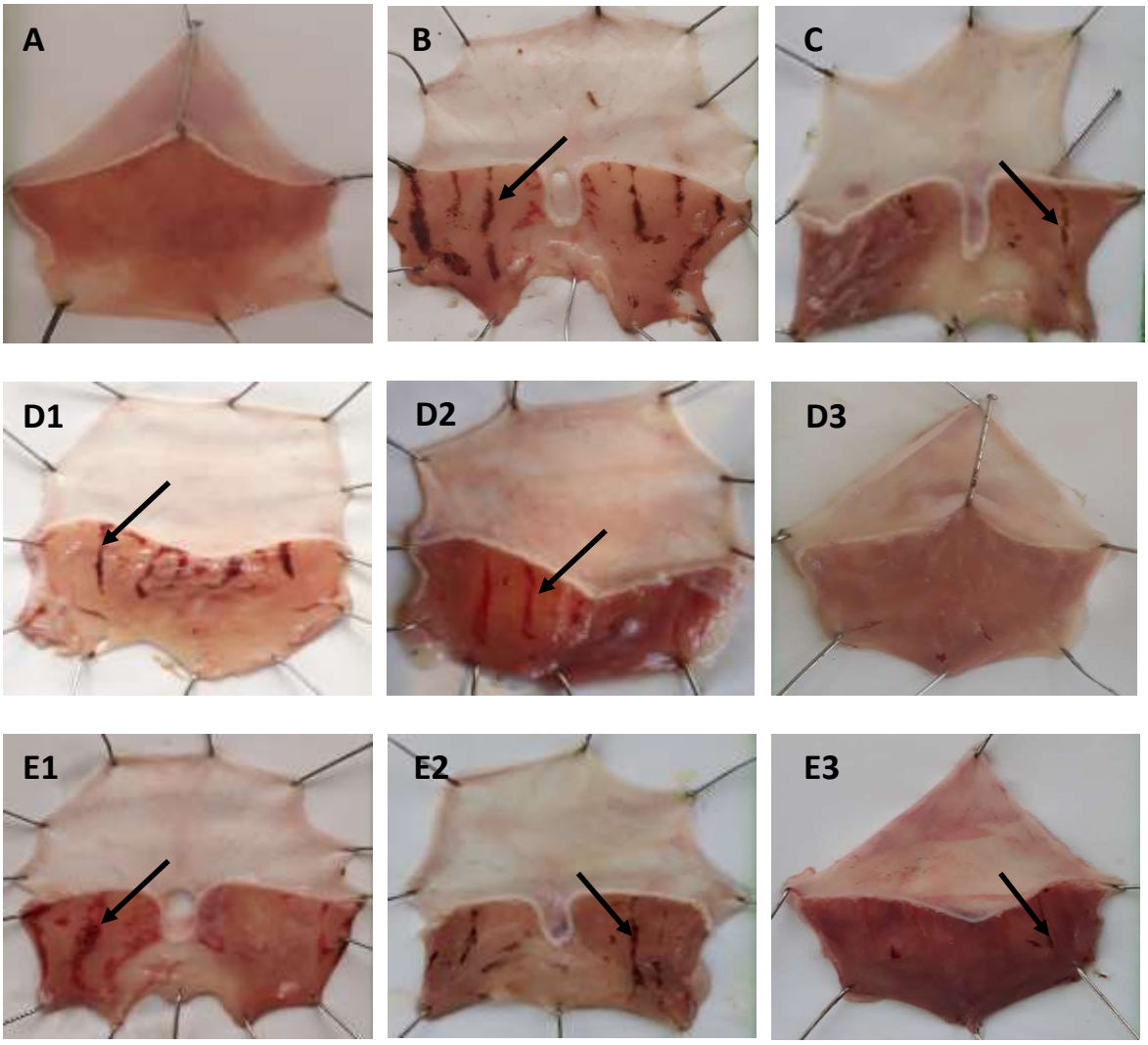

Figure 44: Effects of AACE and ASCE on the macroscopic appearance of the gastric mucosa in ethanol-induced gastric mucosal lesions in rats.

(A) Normal control group. (B) The group pre-treated with CMC vehicle as negative control. (C) The group pre-treated with ranitidine (40 mg/kg) as positive control. (D1, D2 and D3): The group pre-treated with AACE (100, 200 and 400 mg/kg, respectively). AACE: *A. atlantica* crude extract. (E1, E2 and E3): The group pre-treated with ASCE (100, 200 and 400 mg/kg, respectively). ASCE: *A. sicula* L. crude extract. Black arrow: elongated bands of hemorrhagic lesions.

b) Histopathological examination

Histological study of ethanol-induced gastric mucosal damage in rats in the absence and in the presence of ranitidine, AACE and ASCE extracts at different doses are shown in figure 45. Stomach of normal rat (Figure 45 A) showing normal histological structure of the mucosa, submucosa and muscularis. Stomach of vehicle plusethanol-treated rat in ulcer control group (Figure 45 B) showing necrosis in the superficial mucosal epithelium (red arrow) with oedema, inflammatory cell infiltration (blue arrow) and congestion of blood vessels (green arrow) in submucosa. Stomach of ranitidine plus ethanol-treated rat in positive control group (Figure 45 C) showing intact histological structure of the mucosa with mild oedema and inflammatory cell infiltration (blue arrow), and mildly dilated blood vessels (green arrow) in submucosa compared to that in ulcer control rats (Figure 45 B). Rat pretreated with AACE (Figure 45 D) and ASCE (Figure 45 E), have relatively better protection in a dose-dependent manner, as observed by intact gastric epithelium and reduced or complete absence of edema and leucocytes infiltration (blue arrow) was also observed.

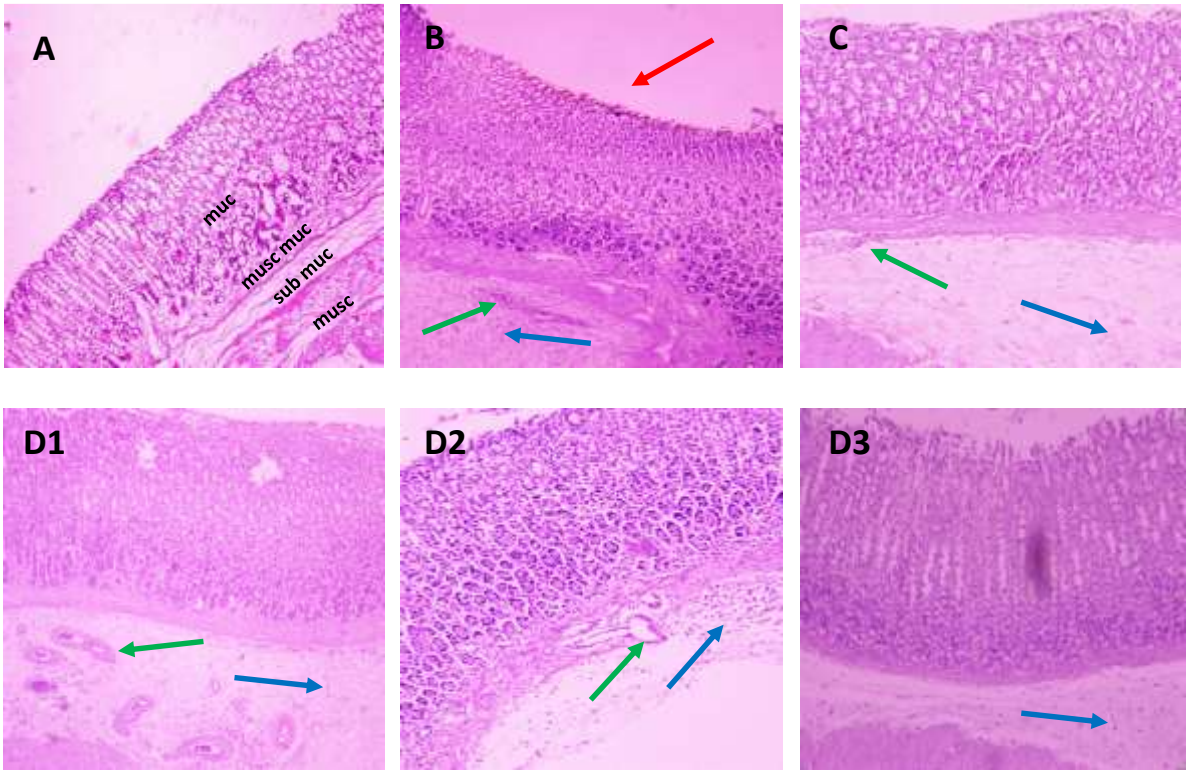

Figure 45 (continued)

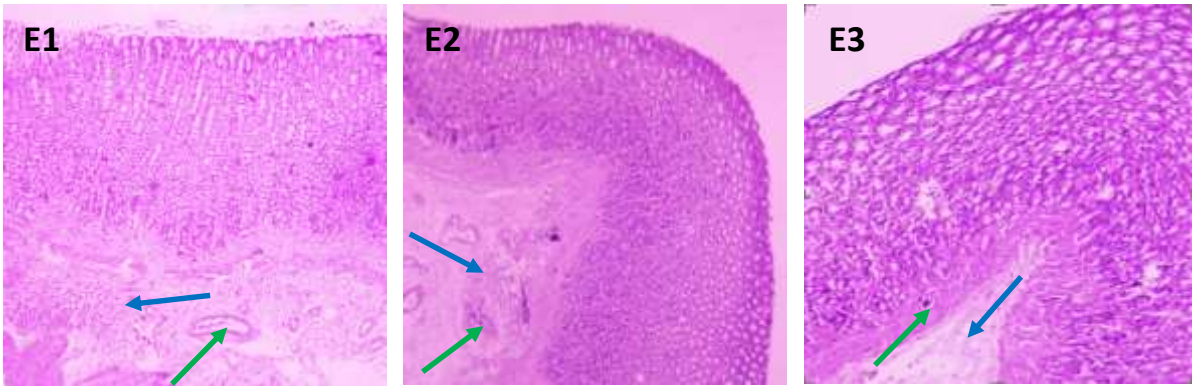

Figure 45: Histological examination for the protective effect of AACE and ASCE against ethanol-induced gastric damage in rat stomach tissue (magnification 100x).

(A) Normal control group. (B) The group pre-treated with CMC vehicle as negative control. (C) The group pre-treated with ranitidine (40 mg/kg) as positive control. (D1, D2 and D3): The group pre-treated with AACE (100, 200 and 400 mg/kg, respectively). AACE: *A. atlantica* crude extract. (E1, E2 and E3): The group pre-treated with ASCE (100, 200 and 400 mg/kg, respectively). ASCE: *A. sicula* L. crude extract. Red arrow: surface epithelium damage and hemorrhagic necrosis penetrating deeply into gastric mucosa. Blue arrow: edema of submucosa and inflammatory cell infiltration. Green arrow: congestion of blood vessels. muc: mucosa, musc muc: muscularis mucosa, sub muc: submucosa, musc: muscularis.

5.3.2.1. Macroscopic and histopathological examination of mechanisms of AACE effect on ethanol-induced gastric mucosa damage in rats

a) Macroscopic examination

Figure 46 showed a macroscopic examination of AACE effect mechanisms on ethanol-induced gastric mucosa damage in rats. The rats pre-treated with the arginine in the absence of AACE (Figure 46 B1) and in its presence (Figure 46 B2) significantly reduced areas of gastric ulcer formation, compared to negative control animals (Figure 46 A1). It appeared similar to the AACE at 400 mg/kg dose (Figure 46 A2). L-NNA either in the absence of AACE (Figure 46 C1) or in its presence (Figure 46 C2) highly increased the gastric ulcer area than negative control animals (Figure 46 A1).Injuries to the gastric mucosa in rats pretreated with atropine are milder (Figure 46 D1) compared to the injuries seen in the negative control rats (Figure 46.A1). However, the atropine in the presence of AACE extract (Figure 46 D2) reduced the formation of gastric lesions compared to atropine effect in absence of AACE (Figure 46 D1). Also, it appeared similar to the AACE at 400 mg/kg dose (Figure 46 A2).

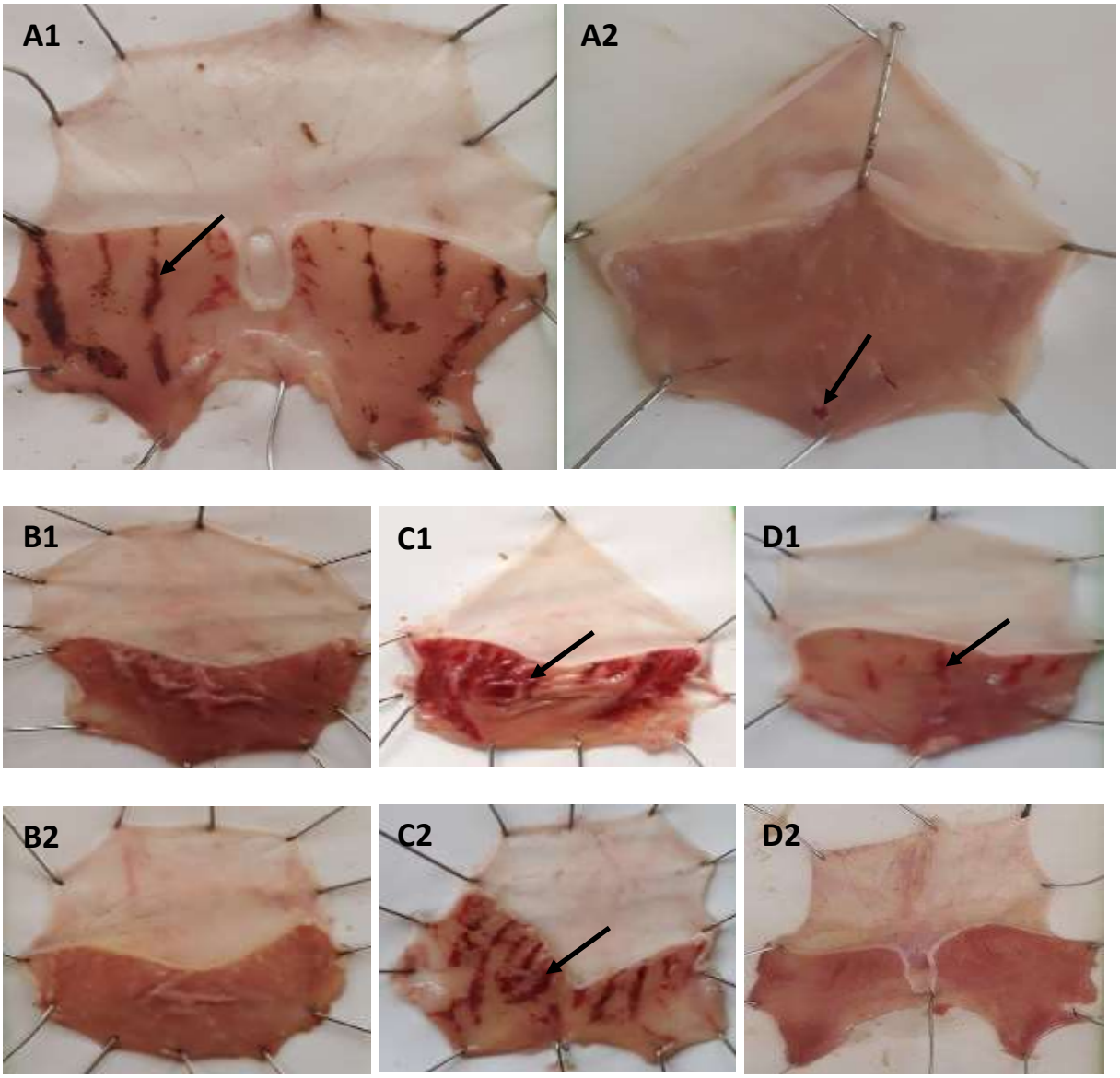

Figure 46: Effect of AACE in absence or presence of different pharmacological substances on the macroscopic appearance of the gastric mucosa in ethanol-induced gastric mucosal lesions in rats.

(A1) The group pre-treated with CMC vehicle as negative control. (A2) The group pre-treated with AACE (400mg/kg). (B1, C1 and D1): The group pre-treated with arginine (1.72 mmol/kg), L-NNA (1.36×10^{-1}mmol/kg) or atropine (3.45×10^{-3}mmol/kg) in absence of AACE. (B2, C2 and D2): The group pre-treated with arginine, L-NNA andatropine, respectively in presence of AACE. AACE: *A. atlantica* crude extract. Black arrow: elongated bands of hemorrhagic lesions.

b) Histopathological examination

Figure 47 showed a histopathological analysis of AACE effect in absence or presence of different pharmacological substances, on the macroscopic appearance of the gastric mucosa in ethanol-induced gastric mucosal lesions in rats. Histological observation confirms the ability of AACE (Figure 47 A2) at its highest dose (400 mg/kg) to prevent ethanol-induce gastric damage in the superficial layer of the gastric mucosa and further highly significantly reduced oedema and leucocytes infiltration (blue arrow) in submucosa compared to the ulcer control group (Figure 47 A1). There is no disruption to the surface mucosal epithelium, no evidence of infiltration, no oedema and no dilated blood vessels in the gastric submucosa of rats treated with the arginine in the absence of AACE (Figure 47 B1) and in its presence (Figure 47 B2). It appeared similar to the AACE alone (Figure 47 A2). There are severe submucosal oedema and leucocytes infiltration (Blue arrow) in rats pretreated with L-NNA (Figure 47 C1) alone and in rats pretreated with both L-NNA and AACE (Figure 47 C2), compared to the negative control (Figure 47 A1). In rats pretreated with atropine (Figure 47 D1); there is no disruption to the surface epithelium and, moderate oedema, leucocytes infiltration (blue arrow) and congestion of blood vessels (green arrow) in the submucosal layer, compared to that seen in the vehicle asnegative control rats (Figure 47 A1). However, the atropine in the presence of AACE extract (Figure 47 D2) significantly reduced the oedema, leucocytes infiltration (blue arrow) and the congestion of blood vessels (green arrow) in the submucosal compared to atropine effect in absence of AACE (Figure 47 D1). AACE effect in presence of atropine appeared similar to its effect in absence of atropine (Figure 47 A2).

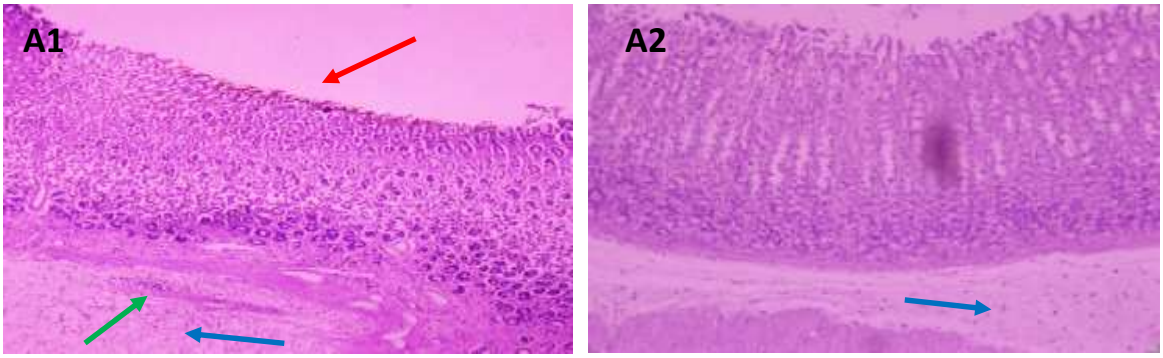

Figure 47 (continued)

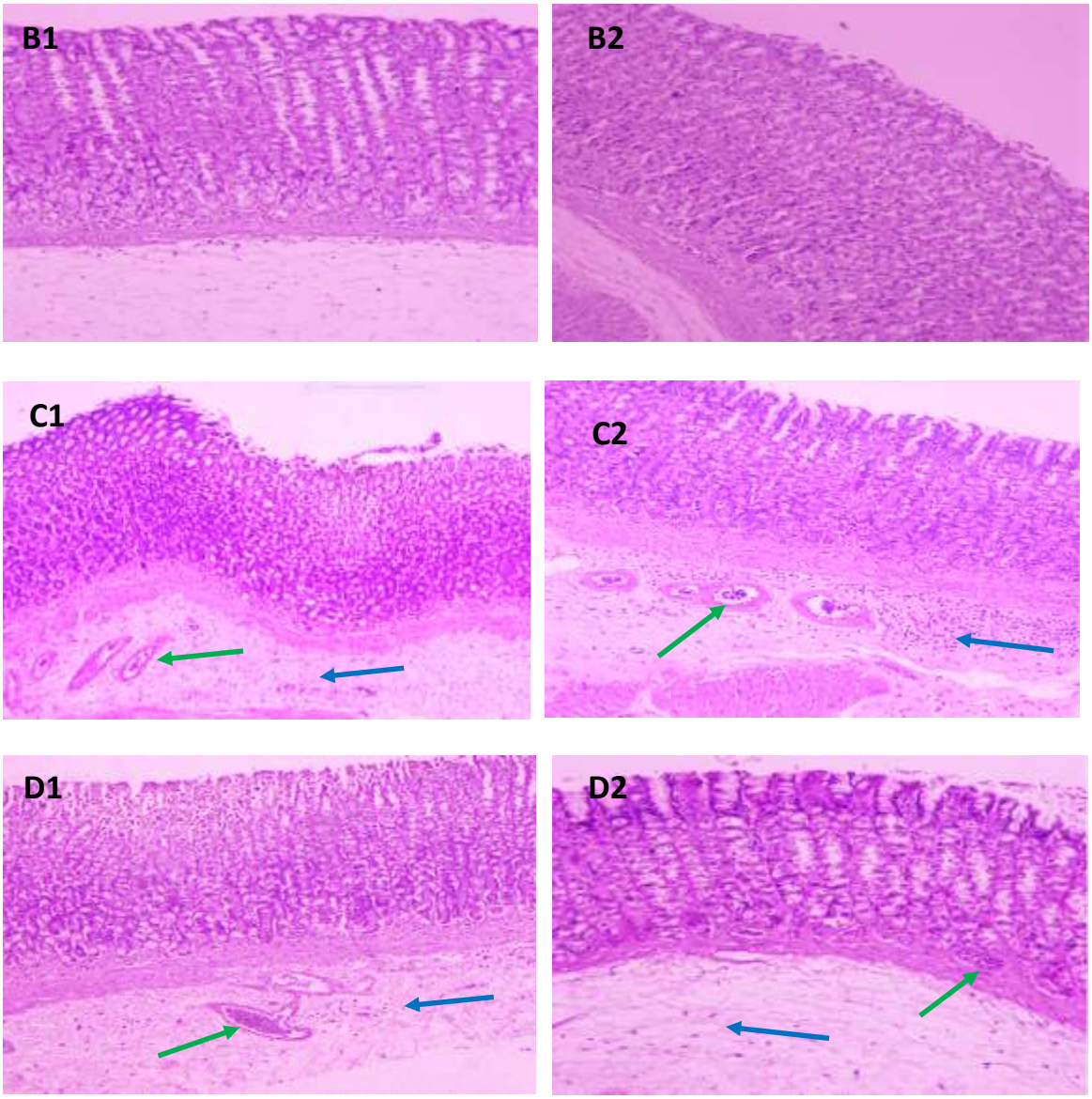

Figure 47: Histological examination for the protective effect of AACE in absence or presence of different pharmacological substances, against ethanol-induced gastric damage in rat stomach tissue (magnification 100x).

(A1) The group pre-treated with CMC vehicle as negative control. (A2) The group pre-treated with AACE (400mg/kg). (B1, C1 and D1): The group pre-treated with arginine (1.72 mmol/kg), L-NNA (1.36×10^{-1}mmol/kg) or atropine (3.45×10^{-3}mmol/kg), respectively in absence of AACE. (B2, C2 and D2): The group pretreated with arginine, L-NNA and atropine, respectively in presence of AACE. AACE: *A. atlantica* crude extract. Red arrow: surface epithelium damage and hemorrhagic necrosis penetrating deeply into gastric mucosa. Blue arrow: oedema of submucosa and inflammatory cell infiltration. Green arrow: congestion of blood vessels.

5.3.2.2. Macroscopic and histopathological examination of mechanismsof ASCE effect on ethanol-induced gastric mucosa damage in rats

a) Macroscopic examination

The macroscopic examination of ASCE effect in absence or presence of different pharmacological substances, on ethanol-induced gastric mucosa damage is shown in figure 48. Intragastric administration of arginine either alone (Figure 48 B1) or with ASCE (Figure 48 B2), ameliorated gastric lesions to minor or or absent injuries than that in vehicle as negative control (Figure 48 A1). It appeared similar to the ASCE effect (Figure 48 A2). Severe injuries are seen in the gastric mucosa with L-NNA either in the absence of ASCE (Figure 48 C1) or in its presence (Figure 48 C2), compared to negative control (Figure 48 A1). On another hand, the atropine provided a gastric protection (Figure 48 D1) against ulcer caused by ethanol (Figure 48 A1). Whereas, the pre-treatment with both atropine and ASCE provides the best protection (Figure 48 D2) than that in presence of atropine alone (Figure 48 D1). It was similar to theASCE effect (Figure 48 A2).

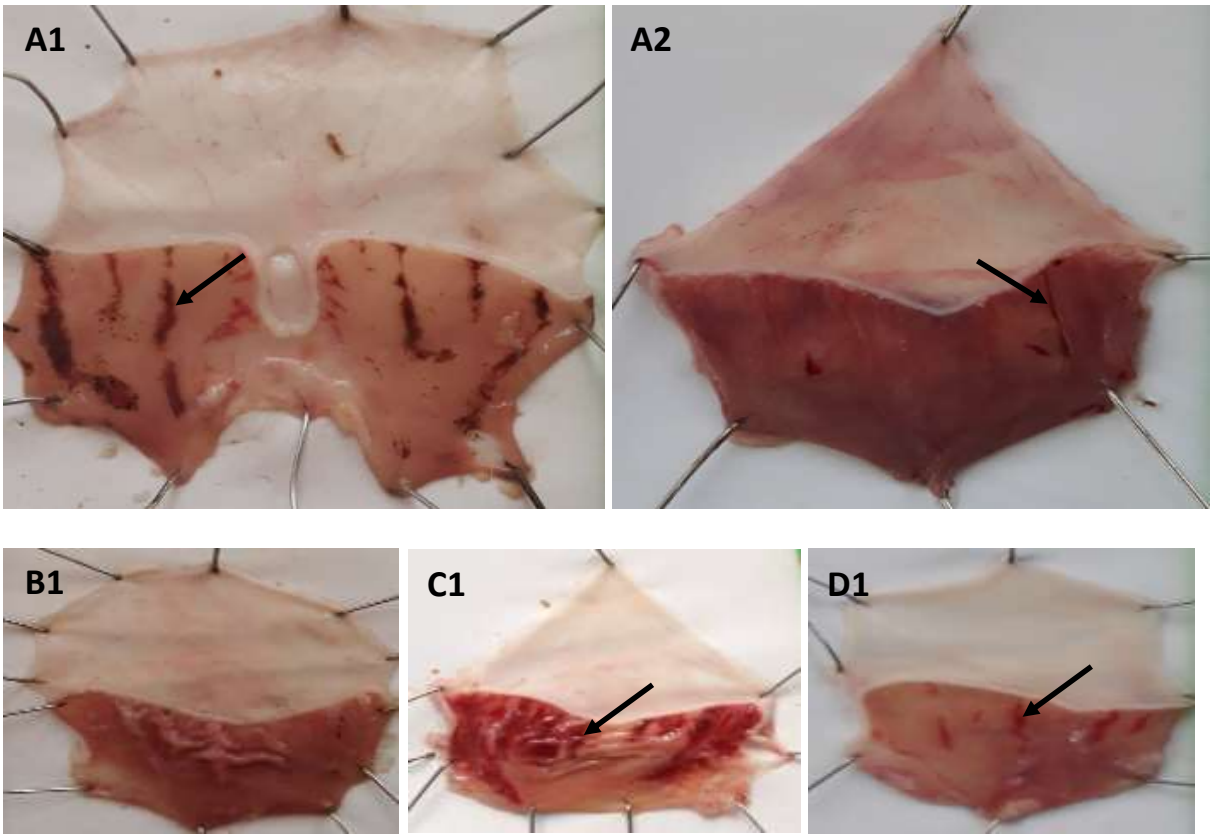

Figure 48 (continued)

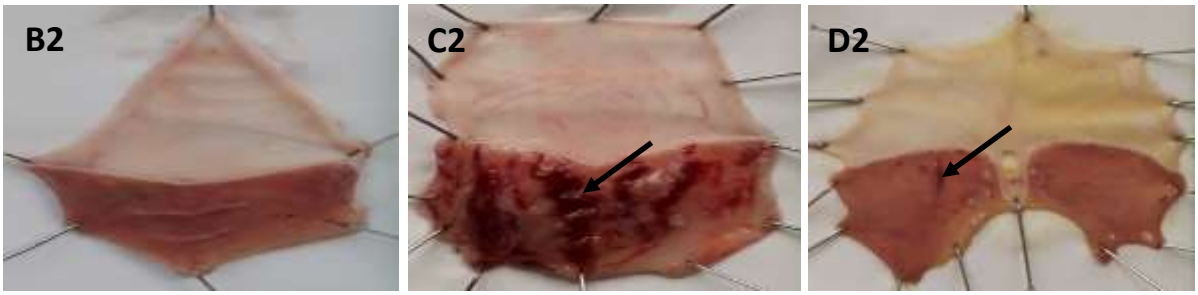

Figure 48: Effect of ASCE in absence or presence of different pharmacological substances on the macroscopic appearance of the gastric mucosa in ethanol-induced gastric mucosal lesions in rats.

(A1) The group pre-treated with CMC vehicle as negative control. (A2) The group pre-treated with ASCE (400mg/kg). (B1, C1 and D1): The group pretreated with arginine (1.72 mmol/kg), L-NNA (1.36×10^{-1}mmol/kg) or atropine (3.45×10^{-3}mmol/kg), respectively in absence of ASCE. (B2, C2, D2 and E2): The group pre-treated with arginine, L-NNA and atropine, respectively in presence of ASCE. ASCE: *A. sicula* L. crude extract. Black arrow: elongated bands of hemorrhagic lesions.

b) Histopathological examination

The histological analysis of ASCE effect in absence or presence of different pharmacological substances, on ethanol-induced gastric mucosa damage is shown in figure 49. The gastric mucosa of the rats pretreated with ASCE at highest dose 400 mg/kg (A2) showed improved histological appearance compared to vehicle as negative control rats (Figure 49 A1) which have necrosis of the gastric mucosa (red arrow) with oedema, leucocytes infiltration (blue arrow) and congestion of blood vessels (green arrow) of submucosa. The animals which received arginine either in the absence of ASCE (Figure 49 B1) or in its presence (Figure 49 B2) were completely protected against the ethanol action, preserving all histological aspects when compared to control animal group (Figure 49 A1). It appeared similar to the ASCE effectin absence of arginine (Figure 49 A2). Pre-treatment with L-NNA either in the absence of ASCE (Figure 49 C1) or in its presence (Figure 49 C2) increased of oedema, leucocytes infiltration (blue arrow) and congestion of blood vessels (green arrow) compared to negative control (Figure 49 A1). Animals pretreated with atropine (Figure 49 D1) showed intact gastric submucosal epithelium, milder oedema and leucocytes infiltration (blue arrow) and moderate congestion of blood vessels (green arrow) of submucosa than that in negative control (Figure 49 A1), whereas, ASCE eliminated these effect completely (Figure 49 D2). These effects were similar to the ASCE effect in absence of atropine (Figure 46 A2).

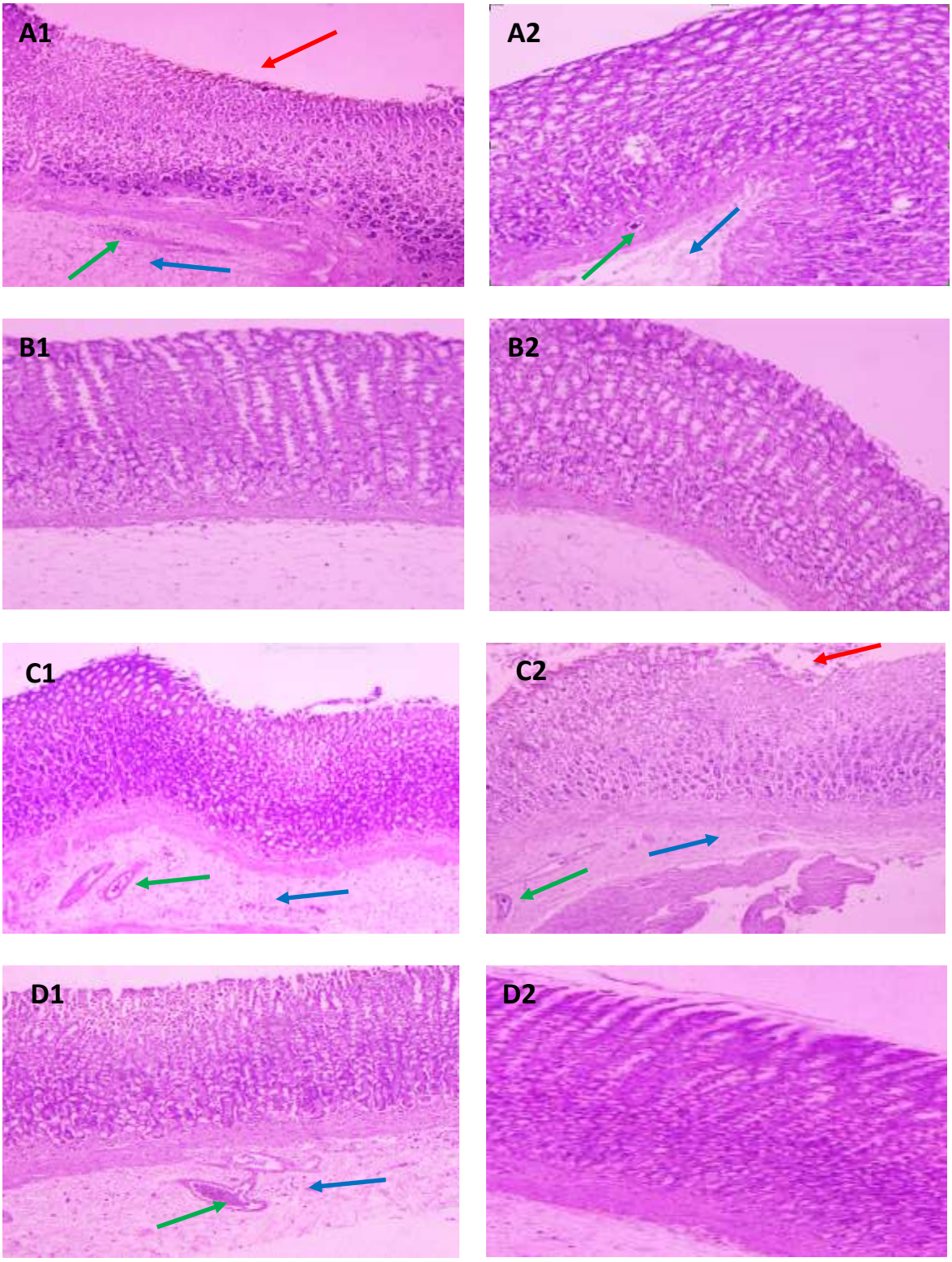

Figure 49: Histological examination for the protective effect of ASCE in absence or presence of different pharmacological substances, against ethanol-induced gastric damage in rat stomach tissue (magnification 100x).

(A1) The group pre-treated with CMC vehicle as negative control. (A2) The group pre-treated with AACE (400mg/kg). (B1, C1 and D1): The group pretreated with arginine, L-NNA, atropine, respectively in absence of

ASCE. (B2, C2 and D2): The group pre-treated with arginine (1.72 mmol/kg), L-NNA (1.36×10^{-1}mmol/kg) or atropine (3.45×10^{-3}mmol/kg), respectively in presence of ASCE. ASCE: *A. sicula* L. crude extract. Red arrow: surface epithelium damage and hemorrhagic necrosis penetrating deeply into gastric mucosa. Blue arrow: edema of submucosa and inflammatory cell infiltration. Green arrow: congestion of blood vessels.

5.3.3. Determination of mucus in gastric layer

The effects of AACE and ASCE on gastric mucus content are shown in figure 50. Compared to the vehicle (180±16 µg of alcian blue/g of wet tissue), AACE and ASCE extracts dose-dependently increased the gastric mucus content. All AACE doses (100, 200 and 400 mg/kg) had very highly significantly ulcer protective (453±39 µg of alcian blue/g of wet tissue, 510±50 µg of alcian blue/g of wet tissue, 570±31 µg of alcian blue/g of wet tissue, respectively; P≤0.0001). ASCE at 100, 200 and 400 mg/kg exhibited good gastric mucus content than to the vehicle (355±32 µg of alcian blue/g of wet tissue, 412±38 µg of alcian blue/g of wet tissueand 433±22 µg of alcian blue/g of wet tissue, respectively; P≤0.05 to P≤0.001). AACE (100, 200 and 400 mg/kg) showed no significant difference on gastric mucus content compared to ranitidine as positive control (522±41 µg of alcian blue/g of wet tissue; P>0.05) and compared to normal control (600±28 µg of alcian blue/ g of wet tissue; P>0.05). Also, at the highest doses (200 and 400 mg/kg), ASCE exhibited no significant difference on gastric mucus content compared to positive control (P≥0.05). The effects at tested doses were not significantly different (P>0.05) in comparison between AACE and ASCE.

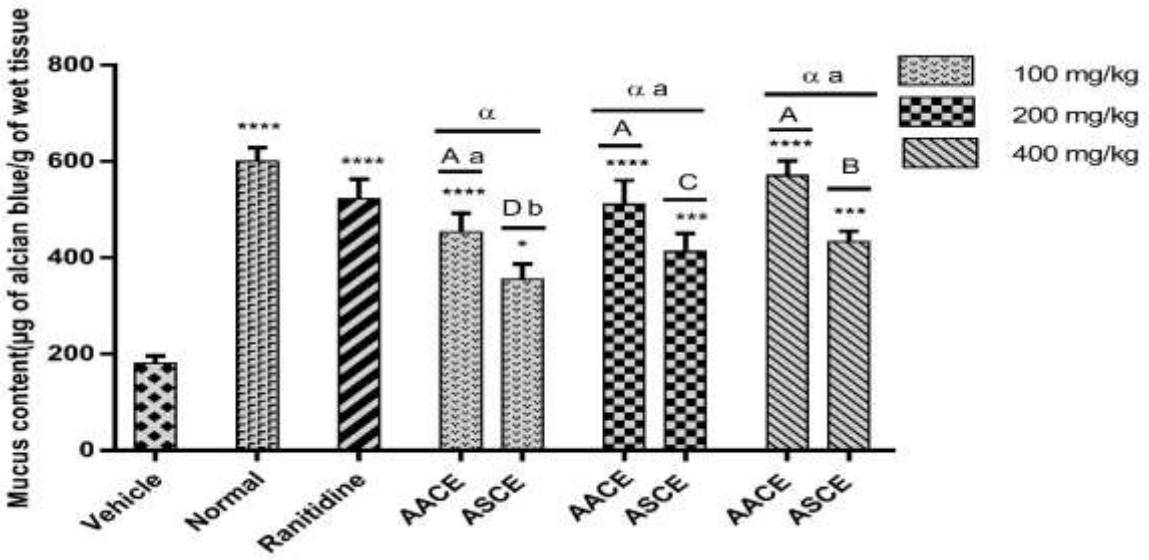

Figure 50: Effects of AACE and ASCE on gastric mucus content in ethanol-induced gastric mucosal lesions in rats.

AACE; *A. atlantica* crude extract, ASCE; *A. sicula* L. crude extract. Bars represent means±SEM (n=8).

*; P≤0.05, ***; P≤0.001 and ****; P≤0.0001 *vs*vehicle as negative control. [Ans]; no significant difference (P>0.05), [B]*; P≤0.05, [C]**; P≤0.01, [D]***; P≤0.001 *vs* normal control. [a]ns; no significant difference (P>0.05), [b]*; P≤0.05*vs* ranitidineas positive control. [α]ns; no significant difference (P>0.05) in comparison between the effects of AACE and ASCE at different doses (100, 200, 400 mg/kg).

5.3.3.1. Mechanisms of the AACE effects on mucus in gastric layer

The mechanisms of AACE effect on gastric mucus content is shown in figure 51. Animals treated with arginineshowed a highly significant increase of mucus content (452±24 and 517±23 µg of alcian blue/g of wet tissue, respectively) comparedto the vehicle (180±16 µg of alcian blue/g of wet tissue; P≤0.0001). There is no significant difference between the two treatment effects of AACE in the absence and in the presence of arginine (P>0.05). L-NNA had a significant effect decrease of mucus content (76±7 µg of alcian blue/g of wet tissue; P≤0.05) compared to the to the vehicle. The mucus content observedwithAACE aloneshowed a very significant affect in the presence of L-NNA (120±10 µg of alcian blue/g of wet tissue; P≤0.0001). Also, the atropinehad significantly increased the mucus contentto 376±17 µg of alcian blue/g of wet tissue (P≤0.0001). The treatment with both atropine and AACE had no significant influences onmucus content to 563±15 µg of alcian blue/g of wet tissue (P≤0.05) compared to the effect of AACE alone.

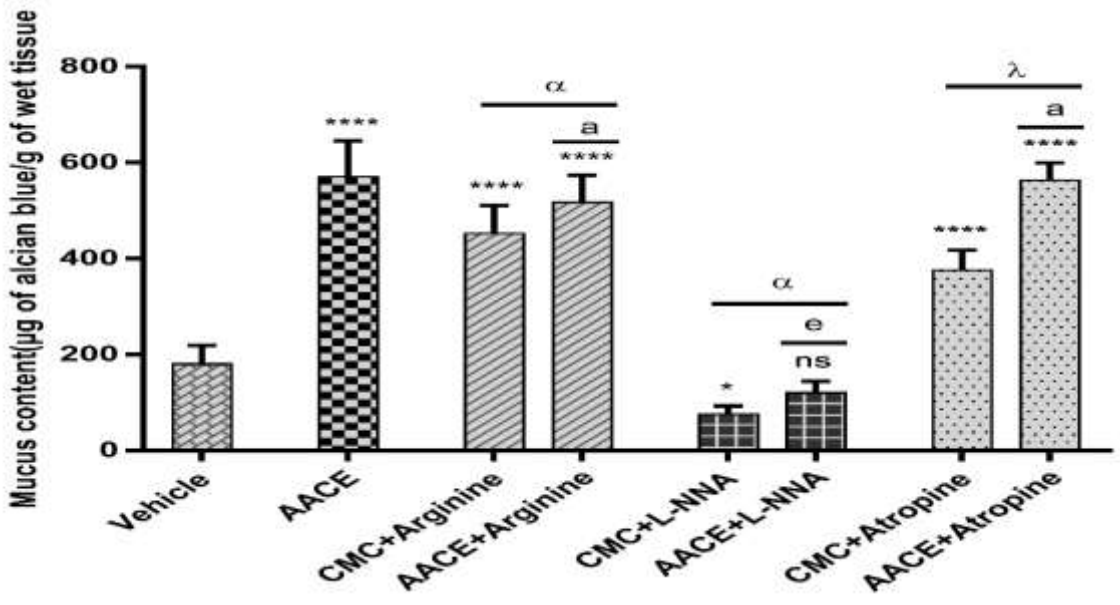

Figure 51: Effect of AACE in absence and in presence of arginine, L-NNA or atropine on gastric mucus content in ethanol-induced gastric mucosal lesions in rats

AACE; *A. atlantica* crude extract. Bars represent means±SEM (n=8). ns; no significant difference (P>0.05), *; P≤0.05, ****; P≤0.0001 *vs*vehicle as negative control. [a]ns; no significant difference (P>0.05), [e]****; P≤0.0001 in

comparison to both effects of AACE in absence and in presence of arginine (1.72 mmol/kg), L-NNA (1.36×10^{-1}mmol/kg) or atropine (3.45×10^{-3}mmol/kg). $^{\alpha}$ns; no significant difference (P>0.05), $^{\lambda}$****; P≤0.0001 in comparison betweenboth effects of arginine, L-NNA or atropine in the absence and in the presence of AACE (400mg/kg).

5.3.3.2. Mechanisms of the ASCE effect on mucus in gastric layer

Figure 52 shows the mechanism of ASCE effect on gastric mucus content. The intragastric administration of both ASCE and arginine showed no significant effect compared to the effect of treatment with AACE alone (489±29 µg of alcian blue/g of wet tissue; P>0.05). A high significant difference between the mucus content in the presence of ASCE either alone or with L-NNA extract was observed (89±8µg of alcian blue/g of wet tissue; P≤0.0001). No significant difference was recorded in a comparison between the ASCE effects in the absence and in the presence of atropine (463±4 µg of alcian blue/g of wet tissue; P>0.05).

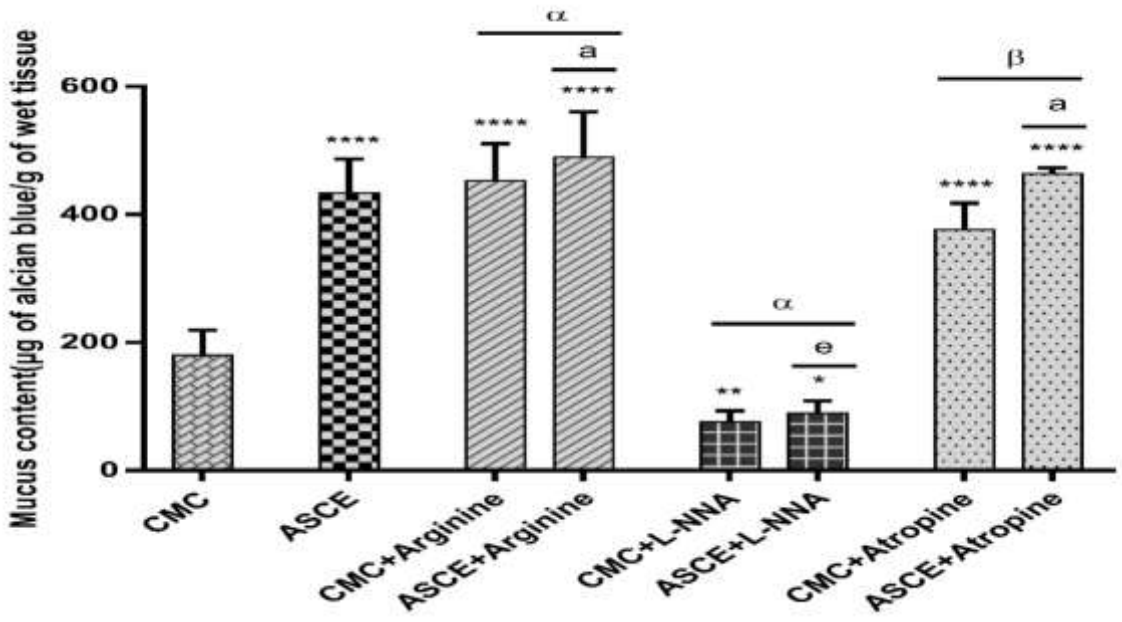

Figure 52: Effect of ASCE in absence and in presence of arginine, L-NNA or atropine on gastric mucus content in ethanol-induced gastric mucosal lesions in rats.

ASCE ; *A. sicula* L. crude extract. Bars represent means±SEM (n=8). *; P≤0.05, **; P≤0.01, ****; P≤0.0001 *vs*vehicle as negative control. ^{a}ns; no significant difference (P>0.05), ^{e}****; P≤0.0001 in comparison to both effects of ASCE in absence and in presence of arginine (1.72 mmol/kg), L-NNA (1.36×10^{-1}mmol/kg) or atropine (3.45×10^{-3}mmol/kg). $^{\alpha}$ns; no significant difference (P>0.05), $^{\beta*}$P≤0.05in comparison betweenboth effects of arginine, L-NNA or atropine in the absence and in the presence of ASCE (400mg/kg).

5.3.4. Evaluation of *in vivo*antioxidant activity of gastric homogenate

5.3.4.1. Estimation of gastric total proteins content

Treatment with AACE (200 and 400 mg/kg) and ASCE (400 mg/kg) resulted in an increase ($P \leq 0.05$; $P \leq 0.0001$ and $P \leq 0.01$ respectively) of total gastric protein level. Whereas, AACE (100 mg/kg) and ASCE (100 and 200 mg/kg) did not show any effect compared to the vehicle ($P > 0.05$). AACE and ASCE at all doses had no significant effect compared to normal control ($P > 0.05$). Also, all doses from AACE and ASCE showed no significant difference in gastric proteins contents compared to ranitidine ($P > 0.05$). All doses showed comparable effects ($P > 0.05$) in comparison between AACE and ASCE (Table11).

a. Mechanisms of the AACE effect on gastric proteins content

Table 12 showed that the intragastric administration of arginine leads to an increase in gastric proteins contents (0.43±0.014 mg/ml; $P \leq 0.0001$) compared to that observed in the vehicle (0.21±0.010 mg/ml). The AACE in the presence of arginine extract showed a significant increase in protein content (0.49±0.02 mg/ml; $P \leq 0.05$) compared to AACE alone. On other hand, L-NNA reduced protein content to 0.14±0.011 mg/ml ($P \leq 0.05$). Whereas, AACE in the presence of L-NNA, had high significant changes of protein content compared to that using AACE alone (0.17±0.02 mg/ml; $P > 0.05$) was observed. The atropine increased the gastric proteins contents to 0.38±0.017 mg/ml ($P \leq 0.0001$) compared to the vehicle, and there was nosignificant difference between both effects of AACE in the presence and in the absence of atropine ($P > 0.05$).

b. Mechanisms of the ASCE effect on gastric protein content

The mechanism of ASCE effect on gastric protein content is shown in table13. No significant difference between two ASCE effects in absence and in presence of argininewas recorded (0.40±0.02 mg/ml; $P > 0.05$). The comparison between the protein content in the presence of ASCE alone and in the presence of ASCE with L-NNAshowed a high significant difference (0.19±0.01 mg/ml; $P \leq 0.0001$). No significant difference between both effects of ASCE in absence and in presence of atropine (0.45±0.01 mg/ml; $P > 0.05$).

5.3.4.2. Estimation of catalase activity

Treatment of rats with the AACE (200 and 400 mg/kg) and ASCE (400 mg/kg) exhibited good effects on CAT activity. AACE at 200 and 400 mg/kg doses increasedthe CAT activity ($P \leq 0.01$ and $P \leq 0.0001$, respectively) compared to the vehicle. Whereas, ASCE only at highest

dose (400mg/kg) showed a significant effect on CAT activity (P≤0.01) compared to the to the vehicle. ASCE (100 and 200 mg/kg) and AACE (100 mg/kg) showed no significant difference in effects compared to the vehicle (P>0.05). At these doses, the value of the CAT activity of AACE and ASCE was between 1.86±0.06 and 2.46±0.08 nmol/min/mg, compared to the negative control (1.32±0.08 nmol/min/mg). All AACE doses exhibited no significant effect compared to the positive control (ranitidine) on one hand (P>0.05), and compared to normal control on other hand (P>0.05). All ASCE doses showed no significant difference (P≥0.05) compared to the ranitidine. ASCE effect (200 and 400 mg/kg) exhibited no significant difference comparedto thenormal control (P>0.05). All doses comparisons were not significantly different (P>0.05) between AACE and ASCE effects (Table 11).

a. Mechanisms of the AACE effect on catalase activity

The mechanism of AACE effects on CAT activity is shown in table 12. The administration of arginine increased the CAT activity (2.41±0.11 µmole/min/mg; P≤0.0001) compared to the vehicle (1.32±0.08 nmole/min/mg). The AACE in the presence of arginine showed no significant changes of CAT activity (2.43±0.09 µmole/min/mg; P>0.05), compared to AACE alone. L-NNA had highly decreasedthe CAT activity to 0.71±0.06 nmole/min/mg (P≤0.001) compared to the vehicle. However, the effect of AACE in the presence of L-NNA had significantly decreased this enzymatic activity to 1.16±0.08 nmole/min/mg (P≤0.0001) compared to the effect ofAACE alone. Also, the atropine significantly increased the CAT activity (2.41±0.09 nmole/min/mg; P≤0.0001) than vehicle, and the effect of AACE plus atropine hadsignificant different effect (2.94±0.12 nmole/min/mg; P≤0.05) compared to the effect of AACE in absence of atropine.

b. Mechanisms of the ASCE effect on catalase activity

Table13 shows the mechanism of ASCE effect on CAT activity. The intragastric administration of ASCE plus arginine showed no significant effect (2.43±0.17 nmole/min/mg; P>0.05) compared to the effect of treatment with ASCE alone. A high significant difference was observed between the CAT activity in the presence of ASCE either alone or with L-NNA (1.11±0.05 nmole/min/mg; P≤0.0001). Also, the ASCE effects werenot significantly different in the absence and/or in the presence of atropine (2.45±0.01 µmole/min/mg; P>0.05).

5.3.4.3. Assessment of reduced glutathione

Table11 showed that, AACE and ASCE extracts (400 mg/kg) increased the GSH levels to 6.89±0.24 nmol/g tissue ($P \leq 0.0001$) and 4.58±0.29 nmol/g tissue ($P \leq 0.05$), respectively, when compared to the vehicle (2.93±0.19 nmol/g tissue). All AACE and ASCE doses showed a similar GSH level compared toranitidine as positive control ($P > 0.05$). AACE (400 mg/kg) had a best effect than ASCE (400 mg/kg) with very highly significant difference ($P \leq 0.001$). Whereas, at lowest doses (100 and 200 mg/kg), AACE and ASCE showed a similar effect ($P > 0.05$).

a. Mechanisms of the AACE effect on reduced glutathione

The table12 showed that the intragastric administration of arginine leads to an increase of GSH level (4.85±0.17 nmol/g tissue; $P \leq 0.0001$) compared to the vehicle (2.93±0.19 nmol/g tissue). Whereas, the AACE in the presence of arginine showed a significant change in GSH level (6.32±0.17 nmol/g tissue; $P \leq 0.05$) compared to AACE effect in absence of arginine. On other hand, L-NNA reducedthe GSH level (1.72±0.13 nmol/g tissue; $P \leq 0.001$) compared to the vehicle. Whereas, AACE in the presence of L-NNAhad a high significant influences on GSH level compared to that using AACE alone (1.98±0.06 nmol/g tissue; $P \leq 0.0001$). The atropine increased the GSH levelto 4.03±0.16 nmol/g tissue ($P \leq 0.001$), compared to the vehicle. No significant difference (5.04±0.22 nmol/g tissue; $P > 0.05$) between both effects of AACE in presence and in absence of atropine was observed.

b. Mechanisms of the ASCE effect on reduced glutathione

The mechanisms of ASCE effect on gastric GSH level are shown in table13. A significant difference between the two ASCE effects in the absence and in presence of arginine (5.51±0.21 nmol/g tissue; $P \leq 0.05$). A high significant difference recorded between the GSH levels in the presence of ASCE alone and in the presence of ASCE with L-NNA (1.32±0.07 nmol/g tissue; $P \geq 0.0001$). On other hand, the ASCE in the presence of atropinehad no significant influences on GSH level compared in its absence (5.01±0.24 nmol/g tissue; $P \leq 0.05$).

5.3.4.4. Determination of superoxide dismutase activity

Oral treatment with AACE and ASCE (200 and 400 mg/kg) extracts inhibited the pyrogallol autoxidation (49.79±5.32% to 60.17±3.01%), when compared to the vehicle (28.18±2.13%, $P \leq 0.05$ to $P \leq 0.0001$). The inhibition capacity of AACE (100, 200 and 400 mg/kg) and ASCE (200 and 400 mg/kg) were not significantly different compared to ranitidine

as positive control (65.47±6.63%; P>0.05). At the highest dose (400 mg/kg), the inhibition capacity of AACE was not significantly different compared to normal control (P>0.05). AACE and ASCE at all doses showed similar SOD activity (P>0.05) (Table11).

a. Mechanisms of the AACE effect on superoxide dismutase

The mechanism of AACE effects on pyrogallol autoxidation inhibition is shown in Table12. The administration of arginine inhibited the pyrogallol autoxidation with 48.18±1.45%; (P≤0.0001) compared to the vehicle (28.18±2.13%). The AACE in the presence of arginine showed no significant influence in this inhibition capacity (57.14±2.19%; P>0.05), compared to the AACE alone effect. L-NNA decreased the pyrogallol autoxidation inhibitionto13.01±0.62%; (P≤0.0001) compared to the vehicle. However, the effect of AACE in the presence of L-NNA showed a high significant increase (22.14±1.42%; P≤0.0001) compared to the effect by the treatment with AACE alone. Also, the atropine increased the SOD activity and thus increase the pyrogallol autoxidation inhibition (51.17±1.67%; P≤0.0001), and the treatment with both AACE and atropine had nosignificant difference effect (61.31±2.31%; P>0.05) compared to the effect of AACE in absence of atropine.

b. Mechanisms of the ASCE effect on superoxide dismutase activity

Table13 shows the mechanism ofASCE effect on pyrogallol autoxidation inhibition, hence on SOD activity. The intragastric administration of both ASCE and arginine showed no significant effect (58.12±1.32%; P>0.05) compared to the effect of treatment with ASCE alone. A high significant difference was observed between the SOD activity in the presence of AACE either alone or with L-NNA extract (19.97±1.03%; P≤0.0001). The ASCE effects were significantly different in the absenceand inthe presence of atropine (61.18±2.13%; P≤0.05).

5.3.4.5. Lipid peroxidation estimation

Lipid peroxidation (LPO) and hence MDA gastric content was markedly elevated following ethanol administration in normal rats (Table11). AACE and ASCE dose-dependently attenuated the damage induced by ethanol (P≤0.01to P≤0.0001). At tested doses, AACE and ASCE reduce the amount of MDA to values (26.13±2.29 to 22.05±1.18 nmol/g tissue) which were lower than that in vehicle (37.18±1.14 nmol/g tissue). The AACE and ASCE at all doses exhibited no significant difference on lipid peroxidation inhibition as positive control, (20.08±3.33 nmol/g tissue; P>0.05). There is no significant difference between AACE and ASCE effects at all doses (P>0.05).

a. Mechanisms of the AACE effect on lipid peroxidation

Table12 showed that the intragastric administration of arginine leads to a highly significant decrease of LPO, and thus a decrease in MDA gastric content (22.97±1.36 nmol/g tissue; $P \leq 0.0001$) than that observed in the vehicle (37.18±1.14 nmol/g tissue). Whereas, the AACE in the presence of arginine showed no significant change in MDA gastric content (19.62±1.23 nmol/g tissue; $P > 0.05$) compared to the AACE effect in absence of the arginine. On other hand, L-NNA significantly increased the MDA gastric content (43.18±1.24 nmol/g tissue; $P \leq 0.05$) than the vehicle. Whereas, AACE in the presence of L-NNA significantly increasedthe MDA gastric content compared to that using AACE alone (37.36±1.20 nmol/g tissue; $P \leq 0.0001$). The atropine decreased the MDA gastric content (24.13±1.26 nmol/g tissue; $P \leq 0.001$) compared to the vehicle, and AACE with atropine no influences on MDA gastric content compared to group treated with AACE alone (17.62±1.05 nmol/g tissue; $P > 0.05$).

b. Mechanisms of the ASCE effect on lipid peroxidation

The mechanism effect of ASCE on LPO, and thus on MDA gastric content is shown in Table13. No significant difference between the two ASCE effects in absence and in presence of arginine (21.07±1.54 nmol/g tissue; $P > 0.05$). There is significant difference between the LPO in the presence of ASCE alone and in the presence of ASCE with L-NNA (46.52±2.29 nmol/g tissue; $P \leq 0.0001$). A significant difference between both effects of ASCE in absence and in presence of atropine (15.23±1.45 nmol/g tissue; $P \leq 0.01$).

Table 11: Effectsof AACE and ASCE on total protein level, CAT activity, GSH level, SOD activity and MDA level of stomach tissuein ethanol-induced gastric mucosal lesions in rats.

Antioxidant parameters	**Extracts**	**Extract doses**			**Normal**	**Rantidine**	**Vehicle**
		100mg/kg	**200mg/kg**	**400mg/kg**			
Total proteins (mg/ml)	AACE	$0.33\pm0.03^{ns\ A\ a\ \alpha}$	$0.36\pm0.03^{*\ A\ a\ \alpha}$	$0.42\pm0.01^{***\ A\ a\ \alpha}$	$0.46\pm0.05^{****}$	$0.38\pm0.04^{*}$	0.21 ± 0.01
	ASCE	$0.32\pm0.04^{ns\ A\ a}$	$0.33\pm0.04^{ns\ A\ a}$	$0.4\pm0.01^{**\ A\ a}$			
Catalase activity (nmol/min/mg)	AACE	$1.97\pm0.08^{ns\ A\ a\ \alpha}$	$2.29\pm0.23^{**\ A\ a\ \alpha}$	$2.46\pm0.08^{****\ A\ a\ \alpha}$	$2.62\pm0.23^{****}$	$2.41\pm0.19^{***}$	1.32 ± 0.08
	ASCE	$1.86\pm0.06^{ns\ B\ a\ \alpha}$	$1.95\pm0.16^{ns\ A\ a\ \alpha}$	$2.17\pm0.11^{**\ A\ a\ \alpha}$			
GSH level (nmol/gtissue)	AACE	$4.29\pm0.23^{ns\ E\ a\ \alpha}$	$4.52\pm0.44^{ns\ E\ a\ \alpha}$	$6.89\pm0.24^{****\ C\ a\ \gamma}$	$9.01\pm0.41^{****}$	$5.82\pm0.49^{****}$	2.93 ± 0.19
	ASCE	$4.21\pm0.37^{ns\ E\ a\ \alpha}$	$4.32\pm0.39^{ns\ E\ a\ \alpha}$	$4.58\pm0.29^{*\ E\ a\ \gamma}$			
SOD activity (**%**)	AACE	$47.14\pm5.26^{ns\ C\ a\ \alpha}$	$51.11\pm5.69^{*\ B\ a\ \alpha}$	$60.17\pm3.01^{***\ A\ a\ \alpha}$	$76.12\pm5.23^{****}$	$65.47\pm6.63^{****}$	28.18 ± 2.13
	ASCE	$39.79\pm4.32^{ns\ E\ b\ \alpha}$	$49.79\pm5.32^{*\ C\ a\ \alpha}$	$53.19\pm2.14^{*\ B\ a\ \alpha}$			
Gastric MDA level (nmol/g tissue)	AACE	$24.79\pm2.31^{**\ E\ a\ \alpha}$	$23.19\pm1.98^{***\ E\ a\ \alpha}$	$22.05\pm1.18^{****\ E\ a\ \alpha}$	$4.23\pm0.52^{****}$	$20.08\pm3.33^{****}$	37.18 ± 1.14
	ASCE	$26.13\pm2.29^{**\ E\ a\ \alpha}$	$24.95\pm1.98^{**\ E\ a\ \alpha}$	$22.87\pm0.97^{***\ E\ a\ \alpha}$			

AACE; *A. atlantica* crude extract, ASCE; *A. sicula* L. crude extract. Bars represent means±SEM (n=8). ^{ns}; no significant difference (P>0.05), *; P≤0.05, **; P≤0.01, ***; P≤0.001, ****; P≤0.0001 *vs*vehicle as negative control. ^{Ans}; no significant difference (P>0.05), ^{B}; P≤0.05, ^{C}; P≤0.01, ^{E}; P≤0.0001 *vs* normal control.^{a}ns; no significant difference (P>0.05),^{b}; P≤0.05*vs*ranitidineas positive control. $^{\alpha}$ns; no significant difference (P>0.05),$^{\gamma}$; P≤0.001in comparison between the effects of AACE and ASCE at different doses (100, 200, 400 mg/kg) .

Table 12: Effect of AACE in absence and in presence of different pharmacological substances on total protein level, CAT activity, GSH level, SOD activity and MDA level of stomach tissuein ethanol-induced gastric mucosal lesions in rats.

Antioxidant parameters	**Extract/ Pharmacological substances**	**Arginine**	**L-NNA**	**Atropine**	**AACE**	**Vehicle**
Total proteins (mg/ml)	Vehicle	0.43±0.014 ****	0.14±0.011 *	0.38±0.017 ****	0.42±0.01 ****	0.21±0.01
	AACE	0.49±0.016 **** b α	0.17±0.017 ns e α	0.45±0.012 **** a β		
Catalase activity (nmol/min/mg)	Vehicle	2.46±0.11 ****	0.71±0.06 ***	2.41±0.09****	2.46±0.08 ****	1.32±0.08
	AACE	2.43±0.09 **** a α	1.16±0.08 ns e β	2.94±0.12**** b δ		
GSH level (nmol/gtissue)	Vehicle	4.85±0.17****	1.72±0.13***	4.03±0.16***	6.89±0.24 ****	2.93±0.19
	AACE	6.02±0.16**** b γ	1.98±0.06** e α	6.32±0.17**** a λ		
SOD activity (%)	Vehicle	48.18±1.45 ****	13.00±0.62 ****	51.17±12.49 ****	60.17±3.01 ****	28.18±2.13
	AACE	57.14±2.19 **** a β	22.14±1.42 ns e β	61.31±2.31 **** a β		
MDA level (nmol/g tissue)	Vehicle	22.97±1.36****	43.18±1.24*	24.13±1.26 ****	22.05±1.18 ****	37.18±1.14
	AACE	19.62±1.23**** a α	37.36±1.20ns e β	17.62 ± 1.05 **** a β		

AACE; *A. atlantica* crude extract. Bars represent means±SEM (n=8). ns; no significant difference (P>0.05), *; P≤0.05, **; P≤0.01, ***; P≤0.001, ****; P≤0.0001 *vs*vehicle as negative control.[a]ns; no significant difference (P>0.05), [b]; P≤0.05, [e]; P≤0.0001 in comparison between the effects of the AACE in absence and in presence of arginine, L-NNA or atropine. [α]ns; no significant difference (P>0.05),[β]; P≤0.05, [δ];P≤0.01, [γ]; P≤0.001,[λ]; P≤0.0001in comparison between the effects of the arginine (1.72 mmol/kg), L-NNA (1.36×10^{-1}mmol/kg) or atropine (3.45×10^{-3}mmol/kg) in absence and in presence of AACE (400mg/kg).

Table 13: Effect of ASCE in absence and in presence of different pharmacological substances on total protein level, CAT activity, GSH level, SOD activity and MDA level of stomach tissuein ethanol-induced gastric mucosal lesions in rats.

Antioxidant parameters	**Extract/ Pharmacological substances**	**Arginine**	**L-NNA**	**Atropine**	**ASCE**	**Vehicle**
Total proteins (mg/ml)	Vehicle	0.43±0.014 ****	0.14±0.011 *	0.38±0.017 ****	0.40±0.01 ****	0.21±0.01
	ASCE	0.40±0.016 **** a α	0.19±0.017 **** e α	0.45±0.014 **** a β		
Catalase activity (nmol/min/mg)	Vehicle	2.41±0.11 ****	0.71±0.06 **	2.41±0.09****	2.17±0.11 ****	1.32±0.08
	ASCE	2.43±0.17**** a α	1.11±0.05 ns e α	2.45±0.01**** a α		
GSH level (nmol/gtissue)	Vehicle	4.85±0.17****	1.72±0.13**	4.03±0.16**	4.58±0.29 ****	2.93±0.19
	ASCE	5.51±0.21**** b α	1.32±0.07****e α	5.01±0.24**** a β		
SOD activity (%)	Vehicle	48.18±1.45 ****	13.00±0.62 ****	51.17±1.67 ****	53.19±2.14 ****	28.18±2.13
	ASCE	58.12±1.32 **** a δ	19.97±1.03 **** e α	61.18±2.13 **** b δ		
MDA level (nmol/g tissue)	Vehicle	22.97±1.36****	43.18±1.24ns	24.13±1.26 ****	22.87±0.97 ****	37.18±1.14
	ASCE	21.07±1.54**** a α	46.52±2.29** e α	15.23 ± 1.45 **** b δ		

ASCE ; *A. sicula* L. crude extract. Bars represent means±SEM (n=8). ns; no significant difference (P>0.05), *; P≤0.05, **; P≤0.01, ****; P≤0.0001 *vs* vehicle as negative control.[a]ns; no significant difference (P>0.05), [b]; P≤0.05, [e]; P≤0.0001 in comparison between the effects of the ASCE in absence and in presence of arginine, L-NNA or atropine.[α]ns; no significant difference (P>0.05), [β]; P≤0.05, [δ]; P≤0.01in comparison between the effects of the arginine (1.72 mmol/kg), L-NNA (1.36×10^{-1}mmol/kg) or atropine (3.45×10^{-3}mmol/kg) in absence and in presence of ASCE (400mg/kg).

Discussion

1. Extraction yields

The results of the present study showed big differences between the yields of various *A. atlantica* and *A. sicula* L. extracts. The nature of the solvents differently affects the yields of the extracts, which is in line with study of Sharma and Cannoo, (2016). The difference in polarities of various compounds present in these extracts is responsible for the difference in yields (Hayouni et *al*., 2007).

2. Phytochemical screening

Determinations of the contents of phenolic and flavonoids compounds are important because, the antioxidant activity of various plant extracts is attributed to the presence of these compounds, particularly the flavonoids (Sousa et *al*., 2015**)**.

In this study, it was found that the *A. atlantica* and *A. sicula* L. hydromethanolic extracts had high phenolics content. We concluded that methanol (80%) was potent to extract phenolics. Moreover, it was reported that ethanol/water has been known as a good solvent for polyphenol extraction because of the combination of organic solvent and water that facilitates the extraction of all compounds that were soluble in both water and organic solvents (Do et *al*., 2014). These results agree with Benteldjoune et *al*., *(*2021), who demonstrated that the hydro-alcoholic extract of *A. atlantica* is a good source of phenolic and flavonoid compounds.

A. atlantica and *A. sicula* L. crude extracts and their sub-fractions contained different levels of total polyphenols, flavonoids, flavonols, tannins, chlorophylls, carotenoids, lycopene and β-carotene. These discrepancies may be attributed to the difference in the properties of these solvents. The results of the present study were in accordance with the findings of Tongpoothorn et *al*., (2012) who claimed that the recovery of phenolic compounds depended on the solvent used and its polarity.

A. atlantica and *A. sicula* L. ethyl acetate extracts had the highest amount of total polyphenols, flavonoids, flavonols and tannins, whereas, the highest amount of chlorophylls and carotenoids quantities were found in AAHE, ASHE, AAChE and ASChE. The high content of phenolic compounds in polar solvents was due to the fact that phenolics have high solubility in polar solvents (Lahmar et *al*., 2017). Bomser et *al*., (1996) found that hexane and chloroform solvents are useful to extract lipophilic compounds.

3. Phytochemical analysis

3.1. UHPLC-MS analysis

Based on literature survey, phytochemicals in the *A. atlantica* and *A. sicula* L. extracts were performed in negative ionization mode since it is more sensitive and selective than positive mode (Fabre et *al*., 2001). The compounds characterized from *A. atlantica* and *A. sicula* L. extracts were according to their retention time (RT) and MS data, available in the literature (Liu et *al*., 2017; Faustinoa et *al*., 2018; Kheyar-Kraouche et *al*., 2018; Rahmouni et *al*., 2018; Boukhalkhal et *al*., 2020; Simões et *al*., 2020). We found two isomers of caffeoylquinic acid in each of the AACE, AAEAE, AABE and AAWE extracts. In addition to the presence of an isomer of dicaffeoylquinic acid in AAEAE. On the contrary, four isomers of caffeoylquinic acid and seven isomers of dicaffeoylquinic acid were previously reported in *A. atlantica* hydromethanolic extract (Benteldjoune et *al*., 2021). Six isomers of caffeoylquinic acid were previously reported in *Daucus muricatus* (Noui et *al*., 2017), which also belong to the family Apiaceae.The main component of the AAWE was kaempferol. In fact, Sharififar et *al*., (2010) also found that *Bunium persicum* (Boiss) which also belongs to the family Apiaceae containsthe kaempferol. There is strong variability of the *A. atlantica*hydromethanolic extract composition compared to those found by Benteldjoune et *al*., (2021). These differences in phytochemical content are likely due to various solvents (degrees of polarity) utilized, extraction and quantification procedures, harvest period, and geographic region (Hayouni et *al*., 2007; Ben Farhat et *al*., 2015). The main constituents of the ASCE or one of its subfractions; ASCE, ASBE, ASEAE, and ASBE were one or several of these compounds; malonyl-3,5-dicaffeoylquinic acid I, astragalin formic acid adduct, astragalin and then cynarin. This is in accordance with another study on Apiaceae species (*Dorema glabrum*) where the cynarin and the astragalin were among of the main compounds (Delnavazi et *al*., 2015).

3.2. GC-MS analysis

Our resultsexhibited that carvacrol (39.24%), linoleic acid (14.09%), oleic acid (9-Octadecenoic acid (Z)) (10.85%), palmitic acid (10.02%) were identified as the major compounds in AAHE. Laouer et *al*., (2008) found that the main constituents of the *A. atlantica* essential oil was thymol (53.2%). On the contrary, we found 3.78% of thymol in AAHE. Sukatta et *al*., (2009) showed that hydro-distillation gave higher oil yields than hexane extraction. The higher yield from hydrodistillation than solvent extraction suggested that conventional solvent extraction is likely to involve losses of more volatile compounds during the removal of the

solvent (Presti et *al*., 2005). The main constituents of the ASHE in present study were carvacrol (15.15%), linoleic acid (11.06%), apiole (10.41%), linolenic acid (10.12%), palmitic acid (8.03%). While the results found by Labed et *al*., (2012) showed that the major components of the essential oil of *A. sicula* L. were germacrene B (88.5%) and apiol (4.9%). This was due to the ability of hydrodistillation to extract only the volatile compounds, whereas solvent extraction of plant materials may produce oleoresin, which contains not only the volatile compounds but also waxesand pigments (Ibrahim, 1997), which have higher molecular weight (Gamarra et *al*., 2006) resulting in a smaller amount of volatile compounds (10.41% apiole) in specific concentration from the hexane extract than the hydrodistillation (4.9% apiole) at the same concentration. However, the chemical constituents obtained using hexane extraction (in this study) and hydrodistillation of *A. atlantica* (Laouer et *al*., 2008) and *A. sicula* L. (Labed et *al*., 2012) were examined by GC-MS and the results were rather similar in major composition but different in minor components. This agrees with the results found by Sukatta et *al*., (2009) with *Zingiber cassumunar* Roxb. These differences in phytochemical content are likely due to various solvents utilized, extraction and quantification procedures, harvest period, and geographic region (Hayouni et *al*., 2007).

4. *In vitro* pharmacological effects

4.1. Antioxidant activities

Testing the antioxidant activity by more than one method is reasonable since different methods measure different characteristics of antioxidants (Erkan et *al*., 2008). It is thus, the effects of the extracts cannot be wholly described with one single method. It is necessary to perform more than one type of antioxidant capacity measurement to get a full understanding of the various mechanisms of antioxidant action (Amira et *al*., 2012). It is important to use several tests to take in consideration the composition of extracts which act through different mechanisms like the prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, reductive capacity, radical scavenging and stop of hydrogen abstraction (Li et *al*., 2008). The antioxidant capacity of *A. atlantica* and *A. sicula* L. extracts was assessed using seven tests.

The extracts are able to neutralize the DPPH free radicals via hydrogen donating (Dao et *al*., 2020). The *A. atlantica* and*A. sicula* L. extracts exhibited strong DPPH scavenging effects. This is probably due to the presence of polyphenols. It is known that the phenolic compounds possess great potential to scavenge free radicals (Naoi et *al*., 2019). This is

confirmed by a good correlation between polyphenols, flavonoids, flavonols and tannins contents and antioxidant activity from *A. atlantica* and *A. sicula* L. extracts using DPPH assay.

The ABTS assay has the same mechanism reaction as DPPH method. Both are based on electron transfer (Huang et *al*., 2005). In our results, the ABTS scavenging power was higher than the DPPH radical scavenging capacity for the two Apiaceae extracts. The solubility of the stereo selectivity of the radicals of the extract in various systems affects the ability of the extract to react against different radicals (Gali and Bedjou, 2018). ABTS scavenging effect of *A. atlantica* and *A. sicula* L. extracts was also correlated with phenolic contents. The high radical-scavenging effects of polyphenols would exhibit antioxidative and other biologic effects (Cheng et *al*., 2017).

The reducing potential of the samples may provide a significant indication of the antioxidant capacity of the plants (Jung et *al*., 2008). The presence of antioxidants in the sample would result in the reduction of ferric ions (Fe^{3+}) to the ferrous ions (Fe^{2+}) (Zengin et *al*., 2011). In this assay, the reducing capacity of two plant extracts at a concentration of 4 mg/ml, reveals the strength of its activity. The reducing potential was considered as excellent at 5 mg/ml (Barros et *al*., 2007). The reducing power of tested extracts was strongly correlated with polyphenols, flavonoids, flavonols and tannins contents. Thus, it is believed that the phenolic contents are related to the reducing power. The degree of hydroxylation and extent of conjugation seemed to be the criteria ruling the reducing power of polyphenols (Pulido et *al*., 2000).

The cupric ion reducing antioxidant capacity assay is based on the reduction of Cu^{2+} to Cu^{+} by antioxidants (Mitic et *al*., 2016). The obtained results showed that the tested extracts had a good cupric ion (Cu^{2+}) reducing ability. This important reducing potential can be attributed to the natural antioxidants that can trap free electrons that cause oxidative reactions. Many studies showed the existence of natural antioxidants especially polyphenols in all parts of plants, which have strong antioxidant properties (Amira et *al*., 2012, Fernandes et *al*., 2015).

The phenanthroline method is built on the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} (Liu et *al*., 2011). Our result showed that *A. atlantica* and *A. sicula* L. extracts had great Fe^{3+} reducing proprieties *via* phenanthroline. A good correlation was found between phenolic contents and this antioxidant capacity. This antioxidant effect is probably due to the presence of polyphenols. The reduction of Fe^{3+} to Fe^{2+} is often utilized as a marker of phenolic antioxidant activity (Stanković et *al*., 2016; Mamache et *al*., 2020).

The antioxidant properties of the *A. atlantica* and*A. sicula* L. extracts were also evaluated based on their ability to chelate metals. The metal chelating capacity is important since it reduces the metal transition. The excessive accumulation of such elements leads to oxidative stress which is responsible for DNA damage, lipid peroxidation, protein modification and other effects (Jomova and Valko, 2011). The high metal chelating activities of *A. atlantica* and*A. sicula* L. extracts are probably due to the presence of compounds that reduce the concentration of the transition of metals. Phenolic compounds have been reported to be chelators of free metal ions (Loucif et *al*., 2020b). No correlation was found between the metal chelating capacity of *A. atlantica* and *A. sicula* L. extracts and their phenolic contents. This relatively high antioxidant activity for an extract with low phenolic content suggests that the type of phenolics may be more of a determinant for these activities than their amounts (Amira et *al*., 2012). On other hand, although phenolic compounds are the most well-known antioxidants in plants, there are also non-phenolic compounds in plants that may be responsible for this activity (Gonçalves et *al*., 2013).

β-Carotene bleaching test can be used to examine the antioxidant potential from plant extracts (Loucif et *al*., 2020a). The ability of *A. atlantica* and *A. sicula* L. extracts to inhibit lipid peroxidation was tested by the β-carotene bleaching test, which evaluates the capacity of antioxidants to retard β-carotene discoloration induced by the conjugated diene hydroperoxides arising from linoleic acid oxidative degradation. The presence of antioxidant compounds prevents this degradation process and reflects the ability to inhibit lipid peroxidation *in vitro* (Benchikh et *al*., 2018; Farahmandfar et *al*., 2018). The tested extracts were effective inhibitors of lipid peroxidation. This activity is most likely due to the presence of antioxidants in the plant extracts (Senguttuvan et *al*., 2014). The non-polar chloroform and hexane extracts were more active than the polar ethyl acetate, crude, butanol and water extracts. This may be attributed to the presence of the highest amounts of lipophilic compounds; chlorophylls, carotenoids, lycopene and β-carotene, in the hexane and chloroform fractions which contribute to the neutralization of free radicals. This test is taught to have high specificity for lipophilic compounds (Kanatt et *al*., 2007). Previous reports showed that chlorophyll and carotenoids had an important antioxidant activity (Kang et *al*., 2018; Moreira et *al*., 2018). Moreover, Arumugam et *al*., (2010) stated that the lipophilic antioxidant activity is generally correlated to the pigment content and in particular, to the carotenoids. In fact, the non-polar extracts exhibit strong antioxidant properties as they are located within the lipid-water interface, thus they can help to prevent the radical formation of β-carotene and lipid oxidation, while, polar extracts are

diluted in the decoction phase and are thus less effective in inhibiting lipid peroxidation (Frankel and Meyer, 2000).

The current study revealed that *A. atlantica* and *A. sicula* L. extracts exhibited different antioxidant activities using various methods. The differences in the antioxidant activities of plant extracts could be due to different qualitative and quantitative compositions of their phenolic constituents (Amira et *al*., 2012). In summary, this study showed that the results further support the view that some plants are promising sources of natural antioxidants (Fernandes et *al*., 2015).

4.2. Enzymatic inhibitory activities

Five enzymatic inhibition assays were used to evaluate the effectiveness of *A. atlantica* and *A. sicula* L. extracts against key enzymes involved in Alzheimer's disease, hyperpigmentation and diabetes mellitus.

Numerous phytochemical substances have demonstrated AChE and BChE inhibitory activities and thus are proposed as being beneficial in the management of Alzheimer's disease (Dastmalchi et *al*., 2007). The *A. atlantica* and*A. sicula* L. extracts exhibited an interesting inhibitory effect against both AChE and BChE. Polyphenol-rich fractions exhibited significant AChE and BChE inhibitory activities, suggesting their possible involvement in these effects (Gali and Bedjou, 2018). This is supported by a strong correlation between total polyphenols, flavonoids, flavonols and tannins content and AChE or BChE inhibitory activities of two plant extracts. A survey of medicinal plants and their extracts in order to evaluate their medicinal and therapeutic potential has revealed that there are many polyphenols that have the potential to act as AChE inhibitors (Roseiro et *al*., 2012).

Tyrosinase plays a major role in the synthesis of melanin, which is involved in determining the color of eyes, hair and mammalian skin providing protection against ultraviolet rays (Kim and Uyama 2005). Although melanin in human skin acts as a major defense mechanism against UV light from the sun, the production of abnormal pigmentation such as melasma, freckles, age spots, liver spots and other forms of melanin hyperpigmentation can be a serious aesthetic problem. The melanin biosynthesis can be inhibited by inhibiting tyrosinase activity (Adhikari et *al*., 2008). All the crude extracts and their fractions from *A. atlantica* and*A. sicula* L. exhibited a tyrosinase inhibitory activity. Many plant extracts are known as inhibitors of tyrosinase (Kamagaju et *al*., 2013). The phenolic compounds from a variety of sources have

been described as potent tyrosinase inhibitors. Flavonoids occupy a prominent role as natural tyrosinase inhibitors (Solimine et *al*., 2016; Panzella and Napolitano 2019). This corresponds to the close correlation between total polyphenol and flavonoidcontents and the inhibition of tyrosinase found in the present study. Moreover, the tyrosinase inhibitory activity correlated with most antioxidant effects of these extracts. It is probable that the same compounds are responsible for both tyrosinase inhibitory and antioxidant activities. This may agree with the findings of Masuda et *al*., (2005) and Kim et *al*., (2018).

Inhibition of enzymes that contribute to the hydrolysis of carbohydrates such as α-amylase and α-glucosidase, is one of the therapeutic methods for controlling hyperglycemia after food intake (Gong et *al*., 2020). Several studies have focused on the use of plant extracts to inhibit these enzymes as a treatment for diabetes (Yang et *al*., 2011; Luyen et *al*., 2013; Ben Sassi et *al*., 2018). The results of this study revealed that α-amylase and α-glucosidase inhibition activities of extracts were most pronounced in the polyphenol-rich extracts; chloroform, ethyl acetate and butanol, with a close correlation found between these enzymatic inhibition activities and total polyphenols, flavonoids, flavonols and tannins contents. α-Amylase and α-glucosidase inhibitory effects are linked with the presence of phenolics (Dey and Mitra, 2013; Yuan et *al*., 2014). In fact, polyphenol-rich extracts affect carbohydrate metabolism and glucose homeostasis by several different mechanisms. The most important of which are inhibiting carbohydrate digestion by inhibiting carbohydrate-hydrolyzing enzymes, thereby blunting post-prandial hyperglycemia (Cazarolli et *al*., 2008; Hanhineva et *al*., 2010). Inhibition of α-amylase and α-glucosidase enzymes using natural products; especially polyphenols are a novel oral policy to regulate carbohydrate metabolism and hyperglycemia (Rasouli et *al*., 2017).

5. *In vivo* biological activities

5.1. Acute oral toxicity

A. atlantica and *A. sicula* L. are important medicinal plants belonging to Apiaceae family. *A. atlantica* and *A. sicula* L. are used in traditional medicine, but to date, there is no documented evidence corroborating their safety. This study determined the toxicity profile in mice after acute oral administration of the AACE and ASCE. The effects of AACE and ASCE after acute oral administration in mice were observied. The findings of present study showed that there are no visible signs of toxicity (behavioral, neurological or physical changes) or mortality was observed at two test doses (2000 mg/kg and 5000 mg/kg) from *A. atlantica* and *A. sicula* L. hydromethanolic extracts within the 14 days of observation. It is concluded that

the minimum lethal dose of the plant extracts tested in this study is over 5000 mg/kg. Overall, the findings of this study indicate that *A. atlantica* and *A. sicula* L. are non-toxic. According to Loomis and Hayes classification (1996), A chemical substance with an LD_{50} within the range of 5000–15000 mg/kg is considered as practically non-toxic.

5.2. AACE and ASCE effects on gastric emptying and intestinal transit in mice and the possible mechanism (s) involved

The present study reveals that *A. atlantica* and *A. sicula* L. dose dependently decreased gastric emptying and intestinal transit. The control of gastric emptying and intestinal transit is a complex process and involves both neural and myogenic mechanisms that are governed by numerous neurotransmitters and mediators. The main excitatory transmitter is acetylcholine, whereas NO is the major inhibitory mediator (Waterman and Costa, 1994). The delaying effects of the extracts on gastric emptying may result from the relaxation of the stomach musculature and/or from the constriction of the pyloric sphincter, while the delay of intestinal transit may involve the inhibition of muscle contraction and/or consolidation of the inhibitory component of the intestinal muscle (Nwinyi et *al*., 2004). The inhibitory effect of AACE and ASCE extracts on GI motility could be attributed to the polyphenolic compounds ; mainly tannins and flavonoids as well as other phytochemicals contained in the extract. According to the literature, flavonoids and Tannins inhibit GI motility at both gastric and intestinal levels. Indeed, several studies have pointed out to the inhibitory effect on the motility of these compounds, whether as pure compounds or as major components of different plant extracts (Amira et *al*., 2008; Rajan et *al*., 2012; Santos et *al*., 2012; Janbaz et *al*., 2013; Yu et *al*., 2017).

Different pharmacological substances (L-NNA (1.36×10^{-1}mmol/kg), atropine (3.45×10^{-3}mmol/kg), or indomethacin (5.58×10^{-2}mmol/kg)) were used to investigate AACE and ASCE mechanisms of action on gastric emptying and intestinal transit.

NO is present in the ENS of the GI tract and acts as NANC transmitter (Sanders and Ward, 1992). The inhibition NO production by the pretreatment with NOS inhinitors, significantly slowed gastric emptying, which in agreement with De Winter, (2002), Amira et *al*., *(*2005) and Idrizaj et *al*., (2021). The retarding effect of AACE and ASCE on gastric emptying is facilitated by the blockade of NOS by L-NNA, thus excluding the involvement of the NO in the mechanism of AACE and ASCE inducing gastric emptying delay.

Atropine injection delayed gastric emptying, which is in line with (Yu et *al*., 2017). In this study, pretreatment with atropine reinforced the decrease in the rate of gastric emptying,

after the administration of ASCE, indicating that muscarinic receptors are not involved in the inhibitory action of ASCE in gastric emptying. By contrast, the inhibitory effect of AACE on gastric emptying is blocked after atropine treatment, which might suggest the involvement of muscarinic receptors in the inhibitory action of AACE on gastric emptying

The results of the present study showed that the pretreatment of mice with indomethacin decreased gastric emptying, which is in agreement with the findings of Stein et *al*., (1994); Corak et *al*., (1997) and Santos et *al*., (2007). The retarding effects of AACE and ASCE on gastric emptying were prevented *via* the blockade of cyclooxygenase by indomethacin, which indicates the involvement of the cyclooxygenase pathway in the mechanism by which the AACE and ASCE delays gastric emptying.

Different results in intestinal transit were obtained under the NOS blockade. Both intestinal transit in dogs (Chiba et *al*., 2002) and colon transit in rats (Mizuta et *al*., 1999) were significantly inhibited after the pretreatment with L-NNA. By contrast, the inhibition of NOS by L-NAME ((L-NG-nitro arginine methyl ester)) did not significantly alter intestinal transit in mice (De Winter et *al*., 2002). In this study, inhibition of NOS by L-NNA reduced intestinal transit in mice. However, administration of AACE or ASCE under inhibition of NOS by L-NNA did not significantly affect the rate of intestinal transit, which may imply that this effect depends on the NO pathway.

In the present experiments, the treatment of mice with atropine significantly decreases intestinal transit which may point to the importance of the cholinergic pathway in the motility of the intestine. This is in accordance with the results of Amira et *al*., (2005) and Yu et *al*., (2017) in mice and Drago et *al*., (2002) in rats. The current findings show that the intestinal transit was not significantly altered in the AACE-treated mice following muscarinic antagonism, whereas, under the same conditions, ASCE treatment consolidated the inhibitory effect of the plant extract, suggesting that the effect of the former plant may involve the the cholinergic pathway, but not the later plant.

Lichtenberger et *al*., (2015) showed that the spontaneous contractile activity in the proximal small intestine of the rat was unaffected by indomethacin treatment, while, indomethacin suppressed contractile activity and frequency in the distal part of the small intestine in the same animal. In these experiments, indomethacin reduced intestinal transit. However, intestinal transit inhibition of AACE or ASCE in mice pretreated with indomethacin

is prevented, which might indicate the participation of the cyclooxygenase pathway in these effects.

5.3. AACE and ASCE effects on ethanol-induced gastric ulceration in rats and possible mechanism (s) of action

Alcohol consumption can produce acute hemorrhagic gastric erosions, and excessive ingestion can result in gastritis characterized by mucosal oedema, sub-epithelial hemorrhages, cellular exfoliation, and inflammatory cell infiltration (Chen et *al*., 2015). Ethanol is well known as a potent necrotizing agent that destroys the defensive factors of the mucosa, leading to the depletion of gastric wall mucus (Wallace, 2001). It is also reported that acute exposure of the gastric mucosa of rats to ethanol can result in gastric lesions similar to those occurring in gastric ulcer in humans; hence, ethanol-induced gastric ulcers have been widely used for the evaluation of gastroprotective activity (Boligon et *al*., 2014).

In the present study, absolute ethanol administration induced severe hemorrhagic lesions. These finding were supported by the histopathological changes, where severe disruption to the surface epithelium, necrotic lesions penetrating deeply into mucosa, extensive oedema of the submucosal layer, inflammatory cells infiltration and and congestion of blood vessels occurred. Oral administration of AACE and ASCE effectively reversed the ethanol-induced gastric injury in a dose-dependent manner, with significant reduction of the gastric ulcer area compared to the negative control. In addition, AACE and ASCE ameliorated the aforementioned gastric histopathological changes induced by ethanol. The mono-caffeoylquinic acid, dicaffeoylquinic acids or caffeoylquinic acid derivatives, were the main constituents of the ASCE and AACE. Previous studies confirmed that dicaffeoylquinic acid was responsible for the effect of *Arctium lappa* (Carlotto et *al*., 2015) and *Ligularia stenocephala* extracts (Lee et *al*., 2010) against ethanol-induced gastric ulcer.

The present study demonstrated that absolute ethanol administration significantly decreased the mucus content in the rat stomach. However, this effect was markedly and dose-dependently increased in all AACE and ASCE treated groups. It is well known that mucus represents the first defensive line of the gastric mucosa that protects the stomach from necrotizing agents (Wallace, 2008). Based on this, it can be suggested that the gastroprotective effect of AACE and ASCE could be partly attributed to their ability to increase the gastric mucus content.

There is growing evidence to suggest that ethanol-induced gastric mucosal injury is closely related to the increased ROS level (Pan et *al*., 2008). Increased ROS provoke oxidative injury, cell death, and epithelial damage (Palle et *al*., 2018). Previous findings have revealed also that ROS augment gastric acid secretion by histamine release and reduces mucus production by decreasing prostaglandin synthesis (Jesus et *al*., 2013; Palle et *al*., 2018).

On the other hand, the body per se has enzymatic and non-enzymatic defenses, including GSH, SOD and CAT against ROS-induced lipid peroxidation (Mates et *al*., 1999). GSH and SOD are known to scavenge superoxide, hydrogen peroxide, hydroxyl and lipid peroxyl radicals, and thus attenuate the tissue damage. CAT as a preventive antioxidant triggers the rapid conversion of peroxyl radical into biologically safe substances, like water (Wong et *al*., 2013). MDA, an index of lipid peroxidation, can usually be quantified to identify lipid peroxidation (Pillai et *al*., 2005). Thus, to address the role of oxidative stress in our model of ulceration, we assessed several oxidant-antioxidant parameters in the gastric tissues of rats. The experimental results showed that ethanol markedly increased MDA level, an effect that was accompanied by a decrease in GSH, CAT and SOD activities, supporting in this way the role of oxidative stress in the pathogenesis of ethanol-induced gastric ulcer. On the contrary, the treatment with AACE and ASCE resulted in a significant increase in the activities of SOD and the levels of GSH, as well as a decrease in MDA formation, indicating their antioxidant activity. This may explain at least in part their gastroprotective effect. The last effect is usually attributed to the chemical constituents found in these plants. In fact, compounds like those ones found in AACE and ASCE (phenolics, tannins and flavonoids) have been reported to possess antioxidant actions and to protect the stomach from ulcerogeny (Zakaria et *al*., 2016, Shin et *al*., 2021).

To further clarify, other gastroprotective mechanism pathways of AACE and ASCE, several pharmacology substances (arginine (1.72 mmol/kg), L-NNA (1.36×10^{-1}mmol/kg) or atropine (3.45×10^{-3}mmol/kg)) were tested.

L-arginine serves as substrate for the synthesis of NO, which is involved in gastroprotection and ulcer healing (Szlachcic et *al*., 2013). In this study, the effects induced by absolute ethanol, including the rise in the levels of MDA and the reduction of SOD, CAT, GSH and total proteins with surface epithelium lesions and submucosal inflammation, were attenuated by concomitant treatment with L-arginine. In contrast, intraperitoneal administration of L-NNA, exacerbated the acute gastric mucosal lesions, reduces SOD, CAT, GSH and increases MDA levels. These findings were corroborated by Szlachcic et *al*., (2013) who found that the administration of non-selective NOS inhibitors, such as L-NNA, L-NAME and L-

NMMA (L-NG-monomethyl-L-arginine), decreased gastric blood flow and aggravated gastric mucosal injury. The anti-ulcer effects of AACE and ASCE are strongly inhibited after pretreatment with L-NNA in the rat, suggesting that the NO pathway is implicated in their mechanisms of protection.

Atropine pretreatment was effective in preventing absolute ethanol-induced gastric damage. This agrees with previous studies (Del Soldato et *al*., 1984; Cho and Ogle, 1991). The administration of AACE or ASCE after blockade of muscarinic receptors by atropine exhibited closer protective effects to that of AACE and ASCE in the absence of atropine, indicating that muscarinic receptors are involved in the protective action of AACE and ASCE against gastric ulcer, which is in agreement with Kolgazi et *al*., (2021).

The gastroprotective effects of *A. sicula* L. and *A. atlantica* seem to have the same pathways, which may be explained by the presence of several in common constituents, suchas, isomer (s) of caffeoylquinic acid, kaempferol, cynarin or astragalin and/or their derivatives. The gastroprotective effect of AACE was greater than ASCE, which may be attributed to the higher phenolic content of AACE compared to ASCE.

Conclusion and future prospects

The results obtained in the present study revealed that *A. atlantica* and *A. sicula* L. are a rich source of phenolic compounds. The level of these chemicals in various extracts from both plants was considerably higher in ethyl acetate extracts than that in other extracts, whereas hexane and chloroform extracts contained a higher amount of chlorophylls and carotenoids.

Three major in common compounds were detected in the AAHE and ASHE extracts; carvacrol, linoleic acid and palmitic acid. Isomers of caffeoylquinic acid and/or its derivative were the main in common constituents in the AACE and ASCE and/or their sub-fractions.

All extracts showed high *in vitro* antioxidant and anti-enzymatic (against key enzymes involved in Alzheimer's, hyperpigmentation and diabetes mellitus diseases) activities being higher in the AAEAE and ASEAE with most tests. A good correlation between the total phenolics content and the majority of the studied antioxidant and anti-enzymatic activities was noted, which may indicate that these activities are probably due to the presence of these compounds. Conversely, a low correlation between phenolic content and metal chelating and β carotene linoleic acid bleaching activities was observed, suggesting that polyphenols might not be the main ion chelators and β-carotene linoleic acid bleaching agents.

No toxic effects at 5000 mg/kg were observed in mice, confirming the safety these plants.

AACE and ASCE extracts decreased gastric emptying and intestinal transit in mice. This antimotility activity involved the cyclooxygenase pathway in ASCE-inducing gastric emptying delay, whereas both cholinergic and cyclooxygenase pathways are implicated in the gastric emptying delay of AACE.

Cholinergic, NO and cyclooxygenase pathways are involved in the intestinal transit delay caused by AACE, While, the cyclooxygenase and NO pathways seem to be responsible for the ASCE inducing intestinal transit delay.

Both AACE and ASCE extracts protected the rat stomach against ethanol-causing gastric ulcer. The protective effect may involve the gastric mucus content, the cholinergic and nitric oxide pathways and the reduction of gastric mucosal stress.

As perspectives, the key research subjects that can be considered in the future include among others:

• To deepen the evaluation of the toxicity of both plant constituents

• To deeply investigate the phytochemical constituents of the plants

• To widen the spectrum of research on the effects of these plants on some chronic diseses, such as, Alzheimer, diabetes, Gastrointestinal ulceration and motility dysfunctions, cancer …. etc

• To investigate the mechanisms of action of the constituents of these plants in these disorders

• To exploit the beneficial effects of both plant constituents in food and pharmaceutical industries.

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Appendices

Appendice 01. Comparative study on percentage of extraction yields obtained from the AACE and ASCE and their sub-fractions.

Extracts		**AACE**	**ASCE**	**AAHE**	**ASHE**	**AAChE**	**ASChE**	**AAEAE**	**ASEAE**	**AABE**	**ASBE**	**AAWE**	**ASWE**
	Extraction yields %	22.1 ±0.6	26.97 ±0.9	0.96 ±0.02	1.77 ±0.07	0.35 ±0.01	0.4 ±0.05	1.49 ±0.2	2.34 ±0.18	2.93 ±0.03	4.1 ±0.04	11.49 ±0.4	17.71 ±0.6
AACE	22.1 ±0.6	-	****	****	****	****	****	****	****	****	****	****	****
ASCE	26.97 ±0.9		-	****	****	****	****	****	****	****	****	****	****
AAHE	0.96 ±0.02			-	ns	ns	ns	ns	**	****	****	****	****
ASHE	1.77 ±0.07				-	**	**	ns	ns	*	****	****	****
AAChE	0.35 ±0.01					-	ns	*	****	****	****	****	****
ASChE	0.4 ±0.05						-	ns	***	****	****	****	****
AAEAE	1.49 ±0.2							-	ns	**	****	****	****
ASEAE	2.34 ±0.18								-	ns	***	****	****
AABE	2.93 ±0.03									-	*	****	****
ASBE	4.10 ±0.04										-	****	****
AAWE	11.49 ±0.4											-	****
ASWE	17.71 ±0.6												-

Appendice 02. Comparative study on total phenolics content of *A. atlantica* and *A. sicula* L. extracts.

Extracts		**AACE**	**ASCE**	**AAHE**	**ASHE**	**AAChE**	**ASChE**	**AAEAE**	**ASEAE**	**AABE**	**ASBE**	**AAWE**	**ASWE**
	TPC (mg GAEq / g DW)	238.31 ± 11.68	149.58 ± 0.77	30.54 ±2.76	67.82 ±3.89	280.96 ±6.24	274.78 ± 2.10	529.49 ±9.01	382.92 ±4.01	280.25 ±5.00	258.01 ±3.54	91.45 ±3.5	70.07 ±4.88
AACE	238.31 ± 11.68	-	****	****	****	****	****	****	****	****	**	****	****
ASCE	149.58 ± 0.77		-	****	****	****	****	****	****	****	****	****	****
AAHE	30.54 ±2.76			-	****	****	****	****	****	****	****	****	****
ASHE	67.82 ±3.89				-	****	****	****	****	****	****	**	ns
AAChE	280.96 ±6.24					-	ns	****	****	ns	**	****	****
ASChE	274.78 ± 2.10						-	****	****	ns	*	****	****
AAEAE	529.49 ±9.01							-	****	****	****	****	****
ASEAE	382.92 ±4.01								-	****	****	****	****
AABE	280.25 ±5.00									-	**	****	****
ASBE	258.01 ±3.54										-	****	****
AAWE	91.45 ±3.5											-	**
ASWE	70.07 ±4.88												-

Appendice 03. Comparative study on total flavonoids content of *A. atlantica* and *A. sicula* L. extracts.

Extracts		**AACE**	**ASCE**	**AAHE**	**ASHE**	**AAChE**	**ASChE**	**AAEAE**	**ASEAE**	**AABE**	**ASBE**	**AAWE**	**ASWE**
	TFC (mg QEq / g DW)	97.81 ±6.98	40.48 ±3.97	15.55 ±0.63	6.94 ±0.36	126.22 ±4.11	83.09 ±1.53	241.16 ±5.17	109.25 ±2.14	62.36 ±3.13	52.15 ±2.82	17.29 ±1.25	9.37 ±0.17
AACE	97.81 ±6.98	-	****	****	****	****	***	****	*	****	****	****	****
ASCE	40.48 ±3.97		-	****	****	****	****	****	****	****	*	****	****
AAHE	15.55 ±0.63			-	ns	****	****	****	****	****	****	ns	ns
ASHE	6.94 ±0.36				-	****	****	****	****	****	****	*	ns
AAChE	126.22 ± 4.11					-	****	****	***	****	****	****	****
ASChE	83.09 ±1.53						-	****	****	****	****	****	****
AAEAE	241.16 ±5.17							-	****	****	****	****	****
ASEAE	109.25 ±2.14								-	****	****	****	****
AABE	62.36 ±3.13									-	*	****	****
ASBE	52.15 ±2.82										-	****	****
AAWE	17.29 ±1.25											-	ns
ASWE	9.37 ±0.17												-

Appendice 04. Comparative study on flavonols content of *A. atlantica* and *A. sicula* L. extracts.

Extracts		**AACE**	**ASCE**	**AAHE**	**ASHE**	**AAChE**	**ASChE**	**AAEAE**	**ASEAE**	**AABE**	**ASBE**	**AAWE**	**ASWE**
	flavonols content (mgQEq/g DW)	32.15 ±0.76	17.37 ±0.76	6.47 ±0.24	4.26 ±0.24	48.19 ±2.13	37.59 ±2.13	70.76 ±3.25	61.11 ±3.25	17.38 ±1.16	21.12 ±1.97	10.02 ±0.21	4.01 ±0.21
AACE	32.15 ± 0.76	-	****	****	****	****	*	****	****	****	****	****	****
ASCE	17.37 ± 0.76		-	****	****	****	****	****	****	ns	ns	**	****
AAHE	6.47 ± 0.24			-	ns	****	****	****	****	****	****	ns	ns
ASHE	4.26 ± 0.24				-	****	****	****	****	****	****	*	ns
AAChE	48.19 ± 2.13					-	****	****	***	****	****	****	****
ASChE	37.59 ± 2.13						-	****	****	****	****	****	****
AAEAE	70.76 ± 3.25							-	****	****	****	****	****
ASEAE	61.11 ± 3.25								-	****	****	****	****
AABE	17.38 ± 1.16									-	ns	**	****
ASBE	21.12 ± 1.97										-	****	****
AAWE	10.02 ± 0.21											-	*
ASWE	4.01 ± 0.21												-

Appendice 05. Comparative study on tannins content of *A. atlantica* and *A. sicula* L. extracts.

Extracts		**AACE**	**ASCE**	**AAHE**	**ASHE**	**AAChE**	**ASChE**	**AAEAE**	**ASEAE**	**AABE**	**ASBE**	**AAWE**	**ASWE**
	Total tannins content (mgTAEq / g DW)	102.38 ±8.46	98.16 ±7.12	12.36 ±1.19	27.82 ±2.14	142.78 ±9.46	134.15 ±6.12	296.18 ±8.74	194.54 ±6.19	121.46 ±7.32	92.47 ± 5.45	57.89 ± 3.48	51.07 ± 2.17
AACE	102.38 ±8.46	-	ns	****	****	****	***	****	****	*	ns	****	****
ASCE	98.16 ± 7.12		-	****	****	****	****	****	****	**	ns	****	****
AAHE	12.36 ± 1.19			-	ns	****	****	****	****	****	****	****	****
ASHE	27.82 ±2.14				-	****	****	****	****	****	****	***	**
AAChE	142.78 ±9.46					-	ns	****	***	*	****	****	****
ASChE	134.15 ±6.12						-	****	****	ns	****	****	****
AAEAE	296.18 ±8.74							-	****	****	****	****	****
ASEAE	194.54 ±6.19								-	****	****	****	****
AABE	121.46 ±7.32									-	***	****	****
ASBE	92.47 ± 5.45										-	****	****
AAWE	57.89 ± 3.48											-	ns
ASWE	51.07 ± 2.17												-

Appendice 06. Comparative study on chlorophylls content of *A. atlantica* and *A. sicula* L. extracts.

Extracts		**AACE**	**ASCE**	**AAHE**	**ASHE**	**AAChE**	**ASChE**	**AAEAE**	**ASEAE**	**AABE**	**ASBE**	**AAWE**	**ASWE**
	Total chlorophylls content (mg / g DW)	2.97 ±0.185	1.79 ±0.04	4.36 ±0.488	12.08 ±0.04	5.78 ±0.365	15.20 ±0.09	0.78 ±0.049	2.09 ±0.03	0.43 ±0.041	1.97 ±0.05	0.41 ±0.036	1.11 ±0.01
AACE	2.97 ± 0.185	-	****	****	****	****	****	****	***	****	****	****	****
ASCE	1.79 ± 0.04		-	****	****	****	****	****	ns	****	ns	****	**
AAHE	4.36 ± 0.488			-	****	****	****	****	****	****	****	****	****
ASHE	12.08 ±0.04				-	****	****	****	****	****	****	****	****
AAChE	5.78 ± 0.365					-	****	****	****	****	****	****	****
ASChE	15.20 ±0.09						-	****	****	****	****	****	****
AAEAE	0.78 ± 0.049							-	****	ns	****	ns	ns
ASEAE	2.09 ± 0.03								-	****	ns	****	****
AABE	0.43 ± 0.041									-	****	ns	**
ASBE	1.97 ± 0.05										-	****	***
AAWE	0.41 ± 0.036											-	**
ASWE	1.11 ± 0.01												-

Appendice 07. Comparative study on carotenoids content of *A. atlantica* and *A. sicula* L. extracts.

Extracts		**AACE**	**ASCE**	**AAHE**	**ASHE**	**AAChE**	**ASChE**	**AAEAE**	**ASEAE**	**AABE**	**ASBE**	**AAWE**	**ASWE**
	Total carotenoids content(mg/g DW)	0.91± 0.087	0.96± 0.08	2.15± 0.195	5.24± 0.09	3.74± 0.264	2.09± 0.07	0.29± 0.021	2.05± 0.02	0.21± 0.002	0.80± 0.05	0.04± 0.003	0.62± 0.02
AACE	0.91± 0.087	-	ns	****	****	****	****	****	****	****	ns	****	ns
ASCE	0.96± 0.08		-	****	****	****	****	****	****	****	ns	****	*
AAHE	2.15± 0.195			-	****	****	ns	****	ns	****	****	****	****
ASHE	5.24± 0.09				-	****	****	****	****	****	****	****	****
AAChE	3.74± 0.264					-	****	****	****	****	****	****	****
ASChE	2.09± 0.07						-	****	ns	****	****	****	****
AAEAE	0.29± 0.021							-	****	ns	***	ns	*
ASEAE	2.05± 0.02								-	****	****	****	****
AABE	0.21± 0.002									-	****	ns	**
ASBE	0.80± 0.05										-	****	ns
AAWE	0.04± 0.003											-	****
ASWE	0.62± 0.02												-

Appendice 08. Comparative study on β-carotene content of *A. atlantica* and *A. sicula* L. extracts.

Extracts		**AACE**	**ASCE**	**AAHE**	**ASHE**	**AAChE**	**ASChE**	**AAEAE**	**ASEAE**	**AABE**	**ASBE**	**AAWE**	**ASWE**
	Total β-carotene content(mg/g DW)	0.21± 0.018	0.19± 0.014	0.22± 0.023	0.28± 0.024	0.27± 0.02	0.27± 0.021	0.12± 0.015	0.12± 0.011	0.14± 0.09	0.13± 0.006	0.06± 0.008	0.03± 0.002
AACE	0.21± 0.018	-	ns	ns	ns	ns	ns	*	*	ns	ns	***	****
ASCE	0.19± 0.014		-	ns	*	ns	ns	ns	ns	ns	ns	**	****
AAHE	0.22± 0.023			-	ns	ns	ns	*	*	ns	*	****	****
ASHE	0.28± 0.024				-	ns	ns	****	****	***	***	****	****
AAChE	0.27± 0.02					-	ns	***	***	**	***	****	****
ASChE	0.27± 0.021						-	***	***	**	***	****	****
AAEAE	0.12± 0.015							-	ns	ns	ns	ns	*
ASEAE	0.12± 0.011								-	ns	ns	ns	*
AABE	0.14± 0.09									-	ns	ns	**
ASBE	0.13± 0.006										-	ns	*
AAWE	0.06± 0.008											-	ns
ASWE	0.03± 0.002												-

Appendice 09. Comparative study on lycopene content of *A. atlantica* and *A. sicula* L. extracts.

Extracts		**AACE**	**ASCE**	**AAHE**	**ASHE**	**AAChE**	**ASChE**	**AAEAE**	**ASEAE**	**AABE**	**ASBE**	**AAWE**	**ASWE**
	Total lycopene content (mg/ g DW	0.34± 0.03	0.33± 0.016	0.38± 0.027	0.36± 0.029	0.35± 0.032	0.35± 0.031	0.16± 0.015	0.34± 0.039	0.21± 0.02	0.14± 0.010	0.10± 0.009	0.24± 0.019
AACE	0.34± 0.03	-	ns	ns	ns	ns	ns	****	ns	****	****	****	**
ASCE	0.33± 0.016		-	ns	ns	ns	ns	****	ns	***	****	****	**
AAHE	0.38± 0.027			-	ns	ns	ns	****	ns	****	****	****	****
ASHE	0.36± 0.029				-	ns	ns	****	ns	****	****	****	***
AAChE	0.35± 0.032					-	ns	****	ns	****	****	****	***
ASChE	0.35± 0.031						-	****	ns	****	****	****	***
AAEAE	0.16± 0.015							-	****	ns	ns	ns	*
ASEAE	0.34± 0.039								-	****	****	****	**
AABE	0.21± 0.02									-	ns	***	ns
ASBE	0.14± 0.010										-	ns	**
AAWE	0.10± 0.009											-	****
ASWE	0.24± 0.019												-

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Open Access Research Article

Total Phenolic Contents, DPPH Radical Scavenging and β-Carotene Bleaching Activities of Aqueous Extract from *Ammoides atlantica*

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ABSTRACT

Phytotherapy has known a great evolution all the world and some medicinal plants are important remedies of some diseases. *Ammoides atlantica* is one of the medicinal plants used in folk medicine. This study aims to estimate the total phenolics and flavonoids contents then to investigate both *in vitro* antioxidant activity models of aqueous extract (AqE) from *Ammoides atlantica*. Total polyphenol contents were determined using Folin Ciocalteu's reagent; flavonoids were quantified employing the $AlCl_3$ Method. The *in vitro* antioxidant property was assessed by DPPH-scavenging radicals and lipid peroxidation assays. The results revealed that *Ammoides atlantica* aqueous extract presented a high total phenolic and flavonoid contents with values of 85.56±4.71 µg GAE (gallic acid equivalent)/mg and 40.55±4.09 µg QE (quercitin equivalent)/mg dry extract, respectively. This extract shows a good DPPH radical scavenging and β-carotene bleaching activities with an IC_{50} of 107.48±5.9 µg/mL and 130.17±5.52 µg/mL, respectively. This study indicates that the aqueous extract from *Ammoides atlantica* has potent antioxidant effects and may prove to be of latent health benefit as well as supplementary sources for natural antioxidants drugs.

Keywords: *Ammoides atlantica*, aqueous extract, antioxidant activity, phenolic compounds.

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1. INTRODUCTION

The excessive production of reactive oxygen species (ROS) overwhelming the antioxidant defense mechanisms of the cells has been shown to oxidize biological molecules and induce damage to the cell membrane, proteins, carbohydrates, and DNA. This oxidative stress is involved in several pathological situations including hypertension, heart failure and diabetes [1]. Antioxidants are compounds that can protect cells against the damage arising from unstable molecules known as ROS and free radicals [2]. The use of synthetic antioxidants in the prevention of free radical damage had many toxicological side effects including carcinogenicity. For this, there is an increasing interest in the potential benefits of natural antioxidants with the potent capacity to inhibit lipid peroxidation and scavenge ROS [3]. Recently, the exploration of natural antioxidant agents from plants is an important and essential step in the evolution of effective alternative medications [4]. Several polyphenols compounds are usually produced in plants and have attracted substantial recognition because of their antioxidant capabilities and free radical scavenging capabilities which are likely to be of concern to human health [5]. The *Ammoides atlantica* (coss. et Dur.) Wolf, of the family Apiaceae, is widespread in the Mediterranean region and it is endemic in Algeria [6]. Traditionally, this plant is known to be used for the therapy of fever and headache, besides its use as antidiarrheic [7]. Given the interest of *Ammoides atlantica* in folk medicine, this study aims to assess the polyphenolic contents of the aqueous extract from aerial parts of *Ammoides atlantica* and evaluate the *in vitro* antioxidant activity using DPPH radical scavenging and β-carotene bleaching assays.

2. MATERIALS AND METHODS

2.1. Plant material

2.1.1. Plant collection and identification

The aerial parts from *Ammoides atlantica* were collected from Jijel North-Eastern part of Algeria, during the flowering stage. The plant was identified and authenticated by Prof.

Laouer H., a botanist at the Department of Biology and Vegetal Ecology, University of Sétif, Algeria.

2.1.2. Extraction procedure

100g of *Ammoides atlantica* powder was mixed with 1L of boiling distilled water (100 °C) and after 20 minutes, it was removed from the heat. The mixture was filtered using Wattman filter paper n°1 and then dried at 45 °C to obtain aqueous extract which was stored at 20°C until further analysis [8].

2.2. Determination of total phenolic and flavonoid contents

2.2.1. Total phenolic content

The total phenolic content was evaluated by utilizing the reagent of Folin-Ciocalteu [9], according to a method of microplate described par Muller et al. [10]. Herein, 20 µl of a sample (1 mg extract/1ml water) were blended with100 µl of Folin-Ciocalteu reagent (1:10) and 75 µl of sodium carbonate solution (7,5%). The microplate was incubated for two hours at room temperature in darkness. Absorbance at 765 nm was measured by using the microplate reader. The total phenolic content was evaluated as micrograms of gallic acid equivalents per milligrams of extract.

2.2.2. Total flavonoids content

Total flavonoids content was determined by the method of Topçu *et al.* [11] with some modifications to adapt it to the microplate. Briefly, 130 µl of methanol were added to 50 µl of a sample (1mg extract/1ml water). Subsequently,10 µl of 1M potassium acetate (CH_3COOK) and 10 µl of 10% aluminum nitrate ($Al(NO_3)_3$, $9H_2O$) were added and the microplate was incubated at room temperature for 40 minutes. Absorbance was read at 415 nm. Data were represented as micrograms of quercetin equivalents per milligrams of extract.

2.3. Antioxidant activity assays

2.3.1. DPPH free radical-scavenging assay

The free radical-scavenging capacity was determined spectrophotometrically employing the DPPH assay [12]. Briefly, 40 µl of the sample at various concentrations was added to 160 µl DPPH (0.1 mM). The reaction mixture was shaken forcefully, and the absorbance of the remaining DPPH was read at 517 nm after 30 min. BHT was used as an antioxidant standard for comparison of the activity. The scavenging capability of DPPH radical was calculated using the following equation: DPPH scavenging effect (%) = $[(A_{Control}-A_{sample})/A_{Control}] \times 100$. The results were given as IC_{50} values (µg/ml) corresponding to the concentration of 50% inhibition.

2.3.2. β-Carotene/linoleic acid assay

The β-carotene bleaching activity was evaluated using the β-carotene-linoleic acid system described by Marco [13]. Thus, a solution of β-carotene (0.5 mg) in 1 ml of chloroform is combined with 25 µl of linoleic acid and 200 µl of Tween 40. After evaporation in vacuo of the chloroform, 50 ml of hydrogen peroxide (H_2O_2) is added under vigorous agitation. The absorbance of the solution is then adjusted to 0.8-0.9 nm. Amounts of 160 µl of this solution are added to 40 µl of a sample at different concentrations. The absorbance was measured at 470 nm, using a 96-well microplate reader. The emulsion system was incubated for 2 h at 50°C. BHT was used as a reference. The bleaching rate (R) was assessed as follows: R = (ln c/d)/t. Where: c= absorbance at time zero, d= absorbance at time 120 min (t) and ln= natural log. The antioxidant activity (AA) was calculated in terms of percent of inhibition relative to the control, using the following equation: AA (%) = $[(R_{Control} - R_{Sample})/R_{Control}] \times 100$.

2.4. Statistical analysis

Results are represented as the mean ± standard deviation (SD) and all measurements were conducted in three determinations (n=3). The statistical interpretation was directed by the help of a one-way analysis of variance (ANOVA) for significance with the aid of Graph Pad Prism 7.00. differences were examined significant at $p \leq 0.05$.

3. RESULTS

3.1. Total phenolics and flavonoids contents

The results showed that the *Ammoides atlantica* aqueous extract (AqE) was rich in polyphenols and flavonoids (141.74±0.44 µg GAE/mg dry extract and 61.87±6.7µg QE/mg dry extract, respectively) as shown in Table 1.

Table 1: Total polyphenols and flavonoids content of *Ammoides atlantica* aqueous extract. AqE: aqueous extract, [a]: µg GAE/mg and [b]: µg QE/mg.

Extract	Total phenolic content[a]	Total flavonoid content[b]
AqE	141.74±0.44	61.87±6.7

3.2 Antioxidant activities

The IC_{50} values of DPPH radical scavenging and β-carotene bleaching activities from *Ammoides atlantica* aqueous extract (AqE) are presented in Table 2. The results revealed that the AqE presented a good scavenging activity against DPPH, with an IC_{50} of 204.22±12.16 µg/mL. As well, AqE displays a strong β-carotene bleaching activity with an IC_{50} of 112.45±6.66 µg/mL. This suggests a significant antioxidant activity from AqE.

Table 2 : Antioxidant activities of *Ammoides atlantica* aqueous extract (AqE). **** $p < 0.0001$ compared to correspondent standards. AqE: aqueous extract, DPPH: 2,2-diphenyl-1-picrylhydrazyl, BHA: butylated hydroxyanisole and BHT: butylated hydroxytoluene.

Extract/ standard	IC_{50} (µg/mL)	
	DPPH scavenging activity	β-carotene bleaching activity
AqE	204.22±12.16****	112.45±6.66****
BHA	5.73±0.41	0.90±0.02
BHT	22.32+1.19	1.05+0.01

4. DISCUSSION

Our results showed that the *Ammoides atlantica* aqueous extract (AqE) was a good source of polyphenol and flavonoid compounds. These results agree with the findings of [14]. Antioxidant capacity from AqE was assessed by DPPH-scavenging radicals and lipid peroxidation assays. In DPPH radical scavenging test, A freshly formulated solution of DPPH shows a dark purple color with maximum absorption at 517 nm. The purple hue usually fades/disappears in the medium contains antioxidants, Thus, antioxidant molecules can neutralize DPPH-free radicals; by provide hydrogen atoms or donate electrons and convert them to a colorless/bleached substance (2,2-diphenyl-1 hydrazine or equivalent hydrazine substituted), resulting in a decrease in absorption at the 517 nm level. The more rapidly absorbance falls, the more efficient the extract's antioxidant function in terms of atomic hydrogen-donating ability [15]. The results revealed that the AqE presented a good DPPH radical scavenging potential. This could be attributed to polyphenols and flavonoids [16]. The ability of A*mmoides atlantica* aqueous extract (AqE) to inhibit lipid peroxidation was tested by the β-carotene bleaching method. β-carotene in the absence of the antioxidant undergoes a rapid decolorization since the free linoleic acid radical attacks the β-carotene, which loses the double bonds and, consequently, its orange color [17]. The presence of a phenolic antioxidant can hinder the extent of b-carotene destruction by "neutralizing" the linoleate free radical (utilizing its redox potential) and any other free radicals formed within the system. Hence, this test can be used to examine the antioxidant potential of *Ammoides atlantica* AqE [18]. The results showed that AqE was able to inhibit lipid peroxidation. These antioxidant activities could be attributed to the richness of this extract in polyphenols and flavonoids. In fact, the literature showed that a good correlation was found between antioxidant activity and the content of polyphenols and flavonoids [19,20]. Our results are in accordance with those of [14, 21], who demonstrated that the *Ammoides atlantica* extracts have potent antioxidant capacities.

5. CONCLUSION

The aqueous extract of *Ammoides atlantica* exhibited good antiradical activities toward 2,2′- diphenyl-1-picrylhydrazyl (DPPH) and acting as inhibiting lipid peroxidation. This may explain the medicinal use of this plant in folk medicine. These results suggest that AqE of *Ammoides atlantica* might be promising for the treatment or prevention of many diseases associated with oxidative damage. Further researches needed to identify and isolate the active principles present in this extract which could be useful for pharmaceutical purposes.

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Metal Chelating and Cupric Ion Reducing Antioxidant Capacities of *Ammoides atlantica* Aqueous Extract

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ABSTRACT

Reactive oxygen (ROS) and nitrogen species (RNS) are produced in all cells and play important roles in physiology. The loss of the redox balance, either by an increase of oxidant molecules ROS and RNS or by decreased antioxidant system activities cause a state of oxidative stress. Several studies are going on worldwide directed towards finding natural antioxidants of plant origin. Plants containing phenolic compounds have been reported to possess strong antioxidant activity. The objective of this study is to evaluate total polyphenols and flavonoids contents (TPC and TFC) as well as examine the *in vitro* antioxidative properties from aqueous extract of *Ammoides atlantica* (AqE). TPC was estimated utilizing Folin-Ciocalteu's reagent. TFC was evaluated utilizing the aluminum chloride method. The antioxidant properties were evaluated using metal chelating and cupric ion reducing antioxidant capacity (CUPRAC) assays. Indeed, results showed that the AqE is rich in polyphenols (141.74±0.44 µg gallic acid equivalents/ mg of dry weight), and flavonoids (61.87±6.7 µg quercetin equivalent/ mg dry weight). These phytochemical compounds possess significant antioxidant activities. The results showed that AqE exhibited a good Metal chelating activity with an IC_{50} of 36.57±4.73 µg/ mL. CUPRAC assay showed that AqE extract exhibited high cupric ion reducing antioxidant capacity with an $A_{0.5}$ of 8.58±0.13 µg/mL. These findings provide evidence that AqE of *Ammoides atlantica* is a potential source of antioxidant which have many benefits towards human health.

Keywords: *Ammoides atlantica*, aqueous extract, phenolic compounds, metal chelating and cupric ion reducing antioxidant capacity.

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1. INTRODUCTION

Oxidative stress is generally characterized by the excess formation of reactive molecules such as ROS (reactive oxygen species). *In vivo*, some of these ROS play a positive role such as energy production, phagocytosis, regulation of cell growth and intracellular signaling [1]. However, ROS are known to be the major cause of various chronic and degenerative diseases, including aging, coronary heart disease, inflammation, diabetes mellitus and cancer [2], and can cause cellular injuries and initiate peroxidation of fatty acids in biological membranes. ROS may damage protein [3], DNA [4], and enzymes [5]. The antioxidant compounds possess anticarcinogenic, antitumor, anti-inflammatory, antiatherosclerotic, antiviral and antibacterial activities [6]. Many plant species have been attractive to scientists as natural sources of compounds that are safer than the synthetic ones. Plant-derived antioxidants, especially, the phenolics have gained considerable importance due to their potential health benefits. Previous studies have shown that plant foods containing antioxidants are advantageous to health as it down-regulates certain degenerative processes and can significantly reduce the occurrence of cardiovascular and cancer diseases [7]. The *Ammoides atlantica* (coss. et Dur.) Wolf, of the family Apiaceae, is widespread in the Mediterranean region and it is endemic in Algeria [8]. Traditionally, this plant is known to be used for the therapy of fever and headache, besides its use as antidiarrheic [9]. This study aims to investigate the *in vitro* antioxidant Activities of

Ammoides atlantica aqueous extract using metal chelating and cupric ion reducing antioxidant capacity (CUPRAC) assays, besides to evaluate total polyphenol and flavonoids contents.

2. MATERIALS AND METHODS

2.1. Plant material

2.1.1. Plant collection and identification

Ammoides atlantica was harvested at the flowering stage from Jijel north-eastern of Algeria during spring. Aerial parts were dried in shadow at room temperature then powdered and stocked in darkness until use. The authenticity was confirmed by Pr Laouar Hocine (Department of Vegetal Biology and Ecology, University Farhat Abbas Setif 1).

2.1.2. Extraction procedure

The extraction process was done according to a method of [8]. 100g of *Ammoides atlantica* powder was mixed with 1L of boiling distilled water (100 °C) and after 20 minutes it was removed from the heat. The mixture was filtered using Wattman filter paper n°1 and then dried at 45 °C to obtain aqueous extract which was stored at 20°C until further analysis [10].

2.2. Determination of total phenolic and flavonoid contents

2.2.1. Total phenolic content (TPC)

The total phenolic content of AqE extract was determined spectrophotometrically using the Folin-Ciocalteu method [11] with some modifications. In a brief description, 100µl of 1:10 Folin-Ciocalteau reagent and 75 µl of sodium carbonate (7.5%) were added to 20 µl of aqueous extract. After 2 h of incubation in the dark at ambient temperature, the absorbance at 765 nm was measured by a microplate reader, against a control. The total polyphenol content was determined as micrograms of gallic acid equivalent per milligram of extract (µg GAE/ mg).

2.2.2. Total flavonoids content (TFC)

TFC was evaluated utilizing the aluminum colorimetric method [12] with some modifications. A volume of 130 µl of methanol was transferred into a micro-plate (96 wells) containing 50 µl of AqE and then 10 µl of potassium acetate (1 M) and 10 ml of aluminum nitrate at 10 % were added. After period incubation for 40 min at ambient temperature, the absorbance was read at 415 nm by a micro-plat reader. The standard calibration curve of quercetin at various concentrations was utilized to calculate total flavonoid concentration. The results were presented as micrograms of quercetin equivalent per milligram of extract (µg QE/ mg).

2.3. Antioxidant activity assays

2.3.1. Metal chelating activity assay

The metal chelating activity by the ferrene-Fe^{2+} complexation assay measured spectrophotometrically [13, 14] with slight modifications. 40 µl of the extract were added to 40 µl of 0.2 mM $FeCl_2$. The reaction was initiated after the addition of 80 µl of ferene solution (0.5 mM). The obtained mixture was shaken then incubated at room ambient for 10 min. The absorbance was read at 593 nm. The metal chelating potential was estimated by the utilize of the following equation. The results were given as IC_{50} value (µg/ml) (50 % inhibition):

$$\text{Metal chelating activity (\%)} = [(A_{Control}-A_{Sample})/A_{Control}]\times 100$$

2.3.2. Cupric reducing antioxidant capacity (CUPRAC) assay

The CUPRAC was determined according to the method of [15]. In each well, the reaction mixture containing 40 µl of sample and 50 µl of a copper (II) chloride solution, 50 µl of a neocuproine alcoholic solution, and 60 µl of ammonium acetate aqueous buffer at pH 7 was combined to give a final volume of 200 µl. After 30 minutes, the absorbance was measured at 450 nm. Results were recorded as absorbance ($A_{0.5}$) compared with the absorbance of BHA and BHT, which were used as antioxidant standards.

2.4. Statistical analysis

All data were the average of triplicate analyses. Data were recorded as the mean ± standard deviation. Analysis of variance was executed using Student's t-test or one-way analysis of variance (ANOVA) with the aid of Graph Pad Prism 7.00. p values < 0.05 were regarded as significant.

3. RESULTS

3.1. Total phenolics and flavonoids contents

Our results showed that the *Ammoides atlantica* aqueous extract (AqE) had high polyphenol (141.74±0.44 µg GAE/ mg dry extract) and flavonoid (61.87±6.7µg QE/ mg dry extract) contents. (Table 1).

Table 1: Total polyphenols and flavonoids content of *Ammoides atlantica* aqueous extract. AqE: aqueous extract, [(a)]: µg GAE/mg and [(b)]: µg QE/mg

Extract	Total phenolic content[(a)]	Total flavonoid content[(b)]
AqE	141.74±0.44	61.87±6.7

3.2 Antioxidant activity

3.2.1. Metal chelating activity

The antioxidative potential was observed in *Ammoides atlantica* aqueous extract (AqE) using a metal chelating test as shown in Table 1. This assay showed that the AqE had a strong antioxidant activity with an IC_{50} of 36.57±4.73 µg/mL (Table 1).

3.2.2. Cupric reducing antioxidant capacity (CUPRAC)

CUPRAC assay showed that the *Ammoides atlantica* AqE exhibited a good effect with an $A_{0.5}$ of 8.58±0.13 µg/mL. This cupric reducing antioxidant capacity from *Ammoides atlantica* AqE was similar to that of BHT synthetic antioxidant ($P>0.05$, no significant difference). But this activity is relatively lower compared to the BHA ($p < 0.0001$) as standard (Table 1).

Table 2: Antioxidant activities of *Ammoides atlantica* aqueous extract (AqE). ns : no significant difference and **** $p < 0.0001$ compared to correspondent standards. AqE: aqueous extract, BHA: butylated hydroxyanisole, BHT: butylated hydroxytoluene, and EDTA: Ethylenediaminetetraacetic acid.

Extract/standard	IC_{50} (µg/mL)	$A_{0.5}$ (µg/mL)
	Metal chelating activity	Cupric reducing antioxidant capacity
AqE	36.57±4.73	8.58±0.13
BHA	/	3.64±0.19 ****
BHT	/	9.62+0.87ns
EDTA	12.11+0.32****	/

4. DISCUSSION

In the current study, the antioxidant activity of *Ammoides atlantica* AqE was evaluated by using metal chelating and CUPRAC assays. Metal ion chelating activity of an antioxidant molecule prevents oxyradical generation and the consequent oxidative damage [16]. Metal ion chelating capacity plays a significant role in antioxidant mechanisms since it reduces the concentration of the catalyzing transition metal in lipid peroxidation [17]. In the presence of chelating agents, the ferrozine-Fe^2 complexes are disrupted, resulting in a decrease in the red color of the complex. *Ammoides atlantica* AqE exhibited a good metal chelating activity. This activity could be attributed to the richness of AqE in polyphenols and flavonoids. Phenolic compounds have been reported to be chelators of free metal ions [18]. CUPRAC method is based on the reaction of an electron transfer, thus the oxidant is reduced, which monitored by a color change [19]. In this assay, the *Ammoides atlantica* aqueous extract demonstrates a strong antioxidant effect. This cupric reducing antioxidant capacity could be due to phenolic and flavonoid contents in AqE. Several authors have reported that the antioxidant capacity depends on the amount of phenolic compounds of plant extracts. [20, 21]. The phenolic compounds acting as hydrogen donors, free radical acceptors, chain oxidation reaction interrupters or metal chelators [22]. This finding of the antioxidant capacity of *Ammoides atlantica* is in agreement with other studies [23, 24, 25].

5. CONCLUSION

This work revealed that aqueous extract of the aerial parts of *Ammoides atlantica* contains high levels of phenolics and flavonoids, and possesses significant antioxidant activities which may due to the presence of polyphenolic compounds. These findings provide scientific support for the traditional uses of *Ammoides atlantica*. It is also suggested that *Ammoides atlantica* be viewed as a potential source of natural antioxidants that can provide precious functional ingredients useful for the prevention of diseases related to oxidative stress.

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الملخص

تعتبر (*A. atlantica*) *Ammoides atlantica* و (*A. sicula* L.) *Athamanta sicula* L. من النباتات الطبية الهامة في الاستخدامات التقليدية للفلورا الجزائرية. تهدف الدراسة الحالية إلى التحليل الكيمياتي وتقييم خارج الحي (في الزجاج) لمضادات الأكسدة ، مضادات الأسيتيل-كولينستراز ومضاد البوتيريل-كولينستراز ، مضادات التيروزيناز ومضادات ألفا-أميلاز ومضادات ألفا-غليكوزيداز لمستخلصات *A. atlantica* و *A. sicula* L. المائية-الميثانولية (AACE و ASCE) وأجزائها الفرعية، الهكسان (AAHE و ASHE) ، الكلوروفورم (AAChE و ASCHE) ، أسيتات الإيثيل (AAEAE و ASEAE) ، البوتانول (AABE و ASBE) والمستخلصات المائية (AAWE و ASWE). علاوة على ذلك، تم تقييم النشاط المضاد للقرحة المعدية و التأثير على الحركية المعدية المعوية (إفراغ المعدة والعبور المعوي) في الحي لكل من المستخلصين AACE و ASCE. أظهر تحليل GC-MS أن الكارفاكرول، وحمض اللينوليك، حمض الأوليك (9-حمض أوكتاديسينويك (Z)) وحمض البالمتيك كانت المركبات الرئيسية في AAHE. في حين كان الكارفاكرول، حمض اللينوليك، الأبيول وحمض البالمتيك المكونات الرئيسية في ASHE. أظهر تحليل UHPLC-MS وجود إحدى أو العديد من ايزومر حمض الكافويلكوينيك و/أو مشتقاته كمكونات رئيسية في AACE و أجزائه الفرعية، في حين أن حمض مالونيل-3،5-ديكافويلكوينيك I، استراغالين حمض الفوريك، استراغالين، و / أو سينارين كانت المكونات الرئيسية في ASCE وأجزائه الفرعية. أظهرت المستخلصات AAEAE و ASEAE أعلى كمية من البوليفينول والفلافونويد والفلافونول والدباغ. أظهرت جميع المستخلصات أنشطة مضادة للأكسدة ومضادة للإنزيمات. كان للمستخلصين AAEAE و ASEAE أعلى نشاطا مع معظم الاختبارات. تسبب كل من AACE و ASCE في تأخير إفراغ المعدة بصورة معتمدة على الجرعة في الفئران و قد تعود هذه التأثيرات الى تدخل مسارات إنزيمات الأكسدة الحلقية في الحيوانات المعالجة بـمستخلصات AACE و ASCE و كذلك المسارات الكولينرجية في الفئران المعالجة بـمستخلص AACE. أدى كل من المستخلصين AACE و ASCE إلى تأخير العبور المعوي بصورة معتمدة على الجرعة. ينتج هذا التأثير عن تدخل مسارات أكسيد النيتريك و إنزيمات الأكسدة الحلقية عند الفئران المعالجة بمستخلص AACE أو ASCE والمسارات الكولينرجية عند الحيوانات المعالجة بمستخلص AACE. قدمت AACE و ASCE حماية معتمدة على الجرعة للغشاء المخاطي ضد القرحة المعدية في الجرذان. و قد تشمل آليات هذا التأثير على الأقل زيادة محتوى مخاط المعدة، المسارات الكولينرجية ، مسارات أكسيد النيتريك وتخفيض الضرر التأكسدي المحدث بالغشاء المخاطي للمعدة. تظهر هذه النتائج لأول مرة بعض المكونات و الأنشطة الجديدة لـ *A. atlantica* و *A.sicula* L. والتي يمكن استغلالها في مجال الصناعات الدوائية والغذائية ومستحضرات التجميل.

الكلمات المفتاحية: *A. atlantica*، *A. sicula* L.، مضادات الأكسدة، مضادات إنزيمية، إفراغ معدي، عبور معوي، القرحة المعدية.

Abstract

Ammoides atlantica (*A. atlantica*) and *Athamanta sicula* L. (*A. sicula* L.) are two important plants in traditional uses of the Algerian flora. The present study aimed to chemically analyze and to evaluate *in vitro* antioxidant**,** anti-acetylcholinesterase and anti-butyrylcholinesterase, anti-tyrosinase, anti-α-amylase and anti-α-glucosidase of *A. atlantica* and *A. sicula* L. crude (hydromethanolic) extracts (AACE and ASCE) and their sub-fractions; hexane (AAHE and ASHE), chloroform (AAChE and ASChE), ethyl acetate (AAEAE and ASEAE), butanol (AABE and ASBE) and water (AAWE and ASWE) extracts. Moreover, the gastro-protective and gastro-intestinal motility (gastric emptying and intestinal transit) effects of the AACE and ASCE were also evaluated *in vivo*. GC-MS analysis revealed that carvacrol, linoleic acid, oleic acid (9-Octadecenoic acid (Z)), palmitic acid were the major compounds in AAHE, whereas, carvacrol, linoleic acid, apiole, palmitic acid were the main constituents in ASHE. The UHPLC-MS analysis showed the presence of one or several of isomer of caffeoylquinic acid and/or its derivative as the main constituent in AACE and its sub-fractions, whereas, malonyl-3,5-dicaffeoylquinic acid I, astragalin formic acid adduct, astragalin and/ or cynarin were the main components in ASCE and its sub-fractions. AAEAE and ASEAE had the highest amount of polyphenols, flavonoids, flavonols and tannins. All extracts showed antioxidant and anti-enzymatic activities. The AAEAE and ASEAE fractions were the most active with the majority of tests. AACE and ASCE induced a dose dependent gastric emptying delay in mice. These effects may involve the cyclooxygenase in AACE and ASCE treated animals as well as cholinergic pathways in AACE treated mice. AACE and ASCE extracts dose dependently decreased the intestinal transit. The effect involves NO, cyclooxygenase pathways in AACE and ASCE treated mice and cholinergic system in AACE treated animals. AACE and ASCE offered a dose-dependent protection to the gastric mucosa against ethanol-induced ulceration in rats. This effect may involve at least in part gastric mucus content, cholinergic and nitric oxide pathways and a reduction in the oxidative damage of the mucosa. These results show for the first time new constituents and activities of *A. atlantica* and *A. sicula* L. that can be exploited in the field of pharmaceutical, food and cosmetic industries.

Keywords: *A. atlantica*, *A sicula* L., antioxidant activities, anti-enzymatic activities, anti-diabetic activity, gastric emptying, intestinal transit, gastric ulcer.

Résumé

Ammoides atlantica (*A. atlantica*) et *Athamanta sicula* L. (*A. sicula* L.) sont deux plantes importantes dans les usages traditionnels de la flore algérienne. La présente étude visait à analyser chimiquement et à évaluer *in vitro* les activités antioxydante, anti-acétylcholinestérase, anti-butyrylcholinestérase, anti-tyrosinase, anti-α-amylase et anti- α-glucosidase des extraits bruts (hydrométhanolique) de A. *atlantica* et *A. sicula* L. (AACE et ASCE) et leurs sous-fractions; extraits d'hexane (AAHE et ASHE), de chloroforme (AAChE et ASChE), d'acétate d'éthyle (AAEAE et ASEAE), de butanol (AABE et ASBE) et d'eau (AAWE et ASWE). De plus, les effets de la gastro-protection et de la motilité gastro-intestinale (vidange gastrique et transit intestinal) des extraits AACE et ASCE ont également été évalués *in vivo*. L'analyse GC-MS a révélé que le carvacrol, l'acide linoléique, l'acide oléique (acide 9-octadécénoïque (*Z*)), l'acide palmitique étaient les principaux composés de l'AAHE, tandis que le carvacrol, l'acide linoléique, l'apiole, l'acide palmitique étaient les principaux constituants de l'ASHE. L'analyse UHPLC-MS a montré la présence d'un ou plusieurs isomères de l'acide caféoylquinique et/ou de son dérivé comme constituants principaux de l'AACE et de ses sous-fractions, tandis que l'acide malonyl-3,5-dicaféoylquinique I, l'adduit d'acide formique d'astragaline, l'astragaline et/ou la cynarine étaient les principaux composants de l'ASCE et de ses sous-fractions. L'AAEAE et l'ASEAE avaient la plus grande quantité de polyphénols, de flavonoïdes, de flavonols et de tanins. Tous les extraits ont montré des activités antioxydantes et anti-enzymatiques. Les fractions AAEAE et ASEAE étaient les plus actives avec la majorité des tests. L'AACE et l'ASCE ont induit un retard, dose-dépendant, de la vidange gastrique chez la souris. Ces effets peuvent impliquer la cyclooxygénase chez les animaux traités par AACE et ASCE ainsi que les voies cholinergiques chez les souris traitées par AACE. Les extraits d'AACE et d'ASCE ont diminué de manière dose-dépendante le transit intestinal. L'effet implique les voies de NO et de la cyclooxygénase chez les souris traitées avec AACE et ASCE et le système cholinergique chez les animaux traités avec AACE. L'AACE et l'ASCE ont offert une protection dose-dépendante de la muqueuse contre l'ulcération induite par l'éthanol chez le rat. Cet effet peut impliquer au moins en partie la teneur en mucus gastrique, les voies cholinergiques et NO et une réduction des dommages oxydatifs de la muqueuse. Ces résultats montrent pour la première fois de nouveaux constituants et des activités d'*A. atlantica* et *A. sicula* L. qui peuvent être explorées dans le domaine des industries pharmaceutiques, alimentaires et cosmétiques.

Mots clés: *A. atlantica*, *A sicula* L., activités antioxydants, activités anti-enzymatiques, vidange gastrique, transit intestinal, ulcer gastrique.