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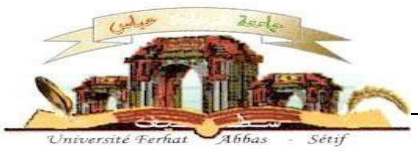

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THESIS

Presented by

Soulaf MEHLOUS

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TOPIC

Chemical profile, gastric ulcer and gastrointestinal effects of *Saccocalyx satureioides* Coss and Dur and *Achillea santolinoides* L. extracts

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Jury

President	Hassiba BENABDALLAH	MCA. UFA Setif 1
Supervisor	Smain AMIRA	Pr. UFA Setif 1
Co-supervisor	Fatima BENCHIKH	MCA. UFA Setif 1
Examiner	Youcef NECIB	Pr. UM Constantine 1
Examiner	Lakhdar GASMI	MCA. UFA Setif 1

Laboratory of Phytotherapy Applied to Chronic Disease

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List of publications

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Mehlous, S., Benchikh, F., Benabdallah, H., Loucif, K., Kaoudoune, C., Laouer, H., & Amira,S. (2020). Total Phenolic Contents, DPPH Radical Scavenging and β-Carotene Bleaching Activities of Ethyl Acetate Extract from *Saccocalyx satureioides* Coss and Dur. *Indian Journal of Novel Drug Delivery*, *12*(4), 196-200.

Kaoudoune, C., Benchikh, F., Benabdallah, H., Loucif, K., **Mehlous, S**., & Amira, S. (2020). Gastroprotective effect and in vitro Antioxidant Activities of the Aqueous Extract from *Artemisia absinthium* L. Aerial Parts. *Journal of Drug Delivery and Therapeutics*, *10*(4), 153-156. DOI https://doi.org/10.22270/jddt.v10i4.4253.

Loucif, K., Benabdallah, H., Benchikh, F., **Mehlous, S**., Souici, C. B., & Amira, S. (2020).Total Phenolic Contents, DPPH Radical Scavenging and β-Carotene Bleaching Activities of Aqueous Extract from *Ammoides atlantica*. *Journal of Drug Delivery and Therapeutics*, *10*(3-s), 196-198. DOI: https://doi.org/10.22270/jddt.v10i3-s.4151

Loucif, K., Benabdallah, H., Benchikh, F., **Mehlous, S**., Kaoudoune, C., Souici, C. B., & Amira, S. (2020). Metal Chelating and Cupric Ion Reducing Antioxidant Capacities of *Ammoides atlantica* Aqueous Extract. *Journal of Drug Delivery and Therapeutics*, *10*(4-s), 108-111. DOI: https://doi.org/10.22270/jddt.v10i4-s.4245

Mehlous, S., Amira, S., Benchikh, F., Benabdallah, H., Mamache, W., Bensouici, C., ... & Loucif, K. (2022). Alkaline DMSO superoxide and radical scavenging, cupric reducing antioxidant capacity (CUPRAC) and polyphenol contents of aqueous and methanol extract from *Achilllea santolinoides* L. aerial parts. *Turkish Journal of Agriculture-Food Science and Technology*, *10*(10), 2045-2050.

List of communications

Mehlous, S., Loucif, K., Amira., S., Khennouf, S., & Dehamna, S., Amira F and Mamache W. Antioxidant activities of the methanolic extract of medicinal plant *in vitro* . The Third Africa International Allelopathy Congress, Blida, Algeria, 24-26, November, 2018, Blida. Algeria.

Mehlous, S., Benchikh, F., Benabdallah, H., Loucif, K. & Amira, S. Polyphenol Contents and the antioxidant activities of the methanol extract from *Saccocalyx satureioides* Coss. and Durieu. Cinquieme Colloque International de Chimie. 04-06, Decembre, 2018. Batna.

Mehlous, S., Benchikh, F., Benabdallah, H., Loucif, K.& Amira, S**.** Assessment of antioxidant activity from *Saccocalyx satureioides* Coss. and Durieu aqueous extract using different methods. The 2nd International Conference on Bioanalysis : Food and Health. 15th, December, 2018, Mahdia- Tunisia.

Mehlous, S., Benchikh, F., Benabdallah, H., Loucif, K.& Amira, S. Assessment of the antioxidant activity of the methanol extract from *Saccocalyx satureioides* Coss. and Durieu by the cupric reducing antioxidant capacity test (CUPRAC). VIIth International Congress of Biotechnology and Valorization of Bio- Resources. 20-23, March, 2019, Tabarka. Tunisia.

Mehlous, S., Benchikh, F., Benabdallah, H., Loucif, K.& Amira, S. Comparison of antioxidant activity of aqueous and methanol extract from *Saccocalyx satureioides* Coss. & and Durieu aqueous extract. The 30th International Congress of the Tunisian Society of Biological Sciences (ATSB). 25-28, March, 2019, Sousse. Tunisia.

Mehlous, S., Benchikh, F., Benabdallah, H & Amia, S. Assessment of antioxidant activity of the methanol extract from *Achillea sallantoiris* using ABTS test. Journée d'étude Zone Humides et Changements Climatiques,1ère édition, 25 février, 2020, Souk Ahras.

Mehlous, S., Benchikh, F., Benabdallah, H., Loucif, K & Amira, S. Antiulcer activity of the aqueous extract from *Saccocalyx satureioides* Coss & Durieu. areal parts.Bio-ressources: Siminaire national : Nutrition, santé et environnement, 17 et 18 Mai 2021, M'sila.

ملخص

تعتبر كلا من *Achillea santolinoides* L. (*A. santolinoides* L.) و *Saccocalyx satureioides* Coss and Dur (*S. Satureioides*) نبتتان مهمتان من النباتات الجزائرية. كان الهدف من الدراسة الحالية تقييم محتوى عديدات الفينول، الفلافونويدات والدباغ ودراسة النشاطية المضادة للأكسدة في المختبر للمستخلص المائي، الميثانولي والهيدروميثانولي لكل من *S. satureioides* (SME، SAE، SHME) و*A. santolinoides* L. (AAE،AME ، AHM) ودراسة التحليل الكيميائي لـمستخلصات SAE، SME، AAE وAME، بالإضافة إلى الإفراغ المعدي، العبور المعوي والتأثير الوقائي المعدي للمستخلصين SAE وAAE. أظهر التحليل الكيميائي النباتي لـ *S. Satureioides* أن SAE وSME يحتويان على أحماض فينولية مشتركة بين المستخلصين (lutéoline-7-O-rutinoside, lutéoline-O-diglucuronide et lutéoline-7-O-glucuronide)، في حين كان SME أكثر احتواء على المركبات الكيميائية مقارنة ب SAE. يكشف التحليل الكيميائي النباتي لنبات *A. santalinoides* L. أن AME أغنى من AAE من حيث المركبات الفينولية. يحتوي AAE على apigening (apigenin-2 ‘-O-pentosyl-8-C-glucoside) وapigenin-*O*-glucuronide) وluteolin-7-*O*-rutinoside. يحتوي AME على apigenin أيضًا (apigenin-2 " -O-pentosyl-8-C-glucoside وapigenin-*O*-glucuronide)، و حمض Dicaffeoylquinic acid) 3,5- Dicaffeoylquinicacid و Dicaffeoylquinicacid-3،4). تنسب أعلى كمية من عديدات الفينول لكل من SME وAME، كما أنه في معظم الاختبارات، أظهرت النشاطية المضادة للأكسدة أفضل نشاط لكلا المستخلصين. تم تقييم تأثير مستخلصات SAE وAAE على الإفراغ المعدي والعبور المعوي لدى الفئران. وجد أن كلا المستخلصين أظهر تأثيرا معنويا في إفراغ المعدة وتأخر العبور المعوي. كان تأثير كلا المستخلصين عبر مسار NO وانزيمات الأكسدة الحلقية بالإضافة إلى تدخل المستقبلات المسكارينية بالنسبة لـمستخلص AAE. في حين لم تتدخل المستقبلات المسكارينية في تأثير SAE لإفراغ المعدة. أعطى كل منAAE و SAEعند الجرعات المختبرة (100 ،200 و400 مجم / كجم) حماية جيدة للغشاء المخاطي ضد تقرح المعدة المحدث بالإيثانول في الجرذان ويتدخل المسارالمسكاريني في التأثير المعدي لـكلا المستخلصين.

الكلمات المفتاحية: *S. satureioides*، *A. santolinoides* L.، مضادات الأكسدة، الدراسة الكيميائية، الإفراغ المعدي، العبورالمعوي، قرحة المعدة.

Résumé

Saccocalyx satureioides Coss and Dur (*S. satureioides*) et *Achillea santolinoides* L. (*A. santolinoides* L.) sont deux plantes importantes de la flore Algérienne. Le but de la présente étude était d'évaluer la teneur totale en phénols, flavonoïdes et tannins et l'activité antioxydante *in vitro* des extraits aqueux, méthanoliques et hydrométhanoliques de *S. satureioides* (SAE, SME et SHME) et d'*Achillea santolinoides* L. (AAE, AME et AHME), et d'établir le profil chimique des extraits SAE, SME, AAE et AME. En plus, la vidange gastrique, le transit intestinal et l'effet gastro-protecteur des extrais SAE et AAE ont été étudiés. L'analyse phytochimique de *S. satureioides* a révélé que SAE et SME contenaient plusieurs acides phénoliques communs aux deux extraits (lutéoline-7-*O*-rutinoside, lutéoline-*O*-diglucuronide et lutéoline-7-*O*-glucuronide). L'extrait SME était plus riche en composés phénoliques que le SAE. L'analyse phytochimique d'*A. santalinoides* L. révèle que l'AME est plus riche en composés phénoliques que l'AAE. L'AME contient de l'apigénine (apigénine-2"-*O*-pentosyl-8-C-glucoside et apigénine-*O*-glucuronide) et de lutéoline-*7*-*O*-rutinoside. L'AME contient aussi de l'apigénine (apigenin-2"-O-pentosyl-8-C-glucoside et apigenin-*O*-glucuronide) et l'acide dicaffeoylquinic (acide 3,5-dicaffeoylquinic et acide 3,4-dicaffeoylquinic). SME et AME présentaient la plus grande quantité de polyphénols. Dans la majorité des tests, la meilleure activité antioxydante a été montrée avec ces extraits. L'effet des extraits SAE et AAE sur la vidange gastrique et le transit intestinal a été étudié chez les souris. Il a été constaté que les deux extraits présentaient un effet significatif sur la vidange gastrique et le retard du transit intestinal. L'effet des deux extraits se fait *via* les voies NO et cyclooxygénase, en plus, les récepteurs muscariniques sont impliqués avec l'extrait AAE, sauf que les récepteurs muscariniques ne sont pas impliqués en présence de l'extrait SAE. SAE et AAE aux doses testées (100, 200 et 400 mg/kg) ont offert une grande protection de la muqueuse contre l'ulcération gastrique induite par l'éthanol chez le rat. La voie muscarinique est impliquée dans l'effet gastroprotecteur des deux extraits.

Mots clés: *S. satureioides*, *A. santolinoides* L., activité antioxydante, analyse phytochimique, vidange gastrique, transit intestinal, ulcère gastrique.

Abstract

Saccocalyx satureioides Coss and Dur (*S. satureioides*) and *Achillea santolinoides* L. (*A. antolinoides* L.) are two important plants of Algerian flora. The aim of the present study is to assess total phenolic, flavonoids and tannins content and the *in vitro* antioxidant activity of the aqueous, methanol and hydromethnol extracts of *S. satureioides* (SAE, SME and SHME) and *A. santolinoides* L. (AAE, AME and AHME), and to establish the chemical profile of SAE, SME, AAE and AME. In addition, to the gastric emptying, intestinal transit and the gastro-protective effect of SAE and AAE extracts were studied. The phytochemical analysis of *S. satureioides* revealed that SAE and SME contained common phenolic acids between the two extracts (luteolin-7-*O*-rutinoside, luteolin-*O*-diglucuronide and luteolin-7-*O*-glucuronide). SME extract was richer in phenolic compounds than SAE. The phytochemical analysis of *A. santalinoides* L. revealed that AME is richer in compounds than AAE. AAE contains apigenin (apigenin-2''-*O*-pentosyl-8-C-glucoside and apigenin-*O*-glucuronide) and luteolin-7-*O*-rutinoside. AAE Also contains apigenin (apigenin-2''-O-pentosyl-8-C-glucoside and apigenin-*O*-glucuronide) and of dicaffeoylquinic acid (3,5-dicaffeoylquinic acid and 3,4-dicaffeoylquinic acid). SME and AME had the highest amount of polyphenols. In the majority of the used tests, the extracts showed the best antioxidant activity. The effect of SAE and AAE on gastric emptying and intestinal transit were studied in mice. It was found that both extracts induced a significant delay in gastric emptying and in intestinal transit. The effect of both extracts are *via* the NO and the cyclooxygenase pathways, in addition to the involvement of muscarinic receptors for the effect of AAE. In contrary, the muscarinic receptors are not involved in gastric emptying in the presence of SAE. SAE and AAE at the tested doses (100, 200 and 400 mg/kg) offered a significant protection of the mucosa against ethanol-induced gastric ulceration in rats. Muscarinic pathway is involved in the gastroprotective effect of both extracts.

Keywords: *S. satureioides*, *A. santolinoides* L., antioxidants activities, phytochemical analysis, gastric emptying, intestinal transit, gastric ulcer.

List of abbreviations

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

AD: Alzheimer's disease

$AlCl_3$: Aluminium tri-chloride

ATP: Adenosine triphosphate

BHT: Butyl hydroxyl toluene

CAT: Catalase

CMC: Carboxymethyl cellulose

CM: Circular muscle

CNS: Central nervous system

CT: Condensed tannins

CUPRAC: Cupric ion reducing antioxidant capacity

DA: Dopaminergic

DMSO: Dimethyl sulfoxide

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

DNA: Deoxyribonucleic acid

DRG: Dorsal root ganglia

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

DW: Dried weight

ECL: Enterochromaffin-like cells

EDTA: Ethylene diamine tetra acetic acid

ENS: Enteric nervous system

FAD: Flavin adenine dinucleotide

FMN: Flavin mononucleotide

GI: Gastrointestinal

GMD: Gastrointestinal motility disorders

GPx: Glutathione peroxidase

GR: GSH reductase

GSH: Glutathione

***H.pylori*:** *Helicobacter pylori*

HT: Hydrolysable tannins

H_2O_2: Hydrogen peroxide

LDL: Low-density lipoprotein

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

MAO-B: Monoamine oxidase-B

MDA: Malondialdehyde

MMC: Migrating myoelectrical complex

NADPH: Nicotinamide adenine dinucleotide phosphate

NO: Nitric oxide

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

$NO_2^{\bullet}$: Nitrogen dioxide

NOS: Nitric oxide synthase

NSAIDs: Non-steroidal anti-inflammatory drugs

$^1O_2$: Singlet oxygen

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

O_3: Ozone

$OH^{\bullet}$: Hydroxyl radical

PD: Parkinson's disease

PUFA: Polyunsaturated fatty acids

PGs: Prostaglandins

PKC: Protein kinase C

RNS: Reactive nitrogen species

ROS: Reactive oxygen species

RS: Reactive species

RT: Retention time

SMP: Submucosal plexus

SNc: Substantia nigra pars compacta

SOD: Superoxide dismutase

SP: P Substance

TBA: Thiobarbituric acid

TCA: Trichloroacetic acid

TFC: Total flavonoid content

TNB: 2-Nitro-5-thiobenzoic acid

TPC: Total phenolics content

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

UV: Ultraviolet

VIP: Vasoactive intestinal peptide

WHO: World Health Organization

XO: Xanthine oxidase

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Introduction

Oxidative stress (OS) is defined as a state of imbalance between excessive oxidant (free) radicals and insufficient degradation of those radicals by antioxidant systems as an in-house defense mechanism. Oxidant compounds such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) are formed under physiological conditions and are removed by several antioxidant defense mechanisms (Daenen *et al*., 2019).

The free radicals induced oxidative stress has been reported to be involved in several diseased conditions such as diabetes mellitus, neurodegenerative disorders (Parkinson's disease-PD and Alzheimer's disease-AD), cardiovascular diseases (atherosclerosis and hypertension), respiratory diseases (asthma), rheumatoid arthritis and in various cancers (Phaniendra *et al.,* 2015; Tan *et al.,* 2018).

The body is equipped to defend against the harmful effects of ROS with the help of antioxidants, collectively called the antioxidant defense system which comprises both enzymatic and nonenzymatic mechanisms. Antioxidants remove free radicals from the system and inhibit oxidation by being oxidized themselves. Dietary intake is another very important source of antioxidants and points to the potential effects of malnutrition or malabsorption of nutrients on the regulation of these mediators (Bhattacharyya *et al*., 2014).

Synthetic chemical antioxidants have been challenged in recent years for their potential harm. As a result, recent research has looked into the potential of plant compounds to act as antioxidants and protect against diseases caused by free radicals. Plant products including phenolics, flavonoids, tannins, proanthocyanidins, and numerous plant or herbal extracts, have been shown to be radical scavengers (Hou *et al*., 2003).

Plants have been widely used for healing diverse diseases, since ancient times. Plants produce an important provenance of efficient natural products which vary vastly in chemical structures, mechanism of actions and biological properties (Farag *et al*., 2020).

In recent times, interest in the application of plant extracts in the food industry has continued to grow, mainly because of their antioxidant properties and associated health-promoting effects for humans (Tajner-Czopek *et al*., 2020), antioxidants are highly able to retard or prevent oxidation of main substances through free radical scavenging (Farag *et al*., 2020).

Polyphenols are secondary metabolites produced by almost every part of plants, including fruits, flowers, leaves and bark. Many common fruits (such as grapes, cherries, apples, pomegranate, oranges), herbs and spices are very rich source of polyphenols. These compounds have potent anti-inflammatory and antioxidant properties. The antioxidants properties of polyphenols depend on polyphenol's ability to scavenge ROS molecules, inhibit

the expression of prooxidant genes and increase the expression of antioxidant genes such as superoxide dismutase (SODs) and catalases (CAT) (Ansari *et al*., 2020).

It has been confirmed by WHO that herbal medicines serve the health needs about 80% of the world's population, especially for millions of people in the vast rural areas of developing countries (Agisho *et al*., 2014). Gastrointestinal motility disorders (GMD) are highly prevalent and responsible for long-term morbidity and sometimes mortality in the affected patients (Wang *et al*., 2017a).

Recently, medicinal plants have attracted attention to treat GI such as digestion and constipation, since there is an increasing evidence that multiple constituents found in medicinal plants have the synergistical potential. Medicinal plants are considered relatively safe and effective in prolonged use, especially in patients with chronic gut motility disorders (Wang *et al*., 2020). Algeria boasts a diverse collection of medicinal plants that are utilized by the local community and traditional healers to treat a variety of ailments (Mouderas *et al*., 2020) and it is one of the richest Arab countries with 3164 plant species (Benarba *et al*., 2016). *Saccocalyx satureioides* Coss and Dur is a tiny aromatic shrub found in the septentrional Sahara region of Algeria. Due to its ancient medical use for stomach issues and spasms, it has gotten a lot of attention (Kherkhache *et al*., 2020), locally known as Zaatar or Zaater, Zaater Armel, and Azir El-Ibel, it has a thyme-like aroma (Souadia *et al*., 2020). The genus *Achillea* comprises many species known for their therapeutic benefits (Fahed *et al*., 2016; Farhang Sardrodi,*et al*., 2017). *Achillea* species are currently known to exhibit a wide range of pharmacological properties including antioxidant, antimicrobial, antibacterial, anti-inflammatory, antispasmodic, diaphoretic and diuretic activities (Mohammadi *et al*., 2021). Thus, the aims of this study are:

- Determination of polyphenols, flavonoids and tannins content in different plant extracts.

- Phytochemical analysis of the plant extracts by UHPLC-MS to identify bioactive compounds in both plants.

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

- Evaluation of the *in vivo* acute oral toxicity of the aqueous extract of the two plants.

-To study the effect of the aqueous extract of *S. satureioides* and *A. santolinoides* L.

on:

* GI motility in mice and the mechanisms involved.

* Ethanol-induced gastric ulceration in rats and the mechanisms involved.

Literature review

1. Oxidative stress

1.1. Definition of oxidative stress

Oxidative stress was defined as the lack of balance between the occurrence of reactive oxygen/nitrogen species (ROS/RNS) and the organism's capacity to counteract their action by the antioxidative protection systems. Oxidative stress emerges from an enhanced ROS/RNS generation or from a decrease of the antioxidant protective ability, being characterized by the reduced capacity of endogenous systems to fight against the oxidative attack directed towards target biomolecules (Pisoschi and Pop, 2015).

1.2. Reactive species (ROS and RNS) or free radicals

Free radicals are highly reactive atoms or molecules with one or more unpaired electron (s) in their external shell and can be formed when oxygen interacts with certain molecules. These radicals can be produced in cells by losing or accepting a single electron, therefore, behaving as oxidants or reductants (Liguori *et al*., 2018). ROS and RNS are free radicals, which are associated with the oxygen atom (O) or their equivalents and have stronger reactivity with other molecules, rather than with O_2·. Generally, ROS/RNS are generated as by-products of cellular metabolism and ionizing radiation (Table 1) (Kurutas, 2015).

Table 1: List of ROS and RNS (Phaniendra *et al*., 2015).

Reactive species	**Radicals**	**Non radicals**
ROS	Superoxide $O2^{\bullet -}$	Hydrogen peroxide H_2O_2
	Hydroxyl $OH^{\bullet}$	Singlet oxygen $^1O_2$
	Alkoxyl radical $RO^{\bullet}$	Ozone O_3
	Peroxyl Radical $ROO^{\bullet}$	Organic peroxide ROOH
		Hypochlorous acid HOCl
RNS	Nitric oxide $NO^{\bullet}$	Peroxynitrite $ONOO^-$
	Nitrogen dioxide $NO_2^{\bullet}$	Nitrosyl cation NO^+
		Nitroxyl anion NO^-
		Dinitrogen trioxide N_2O_3

Dinitrogen tetraoxide N_2O_4

Nitrous acid HNO_2

Peroxynitrous acid ONOOH

1.3. Source of free radicals

The sources for the generation of ROS can be mainly divided into endogenous, which include biological processes that release ROS as by-products, such as the mitochondrial electron transport chain, the endoplasmic reticulum, microsomes and peroxisomes, membrane-bound NADPH oxidase (NOX) family enzymes, and nitric oxide synthases and exogenous, represented by the cellular processes as responses to bacterial invasions, cytokines, and xenobiotics, due to oxidative burst activity in macrophages (Teleanu *et al*., 2019), cigarette smoke, environmental pollutants, radiation, certain drugs, pesticides, industrial solvents (Figure 1) (Fahad and Mohammed, 2020).

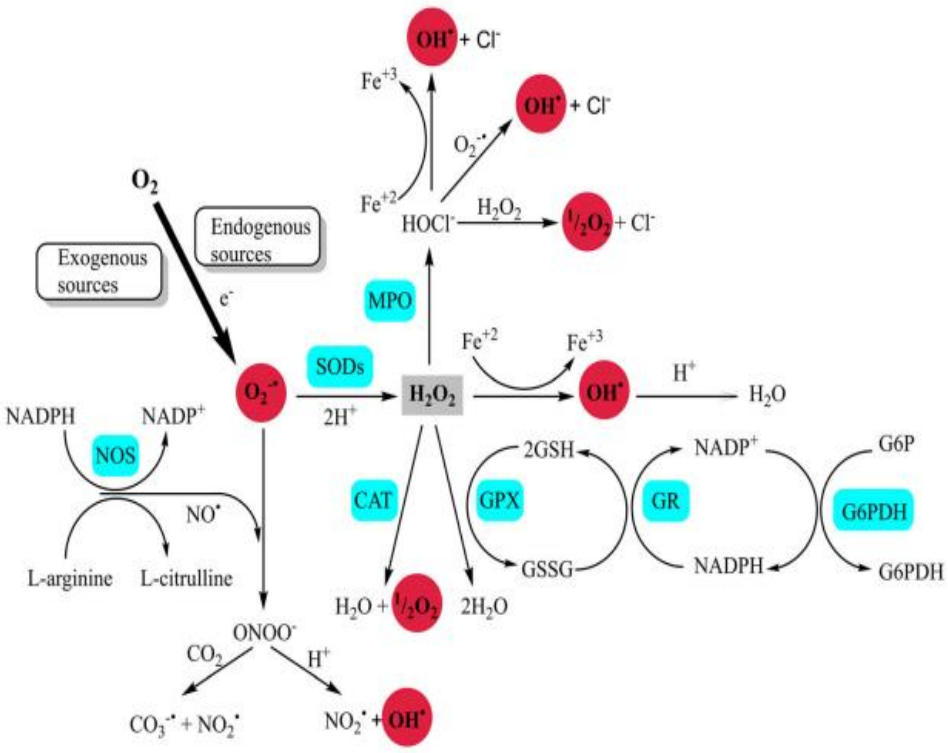


Figure 1: Generation of reactive species (Niedzielska *et al*., 2016).

1.3.1. Endogenous sources

a. ROS generation in the mitochondrial respiratory chain

One of the main functions of the mitochondria is oxidative ATP production, in which oxygen (O_2) is reduced to water, and consequently the major source of intracellular ROS generation is the mitochondrial respiratory chain. During respiration, electrons released from the mitochondrial electron transport chain incompletely reduce O_2 to form supeoxide. Superoxide is converted into H_2O_2 by manganese superoxide dismutase in the mitochondrial matrix (Bae *et al*., 2011).

b. NADPH oxidases

Phagocytic leukocytes play major roles in the innate immune response to pathogens. An important component of this response is the ability of leukocytes to generate ROS via a membrane-associated NADPH oxidase (Nox) (Bokoch and Knaus, 2003). NADPH oxidases are a family of enzyme complexes whose primary function is to catalyze the transfer of electrons from NADPH to molecular oxygen via their "Nox" catalytic subunit, generating $O2^{\bullet\ -}$ and H_2O_2 (Dikalov, 2011). The Nox system of stimulated phagocytic leukocytes catalyzes the one-electron reduction of oxygen to produce superoxide anion using NADPH as substrate (Bokoch and Knaus, 2003).

c. Xanthine oxidase (XO)

Xanthine oxidase (XO) is a flavoprotein enzyme that is widely distributed in various mammalian tissues (Li *et al*., 2004). XO is a homodimer with molecule mass of 290 kDa. Xanthine oxidase belongs to the molybdenum-protein family containing one molybdenum, one of the flavin adenine dinucleotides (FAD), and two iron-sulfur (2Fe-2S) centers of the ferredoxin type in each of its two independent subunits; the enzyme contains two separated substrate-binding sites. XO catalysed the oxidation of hypoxanthine to xanthine and subsequently to uric acid. During the reoxidation of XO, molecular oxygen acts as electron acceptor, producing superoxide radical and hydrogen peroxide. During these reactions, superoxide anion radicals ($O2^{\cdot\ -}$) and H_2O_2 are formed. These reactions can be written as follows:

hypoxanthine + O_2 + H_2O → xanthine + H_2O_2

xanthine + $2O_2$ + H_2O → uric acid + $2O_2^{\cdot\ -}$ + $2H^+$

xanthine + O_2 + H_2O → uric acid + H_2O_2

$2O_2^{\cdot -} + 2H^+ \rightarrow H_2O_2 + O_2$ (Kostić *et al*., 2015)

d. Nitric oxide synthase (NOS)

Nitric oxide (NO) is biologically generated by a family of three nitric oxide synthase enzymes (NOS) isoforms: neuronal nitric oxide synthase (nNOS or NOS1), inducible nitric oxide synthase (iNOS or NOS2), and endothelial nitric oxide synthase (eNOS or NOS3) (Costa *et al*., 2016). All three NOS isoforms, eNOS, nNOS, and iNOS, are self-sufficient enzymes with two major functional domains fused into a single polypeptide. The N-terminal catalytic domain binds the hemeprosthetic group as well as the redox cofactor, tetrahydrobiopterin (H4B), the C-terminal reductase domain has the binding sites for FMN, FAD, and NADPH. NOS produces NO from L-arginine (L-Arg) in the presence of O_2 and NADPH (Li and Poulos, 2005), while, the induction of inducible nitric oxide synthase (iNOS) produces excessive NO accompanied by increased production of reactive oxygen species (ROS), including peroxynitrite ($OONO^-$) and superoxide (Yu *et al*., 2018).

e. Lipoxygenases

The lipoxygenases are nonheme, iron-containing enzymes that catalyze the stereospecific incorporation of molecular oxygen into polyunsaturated fatty acids. Certain lipoxygenase isoforms are thought to promote atherosclerosis by generating ROS and oxidatively modified lipids, such as oxidized low-density lipoprotein (ox-LDL). The 12/15-lipoxygenase (12/15-LO; also known as the leukocyte-type 12-lipoxygenase and 15-lipoxygenase-1) and 5-lipoxygenase (5-LO) are most studied because of their expression pattern in inflammatory cells and endothelial cells. Lipoxygenases are the key enzymes in the biosynthesis of leukotrienes, which play an important pathophysiological role in inflammatory diseases. ROS are generated as a by-product during the biosynthesis step of leukotrienes by lipoxygenases (Sugamura and Keaney, 2011).

1.3.2. Exogenous sources

Many factors have been linked with the increasing of risks that arise from oxidative stress such as lifestyle, cigarette smoking, drugs, exposure of chemicals, stress, illness, pollution, and many other factors (Fahad and Mohammed, 2020).

a. Ionizing radiation

Ionizing radiation, in the presence of O_2, converts hydroxyl radical, superoxide, and organic radicals to hydrogen peroxide and organic hydroperoxides. These hydroperoxide species react with redox active metal ions, and thus induce oxidative stress (Birben *et al*., 2012; Madkour, 2019).

b. Cigarette smoke

Cigarette smoke is a complex mixture of numerous free radicals and ROS that can be divided into two phases: tar (particle) and gas. Tar phase covers about 1017 relatively long-lived radical molecules per gram, for example, quinone/hydroquinone (Q/QH2) radicals that can reduce oxygen to produce superoxide anion ($O2^{\bullet -}$) leading the generation of H_2O_2 and hydroxyl radical ($OH^{\bullet}$). Gas phase of cigarette smoke contains much more reactive molecules than tar. This phase consists of 1015 organic and inorganic radicals per puff, for example, nitric oxide (NO•), nitrogen dioxide, and peroxynitrite ($ONOO^-$) (Boukhenouna *et al*., 2018).

c. Pollution

Many environmental pollutants are sources of several reactive species (RS). Environmental pollutants stimulate a variety of mechanisms of toxicity on molecular level and oxidative stress seems to be the common denominator leading to the damage to cellular membrane lipids, DNA, and proteins, as well as modulation of antioxidant enzymes. RS are, due to their high reactivity (e.g., hydroxyl radical formation), prone to cause damage to any type of molecule within the cell, for example, polyunsaturated fatty acids, glutathione, certain amino acids, and so forth (Poljšak and Fink, 2014).

d. Lifestyle

The modern lifestyle associated with an unhealthy diet, lack of physical exercise, exposure to a combination of chemicals from different sources of pesticides. Heavy metals, food additives, alcohol and drugs can influence the appearance of oxidative stress and it can contribute to the increasing burden of chronic diseases (Sharifi-Rad *et al*., 2020).

1.4. Effect of oxidative stress

Free radicals are able to oxidize biomolecules and may cause protein oxidation, DNA damage, and lipid peroxidation in living cells leading to mutagenic changes, tissue damage and cell death (Sen *et al.*, 2013).

a. Effect of oxidative stress on DNA

ROS can lead to DNA modifications in several ways, which involve degradation of bases, single or double stranded DNA breaks, purine, pyrimidine or sugar-bound modifications, mutations, deletions or translocations, and cross-linking with proteins. Most of these DNA modifications are highly relevant to carcinogenesis, aging, and neurodegenerative, cardiovascular, and autoimmune diseases (Birben *et al*., 2012; Al-Dalaen and Al-Qtaitat, 2014). Formation of 8-OH-G is the best-known DNA damage occurring via oxidative stress and is a potential biomarker for carcinogenesis. Formation of 8-OH-G DNA in transcription factor binding sites can modify binding of transcription factors and thus change the expression of related genes (Birben *et al*., 2012).

b. Effect 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 degradation by specific proteases. Cysteine and methionine residues in proteins are particularly more susceptible to oxidation. Oxidation of sulfhydryl groups or methionine residues of proteins cause conformational changes, protein unfolding and degradation. Enzymes that have metals on or close to their active sites are especially more sensitive to metal catalyzed oxidation. Oxidative modification of enzymes has been shown to inhibit their activities (Ozougwu, 2016).

c. Effects of oxidative stress on lipids

The hydroxyl radical plays an important role in the reactions of lipid peroxidation. It oxidizes lipids containing carbon-carbon double bonds, especially polyunsaturated fatty acids (PUFAs). In PUFAs, the –C=C– units are separated by a single-bonded –C– atom. The hydrogen atoms attached to these –C– atoms (bis-allylic hydrogens) are susceptible to attack by hydroxyl radical, various bioactive aldehydes, such as MDA, 4-hydroxynonenal (4-HNE), and acrolein, are generated by free radical-mediated lipid peroxidation of PUFA, including arachidonic and linoleic acid, all these aldehydes have been found to play a role in the toxic effects of lipid peroxidation. Aldehyde toxicity is based on the alterations of several cell functions, which mostly depend on the formation of covalent adducts with cellular proteins (Ito *et al*., 2019).

1.5. Oxidative stress and pathologies associated with free radicals

a. Cardiovascular disease

During the last years, research data pointed out that oxidative stress should be considered either a primary or a secondary cause for many Cardiovascular disease. Oxidative stress acts mainly as a trigger of atherosclerosis. It is well known that atheromatous plaque formation results from an early endothelial inflammation, which in turn leads to ROS generation by macrophages recruited in situ. Circulating LDL are then oxidized by ROS, thus leading to foam cell formation and lipid accumulation. The result of these events is the formation of an atherosclerotic plaque. Both *in vivo* and *ex vivo* studies provided evidence supporting the role of oxidative stress in atherosclerosis, ischemia, hypertension, cardiomyopathy, cardiac hypertrophy, and congestive heart failure (Pizzino *et al*., 2017).

b. Aging

The age-related redox imbalance is more likely triggered by the net effect of low antioxidative defense systems and incessantly produce of reactive species (Tan *et al*., 2018).

c. Neurodegenerative diseases

Neurodegenerative diseases, as a heterogeneous group of disorders, are characterized by slowly progressive losses of neurons; oxidative stress has been suggested as one of the potential common etiology in various neurodegenerative diseases. Cumulative oxidative stress may induce cellular damage, impairment of the DNA repair system, and mitochondrial dysfunction, all of which have been known as key factors in acceleration of aging process and the development of neurodegenerative disorders. Parkinson's disease (PD) is a neurodegenerative disorder characterized by selective neuronal loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNc) and decreased DA levels in the nigrostriatal DA pathway in the brain. Although the exact mechanism still remains unclear, oxidative stress has been considered as one of major pathophysiological mechanisms underlying PD. Previous studies have found the reduced activity in complex I of the respiratory chain in SNc of patients with PD, which may contribute to the generation of excessive ROS and, in turn, induce apoptosis. Changes in the antioxidant molecules have also been reported even in the early stage of PD. For example, the levels of glutathione, a major antioxidant molecule, have been reduced in the SNc of PD. Although this finding is not specific for PD, it has also been reported that iron levels were increased in DA neurons in PD,

which may allow easier interaction of ferrous iron with H_2O_2 and enhance production of highly toxic hydroxyl radicals (OH). High levels of iron in the SNc, therefore, may potentially exert harmful effects on the survivals of DA neurons (Kim *et al*., 2015).

d. Diabetes

Diabetes mellitus associated with the increased production of free radicals or decreased activity of the antioxidant systems, which leads to development of oxidative stress (Phaniendra *et al*., 2015). In the presence of uncontrolled hyperglycemia excess generation of free radicals occurs due to autoxidation of glucose and glycosylation of proteins, the marked increase in ROS accompanied by a decrease in antioxidant activity causes the structural deterioration of macromolecules (carbohydrates, proteins, lipids, and DNA) leading to their instability and consequently loss of function (Fatani *et al*., 2016).

e. Inflammatory diseases

The imbalance between the oxidative species' activity, and the antioxidant defence, is involved in asthma and allergic rhinitis. The enhanced occurrence of hydroxyl radicals, superoxide radical anions and peroxides, may initiate a series of alterations in nasal and airway mucus as: lipid peroxidation, marked airway reactivity, nasal mucosal sensitivity and secretions, as well as generation of chemo attractant molecules and high vascular permeability (Owoade *et al*., 2019). The enhanced occurrence of hydroxyl radicals, superoxide radical anions and peroxides, may initiate a series of alterations in nasal and airway mucosas: lipid peroxidation, marked airway reactivity, nasal mucosal sensitivity and secretions, as well as generation of chemoattractant molecules and high vascular permeability, it has been noticed that reactive species and antioxidants influence the immune system. Oxidative stress causes disruption in cell signaling and impairs arachidonic acid metabolism, enhances airway and systemic inflammation (Pisoschi and Pop, 2015).

1.6. Antioxidants

Antioxidants are chemical molecules responsible for protecting the body against oxidative stress by preventing or reducing the oxidation process of macromolecules. Specifically, the toxicity of ROS/RNS is counteracted by the action of a diverse group of bioactive molecules that inhibit oxidation reactions by being oxidized themselves. Antioxidants can be classified based on several factors, namely their source, i.e., natural and synthetic antioxidants, their solubility, i.e., oil-soluble and water-soluble antioxidants, and

their mechanism of action, i.e., primary or radical scavengers, secondary or peroxide decomposers, and metal deactivators. However, antioxidants are generally categorized into endogenous compounds and exogenous (Figure 2) (Teleanu *et al*., 2019).

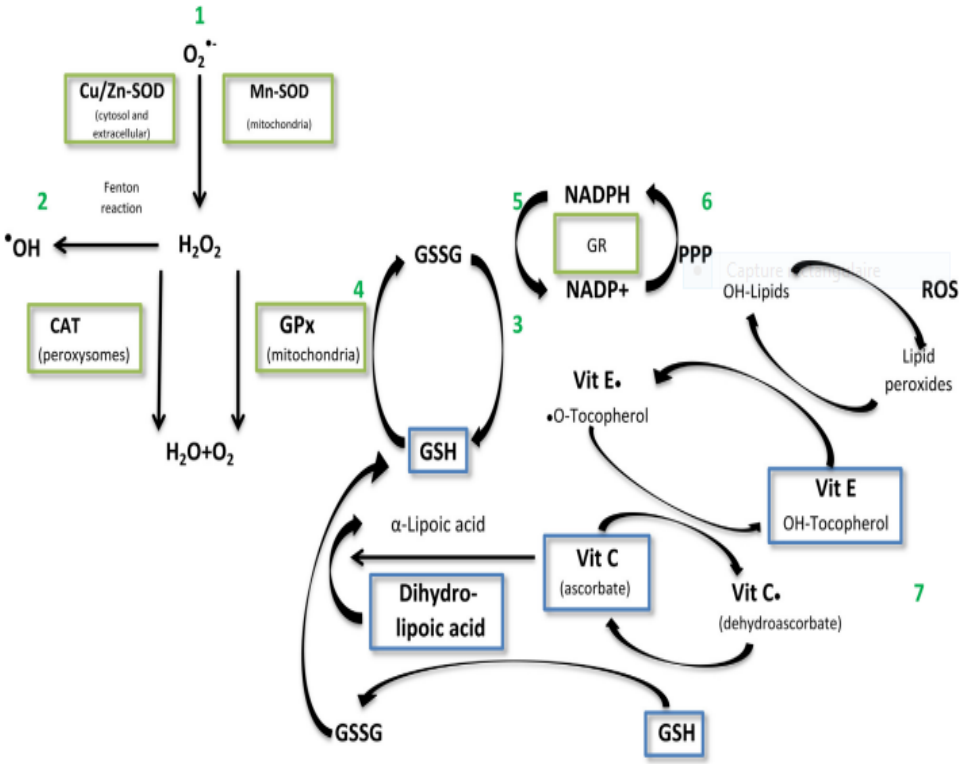


Figure 2: The mechanism of antioxidant protection (Mirończuk-Chodakowska e*t al*., 2018).

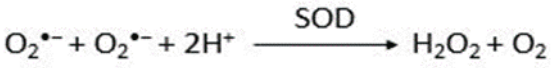
1.6.1. Antioxidant enzymes

Cellular antioxidant enzymes are the primary defense against reactive oxygen species, with the most important enzymes being SOD, CAT, glutathione peroxidases, glutathione reductase. The major function of antioxidant enzymes is averting reactive oxygen radical-induced tissue damage by preventing formation of reactive oxygen radical species or by scavenging the highly reactive oxygen radical species and neutralizing them to inactive compounds (Hrycay and Bandiera, 2015).

a- Superoxide dismutase

SOD destroys the free radical superoxide by converting it to peroxide that can in turn be destroyed by CAT or GPx reactions. A low level of superoxide is constantly generated by aerobic respiration. The electron-transport chain of mitochondria, which is meant to escort four electrons to molecular oxygen to form water, occasionally leaks a single electron. Superoxide reduces Fe^{+3} to Fe^{+2}, releasing the iron from storage sites so that it can react with

hydrogen peroxide and produce hydroxyl radicals. SOD converts superoxide to hydrogen peroxide and molecular oxygen (Mates, 2000).



b- Catalase

CAT is a very important enzyme of all living organisms which catalyzes the decomposition of hydrogen peroxide to water and oxygen (Asaduzzaman Khan *et al*. 2010). CAT is a 240 kilodaltons tetrameric protein with four similar subunits. CAT is a common antioxidant enzyme present almost in all living tissues that utilize oxygen. The enzyme uses either iron or manganese as a cofactor and catalyzes the degradation or reduction of hydrogen peroxide (H_2O_2) to water and molecular oxygen. CAT is highly efficient; it can breaks down millions of hydrogen peroxide molecules in one second. The enzyme is located primarily in the peroxisomes (Ighodaro and Akinloye, 2018)

c- Glutathione peroxidase (GSH-Pxs)

Enzymes in the redox cycle responsible for the reduction of H_2O_2 and lipid hydroperoxides (generated as a result of membrane lipid peroxidation) include the GSH-Pxs. The GSH-Pxs are a family of tetrameric enzymes that contain the unique amino acid selenocysteine within the active sites and use low-molecular-weight thiols, such as GSH, to reduce H_2O_2 and lipid peroxides to their corresponding alcohols. Four GSH-Pxs have been described, encoded by different genes: GSHPx-1 (cellular GSH-Px) is ubiquitous and reduces H_2O_2 and fatty acid peroxides, but not esterified peroxyl lipids (Birben *et al*., 2012).

1.6.2. Non enzymatic antioxidants

a. Glutathione (GSH)

Glutathione, the GST co-substrate is synthesized by two ATP dependent enzymatic steps in the cytosol and chloroplasts (Diaz-Vivancos *et al*., 2015). First, γ-glutamyl-cysteine is formed by the plastidic glutamate-cysteine ligase, also known as γ-glutamylcysteine synthetase (γ-ECS or GSH1), which is the rate-limiting reaction. Glutathione synthetase (GSH2) catalyzes the addition of glycine to γ-glutamyl-cysteine (Noctor *et al*., 2011). Both GSH_1 and GSH_2 genes respond to light and some stress conditions, such as drought, heavy

metals, and certain pathogens. GSH is able to control directly or indirectly the level of ROS; thus, it is considered to be one of the most important cellular antioxidants (Gallé *et al*., 2019).

b. Vitamin E

Vitamin E, fat-soluble antioxidants, is a family of α-, β-,γ-, and δ-tocopherols and corresponding four tocotrienols. The most active form of the vitamin E homologues is -tocopherol which protects cell membranes from oxidation by reacting with ROS and lipid radicals produced in the lipid peroxidation chain reaction (Al-Gubory *et al*., 2010).

c. Vitamin C

Ascorbic acid, generally known as vitamin C, is a widely studied antioxidant molecule which plays a crucial role as a premier substrate for the ROS detoxification. The regenerative behavior and the electron donating ability to different enzymatic and non-enzymatic reactions make ascorbic acid a powerful antioxidant. In addition, ascorbic acid keeps the ROS levels within an acceptable non-toxic limit (Zulfiqar and Ashraf, 2021). It was reported that ascorbic acid is an effective scavenger against $O2^{\cdot -}$, H_2O_2, $OH^{\cdot}$, $^1O_2$ and reactive $NO_2^{\cdot}$ (Gulcin, 2020).

d. Carotenoids

Carotenoids are a group of natural tetraterpenoid pigments distributed widely in plants, algae, fungi, and bacteria, they are responsible for the orange, yellow, and red colours found in many flowers, fruits, and roots. Carotenoids play essential roles in photosynthesis and photoprotection. They provide dietary sources of provitamin A and serve as antioxidants to reduce the onset of some chronic diseases, such as cardiovascular diseases, cancers, and age-related eye diseases (Sun *et al*., 2018).

e. Coenzyme Q10

Coenzyme Q10 is an essential cofactor for mitochondrial electron transport. It exists in oxidized (ubiquinone) and reduced (ubiquinol) forms. Reduced CoQ10 (ubiquinol) acts as a lipophilic antioxidant. Both forms are used as dietary supplements, but reduced CoQ10 (ubiquinol) has greater bioavailability compared to oxidized CoQ10 (ubiquinone) (Nakazawa *et al*., 2019). Coenzyme Q10 (CoQ10) is an endogenous lipid-soluble benzoquinone compound that functions as a diffusible electron carrier in the mitochondrial respiratory chain. CoQ10 acts as a powerful antioxidant that scavenges free radicals, prevents lipid peroxidation in cellular biomembranes, and promotes a-tocophrol regeneration (Yu *et al*., 2019).

f. Uric acid

Uric acid (UA) is one of the low molecular weight organic compounds, which is generated during the metabolism of purines. UA is a hydrophilic antioxidant, which accounts for two thirds of the total oxygen scavenging activity in the blood serum. UA is a scavenger of various ROS, for example peroxynitrite, hydroxyl radical, singlet oxygen and lipid peroxides. It can probably scavenge nitrogen dioxide and carbonate ions as well, and form stable complexes with iron ions and copper ions, leading to the inhibition of free radical reactions, such as the Fenton reaction and the Haber-Weiss reaction. In addition, UA contributes to the protection of such antioxidant enzymes as intracellular superoxide dismutase 1 (SOD1) and extracellular superoxide dismutase 3 (SOD3). Physiological UA levels can modulate activities of SOD1 and SOD3, by postponing inactivation of these enzymes by H_2O (Mirończuk-Chodakowska *et al*., 2018).

g. Poloyphenols

Polyphenols are natural compounds synthesized exclusively by plants, with chemical features related to phenolic substances and strong antioxidant properties. These molecules or classes of substances are mainly present in fruits, vegetables, green tea, and whole grains. In detail, polyphenols are a well-known group of phenolic systems characterized by at least two phenol rings and one or more hydroxyl substituents. Polyphenols can be simply classified into flavonoids and nonflavonoids or subdivided into many subclasses, depending on the number of phenol units within their molecular structure, substituent groups, and/or the linkage type between phenol units (Singla *et al*., 2019).

- **Flavonoids**

Flavonoids are a family of phenolic compounds with strong antioxidant activity present in fruits, vegetables, and other plant foods. More than 5000 distinct flavonoids have been identified, in plants, and several hundreds are known to occur in commonly consumed foods. Flavonoids comprise a large family of compounds synthesized by plants that have a common basic chemical structure. Structurally, flavonoids consist of 2 aromatic rings (A and B rings) linked by a 3-carbon chain that forms an oxygenated heterocyclic ring (C ring). Differences in the generic structure of the heterocyclic C ring, as well as the oxidation state and functional groups of the C ring, classify flavonoids as flavonols, flavones, flavanones, flavan-3-ols (flavans), and anthocyanins. The isoflavones are characterized by attachment of the B-ring at the 2-position instead of the 3-position. The proanthocyanidins are oligomers of

flavan-3-ols. Anthocyanidins are distinguished from other flavonoids as a separate class by virtue of their ability to form flavylium cations. Flavonols are the most widespread flavonoids in foods, and the most prominent flavonols in food are quercetin and kaempferol (Erdman Jr *et al*., 2007).

Many studies have shown that flavonoids exhibit biological and pharmacological activities, including antioxidant, cytotoxic, anticancer, antiviral, antibacterial, cardioprotective, hepatoprotective, neuroprotective, antimalarial, antileishmanial, antitrypanosomal and antiamebial properties. These biological and pharmacological properties are usually attributed to their free radical scavenging efficacies, metal complexion capabilities, and their ability to bind to proteins with a high degree of specificity (Alzand and Mohamed, 2012).

The antioxidant activities of flavonoids include scavenging; suppressing generation of ROS by inhibition of enzymes and chelating trace elements; and up regulating antioxidant defenses. The low redox potential of flavonoids enables the reduction of highly oxidized free radicals such as superoxide, alkoxyl, hydroxyl, and peroxyl radicals by proton donation. Flavonoids inhibit the enzymes such as xanthine oxidase and protein kinase C, which are responsible for the generation of superoxide anion. Flavonoids have also been reported to inhibit other ROS generating enzymes including cyclooxygenase (COX), microsomal monooxygenase, lipoxygenase, mitochondrial succinoxidase, and NADH oxidase. Therefore, the ability of flavonoids in chelating trace metals plays an important role in the oxygen metabolism (Tan *et al*., 2018).

- **Tannins**

Tannins are defined as phenolic compounds of high molecular weight ranging from 500 Da to more than 3000 Da which are found in plants leaves, bark, fruit, wood and roots located basically in the tissues in the vacuoles. Except of some higher molecular weight structures, tannins are soluble in water (20- 35°C). In the plant kingdom tannins are found in both flowering plants and non-flowering plants. Tannins are usually defined as water-soluble polyphenolic substances and have ability to bind to proteins that form insoluble or soluble tannin-protein complexes. As a consequence, tannins are able to make complex with polysaccharides (cellulose, hemicelluloses and pectin) and nucleic acids, steroids, alkaloids, and saponins. Tannins are divided into two main groups: hydrolysable (HT) and condensed

tannins (CT), the characteristics of the two groups are different in molecular weight and structure (Hassanpour *et al*., 2011).

a. Hydrolysable tannins

HT called also gallotannins are the simplest hydrolysable tannins, (Khanbabaee and Van Ree, 2001). HT are polyesters of a sugar moiety or other non-aromatic polyhydroxy compounds and organic acids (Bilić-Šobot *et al*., 2016).

b. Condensed tannins

CT are the most common type of tannins found in forage legumes, trees and stems. They consist of flavanoid units (flavan-3-ol) linked by carbon-carbon bonds. The complexity of CT depends on the flavanoid units which vary among constituents and within sites for interflavan bond formation.The term proanthocyanidins (PAs) is derived from the acid-catalyzed oxidation reaction producing red anthocyanidins upon heating PAs in acidic alcohol solutions (Hassanpour *et al.*, 2011).

- **Phenolic acids**

The term "phenolic acids" generally describes the phenolic compounds having one carboxylic acid group. Phenolic or phenolcarboxylic acids (a type of phytochemical called a polyphenol) are one of the main classes of plant phenolic compounds. They are found in the variety of plant based foods inseeds, skins of fruits and leaves of vegetables. Phenolic acids are mainly divided into two sub-groups: hydroxybenzoic and hydroxycinnamic acid. On the other hand, hydroxybenzoic acids possess a common structure of C6-C1 and derived from benzoic acid. They are found in soluble form (conjugated with sugars or organic acids) and bound with cell wall fractions as lignin (Kumar and Goel, 2019). Phenolic acids have gained momentum owing to their immense dietary health benefits and functionalities like antioxidant, anti-inflammatory, immunoregulatory, anti-allergeric, anti-atherogenic, anti-microbial, anti-thrombotic, cardioprotective, anti-cancer activities and anti- diabetic properties (Rashmi and Negi, 2020).

- **Lignans**

Lignans are a type of secondary plant metabolite exhibiting diverse structures. Biosynthesis of lignans is characterized by a remarkable increase in molecular complexity. Lignans share common biosynthetic pathways, consist of two propyl-benzene units coupled by β, β′–bond and thus belong to the group of dephenolic compounds. Lignans may be

organized into eight structural subgroups (according to the manner in which oxygen is incorporated and the pattern of cyclization): dibenzylbutyrolactol, dibenzocyclooctadiene, dibenzylbutyrolactone, dibenzylbutane, arylnaphthalene, aryltetralin, furan and furofuran (Figure 3). Each subgroup can be further subdivided according to lignan molecule oxidation level and identities of non-propyl aromatic rings present on side chains (Rodríguez-García *et al*., 2019). Lignans have been found in more than 70 plant families and an extensive range of localities within plants, from roots to leaves, seeds and flowers. Most importantly, this class of compound has exhibited several potent, significant, biological activities, including anticancer antimicrobial, antiviral, immunosuppressive, anti-inflammatory, antioxidant, and hepaprotective actions as well as cancer and osteoporosis prevention properties (Pilkington, 2018).

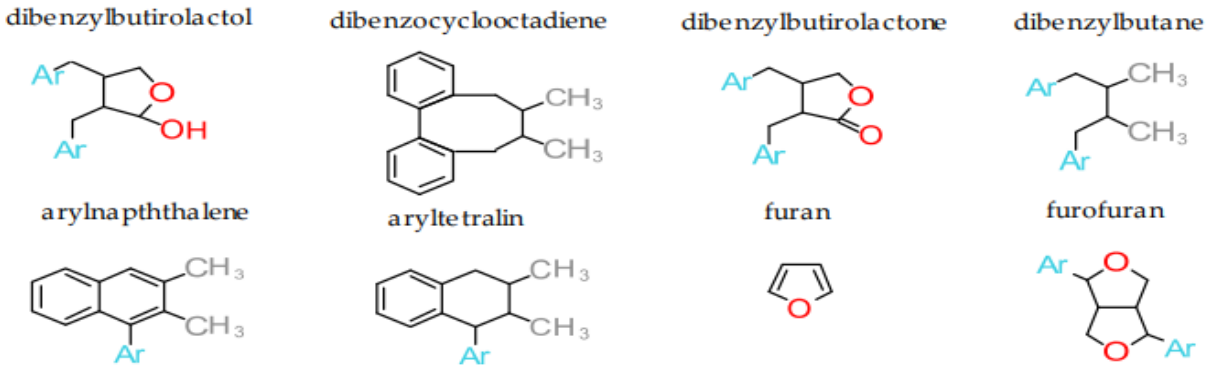


Figure 3: Structural subgroups of lignan (Ar = aryl) (Rodríguez-García *et al*., 2019).

- **Stilbenes**

Stilbenes refer to a large group of nonflavonoid polyphenols that consist of either trans- or cis-ethene double bond with phenol groups substituted on both ends (Peng *et al.*, 2018). Stilbenes are found in various families of plants. Some of these secondary metabolites have been recognized as phytoalexins and associated with the defense mechanisms of plants as they are produced after infection by pathogens or exposure to UV radiation and present antifungal activities. The stilbene structure is based on the C6–C2–C6 backbone, defined by two aromatic rings linked by an ethylene bridge. The most known and best characterized stilbene is resveratrol (3, 4'-5-trihydroxystilbene). More than 400 stilbene derivatives have been identified, their structures range from monomers to octamers and carry various substituents at different positions, like glycosyl, hydroxyl, methyl or isopropyl groups (Figure

4). The presence of stilbenes in the human diet is however limited to a few foods such as grapes, red wine, peanuts and some types of berries (El Khawand *et al*., 2018).

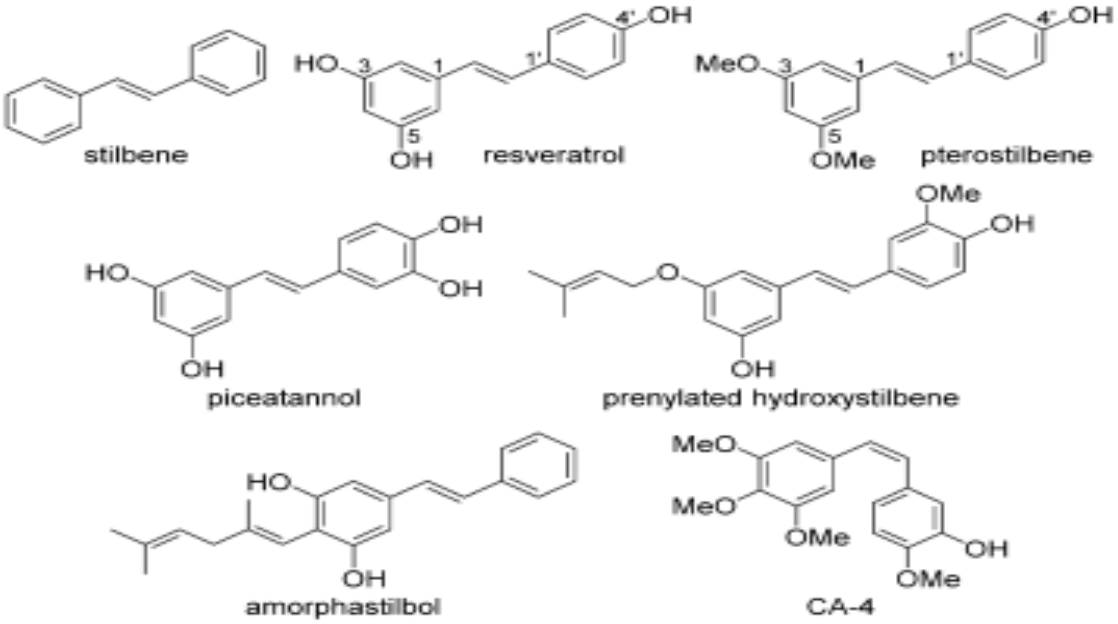


Figure 4: Stilbenes and various derivatives (De Filippis *et al*., 2017).

2. Digestive system

The digestive system includes the gastrointestinal (GI) tract (or alimentary canal) and the accessory organs of digestion including the salivary glands, liver, gallbladder, and exocrine pancreas. GI tract is one of the important parts of the body, it starts from the mouth, includes esophagus, stomach, small and large intestine, and rectum, and finally ends with anus. The human GI tract is a single tube which is approximately nine meters long in relaxed condition (Prasad *et al*., 2015).

GI tract is involved in a number of important tasks. It first and foremost ensures nutritional digestion and absorption. GI tract also acts as a barrier to the directly adjacent external environment, as it is covered in a protective milieu of molecules. GI system is one of the largest endocrine organs, and it regulates satiety and weight control (Urbanska *et al*., 2016). The entire tract is essentially a smooth muscle-enveloped tube with an innermost mucosa (comprising the epithelium, lamina propria, and muscularis mucosae), submucosa, tunica muscularis (also known as the muscularis propria), and a variable outer layer of serosa or adventitia (Figure 5) (Treuting *et al*., 2018).

The mucosa, the innermost layer of the gut wall, lines the entire GI tract and consists of epithelium, lamina propria, and muscularis mucosa. The mucosal epithelium is differentiated along the GI tract; tissue specialization correlates with the regional function of the tract. At the upper and lower ends of the GI tract (the mouth, esophagus, and anal canal) the mucosal epithelium is protective and composed of stratified squamous epithelial cells. On the other hand, the mucosal epithelium in the stomach, small intestine, and colon are composed of simple columnar or glandular epithelial cells. The cells in these regions secrete mucous, enzymes, and other biochemicals that either protect the mucosa or aid in digestion. The lamina propria and muscularis mucosa lie outside the mucosal epithelium. The lamina propria consists primarily of connective tissue, as well as blood and lymphatic vessels. The muscularis mucosa, is a thin layer of smooth muscle that is the boundary between the mucosa and the submucosa. Interstitial cells of Cajal (ICC) lie between and in close proximity in gap junctions between peripheral nerve endings and smooth muscle cells. The submucosa contains blood vessels, lymphatic vessels, submucosal glands, and Meissner's (submucosal) plexus (a nerve network that influences the smooth muscle of the muscularis). The muscularis externa has two smooth muscle layers: an inner circular layer and an outer longitudinal layer. Auerbach's (myenteric) plexus lies between the two layers, and this nerve network coordinates contractions of the layers resulting in rhythmic peristalsis. Adventia (also called serosa) is a connective tissue that has a surface of mesothelium, which is composed of simple squamous epithelial cell (Reed and Wickham, 2009).

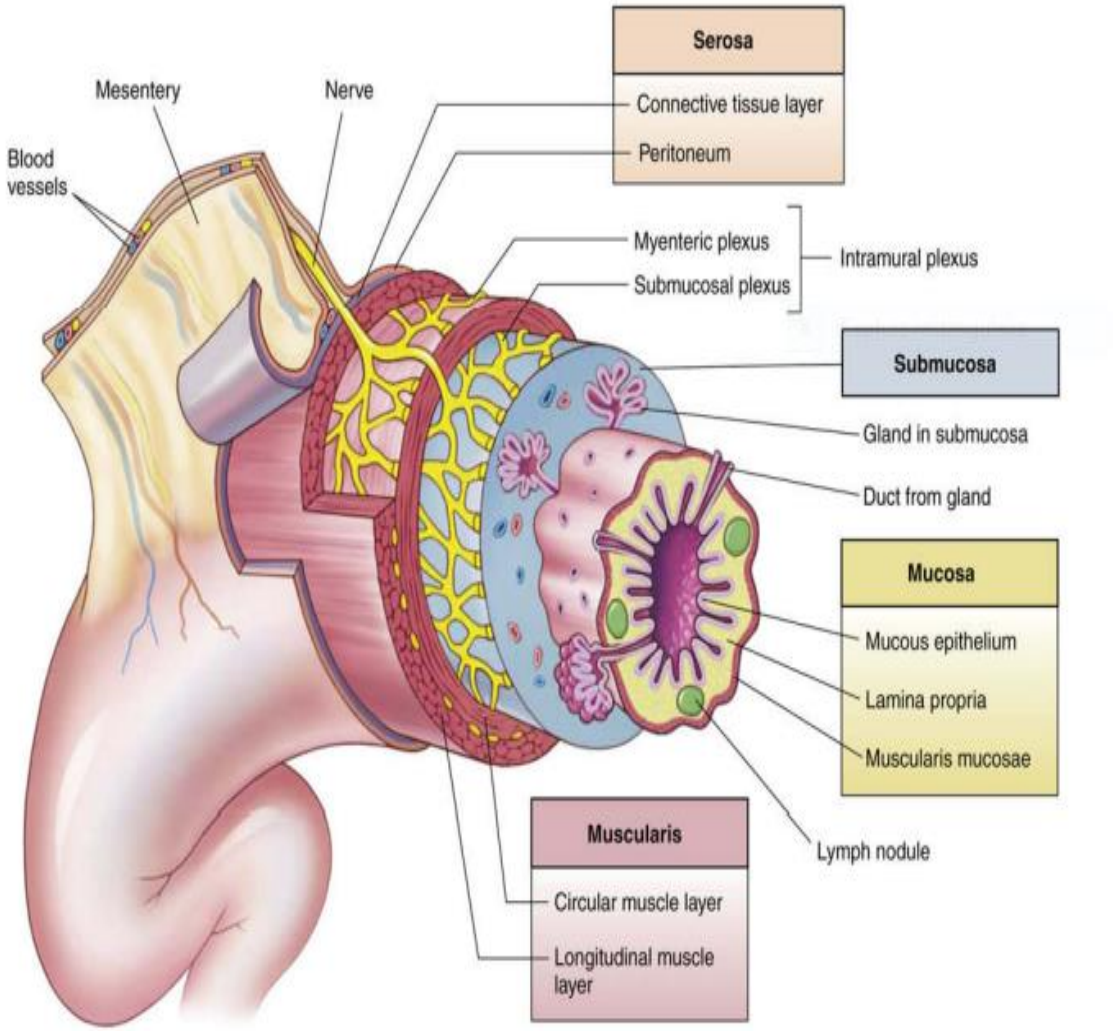


Figure 5: The wall of the GI tract is made up of four layers, shown here in a generalized diagram of a segment of the GI tract (Reed and Wickham, 2009).

2.1. Neural control of the GI tract

The GI tract is innervated by intrinsic neurons of the enteric nervous system (ENS) and by the axons of extrinsic sympathetic, parasympathetic, and visceral afferent neurons (Figure 6) (Phillips and Powley, 2007).

The gut has both an extrinsic and intrinsic innervations. The intrinsic innervation is provided by neurons of the enteric nervous system (ENS), which unlike other divisions of the peripheral nervous system, can regulate the function of its target organ without imput from the central nervous system (Ratcliffe, 2011).

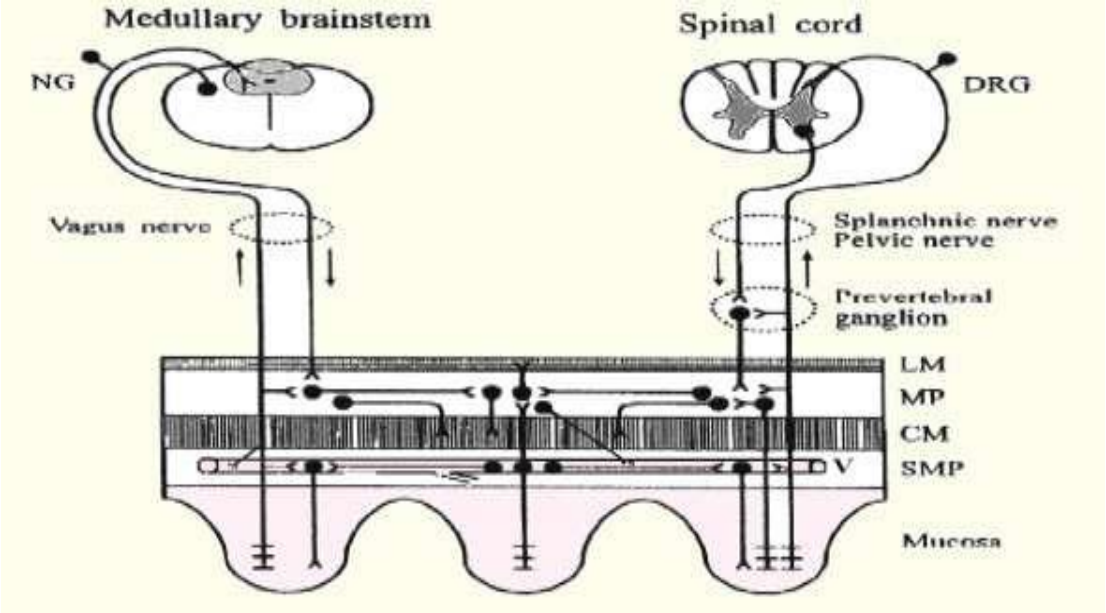


Figure 6: Schematic diagram showing the multiple innervation of the gastrointestinal tract (Holzer *et al*., 2001). LM: longitudinal muscle; CM: circular muscle; MP: myenteric plexux; SMP: submucosal plexus; DRG: dorsal root ganglion; NG: nodose ganglion; V: vessel.

2.2. The extrinsic sensory innervation of the gut

Sensory information from the bowel is conveyed to the CNS by two separate extrinsic pathways. The vagal sensory innervation sends information to the brain via the nodose ganglia. Imput to the spinal cord comes from the sensory innervation provided by the DRG (dorsal root ganglia). Axons from neurons in the thoracic and lumbar DRG travel via the sympathetic chain and grow into the gut along the splanchnic nerves, traversing the celiac ganglion to reach the esophagus, stomach and small intestine and traveling through the mesenteric ganglia to reach the colon. Axons from neurons in sacral DRG follow the parasympathetic pelvic splanchnic nerves to supply the colon and rectum. While these two pathways overlap for the majority of the length of the gut, the vagal afferents are thought to signal mainly from the upper GI regions and the pelvic afferents from the colorectal regionystem (CNS) (Ratcliffe, 2011).

2.3. The enteric nervous system

The enteric nervous system is found in the walls of the entire GI tract from the oesophagus to the anus and associated glands (salivary glands, the pancreas) and the gallbladder. The enteric nervous system is a part of the autonomic nervous system and integrates motility, secretions, blood flow, and immune responses into organised patterns of behaviour through neural reflexes. The enteric nervous system consists of ganglia, primary

interganglionic fiber tracts, and secondary and tertiary fibers projecting to the effector systems (muscle cells, glands, blood vessels, and immune cells). The enteric nervous system has three major ganglionated plexuses (the myenteric – Auerbach's, the submucous – Meissner's, and the mucous) and several aganglionated plexuses. The myenteric plexus is positioned between the outer longitudinal and circular muscle layers throughout the digestive tract, from the oesophagus to the rectum. The submucous plexus is positioned in the submucosa, being only prominent in the intestines. In large mammals, the submucous plexus consists of more than one layer (Hansen, 2003).

2.4. Gastrointestinal circulation

The splanchnic circulation is composed of gastric, small intestinal, colonic, pancreatic, hepatic and splenic circulations, arranged in parallel with one another. The major arteries supplying the splanchnic organs are the celiac artery, the superior mesenteric artery and inferior mesenteric artery, which branch off from the aorta and give rise to smaller arteries that anastomose extensively. Splanchnic blood is drained to the liver via the portal vein, and flows from the liver to the inferior vena cava via the hepatic veins, since the splanchnic circulation receives about 25% of the cardiac output (Morato *et al*., 2008).

2.5. Stomach

The stomach is the most dilated part of the digestive tube, having a capacity of 1000–1500 mL in the adult. It is situated between the end of the oesophagus and the duodenum – the beginning of the small intestine. It lies in the epigastric, umbilical, and left hypochondrial regions of the abdomen, and occupies a cavity bounded by the upper abdominal viscera, the anterior abdominal wall and the diaphragm (Daniels and Allum, 2005). The stomach has five regions; the cardia, the gastroesophageal (GE) junction, the fundus, the corpus, the antrum and the pylorus (Figure 7). The fundus and corpus contain the acid-secreting glands, whereas the antrum contains the alkaline-secreting surface epithelium and the endocrine, gastrin-secreting G-cells (Zhao *et al*., 2008a).

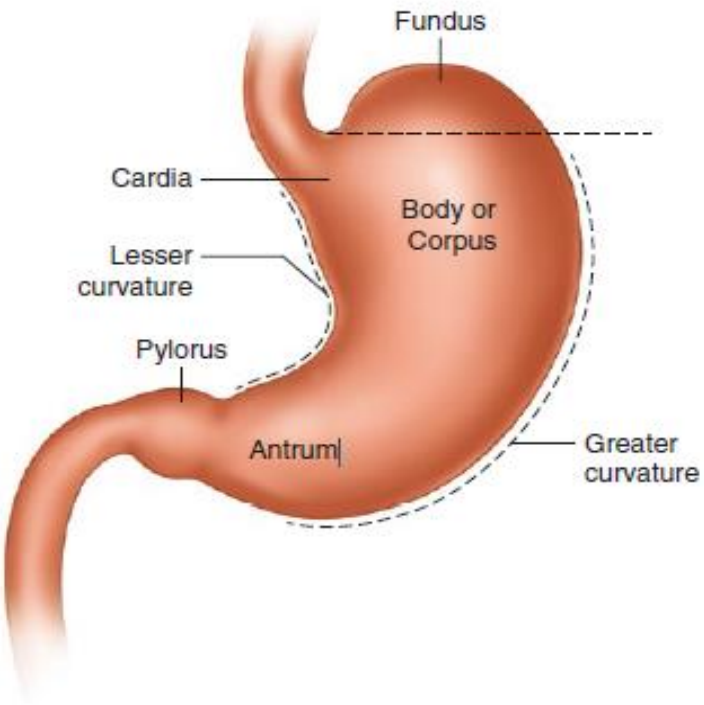


Figure 7: Stomach segmental division (Landa *et al*., 2019).

The stomach has four layers from the outermost to the innermost: the peritoneum or serosa; the muscularis propria, also known as muscularis externa that is composed of three layers of muscles (longitudinal, circular, and oblique), which contains the myenteric plexus of Auerbach; the submucosa, which represents the strongest layer of the stomach; and the mucosa, which is subdivided into muscularis mucosae, lamina propria, and surface epithelium (Landa *et al*., 2019).

2.5.1. Stomach functions

The main function of the stomach is to prepare the ingested food for digestion and absorption. The solid food components need to be broken down to its basic metabolic components in order be absorbed. Thus, the stomach that serves as a storage organ to enable this process that takes approximately 3–4 h also involves the release of hydrochloric acid and other peptides from the gastric glands that mixed with the food content (chyme) and passes from the stomach to the first portion of the small intestine, through the pyloric sphincter to be absorbed (Landa *et al*., 2019).

2.5.2. Gastric acid secretion

The stomach is innervated by the vagus nerve and the sympathetic system through the celiac plexus. Parietal cells produce acid under the influence of gastrin, histamine, and acetylcholine released from the vagus nerve. The vagus nerve has a major influence on acid secretions in the stomach. The vagus nerve innervates the G cells and the Enterochromaffin-like (ECL) cells both of which stimulate acid secretion. Acetylcholine is the principle neurotransmitter modulating acid secretion in the stomach. Somatostatin produced by the D cells reduces the production of acid by inhibiting gastrin production by the G cells and histamine production by the ECL cells. Parietal cells which produce acid have gastrin receptors, muscarinic receptors, histamine receptors, and somatostatin receptors on their cell membrane. Gastrin, produced by the G cells, has a direct effect on parietal cells to increase acid secretion. The production of gastrin is stimulated by proteins and histamine produced by the ECL cells. Acid in the lumen of the stomach inhibits production of gastrin. Somatostatin produced by D cells has a paracrine effect on the G cells and also inhibits the production of gastrin (Figure 8) (Monnet, 2020).

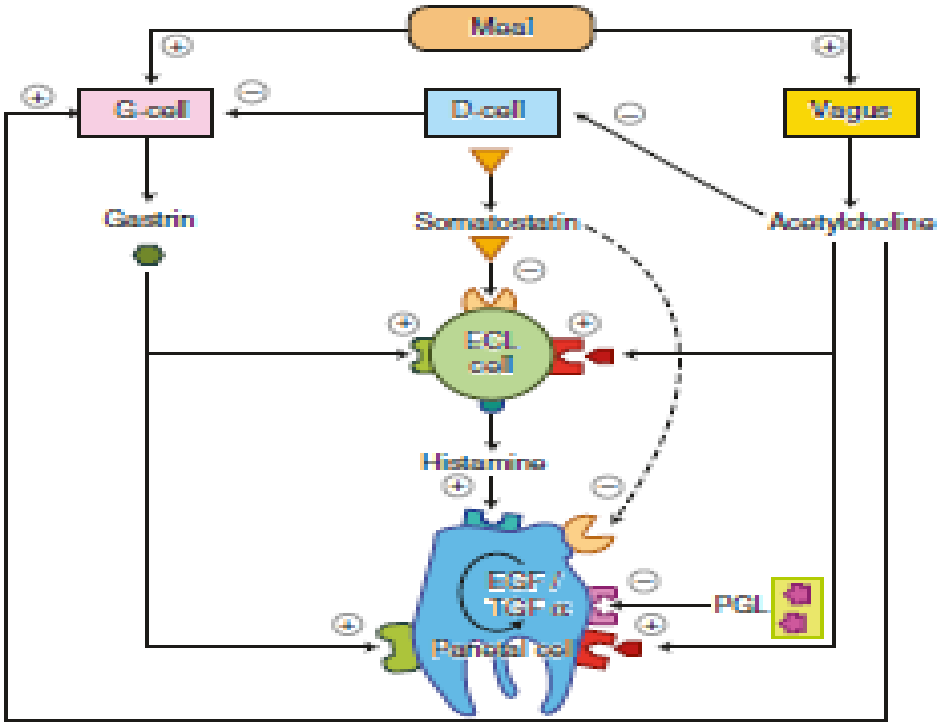


Figure 8: Physiological control of acid secretion. PGL: prostaglandins, EGF: epidermal growth factor,TGFα: transforming growth factor alpha (Landa *et al*., 2019).

2.5.3.Gastric emptying

Flow of chyme from the stomach to the duodenum is pulsatile and is determined by the balance between the strength of antral contraction, the degree of pyloric relaxation, and the duodenal resistance. The pylorus is also involved in regulation of flow to the small intestine, and helps to discriminate fluid, viscous and solid gastric contents. When gastric content is fluid, antral contractions occlude the lumen and liquid is easily transferred to the duodenum. In case of a more viscous or solid gastric content, contractions are not lumen-obliterating, and retropulsion to the proximal stomach serves to mix and grind gastric contents. Duodenal contractions serve to delay further gastric emptying when the duodenum is not empty (Tack, 2007).

2.5.4. Gastric motility

Gastricmotility has two different functional portions. The upper third servesas reservoir of food, and includes the fundus and the proximal one third of the corpus. The distal portion which extends from the distal thirds of the body including the antrum has electric andmotor activity, which results in the mixture of foods with the digestivesecretions and facilitates gastric emptying. The pylorus regulates theoutflow of food to the duodenum.Inthe postprandial state, the proximal and fundus tone relaxes to enable its storagefunction, and the midportion and antrum create repetitive forceful contractions that help to mix and grind the food into small particles (Csendes and Braghetto, 2016). With regard to motility, the proximal stomach is characterised by tonic contractions but no slow wave activity, whereas the distal stomach is characterised by slow wave activity and peristaltic contractions. Two very different motor patterns can be distinguished in the stomach: an interdigestive and a postprandial motor pattern. During the interdigestive phase, the proximal stomach muscle tone is high, whereas the distal stomach is engaged in a recurrent contraction pattern known as the migrating myoelectrical (or motor) complex (MMC). It has been suggested that the MMC serves to clear the stomach of secretions, debris, and microbes during fasting. Upon food intake the motor pattern of the stomach changes drastically: the proximal stomach relaxes and serves initially as a reservoir, after food intake, a tonic contraction of the proximal stomach pushes the food distally, whereas the distal stomach mixes and grinds the food by a powerful and regular peristaltic contraction pattern (Janssen *et al*., 2011). physical nature of food remains crucial in regulating emptying rate. After a liquid meal the gastric emptying begins instantly and emptying half-life is about 30 minutes. Whereas, after a solid meal, the linear emptying occurs after a lag phase and the emptying half-life is about 2 hours.

Therefore, meal over a meal, particularly solid meal, is more likely to delay gastric emptying (Randhawa et al., 2013).

2.6. The intestines

The small intestine is divided into the duodenum, jejunum, and ileum. Although there is a distinct duodenal loop, the yolk stalk (i.e., Meckel's diverticulum) is often used as a landmark to separate the jejunum and ileum. The intestinal wall can contain either folds or villi. The type of mucosal projections are not necessarily consistent between the small and large intestine. Gallus species have villi, which decrease in length from 1.5 mm in the duodenum to 0.4–0.6mm in the ileum and rectum. The intestinal wall contains the same four layers as the remainder of the tract including the mucosal, submucosal, muscle tunic, and the serosal layer. The mucosal layer consists of the muscularis mucosa, lamina propria, and epithelium. The epithelium contains chief cells, goblet cells, and endocrine cells. The crypts of Lieberkuhn are the source of epithelial cells lining the villi. The crypts contain undifferentiated cells, goblet cells, endocrine cells, and lymphocytes. Globular leukocytes and Paneth cells appear near the base of the crypts (Denbow, 2015). The lumen of the small intestine is a complex arrangement of structures that aid in nutrient absorption. Each structure is responsible for increasing the surface area of the intestine to enhance digestion and absorption of nutrients (Campbell *et al*., 2019).

2.6.1. Intestinal motility

Small intestinal motility is regulated through a combination of myogenic, neural, and hormonal factors. Of these three, myogenic factors are the most important. Neural and hormonal factors act to modify myogenic-initiated motor patterns. Intestinal motor activity can persist even with complete blockade of neural signals. Intestinal motor activity exists in two phases: a fed state and a fasting, or interdigestive, state. During the fed state, food is moved along the intestine via segmentation and peristalsis. Segmentation is characterized by a pattern of pressure waves traveling short distances that serve to mix chyme and enhance its contact with the villous surfaces. The peristaltic pattern moves food along the intestine by a muscular contraction proximal to the food bolus and relaxation distal to the bolus (Campbell *et al*., 2019). The MMCs that pass along the intestine at regular intervals in the fasting state and their replacement by peristaltic and other contractions controlled by the BER. In the small intestine, there are an average of 12 BER cycles/min in the proximal jejunum, declining to 8/min in the distal ileum. There are three types of smooth muscle contractions: peristaltic

waves, segmentation contractions, and tonic contractions. It propels the intestinal contents (chyme) toward the large intestines. Segmentation contractions, also described above, move the chyme to and fro and increase its exposure to the mucosal surface. These contractions are initiated by focal increases in Ca^{2+} influx with waves of increased Ca^{2+} concentration spreading from each focus.Tonic contractions are relatively prolonged contractions that in effect isolate one segment of the intestine from another. Note that these last two types of contractions slow transit in the small intestine to the point that the transit time is actually longer in the fed than in the fasting state (Ganone, 2010).

3. Peptic ulcer

Endemic or common peptic ulcer is a complication of an *H. pylori* infection. A peptic ulcer occurs in areas exposed to acid and pepsin and is defined as a break in the mucosa lining the stomach or proximal intestine extending through the muscularis mucosae. Classic peptic ulcer disease is a chronic recurring disease that represented defective wound healing (Graham, 2014). Peptic ulcer disease results from an imbalance between factors that protect the mucosa of the stomach and duodenum, and factors that cause damage to it. Peptic ulcer disease presents with gastrointestinal symptoms similar to dyspepsia and can be difficult to distinguish clinically. It can have potentially serious complications such as bleeding, or perforation, with a high risk of mortality (Sverdén *et al.*, 2019).

3.1. Gastric ulcer

Gastric ulcer, a lesion that penetrates the mucosal muscularis layer characterized by inflammation, irritation, and cell loss in the gastric mucosa, can advance to gastric cancer. Gastric ulcers are prevalent throughout the world, and are considered to be a global health problem (Abdelfattah *et al*., 2019). The aetiology of the gastric ulcer is not completely understood, but is has been established that it may results from an imbalance between the protective factors and aggressive factors in the gastric mucosa. Among the defensive factors, we can include mucus, bicarbonate production, cellular regeneration and adequate blood flow, while the main aggressive factors comprise gastric acid, pepsin secretion, and reactive oxygen species. Furthermore, the gastric ulcer may be triggered by *H. pylori* infection, stress, or chemical agents (excessive alcohol intake or chronic treatment with non-steroidal anti-inflammatories) (Boeing *et al*., 2016).

3.1.1. Aetiology of gastric ulcer

Gastric ulcer is a global concern. Common causes of gastric ulcer include *Helicobacter pylori* infection and excessive use of non-steroidal anti-inflammatory drugs. Modern lifestyles and dietary habits, alcohol consumption, cigarette smoking, and stress are the less common causes of gastritis. Inflammation and oxidative damage are commonly associated with gastric ulcer. Oxidative damage is a serious problem in gastric ulcer. Excessive production of ROS stimulates pro-inflammatory cytokine production, induces cellular protein damage, and disrupts the stomach lining, thereby causing further injury to the gastric mucosa (Abdelfattah *et al*., 2019).

3.1.2. Pathophysiology of ulcers

Pathophysiology of ulcer is due due to imbalance between constructive and destructive mechanisms of gastric system. Constructive factors include mucin and peptide secretions, prostaglandin secretion, and blood flow, while destructive factors comprise gastric acid, pepsin secretion, and *Helicobacter pylori* (Aziz *et al*., 2019). The mucus-bicarbonate-phospholipid "barrier" constitutes the first line of mucosal defense. This barrier is formed by mucus gel, bicarbonate, and surfactant phospholipids, which cover the mucosal surface. This unstirred layer retains bicarbonate secreted by surface epithelial cells to maintain a neutral microenvironment (Ph=7.0) at the surface epithelial cells and prevents penetration of pepsin and thus proteolytic digestion of the surface epithelium. The mucus gel contains phospholipids, and its luminal surface is coated with a film of surfactant phospholipids with strong hydrophobic properties (Laine *et al*., 2008).

3.1.3. Models of gastric ulcer drugs/agents

Peptic ulcers can be induced by physiological, pharmacological or surgical manipulations. Several models are used experimentally fortesting or evaluating antipeptic ulcer activity of drugs/agents (Adinortey *et al*., 2013).

Peptic ulcers generally induced in rodents by physiological, pharmacological or surgical treatments have etiological importance for induction of peptic ulcers. several models that are used to evaluate antiulcer drugs:

- Ethanol induced mucosal damage
- Water-immersion stress or cold-water-restraint
- NSAIDs-(indomethacin, aspirin, and ibuprofen) induced gastric ulcers

- Acetic acid induced gastric ulcers
- Histamine induced gastric ulcers
- Reserpine induced gastric ulcers
- Serotonin induced gastric ulcers
- Pylorus-ligated-induced peptic ulcers
- Ischemia-reperfusion induced gastric ulcers
- Cysteamine induced duodenal ulcers (Qazi and Molvi, 2021).

3.1.4. Ethanol-induced gastric ulcer model

Ethanol is mostly used to induce gastric ulcer in animal models. Ethanol ingestion causes gastric cell necrosis and vascular injury, and consequently ulceration. These effects are attributed to the generation of hydroperoxy free radicals. The stomach and intestine quickly absorb ethanol taken orally into the bloodstream. Ethanol in high concentration induces acute gastritis by eroding the stomach mucosa. Excessive ethanol consumption disposes to the development of acute gastric ulcers through neutrophil infiltration and upregulation of nuclear factor-B (NF-B) (Zheng *et al*., 2016). Acute hemorrhagic stomach erosions, as well as gastritis with mucosal edema, sub-epithelial hemorrhages, cellular exfoliation, and inflammatory cell infiltration (Chen *et al*., 2015).

Ethanol-induced gastric ulceration has been linked to an anti-oxidant effect, enhanced lipid peroxidation, and free-radical production (Al-Qarawi *et al*., 2005). Ingesting ethanol may activate the innate immune system, causing pro-inflammatory cytokines like TNF- α and IL-6 to be released. TNF-α, a typical inflammatory cytokine with several activities, is important in the inflammatory process. TNF- α is widely known for its negative effects, such as producing tissue injury, inflammation, and bacterial and viral infection at high concentrations (Li *et al*., 2013).

Ethanol has been shown to induce oxidative stress that manifests as an increase in malondialdehyde and decrease in CAT and glutathione activities (Albaayit *et al*., 2016). The mechanism of ethanol-induced damage is complex and not fully understood. Ethanol produces a disruption in the integrity of the gastric mucosal barrier through exfoliation of cells, thus increasing mucosal permeability and in some cases provoking bleeding. The extravasation of neutrophils to the site of injury triggers elevated concentrations of ROS and other mediators ofinflammation, causing oxidative damage with deleterious effects on cells.

Oxidative stress has been shown to play a role in alcohol-induced gastric mucosal damage (Guzmán-Gómez *et al*., 2018).

3.1.5. Treatment of gastric ulcer

The drugs used in the treatment of ulcer include proton pump inhibitors, receptor blockers, and drugs affecting the mucosal barrier and acting on the central nervous system. The modern approach used to control gastric ulceration is to inhibit gastric acid secretion, promote gastro protection, block apoptosis and stimulate epithelial cell proliferation for effective healing (Idayat and Mubo, 2019).

3.1.6. Herbal therapeutic of gastric ulcer

Studies in humans and animal models suggest that herbal medicines exert their beneficial effects on gastric ulcer via multiple mechanisms (Figure 09), including antioxidant activity, stimulation of mucosal proliferation, inhibition of acid production and secretion, increased mucus production, as well as inhibition of inflammation (Bi *et al*., 2014).

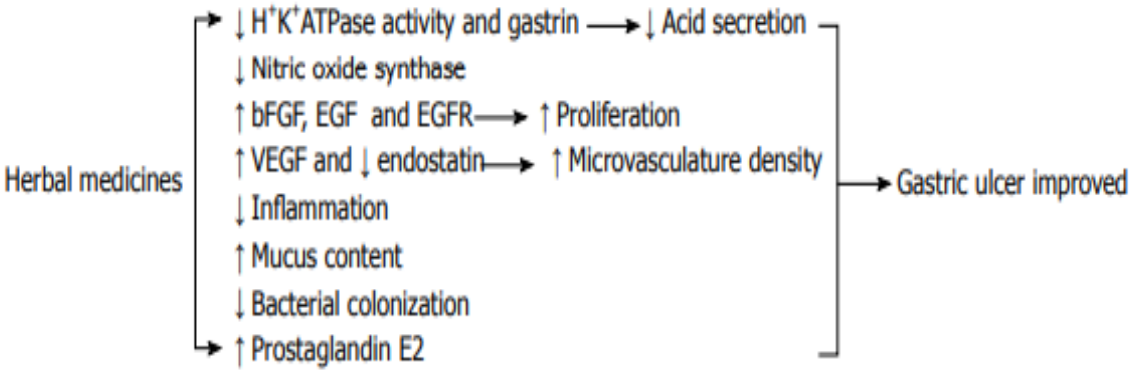


Figure 9: Schematic diagram of possible mechanisms by which herbal medicines benefit gastric ulcer. bFGF: Basic fibroblast growth factor; VEGF: Vascular endothelial growth factor; EGFR: Epidermal growth factor receptor (Bi *et al*., 2014).

4. The plants in study

- ✓ **Lamiaceae family**

Lamiaceae family are popular aromatic plants growing in many regions of the world. Some of them are extensively used to enhance the flavor and aroma of foods, and to improve the overall quality of the product, they are also the basic source of phytochemical compounds which have a beneficial effect on health or play an active role in amelioration of diseases. Many studies showed that herbs from the Lamiaceae family have a potent antioxidant and

antibacterial activities, mostly due to the quantity and quality of phenolic compounds present in them (Kozlowska *et al*., 2015). The Lamiaceae family, one of the most important herbal families, incorporates a wide variety of plants with biological and medical applications. The most known members of this family are a variety of aromatic spices like thyme, mint, oregano, basil, sage, savory, rosemary, self-heal, hyssop,lemon balm, and some others with more limited use. This led us to choose the plant *Saccocalyx satureioides* (coss and Dur) in our study. The Lamiaceae family represents the most important source of plants that have a calming and relaxing effect. Its members also strengthen and stimulate, and have specific effects associated with a particular organ or system. It has been demonstrated that the active compounds present in plants from the Lamiaceae family have natural antibacterial, antioxidant, antifungal and antitumor effects, which suggests that they may be viable alternatives to synthetic products in the therapy of various diseases (Cocan *et al*., 2018).

4.1. *Saccocalyx satureioides* (Coss and Durieu.)

- ✓ **Botanical description**

Saccocalyx satureioides (Lamiaceae) is an Algerian endemic species, which grows in the dunes of the predesert area. This plant is 20–100 cm high, its flowers can be white, rose or crimson (Benahmed *et al*., 2016). Locally, it is known as Zaatar or Zaater, Zaater Armel, and Azir El-Ibel, it has a thyme-like aroma (Souadia *et al*., 2020).

Figure 10: *Saccocalyx satureioides* Coss and Durieu (Picture photographed by Pr. Amira., 1017).

Systematic of *Saccocalyx satureioides*

Family: Lamiaceae
Genus: *Saccocalyx*
Species: *Saccocalyx satureioides*
Name: *Saccocalyx satureioides* Coss and Durieu

- ✓ **Tradutional uses**

Saccocalyx satureioides Coss and Dur, is a tiny aromatic shrub found in the Sahara septentrional region of Algeria. Due to its ancient medical use for stomach issues and spasms, it has gotten a lot of attention (Mohamadi *et al*., 2015; Kherkhache *et al.*, 2018; Ziani *et al*., 2018).

- ✓ **Chemical composition**

In the phytochemical study of Kherkhache *et al*. (2018), the ethyl acetate extract obtained from the aerial parts of *S. satureoides* afforded indole-3-carboxylic acid-(6'-O-caffeoyl)-b-D-glucoside 1, a new indole derivative, together with eight know compounds 2-9 consisting of two indoles, five methylated flavone aglycones and one monoterpene glucoside, detected for the first time in Saccocalyx genus. The two isolated indoles 2 and 3 were identified for the first time from the family Lamiaceae.

Twenty compounds and their corresponding antioxidant activity were identified by Mohamadi and their collabators by HPLC in chloroform, ethyl acetate and n-butanol extracts: Piceol 1, vanillin 2, ferulic aldehyde 3, 3,3'-bis(3,4-dihydro-4-hydroxy-6,8-dimethoxy-2H-1-benzo-pyran)4,3,3'-bis(3,4-dihydro-4-hydroxy-6-methoxy-2H-1-benzopyran)5, Dimethyl-caffeic acid 6, balanophonin 7, 7-methylsudachitin 8, caffeic acid 9, p-coumaric acid 10, isoscutellarein-7-O allosyl(1→2)glucoside 11, isoscutellarein-7-O-(6"-O-acetylallosyl) (1→2)glucoside 12, isoscutellarein-7-O-(6"'-O-acetylallosyl) (1→2)glucoside 13, quercetin 14, isoscutellarein-7-O-(6"'-O-acetyl--d-allopyranosyl(1→2)- -d-6"-O-acetyl--d-glucopyranoside) 15, apigenin 7-O-(6"-trans-p-couma-royl--d-glucopyranoside) 16, sideritiflavone 17 (Mohamadi *et al*., 2015).

- ✓ **Asteraceae family**

The Asteraceae is the richest vascular plant family in the world, with 1600–1700 genera and 24,000-30,000 species. They are easily distinguished by the florets grouped in

capitula. Asteraceae taxa can assume almost every life herbs, succulents, epiphytes, trees, or shrubs (Muhammad *et al*., 2016).

The majority of Asteraceae family members have therapeutic applications, and have a long history in traditional medicine: some members have been cultivated for more than 3000 years for edible and medical purposes. They are most common in arid and semiarid regions of subtropical areas, but are known and distributed throughout the word. The Asteraceae family members show a wide range of anti-inflammatory, antimicrobial, antioxidant and hepatoprotective activities (Rolnik and Olas, 2021).

4.2. *Achillea santolinoides* Lag.

It is a plant of 30-60 cm with leaf-bearing brancherand trilobed segments (Figure 11) and slender peduncles, the flower heads are globose in a compound corymb and loose. The ligules are yellowish, pale and crenellated.

Figure 11: *Achillea santolinoides* Lag plant (Picture photographed by Pr. Amira., 2017).

Systematic of *Achillea santolinoides* Lag.

Kingdom: Plantae
Phyllum: Tracheophyta
Subdivision: Spermatophytina
Class: Magnoliopsida
Superordre: Asteranae
Order: Asterales

Family: Compositae Gieske

Genus: *Achillea*

Species: *Achillea santolinoides* Lag

- ✓ **Chemical composition of *Achillea santolinoides* L.**

The GC-MS analysis of essential oils from different parts of *A. santolinoides* revealed the presence of camphor (60.8% - 62%) and 1-8 cineole (eucalyptol) (14.1% - 15.7%) as major components, in addition to the presence of 30 compounds were characterized; tricyclene, α-pinene, α-thujene, camphene, β-pinene, sabinene, α-terpinene, dehydro-1, 2-methylbutyl isobutyrate, limonene, 1,8-cineole, γ-terpinene, p-cymene, terpinolene, 2-methylbutyl isovalerate, trans-sabinene hydrate, α-campholene aldehyde, benzaldehyde, cis-sabinene hydrate, trans-p-menth-2-en-1-ol, pinocarvone, bornyl acetate, terpinen-4-ol, trans-pinocarveol, δ-terpineol, α-terpineol, borneol, α-campholene alcohol, myrtenol, trans-carveol, p-cymen-8-ol mono hydro, mon oxy (Lamamra, 2016).

Materials and methods

1. Materials

1.1. Plants

The aerial parts of *S. satureioides* and *A. santolinoides* L. were harvested in June, from Djelfa, located at an elevation of 3.734 1.138 m in the Ouled Naïl Range of North-Central Algeria. Both plant's taxonomic identification and classification were established by Prof Laouer H, a botanist at the Department of Biology and Vegetal Ecology, University of Sétif, Algeria.The collected plants were parched in dimness at room temperature. After drying, plant material was ground to a fine powder using electric grinder. Two vouchers numbers; 301 AS 23/05/17 Djel/SA/HL and 206 SS 23/05/17 Djel/SA/HL for *A. santolinoides* L. and *S. satureioides*, respectively, were deposed at the laboratory of Phytotherapy Applied to Chronic Diseases.

1.2. Animals

Adult female wistar albino rats by weighting of 150-200 g and Swiss albino mice weighing between 25 and 30 g obtained from Pasteur Institut, Algiers, Algeria, were used for the present study. They were housed to groups in cage; water and food were freely available. In this study, the animals were deprived of food for 18-20 hours with free access to water until 1 hour before the start of the experiment. During the fasting period, the animals were placed individually in cages with wide-mesh wire bottoms to prevent coprophagy**.**

2. Methods

2.1. Preparation of the extracts

2.1.1. Preparation of the aqueous extract

Aqueous extracts were prepared according to Gharzouli *et al.* (1999) with slight modification, by boiling fifty grams of the powder material of each plant (*S. satureioide*s and *A. santolinoides* L.) in two liters of distilled water for five minutes, then the resulting mixture was filtered and dried at 40°C. *S. satureioide*s aqueous extract (SAE) and *A. santolinoides* L. aqueous extract (AAE) were stored at 4°C until uses.

2.1.2. Preparation of the methanol extract

The methanol extract was prepared according to the method disrcibed by Markham (1982) with some modification. One hundred grams of the plants powder were extracted with one liter of methanol (100 %) at room temperature for three days. The resultant suspension was filtered and concentered using evaporation at 45°C, then dried at 40°C. *S. satureioides*

methanol extract (SME) and *A. santolinoides* L. methanol extract (AME) were saved at 4°C until uses.

2.1.3. Preparation of the hydromethanol extract

The hydromethanol extract was prepared according to the method used by Markham (1982). 100 grams of the plant powder was mixed with 1000 mL of methanol (85 %) at room temperature for 3 days. The resultant suspension was filtered and concentered using evaporation at 45°C. *S. satureioides* hydromethanol extract (SHME) and *A. santolinoides* L. hydromethanol extract (AHME) were saved at 4°C until uses.

2.2. Polyphenols content determination

Total phenolics were determined using the Folin-Ciocalteu reagent according to Li *et al*. (2007). A volume of 100 µL of each extract (or Gallic acid) was added to 500 µL of Folin-Ciocalteu reagent (diluted 10 times). After 4 min, 400 µL of Na_2CO_3 (7.5 %) solution was added. The final mixture was shaken and incubated in dark at room temperature for 90 minutes. The absorbance of all samples was measured at 760 nm. Total phenolic content of the extracts was estimated using the calibration curve of gallic acid and the results were expressed as µg of gallic acid equivalent per mg of dried plant extract (µg GAE/mg DW).

2.3. Flavonoids content determination

Total flavonoids content was estimated using aluminum chloride assay (Bahorun *et al*., 1996). 1 mL of the extract or standard (quercetin) was mixed with 1 mL of $AlCl_3$ solution (2%). The mixture was incubated for 10 minutes in dark at room temperature and then the absorbance was read at 430 nm against the blank. The flavonoids content was expressed as µg of quercetin equivalent per mg of dried plant extract weight (µg QE/mg DW) using the calibration curve of quercetin.

2.4. Tannin content determination

This method is based on the capacity of extracts to precipitate hemoglobin from fresh bovine blood according to the method described by Bate-Smith (1973). Briefly, a volume of each sample was mixed with the same volume of hemolysed bovine blood (absorbance =1.6). After incubation at room temperature for 20 minutes, the mixture was centrifuged at 4000 rpm for 10 minutes and the absorbance of the supernatant was read at 576 nm. The results were expressed as µg equivalent tannic acid per mg of plant extract dry weight (µg TAE/mg DW) using a calibration curve of tannic acid.

2.5. Phenolic compounds characterization by UHPLC-DAD-ESI/MS^n

For the UHPLC, 15 mg of the aqueous extract of the two plants were dissolved in 2 mL of distilled water and 15 mg of methanol extract of each plant were dissolved in 1 mL of methanol, the resultant solutions were filtered through a 0.2 micron nylon membrane (Whatman). For the sake of reproducibility, three separate analyses were conducted. A Thermo Scientific Ultimate 3000RSLC (Dionex) outfitted with a Dionex UltiMate 3000 RS diode array detector and linked to a mass spectrometer was used to accomplish this procedure. The column used was a thermal scientific hypersil gold column (1000 mm x 20 mm) with a 1.9 µm part size and its temperature was maintained at 30 °C. The mobile phase was made up of (A) acetonitrile and (B) 0.1% formic acid (v/v), both degassed and filtered before usage. The flow rate was 0.2 mL/min andthe elution gradient was initiated with 5% of (solvent A) over 14 minutes, 40% of (solvent B) over 2 minutes, 100% (solvent A) over 7 minutes, and 5% over the last 10 minutes re-equilibration of the column. 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 280 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 a negative-ion mode with an electrospray ionization source of 5.00 kV and ESI capillarity temperature of 275°C. The full scan covered a mass range of 50 to 2000 m/z. Collision-induced dissociation MS/MS and MSn experiments were simultaneously acquired for precursor ions.

2.6. Estimation of *in vitro* antioxidant activity

2.6.1. DPPH scavenging activity

The DPPH assay is based on the reduction of DPPH (1,1'-diphenyl-1-picrylhydrazyl), a stable free radical. The free radical DPPH with an odd electron gives a maximum absorption at 517 nm (purple color) (Yaseen *et al*., 2017).

The free radical scavenging activity of each extract was determined spectrophotometrically using the DPPH assay according to Blois (1958). Briefly, 40 µL of samples at different concentration were added to 160 µL of DPPH solution (0.004 %) in 96-well microplates. The reaction mixture was incubated in the dark at room temperature for 30 minutes, and then the absorbance was measured at 517 nm. Butylhydroxytoluene (BHT) used

as standard antioxidant. The percentage of radical scavenging was calculated using the following equation:

$$\% \text{ inhibition} = [(\text{Abs control} - \text{Abs sample})/\text{Abs control}] \times 100$$

Abs control: was the absorbance of the control (containing all reagent except the sample).

Abs sample: was the absorbance of the sample.

2.6.2. ABTS radical scavenging assay

The radical scavenging activity against ABTS (2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) was estimated according to Re *et al*. (1999). A solution of ABTS (7 mM in water) was mixed with potassium persulfate (2.45 mM), and allowed in the dark at room temperature for 12-16 hours. After this incubation, the mixture was diluted with methanol to obtain an absorbance of 0.7± 0.02 at 734 nm. Then 40 µL of samples were mixed with 160 µL of ABTS mixture in 96-well microplate, and incubated for 10 minutes in the dark at room temperature. The absorbance was read at 734 nm. BHT was used as standard antioxidant. The ABTS scavenging capability was calculated as follows:

$$\% \text{ inhibition} = [(\text{Abs of control} - \text{Abs of sample}) / \text{Abs control}] \times 100$$

2.6.3. Ferrous ion chelating of the extracts

The method described by Decker (1990) was used to investigate the ferrous ion chelating ability of different extracts, which is based on the inhibition of the formation of Fe^{2+}-ferrozine complex after treatment of samples with Fe^{2+} ions. The mixture contained 250 µL of sample or EDTA, 50 µL $FeCl_2$ (0.6 mM in water) and 450 µL methanol. Same mixture without the extract or EDTA was considered as a control. The mixture was shaken well and allowed to react at room temperature for 5 minutes; 50 µL of ferrozine (5 mM in methanol) was then added. The absorbance of the Fe^{2+}-ferrozine complex was measured at 562 nm. The chelating effect was calculated as a percentage, using the following equation:

$$\text{Chelating activity (\%)} = [(\text{Abs of control} - \text{Abs of test sample})/\text{Abs of control}] \times 100.$$

2.6.4. Superoxide radical scavenging activity by alkaline DMSO

The alkaline DMSO superoxide activity was determined by the method of Kunchandy and Rao (1990). Briefly, 40 µL of sample (or standard) was mixed with 130 µL of alcalin DMSO (20 mg of NaOH was dissolved in 100 mL DMSO), then 30 µL NBT (1 mg/ mL).The

absorbance of the mixture was read at 560 nm. The scavenging activity of extracts was calculated from the equation below:

$$\% \text{ inhibition} = [(\text{Abs of control} - \text{Abs of sample}) / \text{Abs control}] \times 100$$

2.6.5. Reducing power

The reducing power of the plants extracts was determined following Benchikh *et al*. (2018). A volume of 0.1 mL of extract or standard (BHT) was added to 0.1 mL of phosphate buffer (0.2 M, PH 6.6) and 1% potassium ferricyanid. The mixture was incubated for 20 minutes at 50ºC to reduce ferricyanide into ferrocyanide. After that, 0.25 mL of trichloroacetic acid (10%) was added to stop the reaction and the mixture was centrifuged at 3000 rpm for 10 minutes. Finally, 0.25 mL of the supernatant was mixed with 0.25 mL of distilled water and 0.5 mL $FeCl_3$ (0.1%), then the absorbance was measured at 700 nm. BHT was used as standard. The results were represented as $A_{0.5}$, which stands for effective concentration equal to 0.5 absorbance.

2.6.6. Cupric reducing antioxidant capacity (CUPRAC)

Cupric reducing antioxidant capacity of the extracts was determined according to the method of Apak *et al*. (2004). A volume of 50 µL of $CuCl_2$ (10 mM), 50 µL of 7.5 mM neocupronin, and 60 µL 1M ammonium acetate solution, were added to 40 µL of the extract or the standard, respectively. After 60 minutes, the absorbance of reaction was measured at 450 nm. BHT was used as a standard and the results were given as $A_{0.5}$ (µg/mL) corresponding to the concentration indicating 0.5 absorbance.

2. 7. *In vivo* activity

2.7.1. Acute oral toxicity

Acute oral toxicity of the aqueous extract from *S. satureioides and A. santolinoides* L. was performed using female mice according to the recommendation of the Organization of Economic Co-operation and Development (OECD), guideline 423 (2001). For each extract, a single dose (2000 mg/kg) is administered orally to the first animal. The animal is still fasted for 2 hours after the administration of plant extract. The animal is observed at least once during the first 30 minutes following administration of the product and regularly during the first 24 hours (with particular attention during the first 4 hours; restlessness, agitation, dullness, writhing etc.). If the animal survives, two other animals are treated in the same way. Observation continues daily thereafter, the observation period

totaling 14 days, with the animal weighed weekly. Afterwards, the mice are sacrificed and the organs are weighed and examined. The same previous test is carried out using a dose of 5000 mg/kg.

2.7.2. Gastric emptying and small intestine transit in mice

Gastric emptying was measured using the method published by Amira *et al*. (2005). In this study, after 18-20 hours of fasting, mice (n= 6-8) were orally pretreated with SAE and AAE (100, 200 and 400 mg/kg) and atropine (1 mg/kg) as reference drug. After one hour, each animal received orally 0.2 mL of the test meal (test meal made up of 0.1 % phenol red (a non-absorbable and easily detectable marker) dissolved in CMC 1.5 %) and was sacrificed 20 minutes later.

Under a laparatomy, the stomach and the small intestine were excised after ligation of the pylorus and the cardia. The stomach was homogenized with its contents in 25 mL NaOH (0.1 N). The homogenate was allowed to settle for 1 hour at room temperature. Then, 8 mL of the supernatant were added to 1 mL of trichloroacetic acid (33%) to precipitate proteins. After centrifugation (1600 g for 30 minutes), 1 mL of 2 N NaOH were added to the supernatant. The mixture was homogenized and its absorbance was read at 560 nm. The gastric emptying (GE) rate in the 20-minutes period was calculated according to the following formula:

$$GE\ (\%) = (Abs_{control} - Abs_{test}/Abs_{control}) \times 100.$$

Immediately after the excision of the stomach of the same mice used for gastric emptying model, the whole small intestine was removed for the evaluation of the intestinal transit. The intestine was grossly freed from its mesenteric attachments and its length was 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 of NaOH. The rate of intestinal transit was expressed as the ratio between the distance travelled by the test meal and the total length of the small intestine.

The chosen dose (400 mg/kg) was used in a second series of experiments with atropine (a non specific muscarinic receptor inhibitor), L-NNA (a non specific inhibitor of NO synthase) and indomethacin (a non specific inhibitor of synthesis of prostaglandins) to assess a potential interference of the extract with neurotransmitters that regulate gastrointestinal motility. This was done after determining the most effective doses of the extract on the rate of gastric emptying and intestinal passage. The pharmacological agents are given just before to the force-feeding of the test plant extracts.

2.7.3. Ethanol-induced gastric ulceration in rats

Ethanol-induced gastric lesion was carried out according to Benchikh *et al*. (2017) by oral administration of the ulcerogenic agent (100% ethanol) one hour after the administration of test solutions. The animals were separated into 8 groups, each one consisted of six rats. Each rat of each group was placed in a cage. The animals were fasted for 18-20 hours, with free access to water until 1 hour before the start of the experiment. Group one was treated orally with CMC (1.5 %) as a negative control, group two (positive control) received ranitidine (50 mg/kg). The remaining six groups are treated with aqueous extract of each plant at a dose of 100, 200 and 400 mg/kg. After 60 minutes, all animals received orally 100% ethanol (1 mL /200 g). Thirty minutes after that, the animals were sacrificed with a cervical dislocation, and each stomach was incided, photographed in order to calculate the area of gastric ulcer.

After determining the most protective doses against the ulcer, another series of experiments were applied using the chosen dose (200 mg/kg) with atropine or L-NNA to assess possible interference of the extract with neurotransmitters that control gastroprotective effect. Ulceration percent, histological analyzes of the glandular gastric mucosa, mucus in gastric layer and antioxidant activities were determined.

2.7.4. Histopathological examinations

For microscopic analysis, a portion of each animal stomach was fixed in 10 % formalin and dehydrated with mixtures of increasing grades of ethanol, clarified in xylene using a tissue processor (MTP-SLEE, Mainz, Germany) and embedded in paraffin. After processing, sections of 5 µm were obtained with a standard microtome and were stained with heamatoxylin and eosin. The tissue sections were examined by a pathologist without knowledge of the experimental groups for presence of any negative features, such as edema, erosion, ulceration and necrosis. The slides were later photographed.

3.7.5. Determination of mucus in gastric layer

Adherent mucus measurement was carried out in rats according to the alcian blue dye method described by Corne *et al*. (1974). This cationic dye binds to glycoproteins and soluble mucopolysaccharides into insoluble complexes without penetrating mucosal cells. The animals grouped into 8 groups as described in the previous experimentl design. Each glandular portion of the stomach was immediately immersed in 10 mL of the 0.1 % alcian

blue solution (0.16 M sucrose/0.05 M sodium acetate, pH 5.8). At the end of the 2 hours-period, the unbound dye was removed by two successive washings, first for 15 minutes and then for 45 minutes with 10 mL of 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 hours. Four milliliters of the blue extract was then briefly shaken with an equal volume of 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-1601Spectrophotometer, 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.

2.7.6. Evaluation of *in vivo* antioxidant activity

2.7.6.1. Preparation of homogenate

After the sacrifice of animals, the homogenate of the stomach was prepared according to Okhawa *et al*. (1979), by homogenizing 0.5 g of the glandular part in 4.5 mL (10 % (w/v)) of 50 mM Tris HCl buffer (pH 7.4) using dounce homogenizer in ice-cold. The result homogenate was centrifuged at 4000 g at 4°C for 15 minutes, then the supernatant was used to the determination of total proteins, reduced glutathione (GSH) and lipid peroxidation (MDA).

2.7.6.2. Assessment of total proteins content

Total protein content was measured according to the method of Gornall *et al*. (1949), using the biuret kit total protein reagent. In brief, 1 mL of biuret kit was added to 25 µL of the tissue homogenate or standard (bovine serum albumin (BSA)). The absorbance of the mixture was read at 540 nm after incubation of 10 minutes at room temperature.

The level of protein was estimated from the following formula:

$$\text{Total protein (mg/mL)} = (\text{abs of sample} / \text{abs of standard}) \times n$$

2.7.6.3. Assessment of reduced glutathione (GSH) concentration

Reduced glutathione (GSH) was measured according to the method of Ellman (1959). Briefly, 50 µL of the tissue homogenate was diluted in 10 mL of phosphate buffer (0.1 M, pH 8), then 3 mL of the dilution mixture was mixed with 20 µL of DTNB (0.01 M). After 5

minutes of incubation, the absorbance was read at 412 nm. The concentration of GSH was calculated using the molecular absorption coefficient (ε TNB: 13.6 .103 $M^{-1}.cm^{-1}$) (Razinger et *al*., 2008). The results were expressed as nmoL/mg of tissue.

2.7.6.4. Assessment of lipid peroxidation (LPO)

Level of lipid peroxidation was determined by the method of Olkawa *et al*. (1979). In this assay, the malondialdehyde (MDA) reacts with thiobarbitiruc acid (TBA). In brief, 0.5 mL trichloroacetic acid TCA (20 % w/v) was added to 0.5 of stomach homogenate and then 1 mL of TBA (0.67 % w/v) was added. The mixture was incubated for 15 minutes in boiling water (100°C), then cooled and 4 mL of n-butanol was added and centrifuged at 3000 rpm for 15 minutes. The absorbance of the supernatant was measured at 532 nm against the 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).

2.8. Statistical analysis

In vitro results were represented as the means ± standard deviation (SD) (n=3). All measurements were conducted in three determinations (n=3). The pharmacological results were presented as mean ± i§standard error of mean (S.E.M.) of six-eight experiments. The statistical interpretation was done by one-way analysis of variance (ANOVA) followed by Tukey's test with the aid of Graph Pad Prism 7.00. Differences were considered significant at $P \leq 0.05$.

Results

1. Total polyphenols, flavonoids and tannins content

❖ Total polyphenols, flavonoids and tannins content of *S. satureioides*

Total polyphenols, flavonoids and tannins contents of SAE, SHME and SME extracts are presented in the table 2. Results were expressed in term of µg GAE/mg DW, µg QE/mg DW and µg µg TAE/mg DW, respectively.

These results demonstrate that the greatest total of polyphenols were found in SME (352.08 ± 0.003 µg GAE/mg DW) followed by SAE (209.15 ± 0.001 µg GA/g DW) and SHME (171.37 ± 1.43 µg GAE/mg DW). On the other hand, the highest contents of flavonoids are obtained with both extract SME (38.11 ± 0.000 µg QE/mg DW) and SHME (18.6 ± 0.46 µg QE/mg DW), followed by SAE (11.27 ± 0.06 µg QE/mg DW).

The quantification of the tannins present in the different extracts using the hemoglobin precipitation method showed that SME contains 322.43 ± 0.014 µg TAE/mg DW of extract as the highest content, while the lowest content was obtained with the SAE (131.78 ± 0.035 µg TAE/mg DW).

Table 2: Total polyphenols, flavonoids and tannins contents of *S. satureioides*.

Extract	Total phenolics (TPC) (µg GAE/mg Dw)	Total flavonoids (TFC) (µg QE/mg DW)	Total tannins (TTC) (µg TAE/mg DW)
SAE	209.15 ± 0.001	11.27 ± 0.06	131.78 ± 0.035
SME	352.08 ± 0.003	38.11 ± 0.000	322.43 ± 0.014
SHME	171.37 ± 1.43	18.6 ± 0.46	-

Abbreviations: DW: dry weight, GAE: gallic acid equivalent, QE: quercetin equivalent, SME: *S. satureioides* methanol extract, SHME: *S. satureioides* hydromethanol extract. SAE: *S. satureioides* aqueous extract.

❖ Total polyphenols, flavonoids and tannins content of *A. santolinoides* L.

The total phenolics, flavonoids and tannins contents of the plant extracts were presented in table 3. AME had the highest content of total polyphenols (210.78 ± 0.001µg GAE/mg DW), AHME had the highest content of total flavonoids (31.09 ± 2.7 µg QE/mg DW) compared with AME (21.18 ± 0.025 µg QE/mg DW) and AAE (10.68 ± 0.01 µg QE/mg DW). The greatest amount of tannins was found in AME (198.73 ± 0.014 µg TAE/mg DW) (Table 3).

Table 3: Total polyphenols, total flavonoids and tannins of *A. santolinoides* L.

Extract	Total phenolics (TPC) (µg GAE/mg Dw)	Total flavonoids (TFC) (µg QE/mg DW)	Total tannins (TTC) (µg TAE/mg DW)
AAE	142.26 ± 0.003	10.68 ± 0.01	97.89 ± 0.014
AME	210.78 ± 0.001	21.18 ± 0.025	198.73 ± 0.014
AHME	96.59 ± 3.5	31.09 ± 2.7	-

Abbreviations: DW: dry weight, GAE: gallic acid equivalent, QE: quercetin equivalent, AAE: *A. santolinoides* L. aqueous extract, AME: *A. santolinoides* L. methanol extract, AHME:*A. santolinoides* L. hydrometnanol extract.

2. Phytochemical characterization

❖ Phytochemical characterization of SAE and SME

The comparative study of the aqueous and methanol extract obtained from aerial parts of *S. satureioides* was performed using UHPLC-DAD-ESI-MS/MS operated in negative ionization mode, as shown in figure 12. Table 4 lists all of the compounds identified in both extracts and their important data (Retention time (Rt)), wavelength of maximum absorption in the UV-Visible region (λmax), pseudomolecular ($[M-H]^-$), MS^2 fragment ions in m/z and quantification) during chromatographic analysis. The results revealed that the SAE is less rich in compounds that the SME, phytochemical analysis of SAE reveals the presence of three isomer of luteolin (luteolin-7-*O*-rutinoside, luteolin-*O*-diglucuronide and luteolin-7-*O*-glucuronide), these three compounds are also found in SME, while salvianolic acid, sagerinic acid, rosmarinic acid formic adduct, lithospermic acid A, kaempferol, 3-acetyl-5-caffeoylquinic acid, rosmarinic acid, kaempferol-3-O-glucoside are just found in SME.

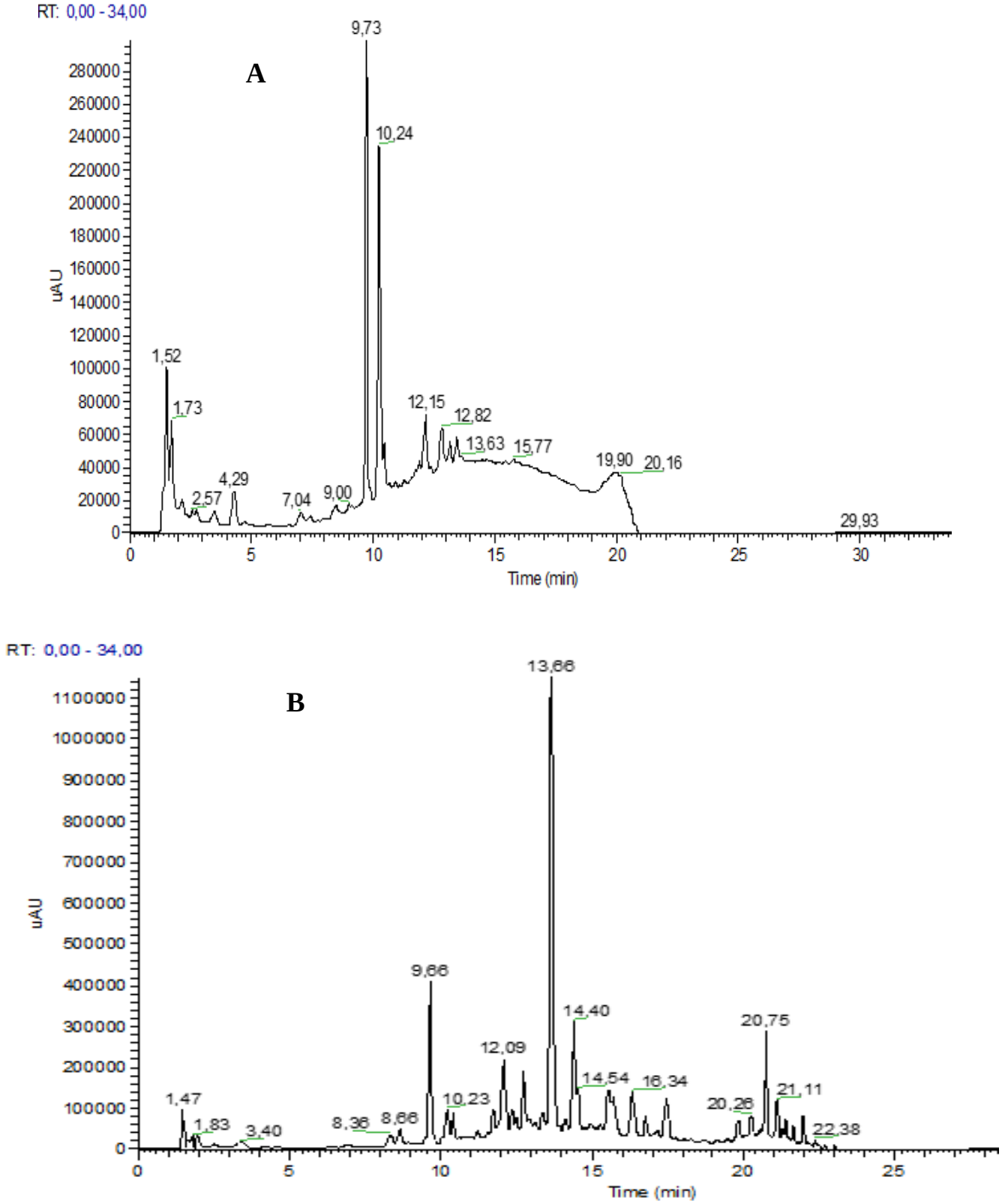


Figure 12: UHPLC chromatogram of *S. Saccocalyx* aqueous extract (A) and methanol extract (B) recorded at 280 nm.

Table 4: Retention time (Rt), wavelength of maximum absorption in the UV-Visible region (λmax), pseudomolecular and MS^n fragment ions, quantification (mean ± SD) and tentative identification of the phenolic compounds in *S. satureioides* aqueous and methanolic extract of the aerial parts.

RT (min)	λ max (nm)	Molecular ion $[M-H]^-$, (m/z)	MS^2	Possible compound	Quantity (mg)*	
					SAE	SME
9.66	221, 270, 343	593	575, 461, 285	Luteolin-7-*O*-rutinoside	-	0.495 ± 0.006
9.73	216, 271, 342	593	575, 461, 28	Luteolin-7-*O*-rutinoside	0.404 ± 0.002	-
10.23	210, 231, 282	637	619, 593, 461, 285	Luteolin-*O*-diglucuronide	-	0.082 ± 0.00
10.24	203, 268, 33	637	619, 593, 461, 285	Luteolin-*O*-diglucuronide	0.390 ± 0.001	-
10.43	203, 226, 281, 321	555	537, 511, 311, 179, 16	Salvianolic acid	-	tr
12.09	204, 220, 253, 266, 344	461	443, 285	Luteolin-7-*O*-glucuronide	-	0.277 ± 0.010
12.15	200,229, 265, 344	461	443, 285	Luteolin-7-*O*-glucuronide	0.089 ± 0.006	-
12.75	201, 223, 253, 282, 329	719	701, 539, 359, 179, 161	Sagerinic acid	-	0.225 ± 0.002
13.66	226, 325	404	386, 359, 179, 161, 135	Rosmarinic acid formic adduct	-	1.521 ± 0.004
14.40	200, 232, 328	537	519, 493, 359, 313, 179, 161	Lithospermic acid A	-	nq
15.55	200, 227, 287	332	314, 304, 232	Unknown	-	nq
15.71	204, 239, 261, 298	285	267, 257, 217	Kaempferol	-	nq
16.34	219, 234, 283, 332	39	353, 191, 179	3-Acetyl-5-caffeoylquinic acid	-	nq
17.47	230, 289, 328	359	197, 179, 161, 13	Rosmarinic acid	-	0.161 ± 0.001
20.75	205, 267, 342	447	285, 227	Kaempferol-3-*O*-glucoside	-	0.372 ± 0.004

SAE: *S. satureioides* aqueous extract, SME: *S. satureioides* methanolic extract.

*This value is in mg of compound / sample in the vessel, using the three replicates for the error calculation; nq = not quantified, tr = traces.

❖ **Phytochemical characterisation of AAE and AME**

The comparative study of the aqueous and methanol extract obtained from aerial part of *A. santolinoides* L. was performed using UHPLC-DAD- ESI-MS/MS, operated in negative ionization mode, as shown in figure 13. Table 5 lists all of the compounds identified in both extract and their important data (Retention time (Rt), wavelength of maximum absorption in the UV-Visible region (λ max), pseudomolecular [M^-H^-], MS^2 fragment ions in m/z and quantification) during chromatographic analysis. The results revealed that AAE is less rich in compounds that AME. Phytochemical analysis reveals that AAE contains two isomers of apigening (apigenin-2''-*O*-pentosyl-8-*C*-glucoside and apigenin-*O*-glucuronide) and luteolin-7-*O*-rutinoside. AME contains two isomers of apigenin also (apigenin-2''-O-pentosyl-8-C-glucoside and apigenin-O-glucuronide), two isomers of dicaffeoylquinic acid (3,5-dicaffeoylquinic acid and 3,4-dicaffeoylquinic acid), in addition to 5-O-caffeoylquinic acid, kaempferol-O-glucuronide, sinapic acid hexoside derivative, cirsimaritin, caffeoyl-D-glucose formic adduct.

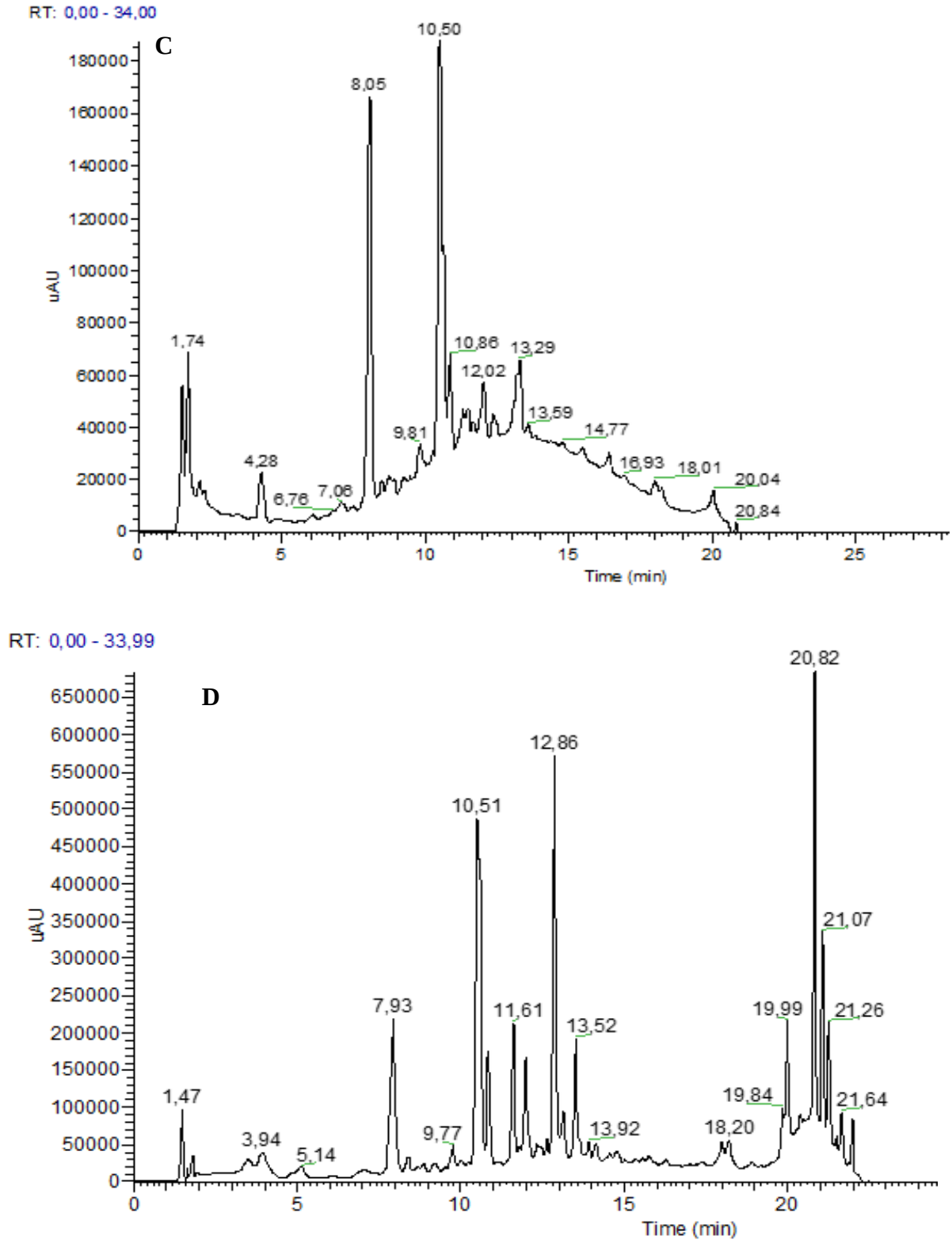


Figure 13: UHPLC chromatogram of *A. Santolinoide* L. aqueous extract (C) and methanol extract (D) recorded at 280 nm

Tables 5: Retention time (Rt), wavelength of maximum absorption in the UV-Visible region (λmax), pseudomolecular and MS^n fragment ions, quantification (mean ± SD) and tentative identification of the phenolic compounds in *A. santolinoides* L. aqueous and methanolic extract of the aerial parts.

RT (min)	λ max (nm)	Molecular ion [M-H]⁻, (m/z)	MS^2	Possible compound	Quantity (mg)*	
					AAE	AME
7.93	218, 236, 299, 325	353	191, 179, 161, 135	5-*O*-Caffeoylquinic acid	-	0.358 ± 0.001
8.05	228, 368	483	465, 439, 421, 309	Unknown	nq	-
10.50	227, 271, 334	563	443, 431, 283, 269	Apigenin-2''-*O*-pentosyl-8- *C*-glucoside	0.391 ± 0.010	-
10.51	218, 270, 339	461	443, 433, 285	Kaempferol-*O*-glucuronide	-	1.110 ± 0.027
10.85	213, 271, 344	563	545, 443, 401, 283, 269	Apigenin-2''-*O*-pentosyl-8- C-glucoside	-	0.204 ± 0.007
10.86	225, 271, 340	593	575, 461, 285	Luteolin-7-*O*-rutinoside	0.068 ± 0.001	-
11.61	227, 249, 307	431	413, 385, 223	Sinapic acid hexoside derivative	-	0.206 ± 0.006
11.99	213, 270, 338	445	427, 417, 269	Apigenin-*O*-glucuronide	-	0.203 ± 0.002
12.02	232, 270, 332	445	427, 417, 269	Apigenin-*O*-glucuronide	0.060±0.001	-
12.86	219, 241, 327	515	497, 353, 179	3,5-Dicaffeoylquinic acid	-	0.719 ± 0.008
13.52	219, 245, 327	515	497, 353, 191	3,4-Dicaffeoylquinic acid	-	0.182 ± 0.003
19.85	214, 273, 334	313	298, 295, 283, 196	Cirsimaritin	-	nq
19.99	240, 294, 330	386	368, 341, 179	Caffeoyl-D-glucose formic adduct	-	0.269 ± 0.002
19.99	240, 294, 330	386	368, 341, 179	Caffeoyl-D-glucose formic adduct	-	0.269 ± 0.002
20.82	217, 242, 275, 340	819	801. 775, 745,563, 478, 355, 313, 298, 295	Unknown	-	nq
21.07	216, 240, 254 271, 344	819	801, 775, 749, 625, 525, 313, 298, 295	Unknown	-	nq
21.26	219, 231, 276, 329	819	801, 783, 775, 643, 572, 561, 470, 415, 288, 269	Unknown	-	nq

AAE : *A. santolinoides* L. aqueous extract, **AME :** *A. santolinoides* L. methanol extract.

*****This value is in mg of compound / sample in the vessel, using the three replicates for the error calculation**;** nq = not quantified.

3. *In vitro* antioxidant activity

3.1. DPPH radical scavenging

Antioxidants capacity to scavenge DPPH radicals was thought to be owing to their proton-donating activity. The antioxidants reduced the stable radical DPPH to the yellow-colored diphenylpicrylhydrazine in a DPPH assay.The scavenging ability of the extracts was expressed as IC_{50} values (the concentration of substrate that causes 50 % loss of DPPH activity). Low IC_{50} values indicate strong ability of the extracts to act as DPPH scavenger.

The ability of SME, SHME and SAE to scavenge the DPPH radical are shown in the figure 14. SME exhibited the highest activity with an IC_{50} value of 24.40 ± 1.11 µg/mL ($P \leq 0.05$) followed by SAE (IC_{50} = 37.55 ± 0.74 µg/mL; $P \leq 0.0001$) and SHME (IC_{50} = 31.76 ± 0.61 µg/mL; $P \leq 0.0001$) compared to BHT as standard.

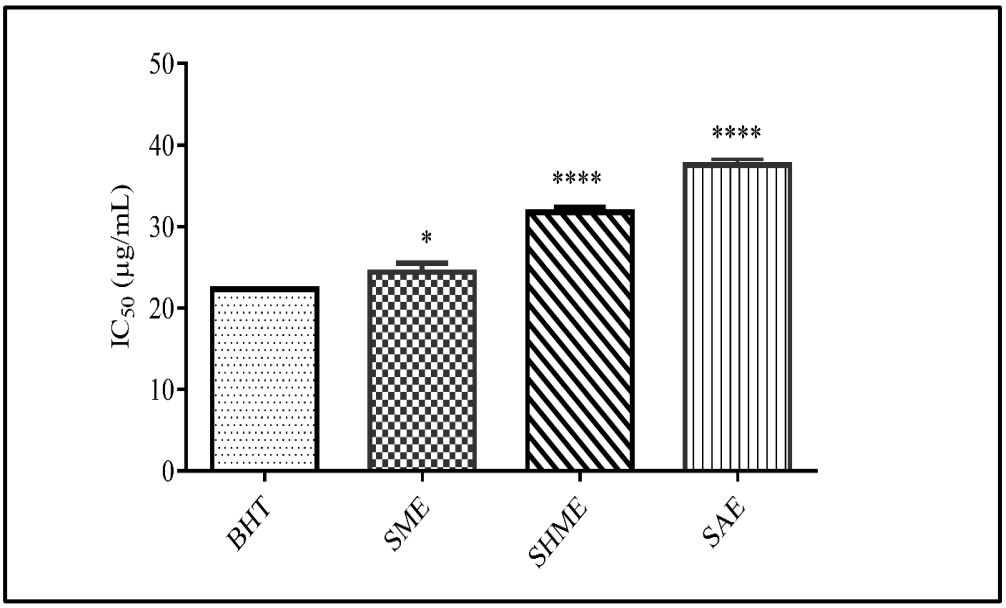


Figure 14: DPPH radical scavenging of *S. satureioides*. SME: *S. satureioides* methanol extract, SHME: *S. satureioides* hydromethanol extract, SAE: *S. satureioides* aqueous extract. Data were presented as means ± SD (n=3). (*P ≤ 0.05; ****P ≤ 0.0001) *vs* BHT as standard.

Figure 15 shows the ability of AAE, AME and AHME to scavenge the DPPH radical. AME showed a strong activity with an IC_{50} value of 24.20 ± 0.93 µg/mL ($P>0.05$), followed by AHME (IC_{50} = 41.66 ± 0.35 µg/mL; $P \leq 0.0001$) and AAE (IC_{50} = 176.43 ± 2.25 µg/mL; $P \leq 0.0001$) compared to BHT. These results are less than those of BHT as a standard (IC_{50} = 22.32 ± 1.19 µg/mL).

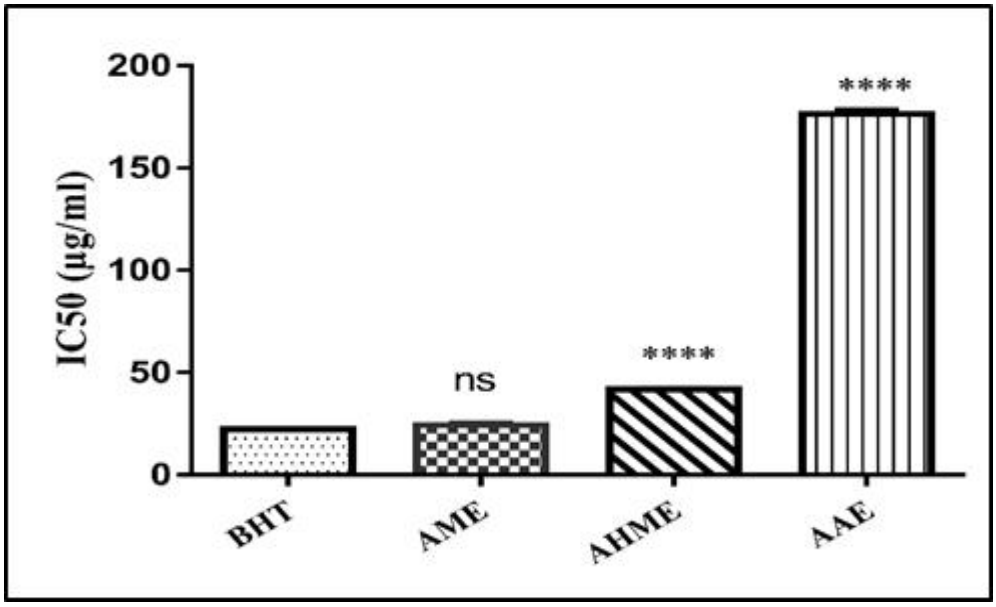


Figure 15: DPPH radical scavenging of *A. santolinoides* L. AME: *A. santolinoides* L. methanol extract, AHME: *A. santolinoides* L. hydromethanol extract, AAE: *A. santolinoides* L. aqueous extract. Data were presented as means ± SD (n=3). (****P ≤ 0.0001; ns: not significant) *vs* BHT as standard.

3.2. ABTS radical scavenging

The ABTS test is a useful method for assessing the antioxidant activity of both hydrogen-donating and chain-breaking antioxidants. As seen in figure 16, *S. satureioides* extracts exhibited high antioxidant activity to scavenge the ABTS radical. The greatest activity was for SME (IC_{50} = 10.55 ± 0.29 µg/mL; P≤0.0001) followed by SHME (IC_{50} = 10.96 ± 0.06 µg/mL; P≤0.0001) then SAE (IC_{50} = 12.72 ± 2.49 µg/mL; P≤0.0001). both extracts were better than the standard BHA to scavenge the ABTS, which had the value of 1.81 ± 0.10 µg/mL.

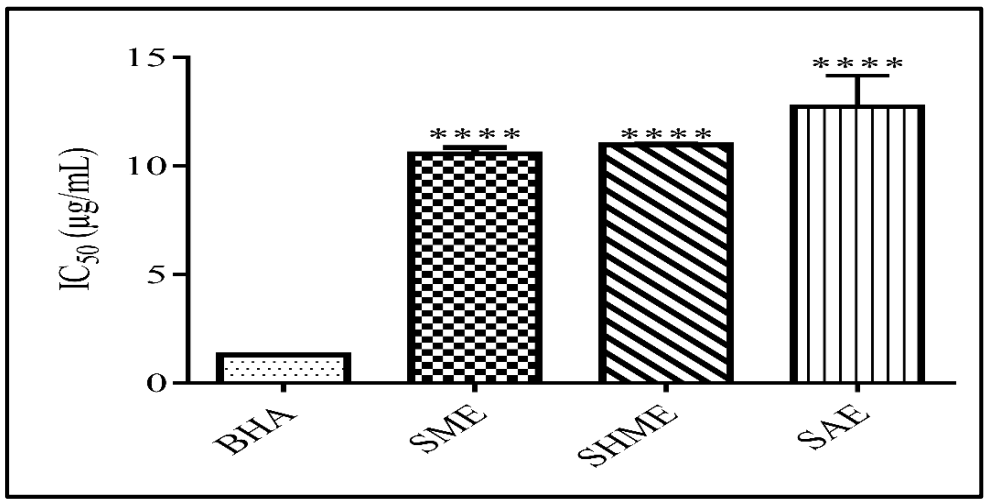


Figure 16: ABTS radical scavenging of *S. satureioides*. SME: *S. satureioides* methanol extract, SHME: *S. satureioides* hydromethanol extrac, SAE: *S.satureioides* aqueous extract. Data were presented as means ± SD (n=3). (****P≤0.0001) *vs* BHA (butylated hydroxyl-anisole) as standard.

For the *A. Santolinoides* L., as indicated in figure 17, in the ABTS experiment, AME had a high scavenging effect with an IC_{50} value of 6.74 ± 0.16 µg/mL (P≤0.001), which was nevertheless lower than the IC_{50} of BHT (IC_{50} = 1.29 ± 0.30 µg/mL). The activity of both AME, AHME (6.73 ± 0.16 µg/mL and 15.07 ± 0.09 µg/mL) and BHT were greater than that obtained with AAE (IC_{50} = 49.75±1.48 µg/mL).

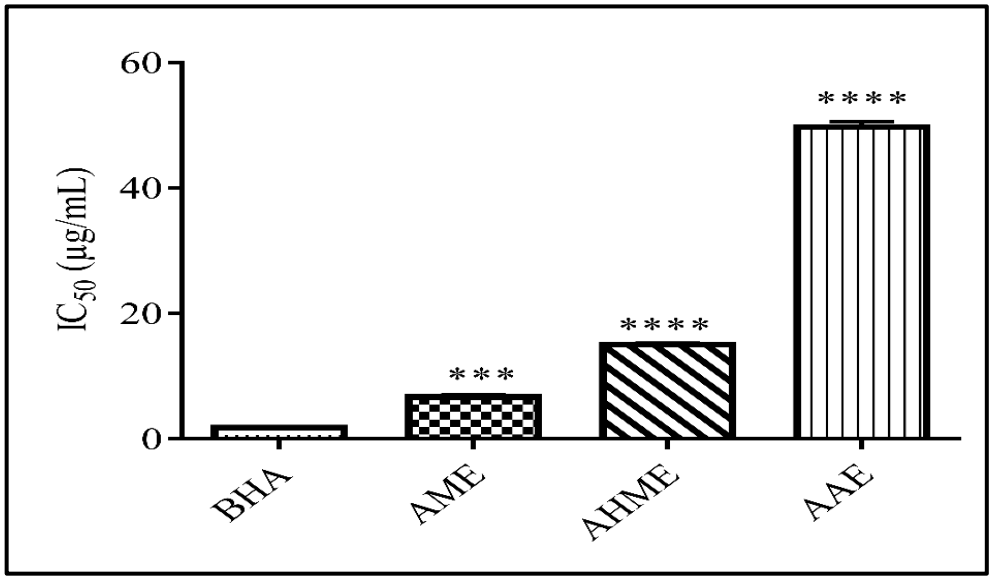


Figure 17: ABTS radical scavenging of *A. Santolinoides* L. AME: *A. santolinoides* L. methanol extract, AHME: *A. santolinoides* L. hydrommethanol extract, AAE: *A. santolinoides* L. aqueous extract. Data were presented as means ± SD (n=3). (***P ≤ 0.001, ****P ≤ 0.0001) *vs* BHA (butylated hydroxyl-anisole) as standard.

3.3. Ferrous ion chelating activity

In the metal chelating assay, ferrozine a chelating agent reacts with ferrous chloride (Fe^{2+}) and can quantitatively form complexes. The ferrosine-Fe^{+2} complexes produced a red chromophore which can be measured and gives maximum absorbance at 562 nm. The presence of chelating agent can disorder the formation of complexes ferrosine-Fe^{+2} and the red color disappears.

As seen in fgure 18, the most important iron chelation activity of *S. satureioides* extracts is noticed in the presence of SHME and SAE with values of IC_{50} = 64.90 ± 0.001 µg/mL and 111.82 ± 0.004 µg/mL (P≤0.0001), respectively compared to EDTA. Whereas the chelation activity obtained by SME is weaker than that observed with the hydromethanol and aqueous extracts with an IC_{50} value of 378.72 ± 0.001 µg/mL (P≤0.0001).

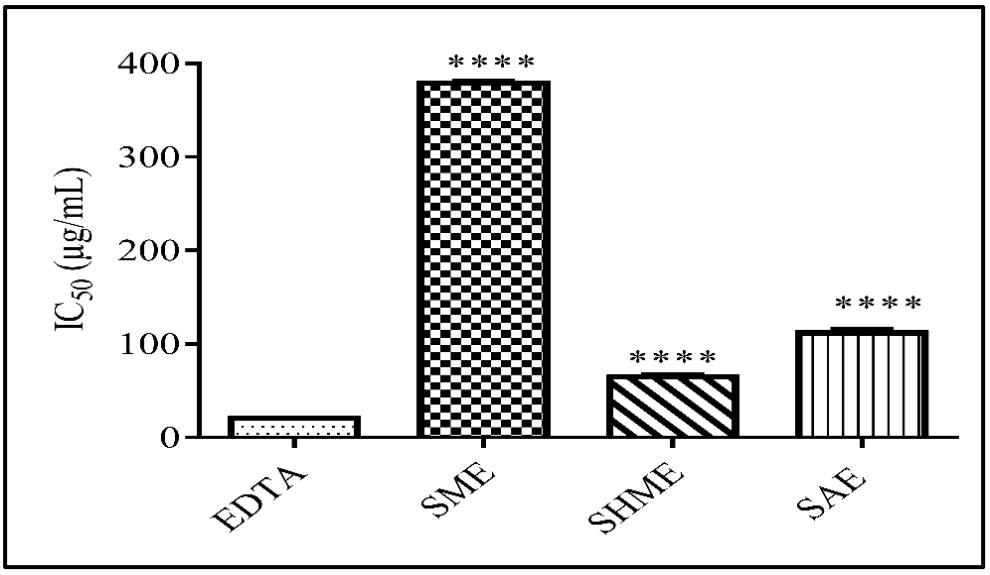


Figure 18: Metal chelating activity of *S. satureioides*. SME: *S. satureioides* methanol extract, SHME: *S. satureioides* hydromethanol extrac, SAE: *S. satureioides* aqueous extract. Data were presented as means ± SD (n=3). (****P ≤ 0.0001) *vs* EDTA as standard.

Figure 19 presented the metal chelating activity of *A. santolinoides* L.. The results showed that EDTA and AHME have strong chelating activity with values of IC_{50} = 20.00 ± 0. 002 µg/mL and 42.00 ± 0.003 µg/mL, respectively with no significant difference. In the other hand, AME and AAE had less activity with IC_{50} values of 247.84 ± 0.05 µg/mL and 127.73 ± 0.003 µg/mL (P≤0.0001; P≤0.01), respectivly compared to standard.

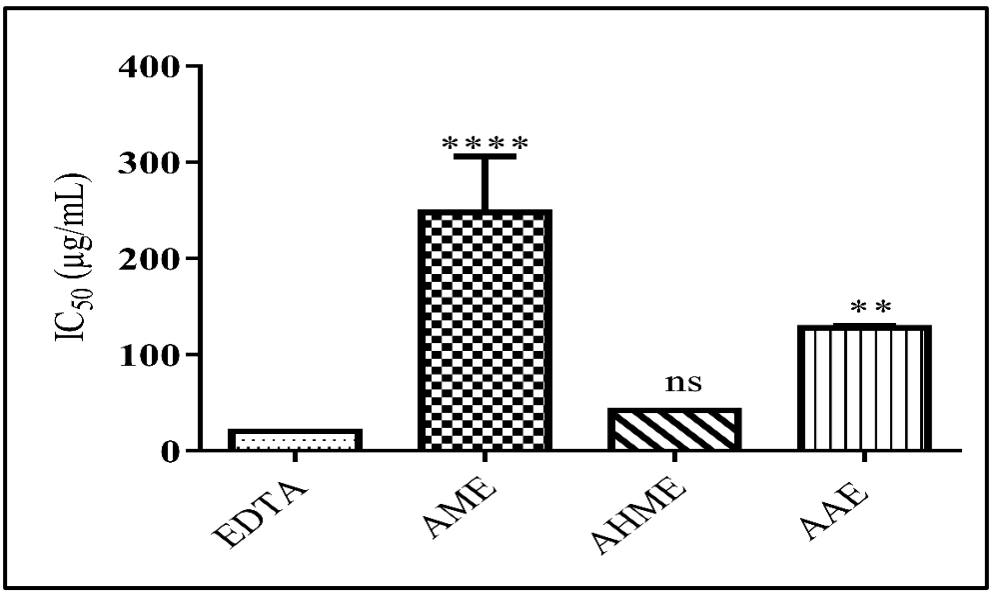


Figure 19: Metal chelating activity of *A. santolinoides* L. AME: *A. santolinoides* L. methanol extract, AHME: *A. santolinoides* L. hydromethanol extract, AAE: *A. santolinoides* L. aqueous extract. Data were presented as means ± SD (n=3). (****P ≤ 0.0001; **P ≤ 0.01; ns: no significant) *vs* EDTA as standard.

3.4. Superoxide DMSO alkaline

The results of anion superoxide radicals scavenging of *S. satureioides* are presented in figure 20. The best scavenging activity was recorded for SME (IC_{50} = 6.21 ± 0.38 µg/mL) which was greater than the standard BHT (IC_{50} = 23.73 µg/mL), SHME (IC_{50} = 10.91 ± 0.11 µg/mL; P≤0.0001) and SAE (IC_{50} = 48.52 ± 1.41 µg/mL).

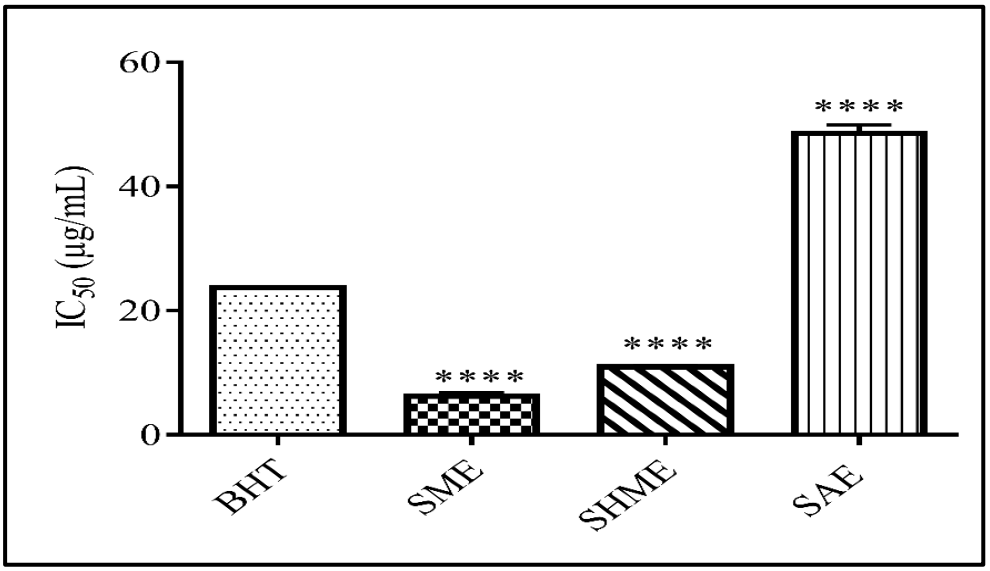


Figure 20: Anion superoxide radical scavenging of *S. satureioides*. SAE: *S. satureioides* aqueous extract, SME: *S. satureioides* methanol extract, SHME: *S. satureioides* hydromethanol extract. Data were presented as means ± SD (n=3). **(******P ≤ 0.0001) *vs* BHTas standard.

The ability of *A. santolinoides* L. extracts to capture the superoxide anion radical was presented in figure 21. AAE possessed a similar scavenging activity (IC_{50} = 23.12 ± 1.61 µg/mL) as BHT (IC_{50}= 23.73 ± 1.11 µg/mL). In the other hand, AME showed a stronger capacity to scavenge the superoxide anion (15.13 ± 0.92 µg/mL; P≤0.0001) followed by AHME with value of IC_{50} = 20.05 ± 0.08 µg/mL; P≤0.01, which was better than AAE and the standard.

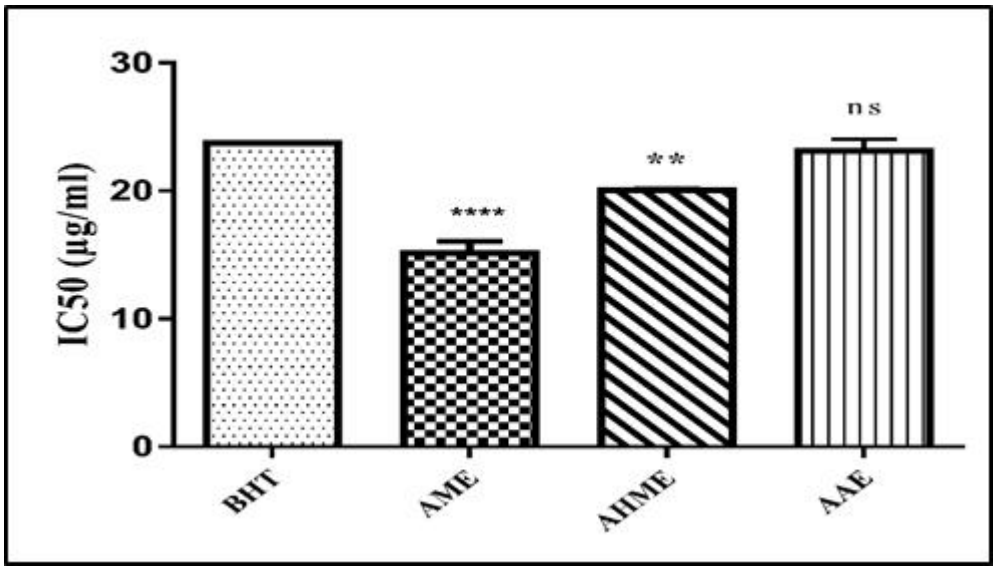


Figure 21 : Anion superoxide radical scavenging of *A. santolinoides* L. AME : *A. santolinoides* methanol extract, AHME : *A. santolinoides* L. hydromethanol extract, AAE : *A. santolinoides* L. aqueous extract. Data were presented as means ± SD (n=3). (****P ≤ 0.0001; **P ≤ 0.01; ns: non significant) *vs* BHTas standard.

3.5. Reducing power

In this assay, the reduction of ferricyanide complex to ferrous form by donating an electron demonstrates the presence of reductant in the extract. The results presented in figure 22 revealed that SME exhibits high reducing power (the effective concentration at which the absorbance was 0.5) with EC_{50} value of 130.89 ± 0.003 µg/mL (P≤0.0001), followed by SAE (EC_{50} = 292.57 ± 0.01 µg/mL; P≤0.0001), then BHT (327.60 ± 0.004 µg/ml) and SHME (543.5 ± 6.25 µg/mL; P≤0.01).

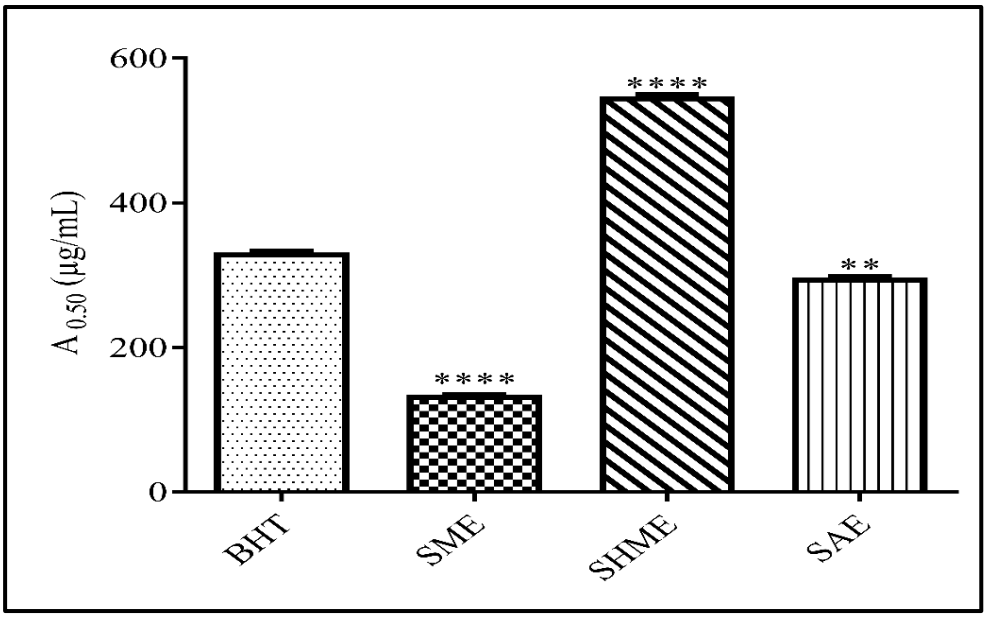


Figure 22: Reducing power activity of *S. satureioides*. SAM: *S. satureioides* methanol extract, SHAM: *S. satureioides* hydromethanol extract, SME: *S. satureioides* aqueous extract. Data were presented as means ± SD (n=3). (****P ≤ 0.0001; **P ≤ 0.01) *vs* BHT as standard.

The results presented in figure 23 revealed that AME exhibits high reducing power with EC_{50} value of 179.71 ± 0.01 µg/mL (P≤0.01), followed by AHME (EC_{50} = 300 ± 0.02 µg/mL), then AAE (EC_{50} = 642.537 ± 0.04 µg/mL; P≤0.0001) compared with BHT (327.60 ± 0.004 µg/mL).

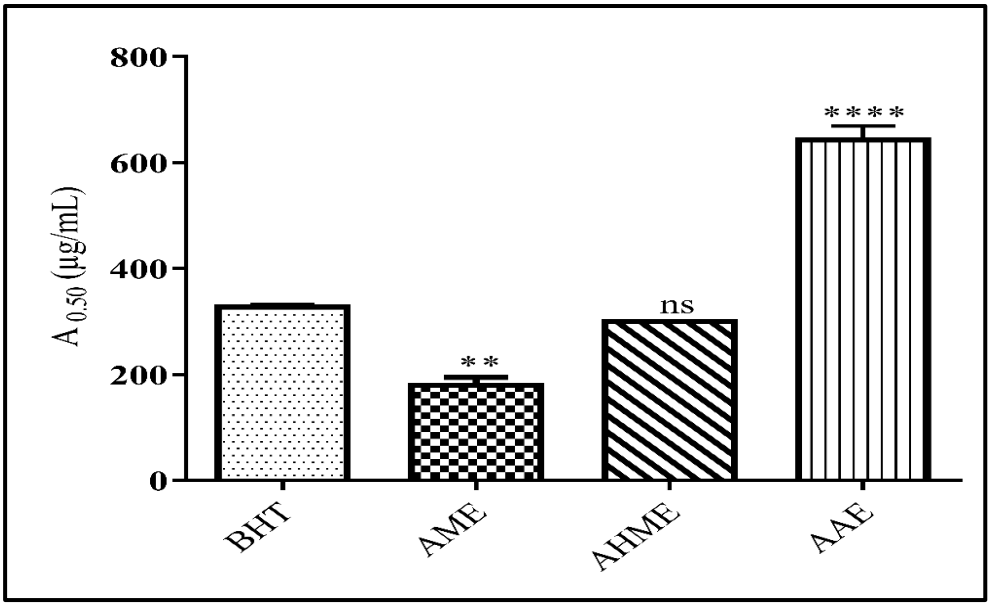


Figure 23: Reducing power activity of *A. santolinoides* L. AME: *A. santolinoides* L. methanol extract, AHME: *A. santolinoides* L. hydromethanol extract, AAE: *A. santolinoides* L. aqueous extract. Data were presented as means ± SD (n=3). (**P≤0.01; ****P≤0.0001; ns:non significant) *vs* BHT as standard.

3.6. Cupric reducing antioxidant capacity (CUPRAC)

As shown in figure 24, we can see that the best cupric reducing antioxidant capacity (the effective concentration at which the absorbance was 0.5) was for SME ($A_{0.50}$ =19.97± 0.38 µg/mL), comparing to SHME ($A_{0.50}$ = 25.07 ± 0.09 µg/mL) and SAE ($A_{0.50}$ = 47.07 ± 1.50 µg/mL). However, the cupric reducing capacity of *S. satureioides* extracts was significantly (P≤0.0001) lower than the standard BHT ($A_{0.50}$ = 9.62 ± 0.87µg/mL).

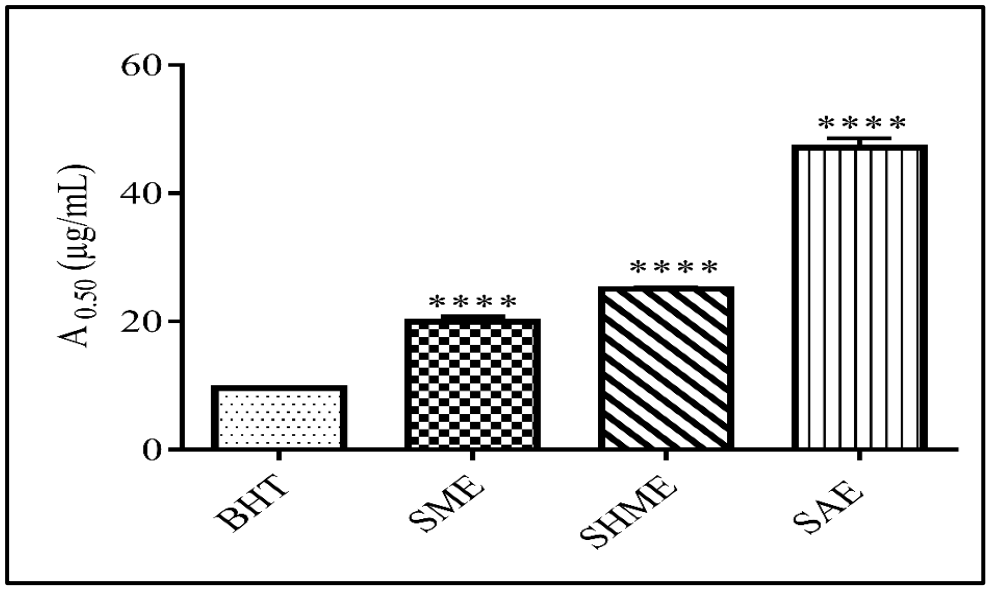


Figure 24: Cupric reducing activity of *S. satureioides.* SME: *S. satureioides* methanol extract, SHME: *S. satureioides* hydromethanol extract, SAE: *S. satureioides* aqueous extract. Data were presented as means ± SD (n=3). (****P≤0.0001) *vs* BHT as standard.

Concerning *A. santolinoides* L. (Figure 25), the highest cupric reducing antioxidant capacity value has been found for AME ($A_{0.50}$ =76.56 ± 2.35 µg/mL; P≤0.0001) followed by AHME ($A_{0.50}$ = 98.46 ± 0.19 µg/mL), then AAE ($A_{0.50}$ =155.47 ± 1.45 µg/mL; P≤0.0001).

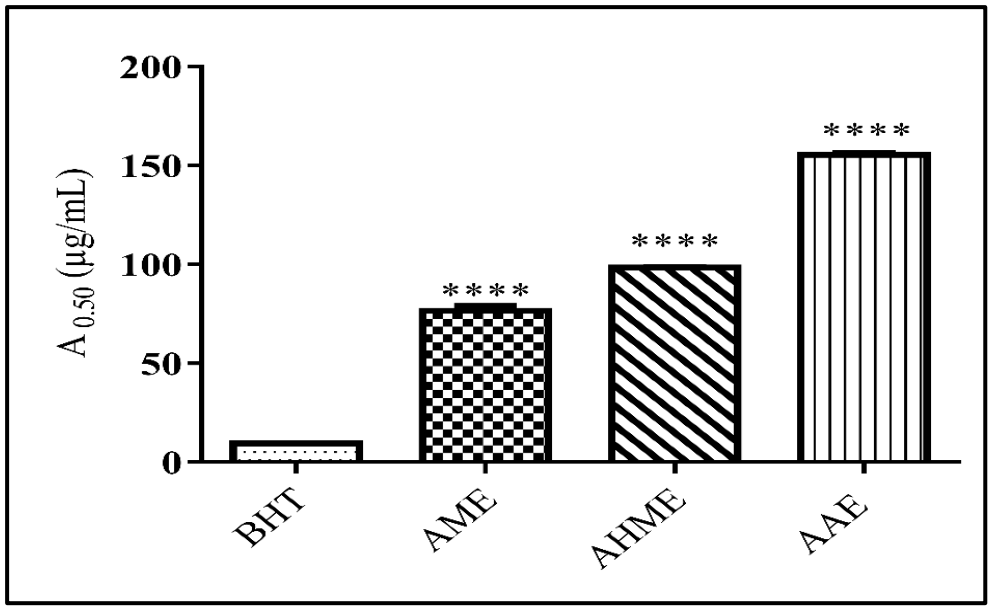


Figure 25: Cupric reducing activity of *A. santolinoides* L. AME: *A. santolinoides* L. methanol extract, AME: *A. santolinoides* L. hydromethanol extract, AAE: *A. santolinoides* L. aqueous extract. Data were presented as means ± SD (n=3). (****P≤0.0001) *vs* BHT as standard.

4. *In vivo* activity of the plants in study

4.1. Toxicity

Oral acute toxicity test was achieved by gavaging mice with SAE and AAE extracts at single doses of 2000 mg/kg and 5000 mg/kg. Animals treated with SAE and AAE extracts were under observation during a period of 14 days. The animals did not also show any behavioral, neurological or physical changes.

4.2. Gastric emptying and small intestine transit in mice

4.2.1. Effect of SAE on gastric emptying in mice

As shown in figure 26, at the lowest dose (100 mg/kg), SAE slightly decreased gastric emptying (73.99 ± 6.96 %) against vehicle treated animals (80.41 ± 1.69 %). However, the highest doses (200 and 400 mg/kg) significantly reduced gastric emptying to 67.90 and 51.89 %, respectively (P≤0.05 and P≤0.0001). The treatment of mice with the aqueous extract of *S. satureioides* did not show any significant difference between the dose of 100 mg/kg and 200 mg/kg, however, the treatment of mice at doses of 200 and 400 mg/kg showed the presence of a significant difference (P≤0.01) between the both doses. The delaying effect of atropine (59.85 ± 4.22 %) as a reference drug and the extract at dose of 200 and 400 mg/kg were not significantly different.

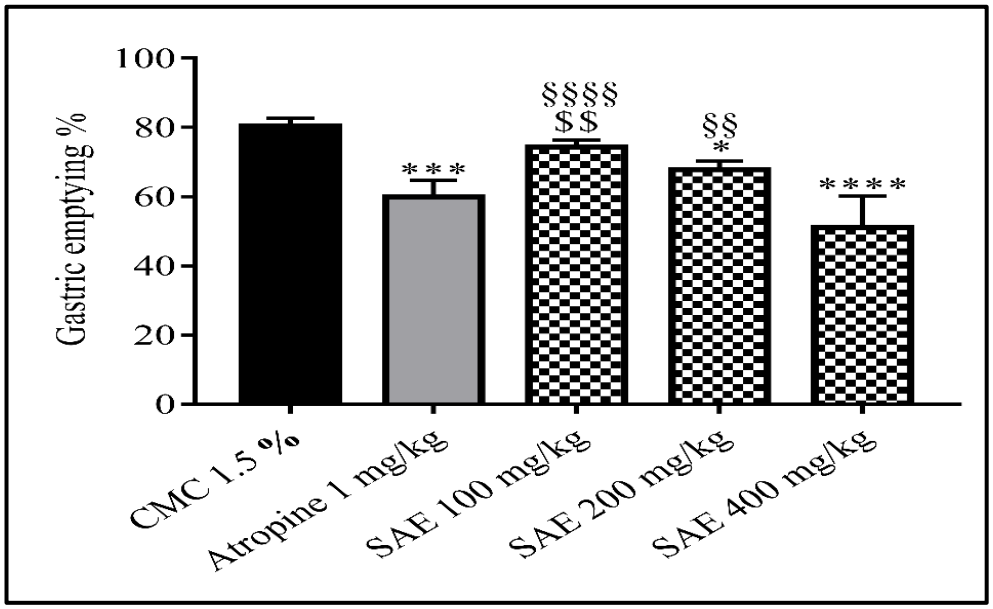


Figure 26: Effect of SAE on gastric emptying in mice. SAE: *S. satureioides* aqueous extract. The values are expressed as means ± SEM (n = 6-8). ns: no significant difference; (*P≤0.05; ***P≤0.001; ****P≤0.0001) comparison vs vehicle treated group (CMC 1.5%). ($^{\$\$}$P≤0.0)1 comparison *vs* atropine as reference drug. (§§P≤0.01; §§§§P≤0.0001) in comparison SAE 100 mg/kg SAE 200 mg/kg *vs* SAE 400 mg/kg, respectively.

4.2.2. Mechanisms of SAE effect on gastric emptying in mice

Atropine reduced the rate of gastric emptying (59.85 ± 4.22; P≤0.001) compared to the vehicle (CMC 1.5 %). The treatment of mice with SAE in the presence of atropine increased the gastric emptying with rate of 66.87 ± 1.14 (P≤0.05) compared to SAE alone (figure 27). Simultaneous use of L-NNA with SAE slightly increased the rate of gastric emptying compared to SAE alone (P>0.05). Indomethacin reduced the rate of gastric emptying (54.22 ±2.35 %; P≤0.0001) compared to the vehicle (CMC 1.5 %). The treatment of mice with SAE in the presence of indomethacin did not show any difference in the rate of gastric emptying with rate of 59.07 ± 2.20 % (P>0.05) compared to animals treated with SAE alone (Figure 27).

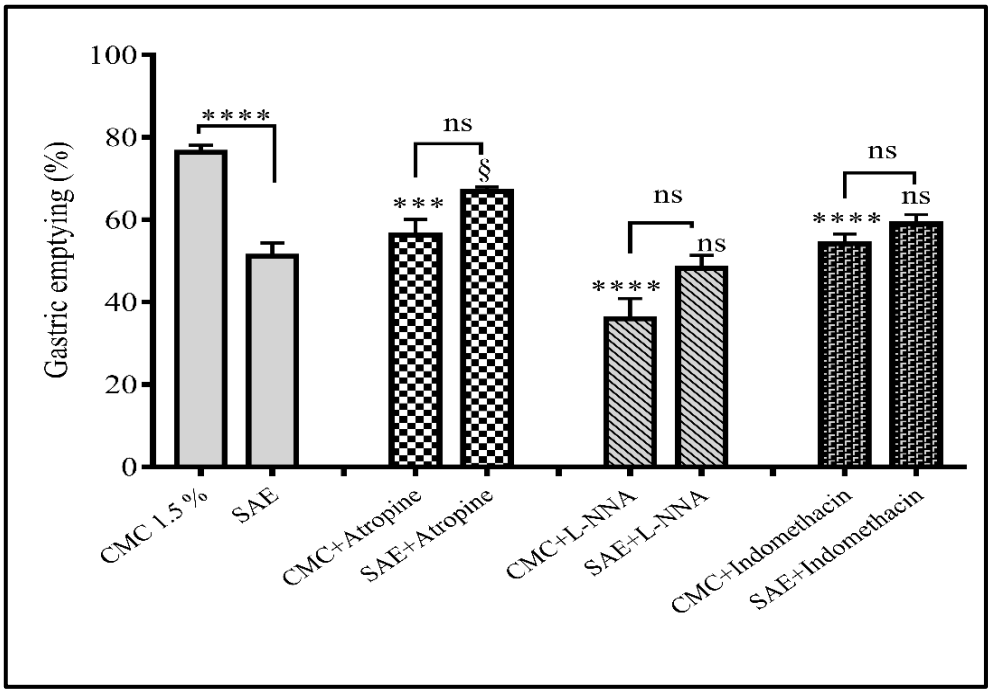


Figure 27: Effects of SAE (400 mg/kg) on gastric emptying in the presence of atropine, L-NNA or indomethacin. SAE: *S. satureioides* aqueous extract. The values are expressed as means ± SEM (n=6-8). ***P≤0.001; ****P≤0.0001 comparison to CMC as negative control. §P≤0.05 in comparison SAE in the presence of atropine, L-NNA or indomethacin *vs* SAE alone. ns: no significant difference.

4.2.3. Effect of SAE on intestinal transit in mice

The effect of SAE on intestinal transit is shown in figure 28. Compared to the vehicle (CMC 1.5 %), SAE at dose of 100 mg/kg and 200 mg/kg did not show any effect on the transit of phenol red through the small intestine. At the highest dose (SAE 400 mg/kg), the rate of intestinal transit decreased to 41 % (P≤0.01). No significant difference was observed between the three doses. All doses showed no significant difference in intestinal transit compared to the reference drug (atropine).

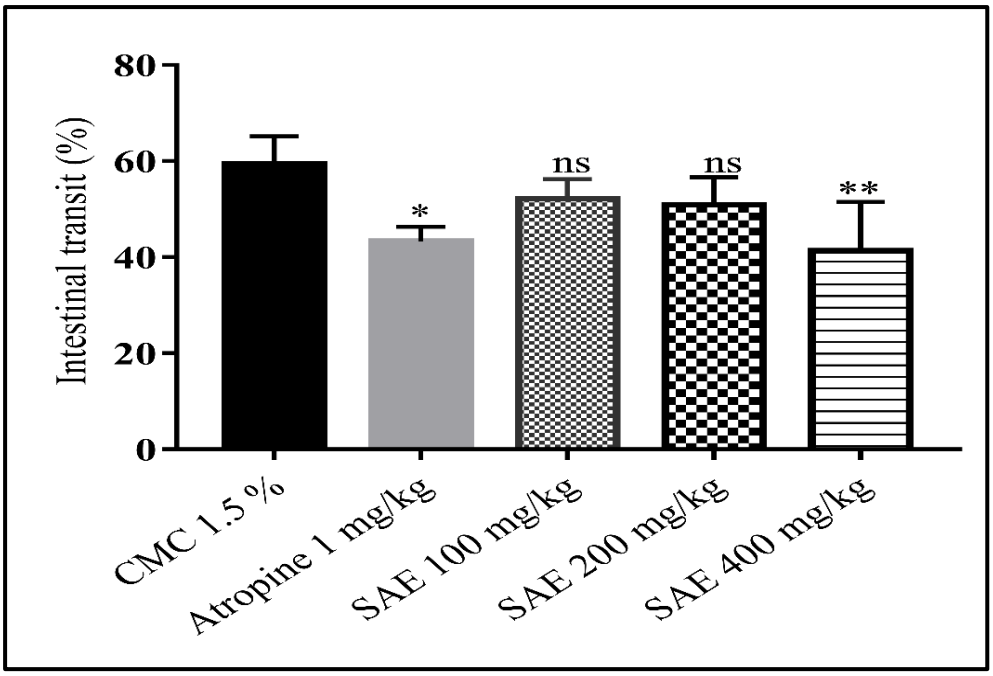


Figure 28: Effect of SAE on intestinal transit in mice. SAE : *S. Satureioides* aqueous extract. The values are expressed as means ± SEM (n=6-8). *P≤0.05; **P≤0.01 *vs* CMC as negative control, ns: no significant.

4.2.4. Mechanisms of SAE effect on intestinal transit

As shown in figure 29, treatment of mice with atropine, L-NNA, or indomethacin decreased the intestinal transit (43.29±3.02 %, 43.83±2.57 %, and 41.60±3.71 %; respectively, compared to vehicle (CMC as control). The effect of SAE in the presence of atropine, L-NNA or indomethacin did not show any difference on the intestinal transit (P>0.05) compared to the treatment with SAE alone, and to the correspondent control (atropine, L-NNA or indomethacin).

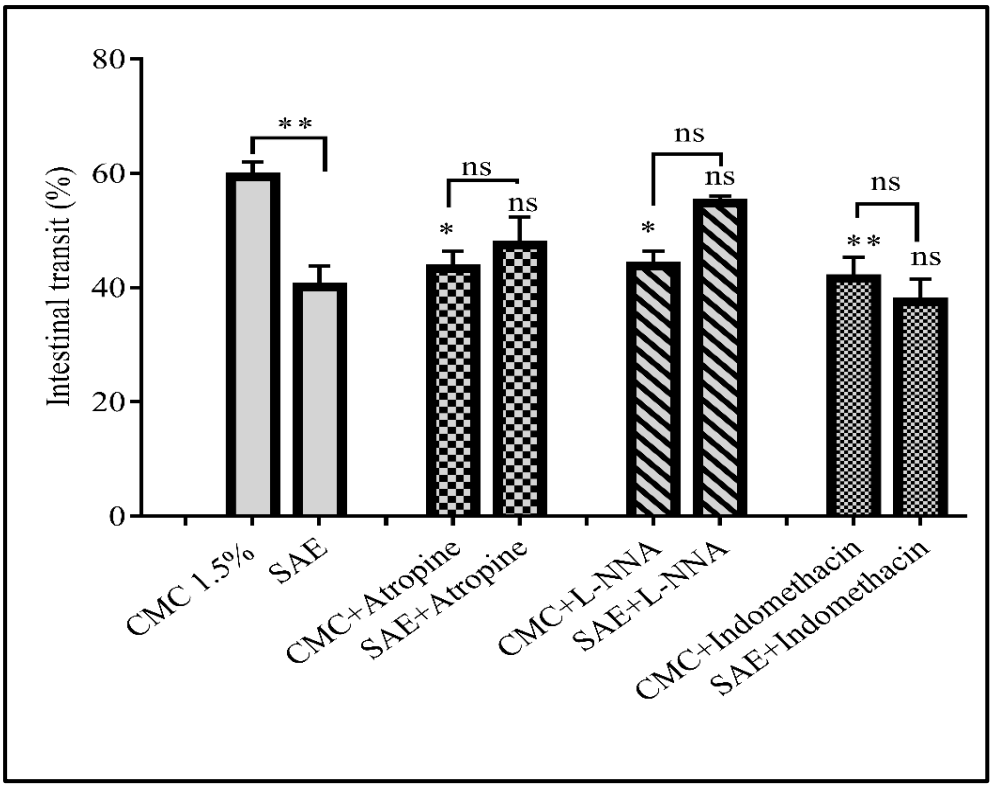


Figure 29: Effect of SAE (400 mg/kg) on intestinal transit in mice in the presence of atropine, L-NNA or indomethacin. SAE: *S. Satureioides* aqueous extract. The values are expressed as means ± SEM (n=6-8). (*P≤ 0.05; **P≤0.01) in comparison with the CMC 1.5 %. ns: no significant difference.

4.2.5. Effect of AAE on gastric emptying in mice

Treatment with the lowest dose of AAE (100 mg/kg) showed no significant decrease in gastric emptying compared to the vehicle treated animals. By contrast, the doses of 200 mg/kg and 400 mg/kg significantly decreased the gastric emptying (P≤0.05). Statistical analysis showed that there is no significant difference between the groups treated with the three doses (100, 200 and 400 mg/kg) (P>0.05). This effect was not significantly different (P>0.05) from the reference drug (atropine) (Figure 30).

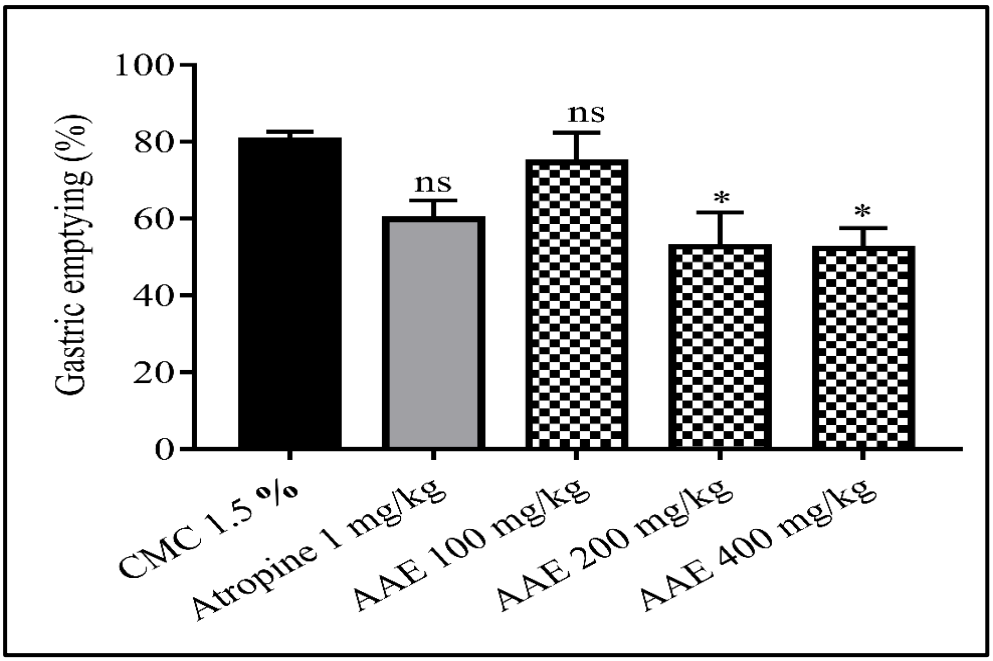


Figure 30: Effect of AAE on gastric emptying in mice. AAE : *A. santolinoides* L. aqueous extract. The values are expressed as means ± SEM (n=6-8). *P≤ 0.05; ns: no significant difference *vs* vehicle treated group (CMC 1.5%).

4.2.6. Mechanisms of AAE effect on gastric emptying

The oral administration of atropine with AAE induced a slight increase in gastric emptying, but not significantly to reach 46.22 ± 4.49 % (P>0.05) (Figure 31). A significant reduction (22.92 %, P≤0.001) in gastric emptying was observed in the simultaneous use of L-NNA with AAE compared to AAE alone, however, this effect did not show any difference on gastric emptying delay (P>0.05) compared to the animals pre-treated only with L-NNA. Treatment of animals with AAE at the same time with indomethacin had slightly decreased the rate of gastric emptying (48.60 ± 2.55 %; P≤0.05) (Figure 31).

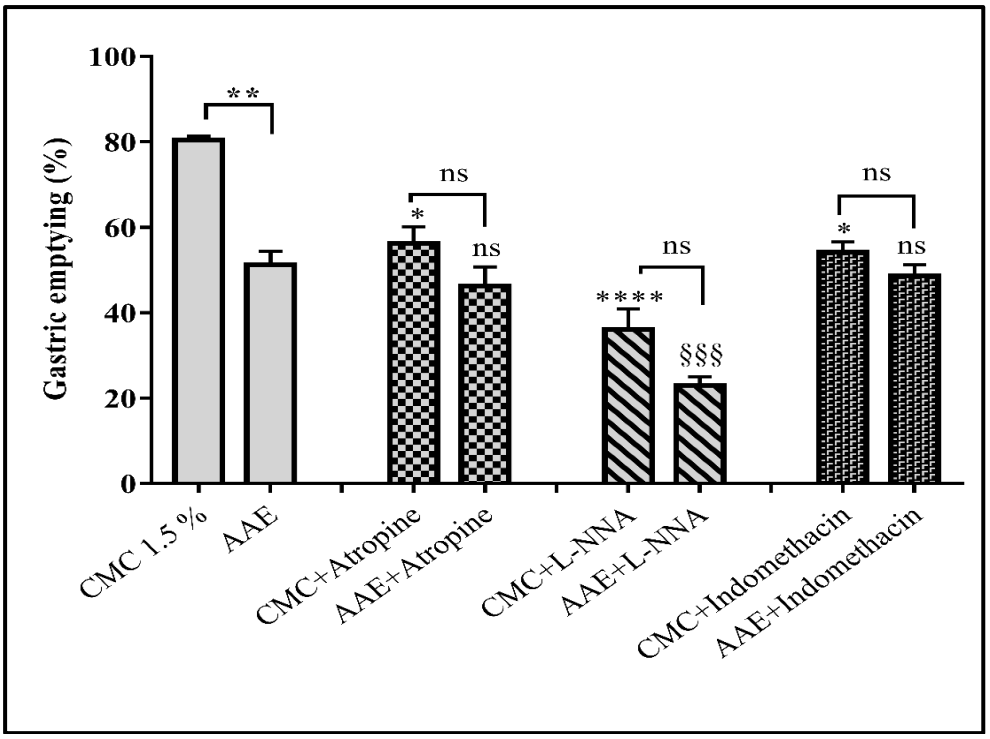


Figure 31: Effect of AAE (400 mg/kg) on gastric emptying in the presence of atropine, L-NNA or indomethacin. AAE: *A. santolinoides* L. aqueous extract. The values are expressed as means ± SEM (n=6-8). *P ≤0.05; **P≤0.01; ****P≤0.0001 comparison to CMC as negative control. $^{\$\$\$}$P≤0.001 in comparison AAE in the presence of L-NNA to AAE alone. ns: no significant difference.

4.2.7. Effect of AAE on intestinal transit in mice

AAE reduced the intestinal transit compared to the vehicle. This effect was significant (39.17 %, 37.58 % and 32.73 %, P≤0.0; P≤0.01 and P≤0.001, respectively) for the three doses (100, 200 and 400 mg/kg) and did not show any significant difference between these doses. AAE at the three tested doses showed no significant difference effects compared to atropine as reference drug (43.29 %; P>0.05) (Figure 32).

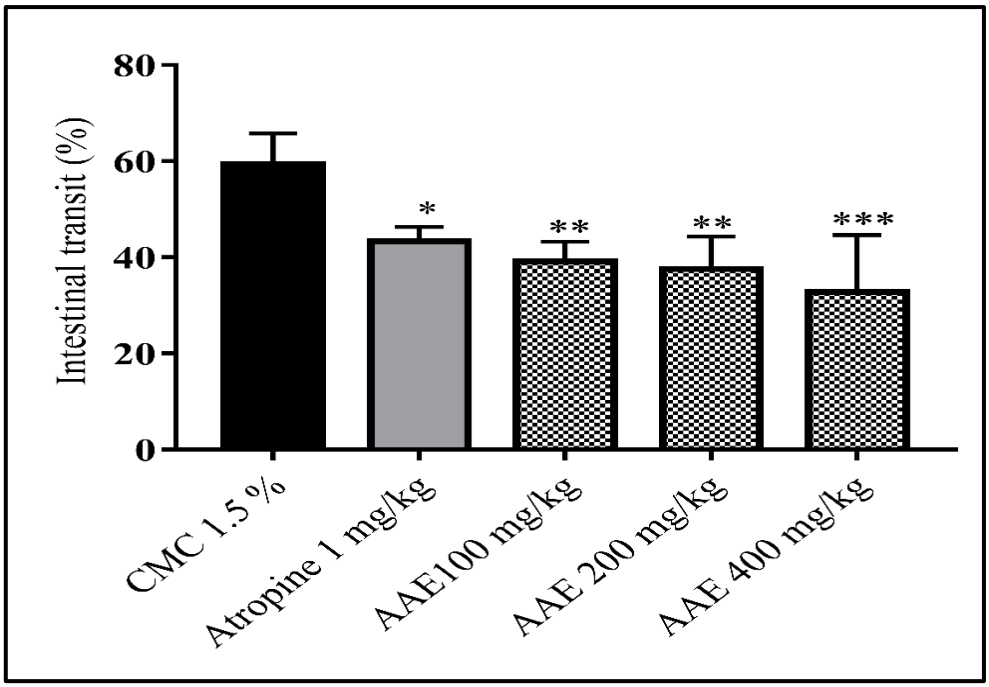


Figure 32: Effect of AAE on intestinal transit in mice. AAE : *A. Santolinoides* L. aqueous extract. The values are expressed as means ± SEM (n=6-8). (*P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001 *vs* CMC as negative control.

4.2.8. Mechanisms of AAE effect on intestinal transit in mice

As shown in figure 33, in the presence of atropine, AAE did not show any difference on the intestinal transit rate (P>0.05) compared to the effect observed with AAE alone or to the effect of atropine. No significant difference was observed in the treatment of mice with L-NNA and AAE simultaneously (P>0.05). Indomethacin, in the presence of AAE increased the intestinal transit compared to AAE alone to rate of 42.49 ± 5.90 %.

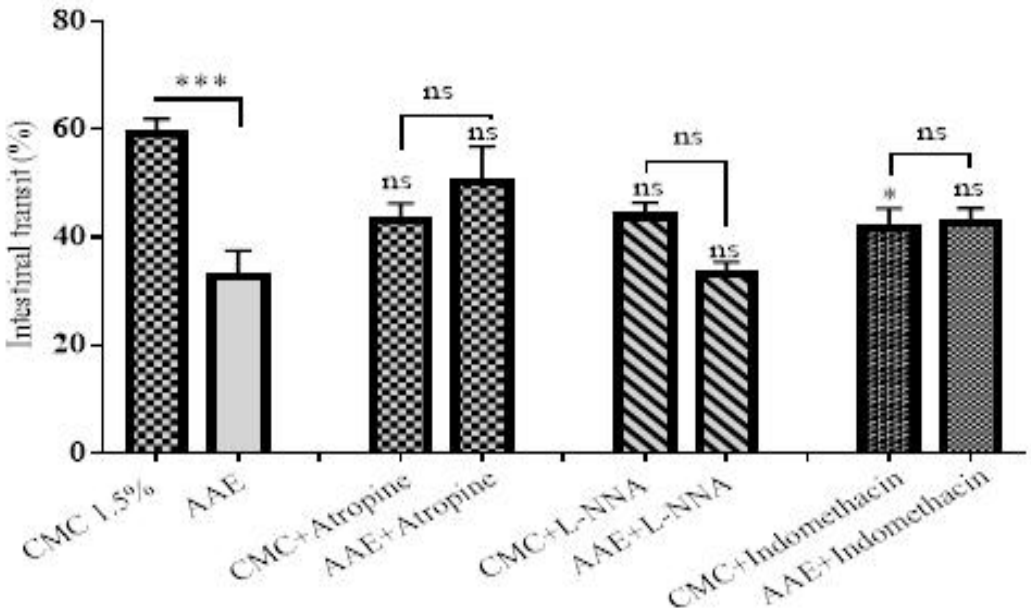


Figure 33: Effect of AAE (400 mg/kg) on intestinal transit in mice in the presence of atropine, L-NNA or indomethacin. AAE: *A. Santolinoides* L. aqueous extract. The values -are expressed as means ± SEM (n=6-8). *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001*vs* vehicle treated group (CMC 1.5%). ns: no significant difference.

4.3. Ethanol-induced gastric ulceration in rats

4.3.1. Macroscopic and histopathological examination of SAE effect on gastric mucosa in rats

The results of macroscopic examination of SAE effect on gastric mucosa in rats are shown in figures 34. The animals that received 100% ethanol developed consistent macroscopic damage, which was evidenced by a loss of normal colour and the presence of hemorrhage and oedema. This damage is reduced by the administration of SAE at different doses (Figure 34). These results were demonstrated by the histopathological analysis, here is less evidence of hemorrhage lesions, less infiltration and oedema in the gastric mucosa of rats treated with SAE at the dose of 100 mg/kg. At the highest doses of SAE (200 and 400 mg/kg), the animals were completely protected against the ethanol action, preserving all histological aspects when compared to control animal group (Figure 35).

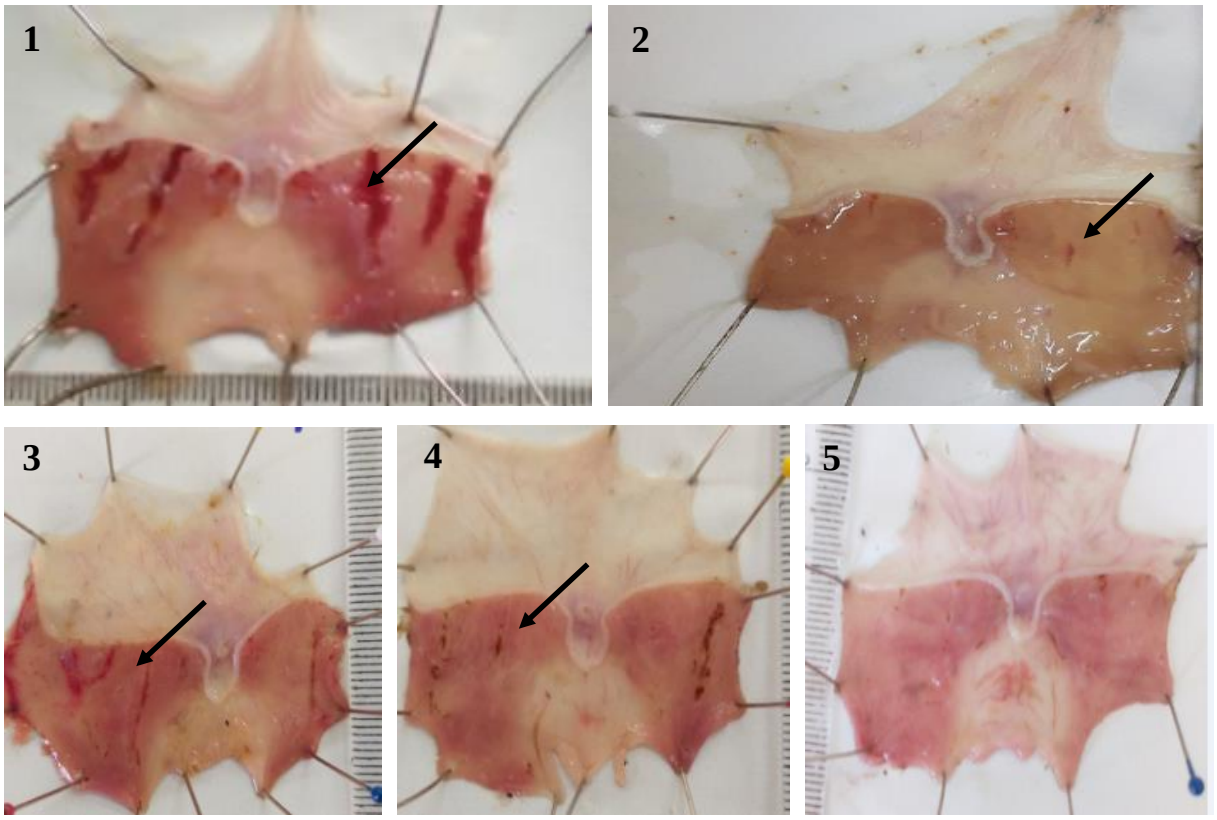


Figure 34: Effects of SAE (100, 200 and 400 mg/kg) on the macroscopic appearance of the gastric mucosa in ethanol-induced gastric mucosal lesions in rats.
(1): The group treated with CMC 1.5% as negative control. (2): The group treated with ranitidine (50mg/kg) as positive control. (3, 4 and 5): The group pre-treated with SAE (100, 200 and 400 mg/kg, respectively).

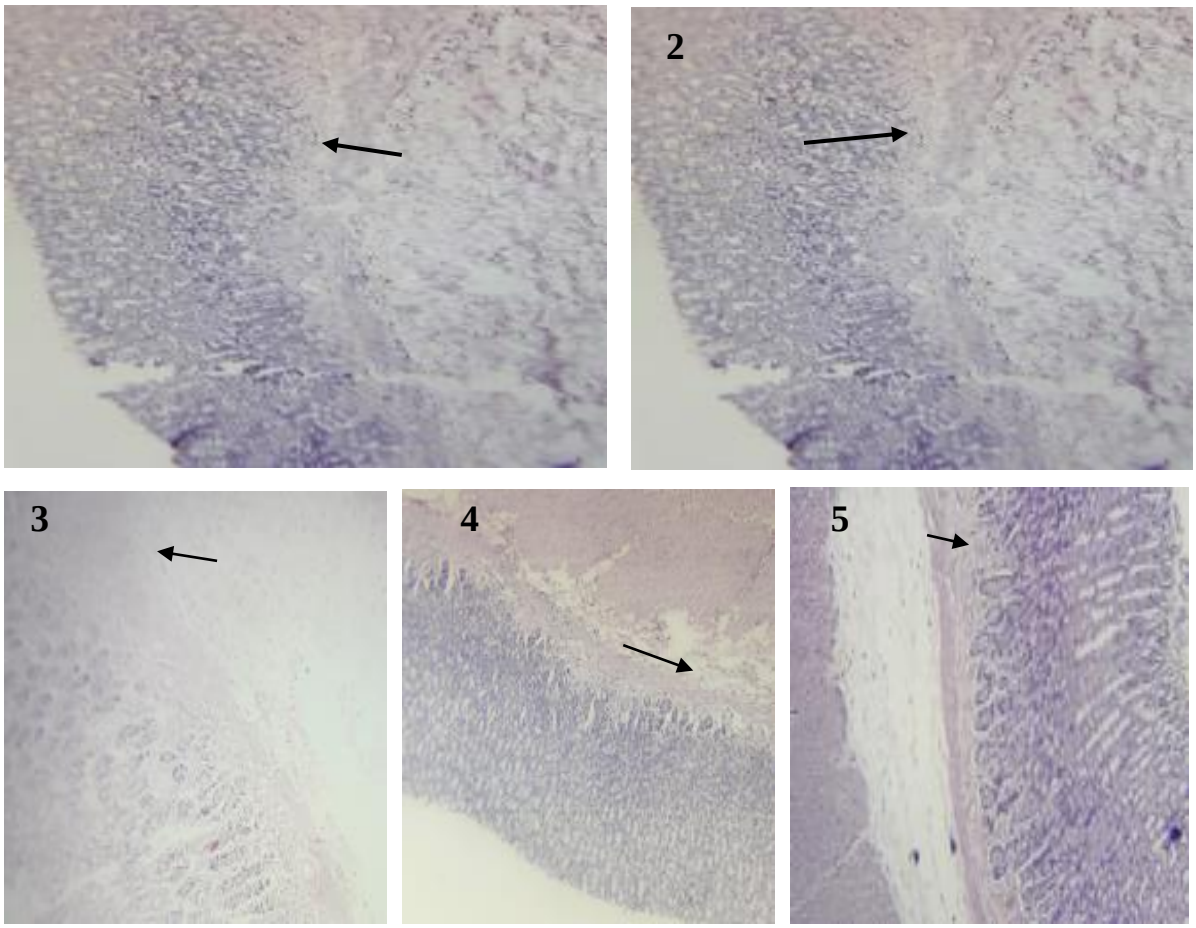


Figure 35: Histological evaluations of the protective effect of SAE (100, 200 and 400 mg/kg) on ethanol-induced gastric damage in rat stomach tissues (magnification 100x).
(1): group treated with CMC 1.5% as negative control. (2): The group pre-treated with ranitidine (50 mg/kg) as positive control. (3, 4 and 5): The group pre-treated with SAE (100, 200 and 400 mg/kg, respectively), Black arrows: inflammatory infiltrate composed of polynuclear eosinophyles.

4.3.2. Effect of SAE on gastric ulcer

The anti-ulcer activity of SAE in ethanol-induced gastric lesion model is presented in figure 36. SAE showed significant gastro protection ($P \leq 0.0001$) in comparison with the vehicle (CMC,1.5% as negative control). SAE at the tested doses (100, 200 and 400) reduced the gastric lesions to rate of 5.94 ± 0.62 %, 4.83 ± 1.34% and 2.39 ± 1.30% ($P \leq 0.0001$), respectively. No significant difference is recorded between the different doses of extract. The highest doses of SAE (200 and 400 mg/kg) did not show any significant difference($P > 0.05$) compared to ranitidine as positive control.

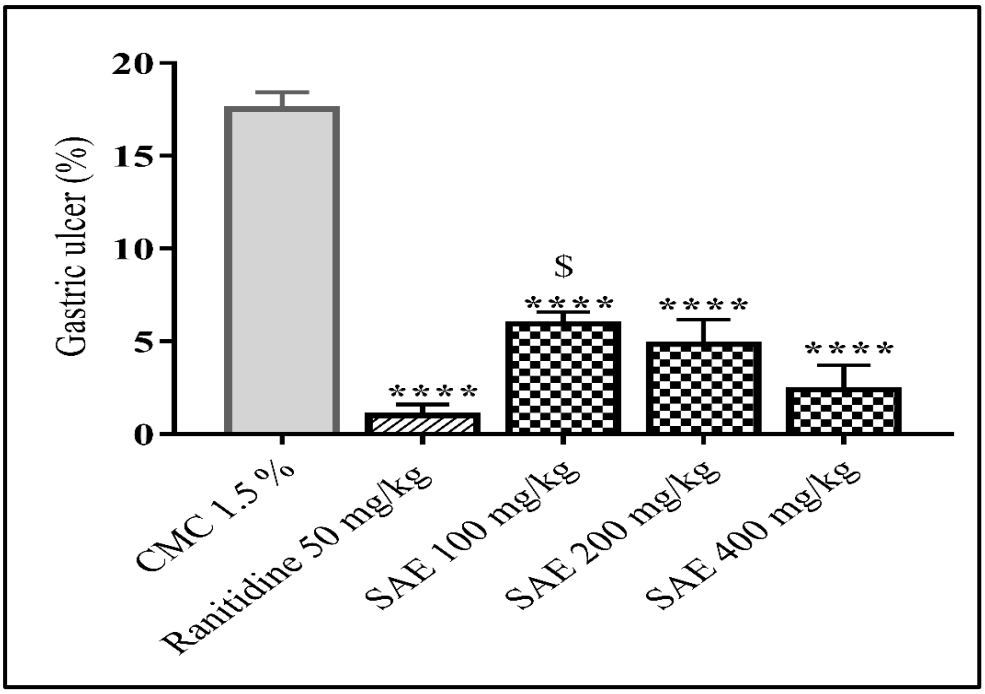


Figure 36: Effect of SAE (100, 200 and 400 mg/kg) on gastric ulcer in rats. SAE: *S. satureioides* aqueous extract. The values are expressed as means ± SEM (n=6). (****$P \leq 0.0001$) *vs* vehicle treated group (CMC 1.5 %), ($^{\$}P \leq 0.05$) *vs* ranitidine as positive control.

4.3.3. Effect of SAE on determination of gastric mucus content in rats

The effects of SAE on gastric mucus content are shown in figure 37. Compared with the vehicle treated animals (CMC 1.5%), SAE at doses (100 mg/kg, 200 mg/kg and 400 mg/kg) significantly ($P \leq 0.01$, $P \leq 0.001$; $P \leq 0.0001$) increased the gastric mucus content. No significant differece was observed in comparison of SAE at different tested doses and ranitidine as positive control ($P > 0.05$) .

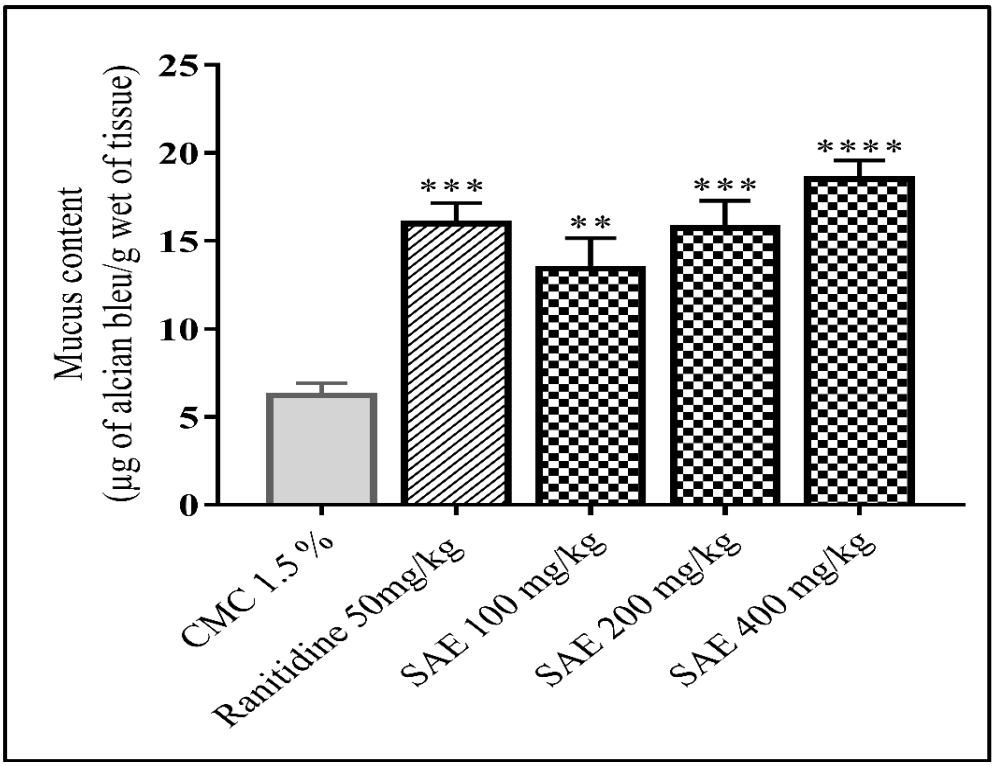


Figure 37: Effect of SAE on gastric mucus content in rats. SAE: *S. Satureioides* aqueous extract. The values of are expressed as means ± SEM (n=6). (**P≤0.01, ***P≤0.001; ****P≤0.0001) *vs* vehicle treated (CMC 1.5 %).

4.3.4. Evaluation of *in vivo* antioxidant activity of SAE gastric homogenate

4.3.4.1. Estimation of gastric total proteins content

As shown in table 6, statistical analysis showed that there is no significant difference in the protein content between the group treated with the three doses (100, 200 and 400 mg/kg) (23.58±0,85 µg/mL; 23.51±0,57 µg/mL and 23.77±0,31 µg/mL; P>0.05,respectively), while SAE increased the protein content (P<0.01) compared to the vehicle 19.44 ± 0.75 µg/mL). The treatment of rats with different doses of SAE (100, 200 and 400 mg/kg) has no effect (P>0.05) on the protein content compared to the group treated with ranitidine as positive controle.

4.3.4.2. Effect of SAE on GSH level

The effect of SAE on GSH levels in stomach tissue homogenates is presented in table 6. We notice that the treatment of rats with SAE caused a significant increase in GSH level at the doses of 200 and 400 mg/kg (24.43 ± 1.02 µmoL/g tissue and 25.03 ± 1.27 µmoL/g tissue, P < 0.0001) compared to the vehicle (8.68 µmoL/g tissue). Whereas, these values were higher than those of the positive control group (11.06 ± 0.64 µmoL/g tissue P<0.05).

4.3.4.3. Effect of SAE on lipid peroxidation

Following ethanol administration, lipid peroxidation and therefore MDA stomach content was significantly increased in with the vehicle treated animal group. On the other hand, the pre-treatment of the rats with different doses of SAE decreased gastric lipid peroxidation. At the highest doses (200 mg/kg and 400 mg/kg), SAE greatly (4.03 ± 0.51; 3.67 ± 0.36 µmoL/g tissue; P≤0.05; P≤0.01) decreased MDA levels (Table 6). SAE at the highest (200 and 400 mg/kg) exhibited significant difference on lipid peroxidation inhibition as positive control, (6.76 ± 0.83 µmoL/g tissue; P≤0.05). There is no significant difference between SAE effect at all doses (P>0.05) (Table 6).

Table 6: Effect of SAE on total protein, GSH and MDA levels of stomach tissue in ethanol-induced gastric mucosal lesions in rats.

Antioxidant parameters	**Proteins (µg/mL)**	**GSH (µmoL /g tissue)**	**MDA (µmoL/g tissue)**
Vehicle	19.44 ± 0.75	8.62 ± 0.65	7.24 ± 0.59
Ranitidine 50 mg/kg	23.42 ± 1.17 **	16.10 ± 0.83 *	6.76 ± 0.83 ^{ns}
SAE (100 mg/kg)	23.58 ± 0,85 **	11.06 ± 0.64 $^{ns\ §§§§}$	4.56 ± 0.27 ^{ns}
SAE (200 mg/kg)	23.51± 0,57 **	24.43 ± 1.02 $^{****\ \$\ §§§}$	4.03 ± 0.51* $^{\$}$
SAE (400 mg/kg)	23.77 ± 0,31**	25.03 ± 1.27 $^{****\ \$}$	3.67 ± 0.36 **** $^{\$}$

SAE: *S. satureioides* aqueous extract. Bars represent means±SEM (n=6). *P≤0.05; ** P≤0.01; ***P≤0.001; ****P≤0.0001 *vs* vehicle as negative control. ($^{\$}$P≤0.05) *vs* ranitidine as positive control. (§§§P≤0.001; §§§§P≤0.0001 in comparison between the effect of SAE at different doses (100, 200, 400 mg/kg). ns:no significant difference.

4.3.5. Macroscopic and histopathological examination of the mechanismes of SAE effect on gastric mucosa in rats

Macroscopic analysis of SAE effect on ethanol-induced gastric ulcer in rats is depicted in figure 38. compared to the injuries seen in the negative control rats, atropine, whether used in the absence or presence of SAE, reduced the stomach mucosal damage (Figure 38:3 and 38: 5). L-NNA either increased the stomach ulcer area in the absence of SAE (Figure 38: 4) or in its presence (Figure 38: 6). Additionally, these results were demonstrated by the histopathological analysis as indicated in figure 39.

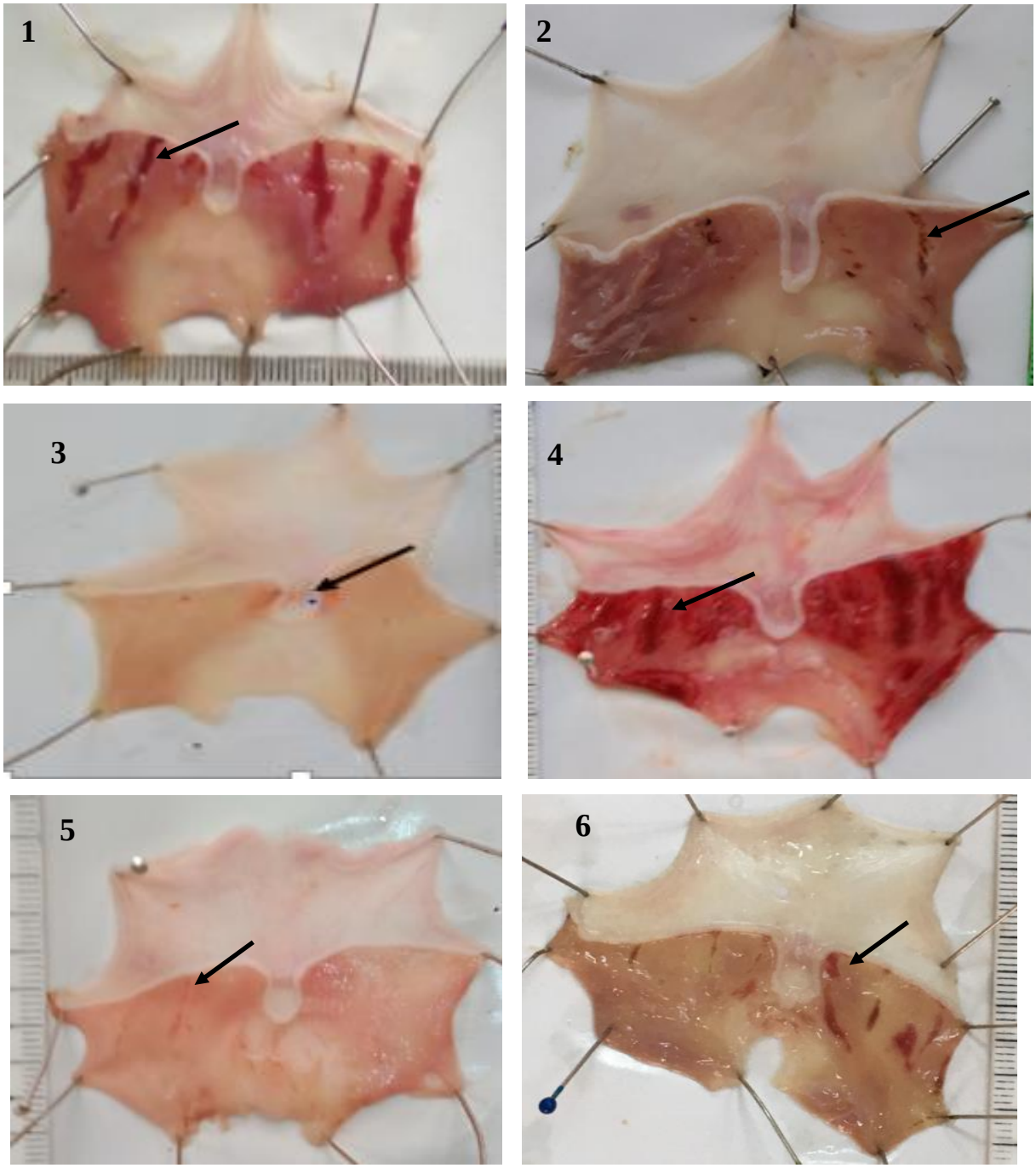


Figure 38: Effects of SAE on the macroscopic appearance of the gastric mucosa in ethanol-induced gastric mucosal lesions in rats in the presence of atropine or L-NNA.
(1): The group pre-treated with CMC vehicle as negative control. (2): The group pre-treated with SAE (200 mg/kg). (3 and 4): The group pre-treated with atropine or L-NNA, respectively in absence of SAE. (5 and 6): The group pre-treated with atropine and L-NNA, respectively in presence of SAE. Black arrow: elongated bands of hemorrhagic lesions.

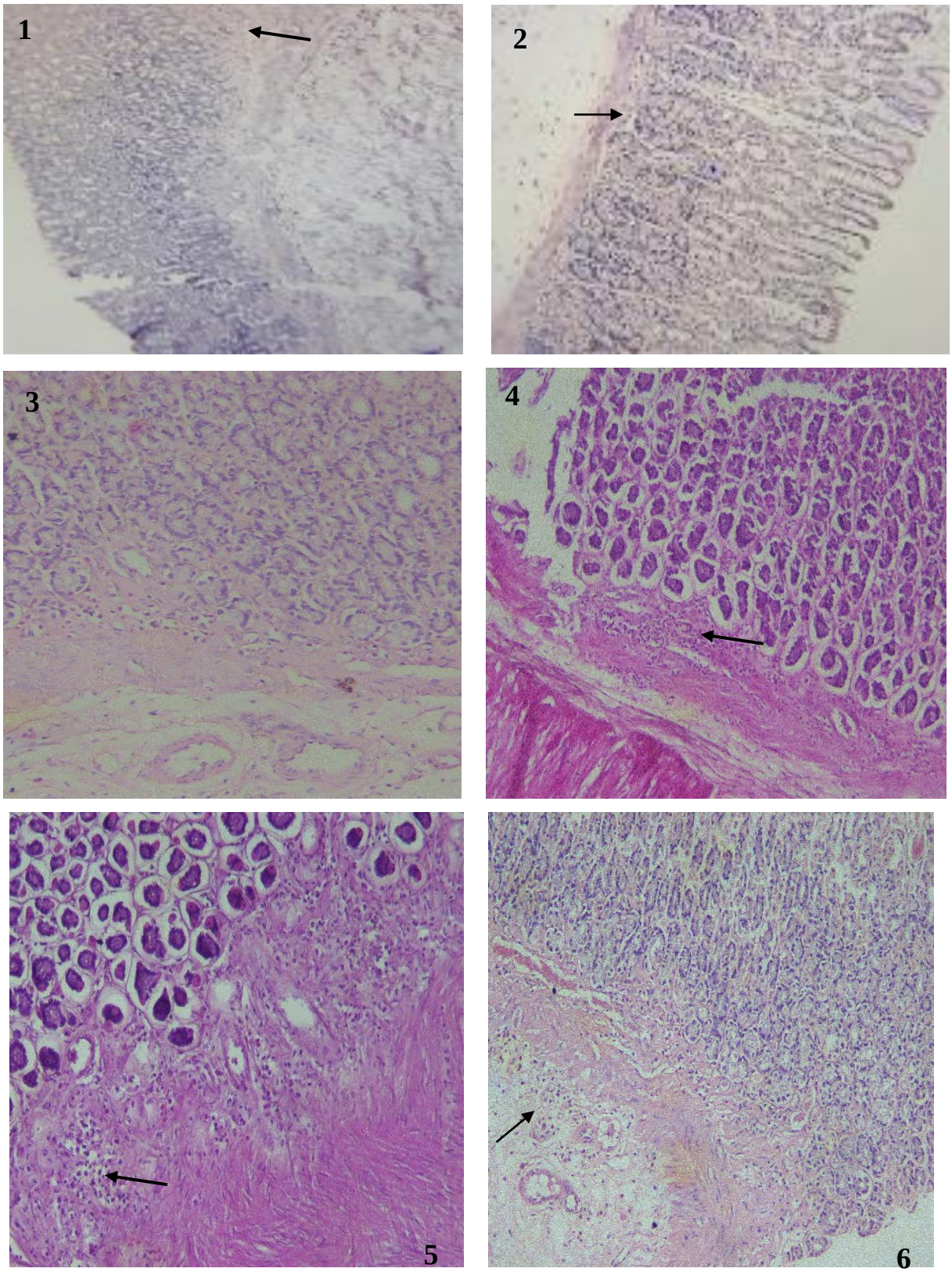


Figure 39: Histological evaluations for the protective effect of SAE in absence or presence of atropine or L-NNA against ethanol-induced gastric mucosal lesions in rats (magnification 100x).

(1): The group treated with CMC 1.5 % as negative control. (2): The group treated with SAE (200 mg/kg). (3 and 4): The groups pre-treated with atropine or L-NNA, respectively, (5 and 6): The groups pre-treated with atropine or L-NNA, respectively in the same time with SAE at dose of 200 mg/kg. Black arrows: inflammatory infiltrate composed of polynuclear eosinophyles.

4.3.6. Mechanisms of SAE on gastroprotective effect

The effect of SAE (200 mg/kg) on gastric lesions induced by ethanol in the presence of L-NNA or atropine is reported in figure 36. Atropine reduced the gastric lesion to 2.85 ± 0.36 %. In contrary, L-NNA increased the gastric ulcer to 17.89 ± 2.61 %. Administration of SAE extract to animals pretreated with atropine decreased the gastric lesions to 1.60 ± 0.97% ($P \leq 0.0001$) compared to the vehicle treated animals. In contrary, treatment of animals with SAE in the presence of L-NNA increased the area of gastric lesion to 5.91 ± 2.35 % (Figure 40). It was found that pre-treatment of animals with SAE alone did not show any significant difference compared to SAE in the presence of atropine or L-NNA.

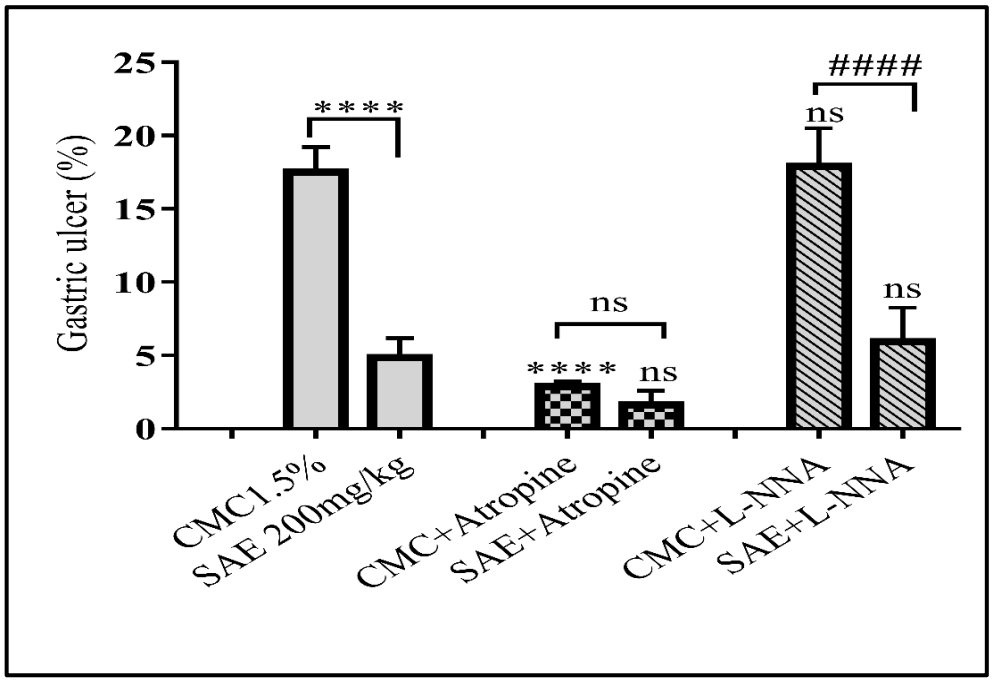


Figure 40: Effect of SAE (200 mg/kg) on gastric ulcer in rats in the presence of atropine or L-NNA. SAE: *S. saturoiedes* aqueous extract. The values are expressed as means ± SEM (n=6). (****$P \leq 0.0001$) in comparison with CMC 1.5 %. ($^{\#\#\#\#}P \leq 0.0001$) in comparison with correspondent control. ns: no significant difference.

4.3.7. Mechanisms of SAE effects on mucus content in gastric layer

The mechanisms of SAE effect on gastric mucus content is shown in figure 41. Atropine significantly increased the mucus content ($P \leq 0.001$) compared to the vehicule. Mucus content observed with SAE in the presence of atropine was significantly increased ($P \leq 0.001$). Treatment of animals with SAE simultanously with L-NNA did not show any difference when compared to SAE alone.

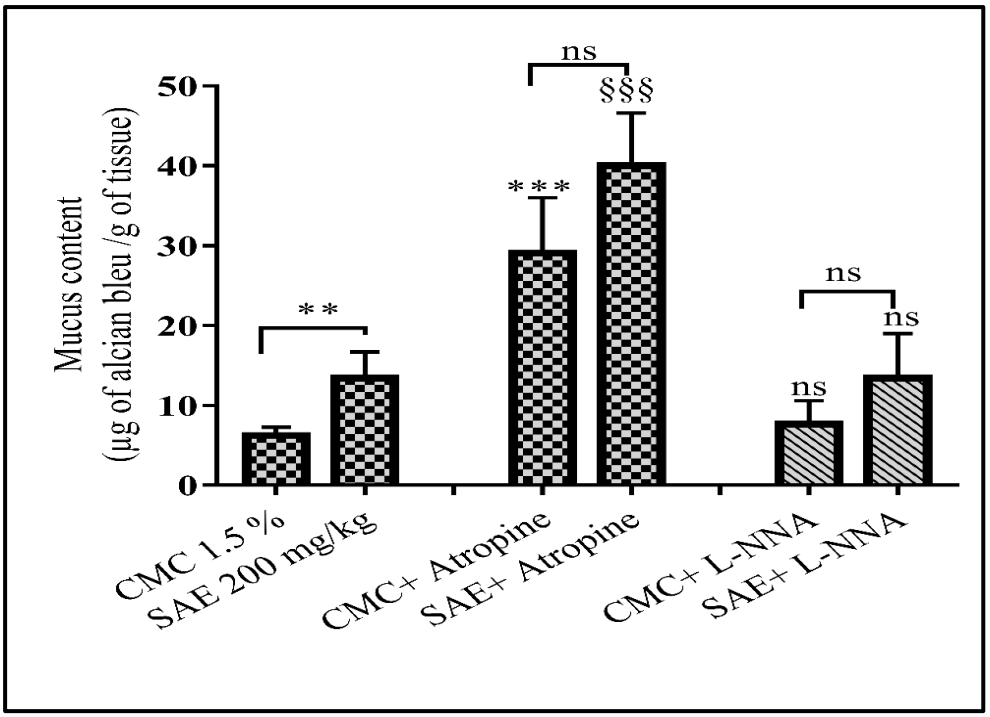


Figure 41: Effect of SAE on gastric mucus content in rats in the presence of atropine or L-NNA. SAE: *S . Satureioides* aqueous extract. The values are expressed as means ± SEM (n=6). (**P≤0.01; ***P≤0.001) *vs* vehicle treated group. §§§P≤0.001 in comparison with SAE in the presence of atropine with SAE alone.

4.3. 8. *In vivo* antioxidant activity of mechanisms of SAE

4.3.8.1. Estimation of gastric total proteins content

The mechanism of SAE effect on gastric protein content is shown in table 7. Treatment of rats with atropine or L-NNA with SAE (200 mg/kg) has no effect (P>0.05) on the protein content compared to the group treated with SAE in presence and in absence of atropine or L-NNA.

4.3.8.2. Effect of SAE on GSH level

Treatment of rats with atropine significantly (P≤0.0001) increased GSH level (42.32 ± 1.78). Whereas, in the presence of L-NNA, the level of GSH decreased to the level of 16 ± 2.22 µmoL/g tissue (P≤0.0001) (Table 7). Treatment of rats with SAE caused a significant increase in GSH level in the presence of atropine compared to the effect by the treatment with SAE alone (46.90 ± 2.28 µmoL/g tissue; P≤0.0001). No significant difference was recorded between SAE in the presence or the absence of L-NNA on the GSH level (Table 7).

4.3.8.3. Effect of SAE on lipid peroxidation

The effect of SAE on MDA levels in stomach tissue homogenates in the presence of atropine or L-NNA is presented in table 7. We observed that the animals treated with atropine showed a decrease in MDA level (4.38 ± 0.85; P≤0.01) compared to the vehicle. MDA level was non significantly increased in the animals treated with L-NNA (7.45 ± 0.16 µmol/g tissue; (P>0.05) compared to the vehicle (7.24 ± 0.59 µmol/g tissue). In the presence of atropine, SAE decreased MDA level (1.61± 0.22µmoL/g tissue) compared to SAE alone (P ≤ 0.05). SAE in the presence of L-NNA significantly increased the MDA gastric content compared to SAE when used alone (P>0.05).

Table 7: Effect of SAE on total protein, GSH and MDA levels of stomach tissue in ethanol-induced gastric mucosal lesions in rats in presence of atropine or L-NNA.

	Vehicle	SAE 200 mg/kg	Vehicle		SAE 200 (mg/kg)	
			Atropine	**L-NNA**	**Atropine**	**L-NNA**
Proteins µg/ml	19.44 ± 0.75	23.51 ± 0.57 **	20.51± 0.52 ^{ns}	19.24± 0.47 ^{ns}	20.87±0.95 ^{ns}	20.03±0.88 ^{ns}
GSH (µmol/g tissue)	8.62 ± 0.65	24.43 ± 1.02 ****	42.32 ± 1.78 ****	16.19 ± 1.83****	46.90 ± 2.28 §§§§	22.41 ± 1.77 ^{ns}
MDA (µmol/g tissue)	7.24 ± 0.59	4.03 ± 0.51 ***	4.38 ± 0.85 **	7.45 ± 0.16 ^{ns}	1.61± 0.22 §	5.05±0.28 ^{ns}

SAE: *S. satureioides* aqueous extract. Values represent means ± SEM (n=6). (** P≤0.01, *** P≤0.001, ****P≤0.0001) *vs* vehicle as negative control. (§≤0.001 ; §§§§ P≤0.0001;) in comparing SAE in presence of atropine or L-NNA *vs* SAE alone. ns; no significant difference.

4.3.9. Macroscopic and histopathological examination of AAE effect on gastric mucosa in rats

Figures 42 displays the findings of a macroscopic analysis of AAE on rat stomach mucosa. A loss of normal color, the presence of bleeding, as well as continuous macroscopic damage, were all signs of treatment with 100% ethanol in the animals. By administering the utilized doses of AAE at various doses, this harm is diminished (Figure 42). These findings were supported by histopathological analysis, which showed that rats treated with AAE at doses of 200 mg/kg and 400 mg/kg exhibites less evidence of hemorrhage lesions, less infiltration, and less oedema in the gastric mucosa. These animals were completely protected from the effects of ethanol, maintaining all histological aspects when compared to the control animal group (Figure 43).

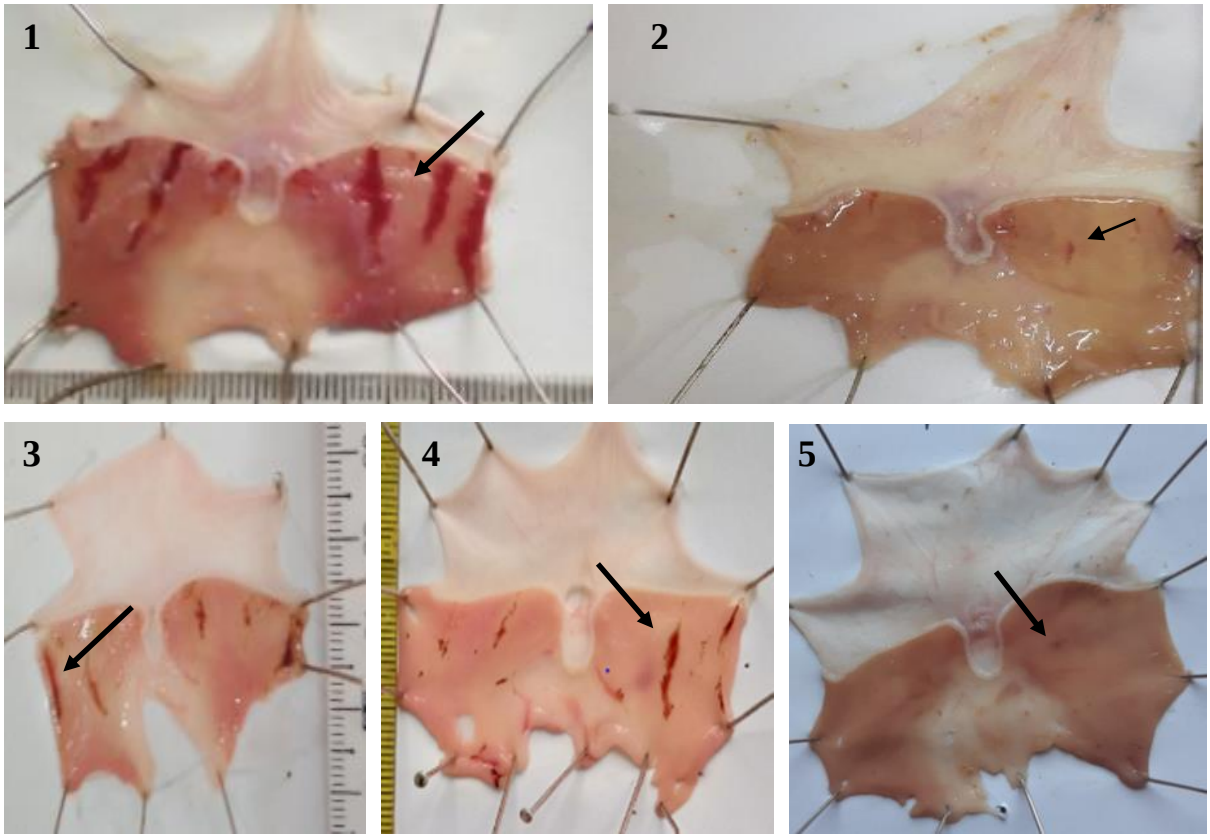


Figure 42: Effect of AAE (100, 200 and 400 mg/kg) on the macroscopic appearance of the gastric mucosa in ethanol-induced gastric mucosal lesions in rats.
(1): The group treated with CMC 1.5% as negative control. (2): The group treated with ranitidine (50 mg/kg) as positive control. (3, 4 and 5): The group pre-treated with AAE (100, 200 and 400 mg/kg, respectively).

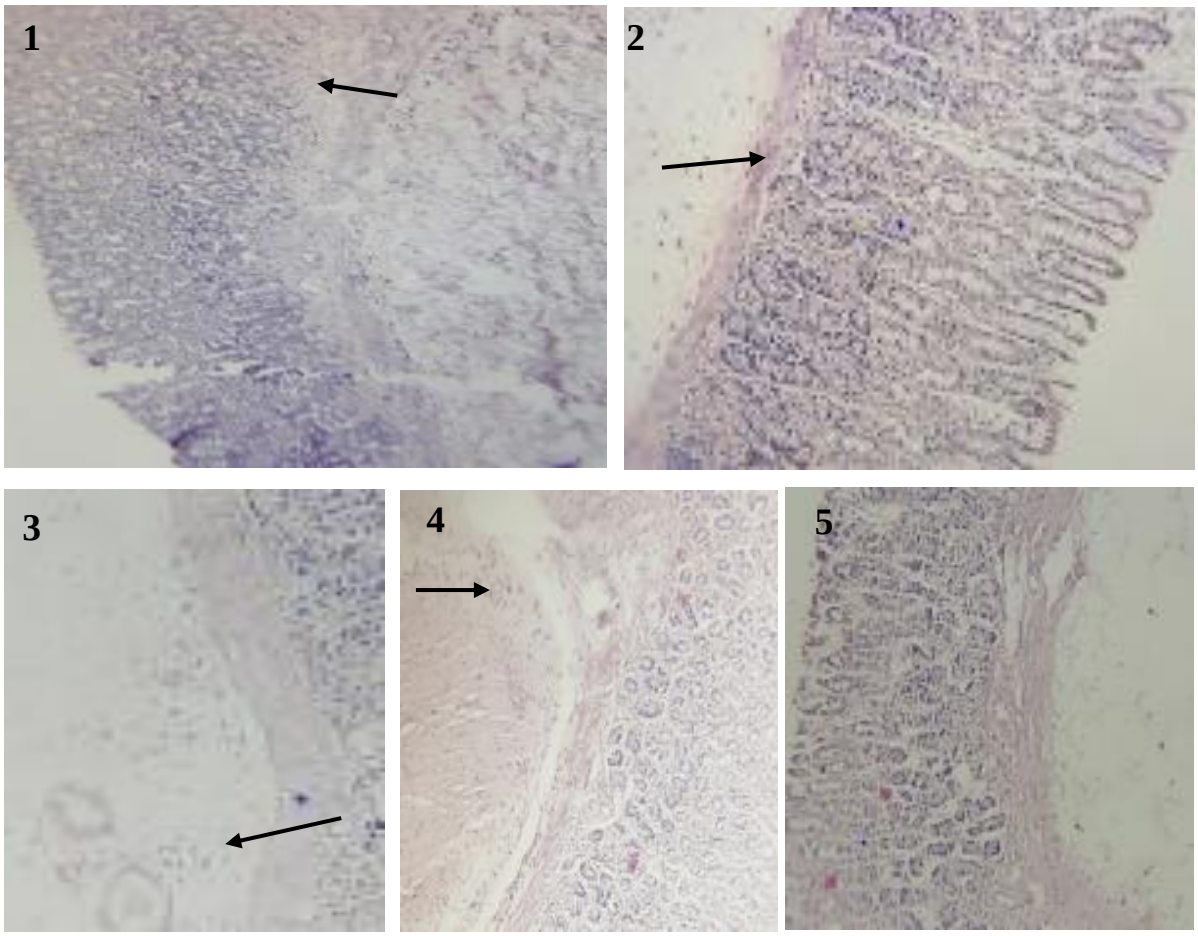


Figure 43: Histological evaluations of the protective effect of AAE (100, 200 and 400 mg/kg) on ethanol-induced gastric damage in rat stomach tissues (magnification 100x).
(1): The group treated with CMC 1.5 % as negative control. (2): The group treated with ranitidine (50 mg/kg) as positive control. (3, 4 and 5): The group pre-treated with AAE (100, 200 and 400 mg/kg, respectively), Red arrow: surface epithlium damage; Black arrows: inflammatory infiltrate composed of polynuclear eosinophyles.

4.3.10. Effect of AAE on gastric ulcer

As shown in figure 44, AAE at the tested doses (100, 200 and 400 mg/kg) significantly reduced the gastric lesions against ethanol-induced gastric ulceration in rats with level of gastric lesions of 7.78 ± 0.62%, 4.91 ± 1.20% and 3.62 ± 1.69% ($P \leq 0.0001$), respectively, compared to the vehicle treated animals (CMC 1.5%). No significant difference is recorded between the different doses of extract. The highest doses of AAE (200 and 400 mg/kg) did not show any significant difference ($P > 0.05$) compared to ranitidine as positive control.

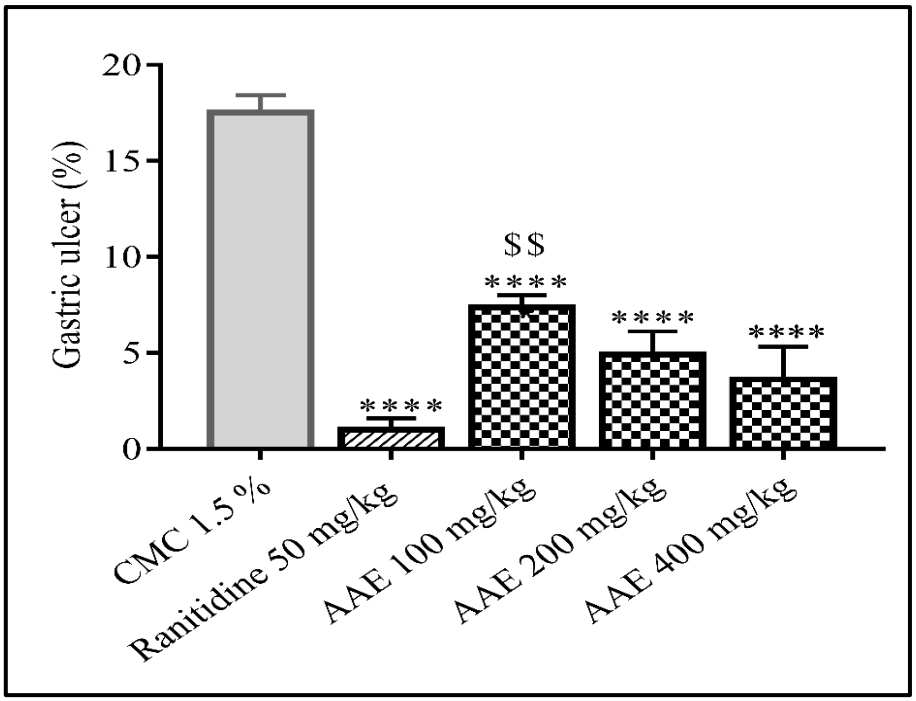


Figure 44: Effect of AAE (100, 200 and 400 mg/kg) on gastric ulcer in rats. AAE: *A. santolinoides* L. aqueous extract. The values are expressed as means ± SEM (n=6). (****P≤0.0001) *vs* vehicle treated group, ($$P≤0.01) *vs* ranitidine as positive control.

4.3.11. Effect of AAE on determination of gastric mucus content

Figure 45 showed the effects of AAE on gastric mucus content. AAE at doses of 100 mg/kg and 200 mg/kg did not show any significant difference compared to the vehicle group. In contrary at the highest dose (400 mg/kg; significantly, AAE increased the gastric mucus content compared to the vehicle group (P≤0.0001). AAE at the lowest doses (100 and 200 mg/kg) showed comparable effects (P≥0.05) as ranitidine as positive control; however at the highest dose, AAE showed a highly significant difference on gastric mucus content compared to ranitidine as positive control. No significant difference was observed in comparison of AAE at 100 mg/kg and 200 mg/kg, and it showed significant difference on gastric mucus content than AAE 400 mg/kg.

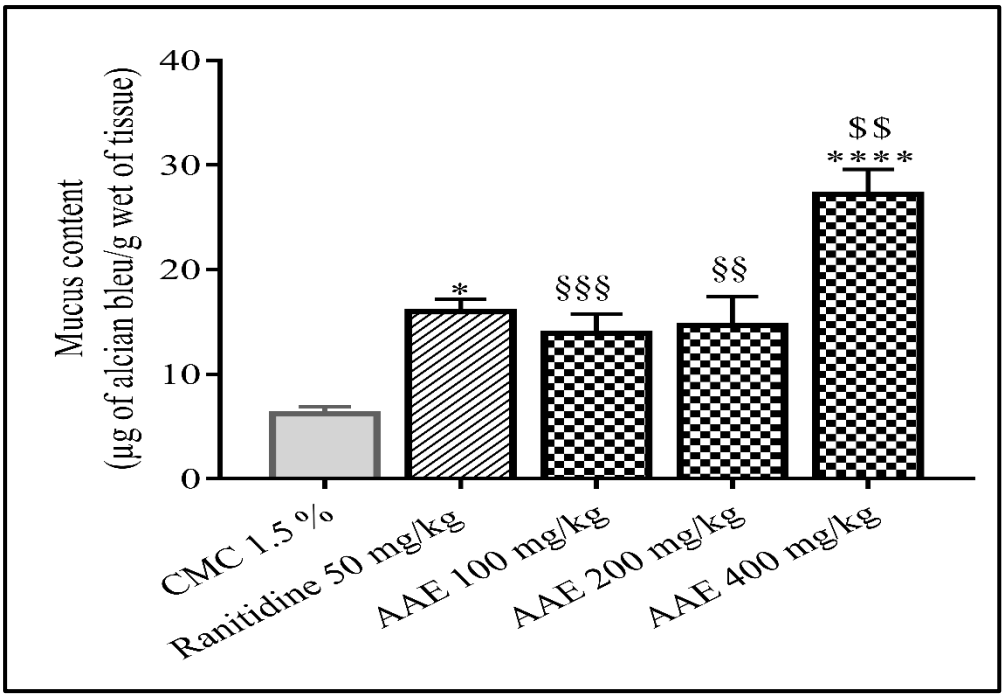


Figure 45: Effect of AAE on gastric mucus content in rats. AAE: *A . Satntolinoides* aqueous extract. The values are expressed as means ± SEM (n=6). (*P≤0.05, ****P≤0.0001) *vs* vehicle treated group. ($^{\$\$}$P≤0.01) *vs* ranitidine as positive control. (§§P≤0.01; §§§P≤0.001) in comparison AAE 100 mg/kg and AAE 200 mg/kg *vs* AAE 400 mg/kg.

4.3.12. *In vivo* antioxidant activity of AAE gastric homogenate

4.3.12.1. Estimation of gastric total proteins content

As shown in table 8, treatment of rats with AAE (100 and 200 mg/kg) did not show any significant difference compared to the vehicle (P>0.05). In contrary, the highest dose (400 mg/kg) showed significant difference (P≤0.001) in protein content. There was no significant difference (P>0.05) between the highest doses (200 and 400 mg/kg) compared to the animal group treated with ranitidine as positive control.

4.3.12.2. Effect of AAE on GSH level

Table 8 presented the effect of AAE on GSH levels in stomach tissue homogenates. We observed that the treatment of rats with AAE caused a significant increase in GSH level, at the highest dose (400 mg/kg), AAE had highly significant difference (31.05 ± 0.56 µmoL/g tisuue; P≤0.0001) compared to the vehicle treated animal group (8.68 µmoL/g tissue. Whereas, AAE at 100 and 200 mg/kg did not have any significant difference when compared to ranitidine as positive control (P>0.05). On the other hand, AAE (400 mg/kg) showed a significant difference compared to ranitidine as positive control (16.10 ± 0.83 µmoL/g tissue P≤ 0.01).

4. 3.12.3. Effect of AAE on lipid peroxidation

Following ethanol administration, lipid peroxidation and therefore MDA stomach content was significantly increased in the vehicle treated animal group. On the other hand, the pretreatment of rats with the different doses of AAE decreased gastric lipid peroxidation. At the highest doses (200 mg/kg and 400 mg/kg), AAE greatly decreased MDA levels (3.88 ± 0.15 and 3.80 ± 0.24 µmoL/g tissue, P≤0.01 and P≤0.05, compared to vehicle and to ranitidine as positive control. There was no significant difference between all the tested doses (P>0.05) (Table 8).

Table 8: Effect of AAE on total protein, GSH and MDA levels of stomach tissue in ethanol-induced gastric mucosal lesions in rats.

Antioxidant parameters	**Proteins (µg/mL)**	**GSH (µmoL/g tissue)**	**MDA (µmoL/g tissue)**
Vehicle	19.44 ± 0.75	8.62 ± 0.65	7.24 ± 0.59
Ranitidine (50 mg/kg)	23.42 ± 1.17 **	16.10 ± 0.83*	6.76 ± 0.83 ns
AAE (100 mg/kg)	20.61 ± 0.95 $ §§	23.15 ± 2.06 **	5.19 ± 0.75 ns
AAE (200 mg/kg)	20.94 ± 1.14	18.85 ± 1.07 * §§	3.88 ± 0.15 ** $
AAE (400 mg/kg)	24.89± 1.78 ***	31.05 ± 0.56 **** $$	3.80 ± 0.24 ** $

AAE: *A. Santolinoides* aqueous extract. Values represent means ± SEM (n=6). (*P≤0.05; **P≤0.01; ***P≤ 0.001;****P≤0.0001) *vs* vehicle as negative control. ($P≤0.05;$$P≤0.01) *vs* ranitidine as positive control. (§§P ≤ 0.01) in comparison with AAE 400 mg/kg. ns: no significant difference.

4.3.13. Macroscopic examination of the mechanismes of AAE effect on gastric mucosa in rats

Macroscopic analysis of AAE effect on ethanol-induced gastric ulcer in rats is depicted in figure 46. Animals pre-treated with atropine in the absence of AAE (Figure 46: 3) and in its presence (Figure 46: 5) reduced the area of gastric ulcer, compared to negative control animals. L-NNA either in the absence (Figure 46: 3) or in the presence of AAE (Figure 46: 6) increased the gastric ulcer area. These results were demonstrated by the histopathological analysis (Figure 47).

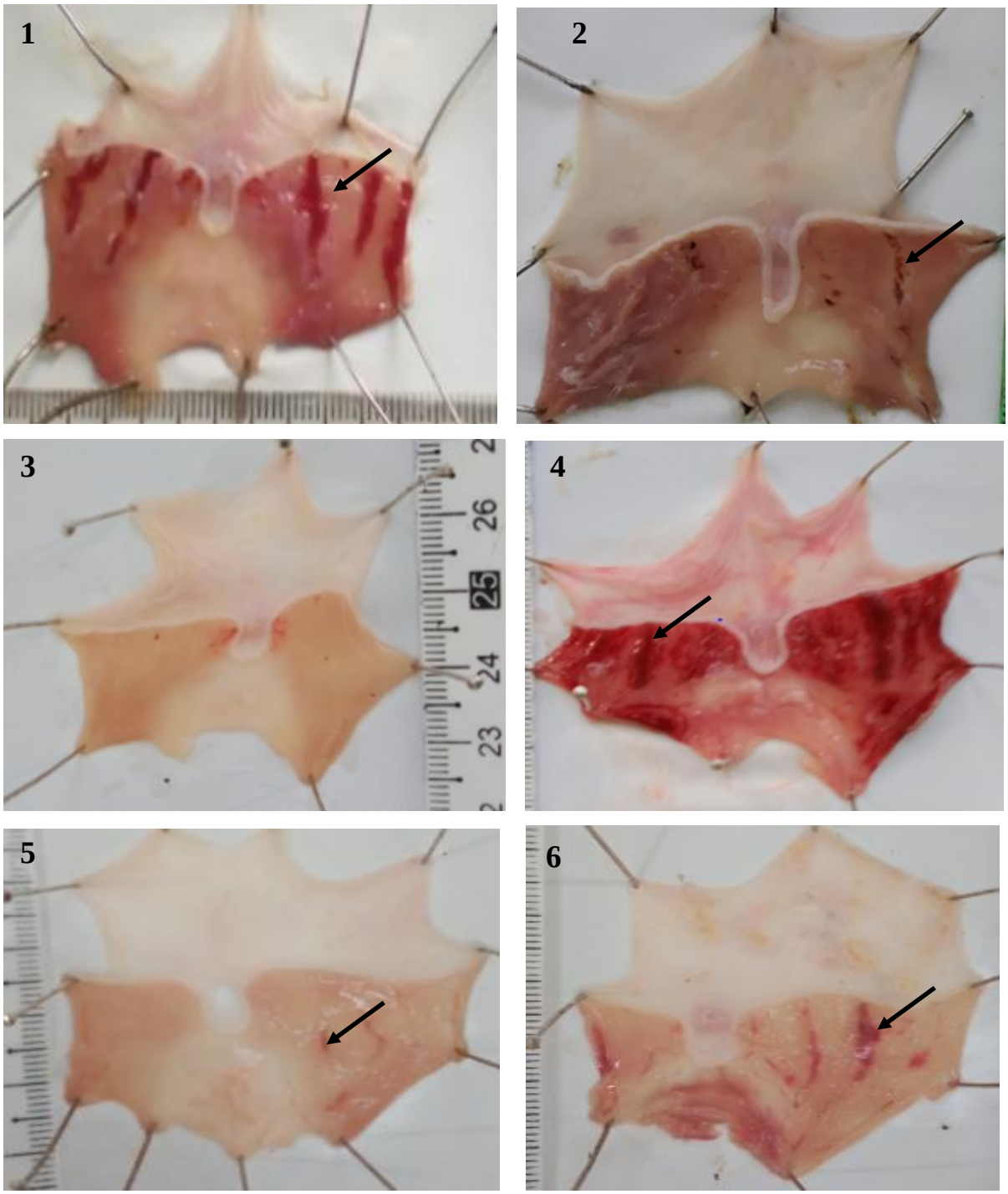


Figure 46: Effect of AAE in absence or presence of atropine or L-NNA on the macroscopic appearance of the gastric mucosa in ethanol-induced gastric mucosal lesions in rats.
(1): The group pre-treated with CMC vehicle as negative control. (2): The group pre-treated with AAE (200 mg/kg). (3 and 4): The group pre-treated with atropine and L-NNA, respectively in absence of AAE. (5 and 6): The group pre-treated with atropine and L-NNA, respectively in presence of AAE. Black arrow: elongated bands of hemorrhagic lesions.

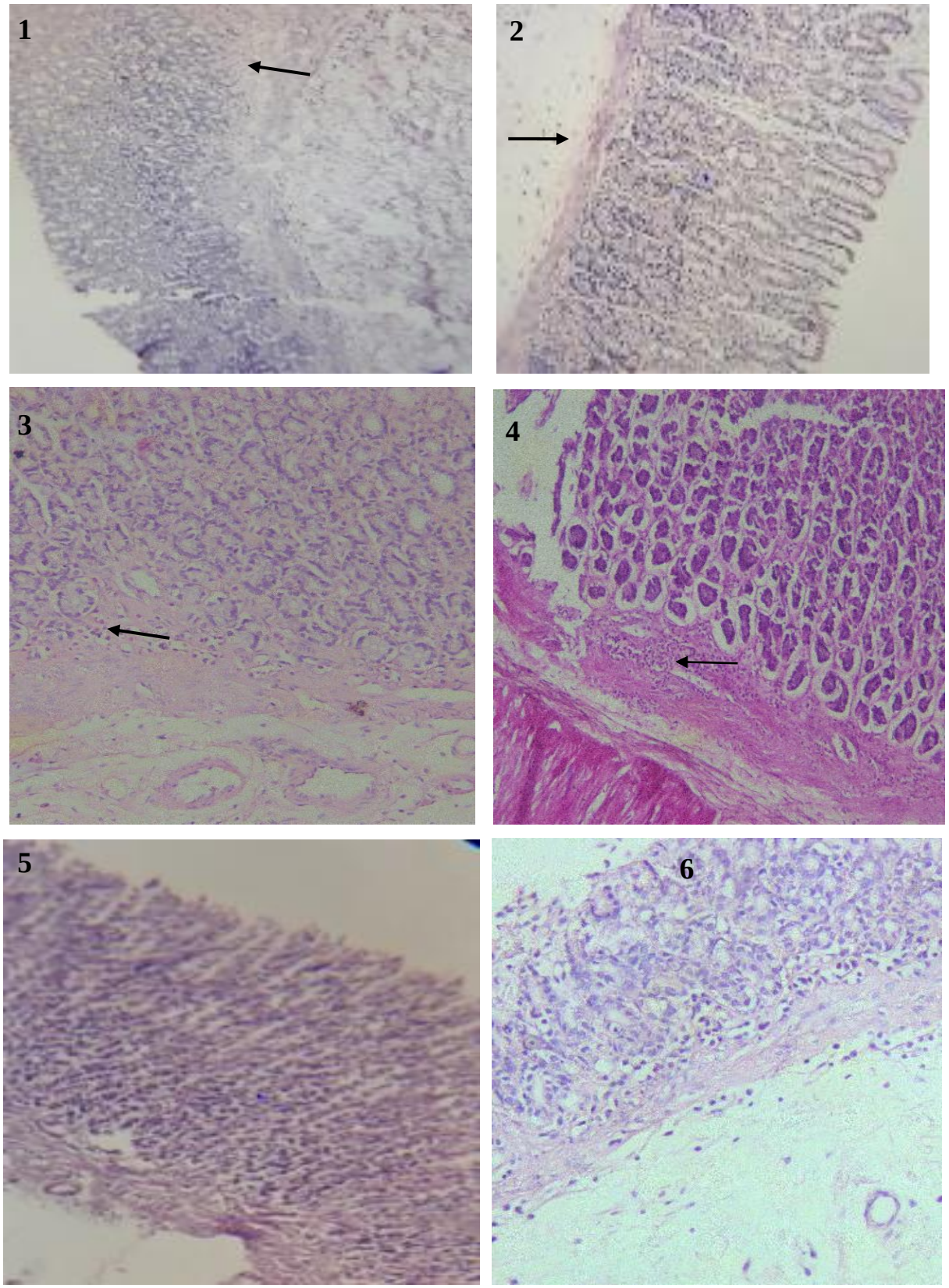


Figure 47: Histological evaluations for the protective effect of AAE in absence or presence of atropine or L-NNA against ethanol-induced gastric mucosal lesions in rats (magnification 100x).
(1): The group treated with CMC 1.5 % as negative control. (2): The group treated with AAE (200 mg/kg). (3 and 4): The groups pre-treated with atropine or L-NNA, respectively. (5 and 6): The groups pre-treated with atropine or L-NNA, respectively in the same time with AAE at dose of 200 mg/kg. Black arrows: inflammatory infiltrate composed of polynuclear eosinophyles.

4.3.14. Mechanisms of AAE on gastroprotective effect

The mechanisms of AAE gastroprotective effect is shown in figure 48. AAE in the presence of atropine showed no significant difference effect (3.78 %, P>0.05) compared to the group treated with atropine or to the group treated with AAE alone. On other hand, AAE with L-NNA slightly incresead the gastric ulcer compared to the AAE alone (P>0.05), but it had provided a high significant difference compared to the group treated with L-NNA (P≤0.0001).

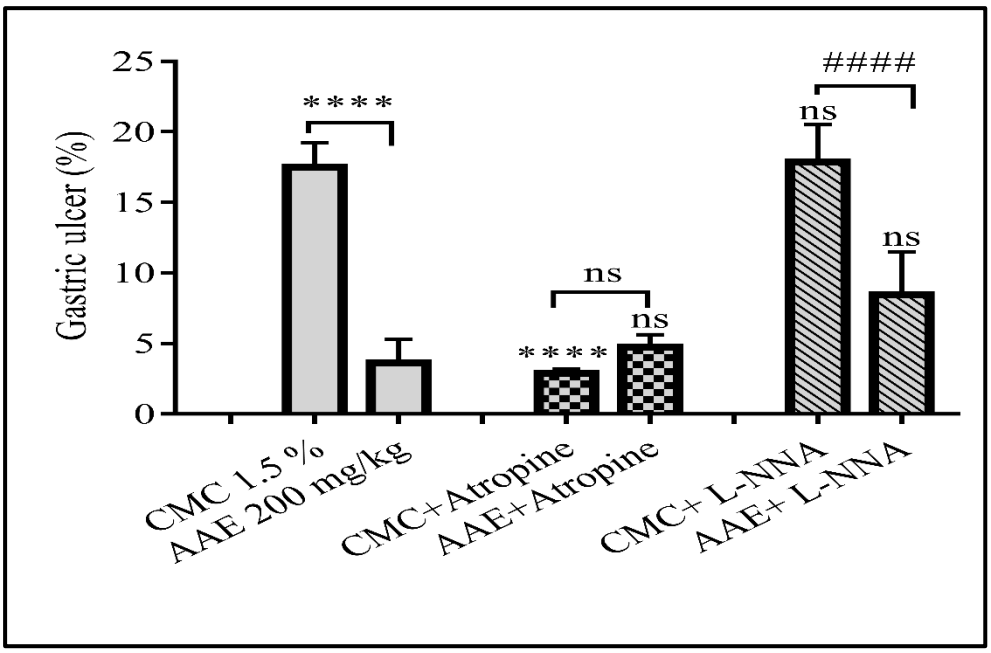


Figure 48: Effect of AAE (200 mg/kg) on gastric ulcer in rats in the presence of atropine or L-NNA. AAE: *A. santolinoides* L. aqueous extract. The values are expressed as means ± SEM (n=6). (****P≤0.0001) in comparison with CMC1.5%. (####P≤0.0001) in comparison with L-NNA as correspondent control. ns: no significant difference.

4.3.15. Determination of mucus content in gastric layer in rats

The mechanisms of AAE effect on gastric mucus content is shown in figure 49. Atropine significantly increased the mucus content (P≤0.001) compared to the vehicule. The mucus content observed with AAE in the presnence of atropine was significantly increased (P≤0.001). Treatment of animals with AAE similtaneously with L-NNA did not show any difference when compared to AAE alone.

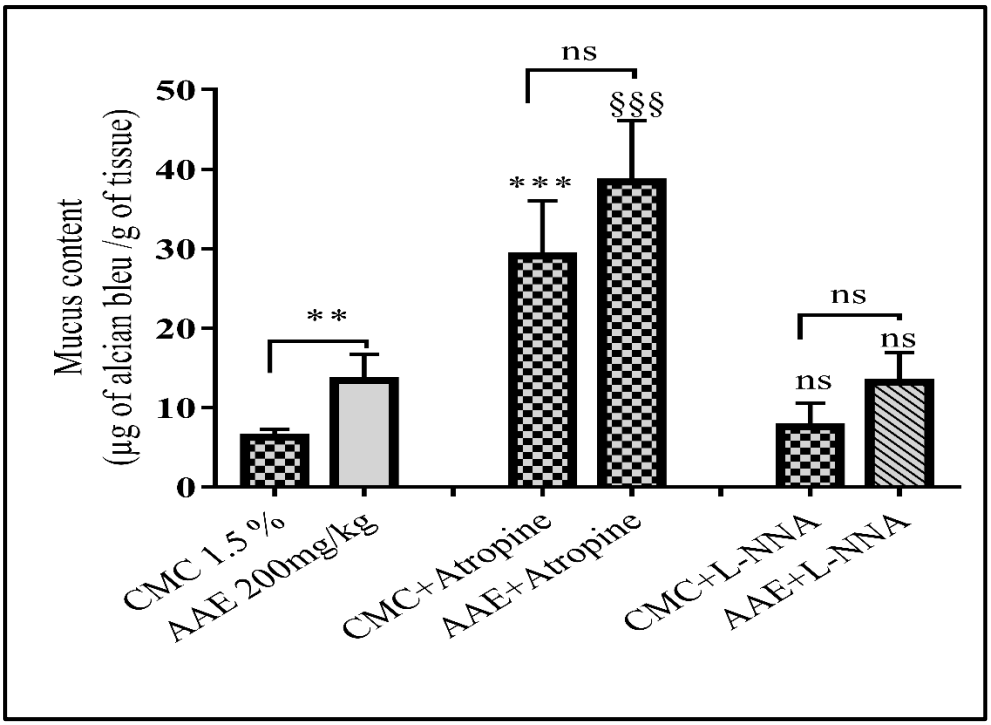


Figure 49: Effect of AAE on gastric mucus content in rats in the presence of atropine or L-NNA. AAE: *A. Santolinoides* L aqueous extract. The values are expressed as means ± SEM (n=6). (**P≤0.01; ***P≤0.001) *vs* vehicle treated group. (§§§P≤0.001) in comparison AAE in the presence of atropine or L-NNA with AAE alone. ns: no significant difference.

4.3.16. *In vivo* antioxidant activity of homogenate on mechanisms of AAE

4.3.16.1. Estimation of gastric total proteins content

treatment of rats with different pharmacological substances (atropine or L-NNA) has no significant effect (P>0.05) on protein content *versus* the vehicle treated animals (Table 9). The use of atropine simultaneously with AAE significantly increased the level of protein (24.32 ± 2.68 µg/mL; P≤0.01) compared to the group treated with atropine alone. Protein content in the animals treated with AAE in the presence of L-NNA decreased (20.94 ± 1.84 µg/mL; P>0.05).

4.3.16.2. Effect of AAE on GSH level

The effect of AAE on GSH levels in stomach tissue homogenates in the presence of atropine or L-NNA is presented in table 9. Treatment of rats with AAE caused a significant increase in GSH level in the presence of atropine compared to AAE alone (45,77 ± 2.02 µmol/g tissue; P≤0.0001). There was no significant change in the effects of AAE in the presence or absence of L-NNA on GSH level (19.22 ± 0.41 µmoL/g tissue; P>0.05).

4.3.16.3. Effect of AAE on lipid peroxidation

The effect of AAE on MDA levels in stomach tissue homogenates in the presence of atropine or L-NNA is presented in table 9. We observed that AAE caused a significant decrease in MDA level in the presence of atropine (1.73 ± 0.22 µmol/g tissue; $P \leq 0.05$) compared to SAE alone. The effect of AAE on MDA level was increased slightly in the presence of L-NNA (2.96 ± 0.27 µmol/g tissue; $P \leq 0.0001$) compared to L-NNA as correspandant control and with no significant difference compared to AAE alone.

Table 9: Effect of AAE on total protein, GSH and MDA levels of stomach tissue in ethanol-induced gastric mucosal lesions in rats in presence of atropine or LNNA.

	Vehicle	**AAE 200 mg/kg**	**Vehicle**		**AAE 200 (mg/kg)**	
			Atropine	**L-NNA**	**Atropine**	**L-NNA**
Proteins µg/ml	19.44 ± 0.75	22,47 ± 1,14 ^{ns}	20.51± 0.52 ^{ns}	19.24± 0.47 ^{ns}	24.32±2.68 $^{ns\ \#}$	20.94±1.84 ^{ns}
GSH (µmol/g tissue)	8.62 ± 0.65	18.85 ± 1.07 ****	42.32 ± 1.78 ****	16.19 ± 1.83 ***	45.77 ± 2.02 §§§§	19.22 ± 0.41 ^{ns}
MDA (µmol/g tissue)	7.24 ± 0.59	3.88 ± 0.15 ****	4.38 ± 0.85 ***	7.45 ± 0.16 ^{ns}	1.73± 0.22 $^{§\ \#\#}$	2.96±0.27 $^{ns\ ####}$

AAE: *A. santolinoides* L. aqueous extract. Values represent means ± SEM (n=6). (**P≤0.01, ***P≤0.001, ****P≤0.0001) *vs* vehicle as negative control. (§P≤0.001 ; §§§§P≤0.0001;) in comparing AAE in the presence of atropine or L-NNA *vs* AAE alone. ($^{\#\#}$ P≤0.01, $^{\#\#\#\#}$P≤0.0001) in comparing AAE in the presence of atropine or L-NNA *vs* atropine or L- NNA as correspandant control. ns: no significant difference.

Discussion

1. **Phytochemical screening**

Phenols are major plant elements that are distributed as secondary metabolites and play an important function as antioxidants and stress defense. Flavonoids are thought to be important in protecting biological systems against the negative effects of oxidative processes on macromolecules (Ito *et al*., 2019)**.** Tannins are another major group of polyphenols in our diets. Researchers and food manufacturers have become more interested in polyphenols due to their potent antioxidant properties, their abundance in the diet, and their credible effects in the prevention of various oxidative stress associated diseases (Dai and Mumper, 2010; Benabdallah *et al*., 2014).

The results of the present work revealed that SME, SHME and SAE were rich in polyphenols. Highest TPC, TFC and TTC have been identified in SME. TPC estimated in SME, SHME and SAE (352.08 ± 0.003 µg GAE/mg Dw, 171.37 ± 1.43 µg GAE/mg Dw, and 209.15 ± 0.001 µg GAE/mg Dw; respectively) were lower than those obtained in the hydromethanolic extract (3.405 µg GAE/mg DW) of Belmekki and Bendimerad (2012), TFC obtained in SME, SHME and SAE (38.11± 0.000 µg QE/mg DW, 18.6 ± 0.46 µg QE/mg DW and 11.27 ± 0.06 µg QE/mg DW) were also lower than those found in the hydromethanolic extract (2.9 µg catechin E/mg DW) of Belmekki and Bendimerad (2012).

In this study, AME had higher content of TPC (210.78 ± 0.001µg GAE/mg DW) and TTC (198.73 ± 0.014 µg TAE/mg DW) than AAE (142.26 ± 0.003 µg GAE/mg DW and 97.89 ± 0.014 µg TAE/mg DW). AHME is richer in TFC (31.09 ± 2.7 µg QE/mg DW) than AME (21.18 ± 0.025 µg QE/mg DW) and AAE (10.68 ± 0.01 µg QE/mg DW).

The differences in phytochemical contents are likely due to the degrees of the used solvents polarity, extract and quantification procedures, harvest period, and geographic region (Al Jahid *et al*., 2016; Benchikh *et al*., 2018; Mamache *et al*., 2020), and therefore affects the biological activities mediated by these metabolites (Kaoudoune *et al*., 2020).

2. UHPLC-MS analysis

Several studies have described *S. satureioides* as being rich in phenolic acids, flavonoids and essential oils (Biondi *et al*,. 2006; Laouer *et al*,. 2006; Bendahou *et al*,. 2008; Mohamadi *et al*., 2015; Ziani *et al*., 2018; Souadia *et al*., 2020). UHPLC-MS analysis (negative mode) identified several compounds responsible for several biological activities. These compounds were identified on the basis of their retention time (RT), UV–vis mass

spectra and MS data. In this study, three compounds (Luteolin-7-*O*-rutinoside,luteolin-7-*O*-glucuronide and luteolin-*O*-*di*glucuronide) were detected in commun in SAE and SME. These results are in accordance with those of Ziani *et al*. (2018) in *S. satureioides* infusions. In contrary, salvianolic acid, sagerinic acid, rosmarinic acid formic adduct, lithospermic acid A, kaempferol, 3-Acetyl-5-caffeoylquinic acid, rosmarinic acid, kaempferol-3-*O*-glucoside are only characterized in SME.

Several studies described that *Achillea* genus are rich in phenolic compounds (Benelli *et al*., 2015; Fahed *et al*., 2016; Farhang Sardrodi *et al*., 2017; Gevrenova *et al*., 2021). Several compounds were identified by Gevrenova *et al*. (2021) in *A. santolinoides* L. (rutin, kaempferol-3-*O*-glucoside and quercetin), several pathways have been shown to be responsible for the antioxidant activity of rutin, it was reported that its chemical structure can directly scavenge ROS, it enhances GSH production, it is thought that it enhanced expression of antioxidant enzymes like CAT and SOD upregulates cellular oxidative defense systems and inhibits the enzyme xanthine oxidase, which is involved in the production of ROS (Enogieru *et al*., 2018). Kaempferol-3-O-glucoside exhibit potent antioxidant activities, the radical scavenging abilities of kaempferol-3-O-glucoside are mainly due to the presence of hydroxyl groups and their redox properties (Taiwo *et al*., 2019). Quercetin (one of *A. santolinoides* components) was found to remove free radicals and strengthen antioxidant defense systems in the body. Thus, quercetin can suppress oxidative stress including the production of ROS (Xu *et al*., 2019).

3. *In vitro* antioxidant activities

Different methods are used for measuring the antioxidant activity. Most of these methods are based on the coloring and discoloration of reagent in the reaction medium. Some assays are used to evaluate lipid peroxidation and others for measuring the free radical scavenging activity (Dasgupta and Klein, 2014). 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).

In the present study, the antioxidant activity of the methanol, hydromethanol and aqueous extract of *S. satureioides* and *A. santolinides* L. was evaluated using DPPH radical scaveging, ABTS, DMSO alcalin, metal chelating, reducing power and CUPRAC test .

The DPPH molecule a stable free radical, has been widely used to assess the ability of antioxidants to scavenge radicals (Atere *et al*., 2018). The addition of the extract to a violet-colored DPPH solution reduces it to a yellow-colored product, diphenylpicrylhydrazine, (Rahman *et al*,. 2015). The purple hue usually disappears in the medium containing antioxidants. Thus, antioxidant molecules can neutralize DPPH-free radicals; by providing hydrogen atoms or donating electrons and converting 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 (Loucif *et al*., 2020).

In this work, DPPH radical scavenging activity of *S. satureioides* was higher in SME (24.40 ± 1.11 µg/mL) than SHME (31.76 ± 0.61 µg/mL) and SAE (37.55 ± 0.74 µg/mL). These results were lower than those obtained in the hydromethanolic extract (7:3) (7.17 ± 0.23 µg/mL) by Belmekki and Bendimerad (2012). However, they were higher than essential oil (1.292 ± 0.002 mg/mL) of Souadia *et al*. (2020).

Concerning *A. santolinoides* L., when comparing the IC_{50} values of the plant extracts, we observed that AME (24.20 ± 0.93 µg/mL) had the highest activity to scavenge DPPH radical compared to AHME (41.66 ± 0.35 µg/mL) and AAE (176.43 ± 2.25 µg/mL). Our results are close to the results reported in other species of this plant; Baris *et al*. (2006) found that the methanolic extract of *A. biebersteinii* reduced the stable of DPPH radicals with an IC_{50} value of 89.9 µg/mL, and to the results of Türkan *et al*. (2020), observed in the methanol and water extracts of A. *schischkinii* (IC_{50} value of 44.5 and 115.2 µg/mL, respectively). The antioxidant activity of the extracts may be due to the neutralization of free radicals (DPPH), either by transfer of hydrogen atom or by transfer of an electron. This maybe because the extract contains the largest amount of tannins compared to the rest extracts (Benchikh *et al.*, 2018).

Similar to DPPH radical, the ABTS radical could react with radical scavengers. DPPH and ABTS radicals are the model free radicals with enough stability for use in laboratory assays of the compounds radical scavenging activity (Xie *et al.*, 2018). ABTS forms a relatively stable free radical, which decolorizes in its non-radical form. When an antioxidant is added to the radicals, there is a degree of decolorization owing to the presence of the antioxidants (Ak and Gülçin, 2008).

Interestingly, the current free radical scavenging of ABTS by SME (IC_{50} = 10.55 ± 0.29 µg/mL) showed a high level activity compared to SAE (IC_{50} = 12.72 ± 2.49 µg/mL) and SHME (IC_{50} = 10.96 ± 0.06 µg/mL). Free radical scavenging of ABTS was investigated using these extracts for the first time in this study.

Concerning *A. santolinoides* L., the obtained results for $ABTS^{\bullet+}$ scavenging activity revealed that AME (6.74 ± 0.16 µg/mL) had a stronger antioxidant ability compared to AAE (IC_{50} = 49.75 ± 1.48 µg/mL), AHME (15.07± 0.06 µg/mL) and to other results using the same species in the methanol extract (42.06 ± 0.40 mg Trolox equivalent/g) and in water extract (95.34 ± 1.15 mg Trolox equivalent/g) (Gevrenova *et al*., 2021). It was reported that high molecular weight of some phenolic compounds such as tannins have more ability to scavenge free radicals such as $ABTS^{\bullet+}$ (Amira *et al*., 2020).

The metal chelating capacity of *S. satureioides* and *A. santolinoides* L. extracts were also evaluated. In the metal chelating assay, ferrozine a chelating agent reacts with ferrous chloride (Fe2+) and can quantitatively form complexes. The Ferrosine-Fe^{+2} complexes produced a red chromophore which can be measured and gives maximum absorbance at 562 nm. The presence of chelating agent can disorder the formation of complexes Ferrosine-Fe^{+2} and the red color disappears (Gupta *et al*., 2014). Metal ion chelating activity of an antioxidant molecule prevents oxyradical generation and the consequent oxidative damage. Metal ion chelating capacity plays a significant role in antioxidant mechanisms, since it reduces the concentration of the catalysing transition metal in lipid peroxidation (Benchikh *et al*., 2018).

In this context, *S. satureioides* extracts exhibited the ability to chelate metal ions. SHME (IC_{50} = 64.90 ± 0.001 µg/mL) among the different sample extracts exhibited higher metal chelating activity and inhibited the formation of ferrozine- Fe^{+2} complex.

The metal chelating activity of *A. santolinoides* in our data showed that AHME (IC_{50} = 42.00 ± 0.003 µg/mL) has strong chelating activity. This activity could be related to the richness of this extract in flavonoides content. The metal chelating activity of *A. santolinoides* L. was estimated for the first time in this study.

The scavenging activity of the extracts against O^{2-} generated in NaOH-DMSO-NBT was measured spectrophotometrically by looking for blue formazan production. The superoxide scavenger can inhibit the production of red formazan color (Mamache *et al.*, 2020). Superoxide anion is a major source of many free radicals, such as peroxyl, alkoxyl, hydroxyl, and NO, which are formed from superoxide anion through fenton reaction and/or

lipid oxidation or nitric oxidation (Zhao *et al*., 2006; Zhao *et al*., 2008b; Lalhminghlui and Jagetia, 2018). It was reported that the superoxide anion scavenging activity could be due to the action of a free hydroxyl group of phenolic compounds (Prasad *et al*., 2009).

The extract's scavenging activity against superoxide radicals formed in the NaOH-alkaline DMSO-NBT system, which resulted in the creation of blue formazan, was investigated in this study. SME exhibited the highest inhibition with values of IC_{50} = 6.21± 0.38 µg/mL in comparison to SHME (IC_{50} = 10.91 ± 0.11µg/mL), SAE (IC_{50} = 48.52 ± 1.41µg/mL) and to BHT (IC_{50} = 23.73 µg/mL) as reference. No reports about O_2 radical scavenging of the extracts were realized before, that means this work investigates for the first time this activity of the extracts in use.

Our results showed that *A. santolinoides* L. ME had the highest superoxide anion radical scavenging activity (15.13 ± 0.92 µg/mL) followed by AHME then AAE and standard BHT with value of IC_{50} = 20.05 ± 0.08 µg/mL, IC_{50} = 23.12 ± 1.61 µg/mL and IC_{50} = 23.73 ± 1.11 µg/mL, respectively.

Reducing power is one of the mechanisms of action of antioxidants and that could be a good indicator of their potential antioxidant effectiveness. In reducing power, the transformation of Fe^{3+} to Fe^{2+} in the presence of the sample extract is used to determine the antioxidant's reductive ability (Vikas *et al*., 2017).

In this study, the plant extracts reduced most of Fe^{3+} ions and had the highest reductive activity compared to the BHT standard. Increased reaction absorbance suggested increased reducing power. The results revealed that SME had the highest reducing capacity (EC_{50} = 130.89 ± 0.003 µg/mL) comparing to BHT (327.60 ± 0.004 µg/ml), SAE (EC_{50} = 292.57 ± 0.01 µg/mL) and SHME (EC_{50} = 543.5 ± 6.25 µg/mL). No reports about reducing power of the extract were realized before, that means this work investigates for the first time this activity for the extracts in use.

For *A santolinoides*, the best reducing power was for AME with EC_{50} value of 179.71 ± 0.01 µg/mL compared to BHT (327.60 ± 0.004 µg/mL), AHME (EC_{50} = 300 ± 0.02 µg/mL) and to AAE (EC_{50} = 642.537 ± 0.04 µg/mL). The reducing power activity of the methanol, hydromethanol and aqueous extracts of *A. santolinoides* L. was reported for the first time in this work.

The CUPRAC method measures electron transferring of the antioxidant (Boudjada *et al*., 2019). It is based on the reduction of the neocuproine-copper complex, which produces a

chromogenic Cu (II)-Nc complex that absorbs at 450 nm (Çekiç *et al*., 2009, Mazouz *et al*., 2020). In this test, a higher absorbance suggests a greater ability to reduce cupric ion (Cu^2) (Cetinkaya *et al*., 2012).

For the first time, the cupric ion reducing capacity of methanol, hydromethanol and aqueous extract of *S. satureioides* was investigated in this study. Our results showed that methanol extract preparations had the highest cupric ion Cu^{2+} reducing activity ($A_{0.50}$ = 19.97± 0.38 µg/mL) following by SHME ($A_{0.50}$ = 25.07 ± 0.09 µg/mL) and SAE ($A_{0.50}$ = 47.07 ± 1.50 µg/mL .

In this test, our results showed that AME had the highest cupric ion Cu^{2+} reducing activity ($A_{0.50}$ =76.56 ± 2.35 µg/mL) in comparison with AHME ($A_{0.50}$ = 98.46 ± 0.19 µg/mL), AAE ($A_{0.50}$ =155.47 ± 1.45 µg/mL) and to the result observed by Gevrenova *et al*. (2021) ($A_{0.50}$ = 98.46 ± 0.19 mg Trolox equivalent/g) using the methanol extract prepared from Turkish *A. santolinoides* L.

4. Effects of SAE and AAE on gastric emptying and intestinal transit in mice and the possible mechanisms involved

The present study reveals that SAE significantly decreased gastric emptying in mice at the highest doses. The inhibitory effect was very close to that of atropine as reference drug. SAE also significantly decreased the intestinal transit when compared to control group (CMC 1.5 %) in the same animal species.

Similarly, AAE significantly decreased the gastric emptying at the highest doses. AAE reduced the intestinal transit compared to negative control, and did not show any difference *versus* atropine as reference drug.

The control of gastric emptying is complex and involves the coordination of motor activity of the proximal stomach, the antrum, the pylorus and the duodenum, as well as the passive forces created by intragastric volume and gravity. The tonus of the pyloric sphincter plays a crucial role in the rate of gastric emptying (Benchikh *et al.,* 2016).

Intestinal transit is controlled by both neural and myogenic mechanisms, several mediators and neurotransmitters govern these motor patterns. Acetylcholine is the main excitatory neurotransmitter in the enteric nervous system, while NO is the major transmitter of the inhibitory motor neurons (Amira *et al*., 2005; Benchikh *et al*., 2016). The latter governs

numerous physiological processes in the body, including neural control of gastrointestinal motor functions (Pimentel *et al*., 2006).

The effect of SAE and AAE on GI motility is probably due to their phytochemical constituents including phenolic compounds. Indeeded, several studies have demonstrated the inhibitory effect of flavonoids on the motility of the GI tract (Gharzouli and Holzer, 2004; Amira *et al*., 2008; Rotondo *et al*., 2009; Kimura and Taniguchi, 2021).

Kimura and Taniguchi (2021) found that gastric emptying and GI motility were stimulated by rosmarinic acid through the inhibition of dopamine D_2 receptors but did not affect 5-HT-induced delais. Flavonoids are known to relax precontracted intestinal smooth muscle and delayed intestinal transit (Gharzouli and Holzer, 2004). In addition, a number of flavonoids have decreased vascular tone (Chan *et al*., 2000) and intestinal smooth muscle contractility. The smooth muscle contraction triggered by several spasmogens (Rotondo *et al*., 2009).

To investigate the mechanism of action of the studied plant extracts on gastric emptying and intestinal transit, atropine (a non specific muscarinic receptor inhibitor), L-NNA (a non specific inhibitor of NO synthase) and indomethacin (a non specific inhibitor of synthesis of prostaglandins) were used.

Atropine decreased the rate of gastric emptying, it is known that atropine reduces GI motility through the delay of gastric emptying and intestinal transit. The effect occurs via the blockade of muscarinic receptors (Collares *et al*., 2017; Wang *et al* (b)., 2017; Wang *et al*., 2020). Both SAE and AAE decreased gastric emptying, while the administration of AAE with atropine did not shows any significant differnce compared to animals treated with atropine alone, suggesting that the cholinergic system is involved in the delaying effect of AAE. The administration of SAE with atropine showed a slight difference compared to animals treated with SAE alone, suggesting that the muscarinic receptors are not involved in the delaying effect of SAE.

L-NAME reduced the rate of gastric emptying. It is validated that NO plays a pivotal role in neural transduction and GI motility (Wang *et al*., 2002). NO was proposed as non-adrenergic, noncholinergic (NANC) transmitter in different parts of GI tract. It mediates the accommodation in the stomach (Desai *et al*., 1991) and it is believed to control NANC relaxation of the pylorus (Amira *et al*., 2005). The administration of SAE and AAE with L-NNA did not show any significant difference, meaning that there is an involvement of NO in the mechanism of SAE and AAE on gastric emptying delay.

Martinez *et al*. (2006) found that in rats injected *i.v.* with vehicle, pretreatment with indomethacin did not alter gastric emptying. The results of the present study showed that the pretreatment of mice with indomethacin decreased gastric emptying; the blocking of cyclooxygenase with indomethacin, is involved in the retarding effects of AAE and SAE on gastric emptying, demonstrating the role of the cyclooxygenase pathway in the mechanism by which the extracts delay gastric emptying.

In the present work, the treatment of mice with atropine significantly decreased intestinal transit. This is in accordance with the results of Eghianruwa *et al*., (2006) and Amira et *al*., (2005) in mice. SAE and AAE extracts in the presence of atropine did not show any difference. These effects may be related to the inhibition of muscarinic receptors.

Inhibition of NOS by L-NNA decreased the rate of intestinal transit in mice, this is in agreement with the results of De Winter *et al*., (2002), who found that L-NAME reduced the intestinal transit in mice. Whereas, the present results reported that SAE and AAE did not significantly affect the rate of intestinal transit under the inhibition of NOS by L-NNA, which may indicates that this effect depends on the NO pathway.

Lichtenberger *et al*., (2015) showed that the spontaneous contractile activity in the proximal small intestine of rat was unaffected by indomethacin treatment; frequency of contractions were unaffected by indomethacin treatment in the proximal part of the small intestine in rats, The present work showed a decrease in the intestinal transit in the animal treated with indomethacin, both SAE and AAE reduced intestinal transit in mice pretreated with indomethacin, this suggests the participation of the cyclooxygenase pathway in these effects.

5. Effect of SAE and AAE on ethanol-induced gastric ulceration in rats

The present study shows for the first time that SAE exhibits a significant antiulcerogenic activity by reducing the gastric mucosa lesion induced by ethanol. The results of other studies have demonstrated that the different extracts or fractions prepared from the plants *S. officinalis* L or *S. plebeian* from the *lamiaceae* famil**y** are able to significantly reduce the lesions caused by ethanol in rats (Mayer *et al.*, 2009; Nugroho *et al.*, 2012).

Also the present work indicated for the first time that AAE exhibits a significant antiulcerogenic activity by reducing the gastric mucosa lesion induced by ethanol. Many studies indicated that the genus *Achillea* like *Achillea millefolium* L. (Potrich *et al*., 2010;

Alomair *et al*., 2022) and *Achillea wilhelmsii* (Niazmand *et al*., 2010) inhibited ethanol-induced gastric lesions.

Gastric ulcer has been linked to an imbalance between aggressive factors (physical, chemical, or psychological) in the lumen (Li *et al*., 2014). The injurius elements include alcohol, nonsteroidal anti-inflammatory drugs, stress, and *H. pylori* infection (Swarnakar *et al*., 2007), while and defensive mechanisms are represented by mucus and bicarbonate secretions, as well as prostaglandins, sulphydryl compounds, polyamines, NO and dopamine (Li *et al*., 2014). Excessive consumption of alcohol can cause acute hemorrhagic stomach erosions, as well as gastritis with mucosal edema, sub-epithelial hemorrhages, cellular exfoliation, and inflammatory cell infiltration (Chen *et al*., 2015).

Orally administered ethanol is rapidly absorbed into the bloodstream of the stomach and intestine and erodes the mucosa of stomach and the duodenum causing acute gastritis (Zheng *et al*., 2016). Excessive ethanol consumption predisposes to the establishment of acute gastric ulcers through neutrophil infiltration and overexpression of nuclear factor-B (NF-B) (Amirshahrokhi and Khalili, 2015). Current medicinal treatments of gastric ulcer generally rely on the inhibition of gastric acid secretion by H_2-antagonists, such as ranitidine and mainly cimitidine or proton-pump inhibitors, such as omeprazole and its derivatives (Zheng *et al*., 2016).

In the current investigation, ethanol administration resulted in both histological and macroscopic erosion of the mucus layer, petechiae, hemorrhage, and oedema and chemical alteration including mainly elements of the antioxidants system of the stomach. The epithelium and vasculature were preserved after treatment with SAE and AAE, which counteracted the negative effects of ethanol. A low ulcer index and a high percentage of protection are indicators of this effect.

Antiulcerogenic activity of both plants is most likely due to their richness in phytochemical compounds; mainly phenolics, such us rosmarinic acid, luteolin and kaempferol. Rosmarinic acid is an important constituent of *S. saturouides*. It is recognised as natural antioxidant, and as an anti-ulcerogenic compound, in deeded to the results found by Amaral *et al*., (2013), who indicated that *Rosmarinus officinalis* L. induced an anti-ulcerogenic effect with antioxidant aspects and NOx levels, whereas the rosmarinic acids are the most abundant constituents of *Rosmarinus officinalis* L.

Luteolin and kaempferol are major constituent of both *S. satureioides* and *A. santolinoides* L., luteolin has been reported to have antiulcer property (Sofidiya *et al*., 2014).

It is a selective inhibitor of COX II; the inhibition of COX II activities blocks prostaglandin induced elaboration of prostanoid mediators of inflammation and hyperalgesia experienced by ulcer patients (Ode and Asuzu, 2014). According to Izzo *et al*., (1994), intraperitoneally administration of kaempferol (25-100 mg/kg) dose-dependently inhibited gastric damage produced by acidified ethanol in rats.

Ethanol-induced gastric ulceration, enhanced lipid peroxidation, and free-radical production (Li *et al*., 2013). CAT has been shown to scavenge superoxide, hydrogen peroxide, hydroxyl, and lipid peroxyl radicals, therby reducing tissue damage. As a preventative antioxidant, CAT causes the peroxyl radical to be rapidly converted into biologically harmless molecules such as water (Chen *et al*., 2015).

The present study demonstrated that absolute ethanol administration significantly decreased the gastric mucus content and GSH level. Treatment with SAE and AAE significantly increased the gastric mucus content and GSH level and significantly decreased lipid peroxidation. The mucus layer lining the entire gastric mucosa acts as a physical barrier against the aggressive effect of gastric juice. Furthermore, mucus has an antioxidant effect. Weakening of the mucus barrier is directly responsible for gastric damage by ulcerogens, such as ethanol, a well known breaker of gastric mucus layer (Sidahmed *et al*., 2019).

6. Mechanisms of SAE and AAE effects on ethanol-induced gastric ulceration in rats

To further clarify the gastroprotective effect of SAE and AAE in rats, the involvement of the cholinergic and the nitrinergic systems were assessed in this action using atropine and L-NNA.

Treatment of rats with atropine, protected the gastric mucosa against damages caused by ethanol, this is in agreement with Bhargava *et al.,* (1973); Ray *et al*., (1990); Rahgozar *et al*., (2001). Administration of both SAE and AAE after intraperitoneal injection of atropine showed a protective effect close to those of atropine, this suggests that the muscarinic receptors are involved in the protective effect of both SAE and AAE against gastric ulcer induced by ethanol.

Nitric oxide plays an important role in many physiological processes, as shown by reports of its protective effect on the gastric mucosa by stimulating mucus production and blood flow, improving nitrogen balance and acceleration of wound healing (Jimenez *et al*., 2004; Sánchez-Mendoza *et al*., 2019). Treatment of animals with L-NNA increased the

gastric ulcer induced by ethanol, the level of MDA and reduced the the GSH level, this is in accord with Kwiecien *et al*., (2004) who demonstrated that in rats with water immersion, L-NNA resulted a significant increase in gastric damage and in the level of MDA and decreased GSH concentration. Treatment of animals with SAE and AAE in the presence of L-NNA decreased the gastric ulcer induced by ethanol, thus suggesting that the NO pathway is not involved in the effect of SAE and AAE in the gastric protection.

Conclusion and future prospects

The results obtained in the present study revealed that *S. satureioides* and *A. santolinoides* L. are a rich source of phenolic compounds. The level of these chemicals in various extracts from both plants was considerably higher in the methanol extract than in the other extracts. The phytochemical analysis of SAE and SME revealed the presence of several phenolic acids, the common ones between both SAE and SME are: Luteolin-7-*O*-rutinoside, luteolin-*O*-diglucuronide and luteolin-7-*O*-glucuronide. The phytochemical analysis of *A. santalinoides* revealed that AAE contains two isomers of apigening (Apigenin-2''-*O*-pentosyl-8-C-glucoside and Apigenin-*O*-glucuronide) and luteolin-7-*O*-rutinoside. AME also contains two isomers of apigenin (apigenin-2''-*O*-pentosyl-8-C-glucoside and apigenin-O-glucuronide) and two isomers of dicaffeoylquinic acid (3,5-Dicaffeoylquinic acid and 3,4-Dicaffeoylquinic acid).

All extracts showed high *in vitro* antioxidant activities being higher in SME and AME with the majority of the tests. Both SAE and AAE extracts delayed gastric emptying and intestinal transit in mice. In addition, both SAE and AAE extracts protected the rat stomach against ethanol inducing gastric ulcer. These effects may be attributed to the presence of several classes of chemicals in the plant extracts.

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 asses the activity of these plant extracts on other experimental models of gastric ulcer and gastro intestinal motility and to isolate the phytoconstients that are responsible for the activites.
- To exploit the beneficial effects of both plant constituents in food and pharmaceutical industries.
- To widen the spectrum of research on the effects of these plants on some chronic diseases, such as, Alzheimer, diabetes, cancer etc.

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

References

References

Abdelfattah, M. S., Elmallah, M. I., Ebrahim, H. Y., Almeer, R. S., Eltanany, R. M., & Abdel Moneim, A. E. (2019). Prodigiosins from a marine sponge-associated actinomycete attenuate HCl/ethanol-induced gastric lesion via antioxidant and anti-inflammatory mechanisms. *PloS one*, *14*(6), e0216737.

Adinortey, M. B., Ansah, C., Galyuon, I., & Nyarko, A. (2013). *In vivo* models used for evaluation of potential antigastroduodenal ulcer agents. *Ulcers*, *2013*.

Agisho, H., Osie, M., & Lambore, T. (2014). Traditional medicinal plants utilization, management and threats in Hadiya Zone, Ethiopia. *Journal of Medicinal Plants*, *2*(2), 94-108

Ak, T., & Gülçin, İ. (2008). Antioxidant and radical scavenging properties of curcumin. *Chemico-biological interactions*, *174*(1), 27-37.

Albaayit, S. F. A., Abba, Y., Abdullah, R., & Abdullah, N. (2016). Prophylactic effects of *Clausena excavata* Burum. f. leaf extract in ethanol-induced gastric ulcers. *Drug Design, Development and Therapy*, *10*, 1973.

Al-Dalaen, S. M., & Al-Qtaitat, A. I. (2014). Review Article: Oxidative Stress Versus Antioxidants. American Journal of Bioscience and Bioengineering. *American Journal of Bioscience and Bioengineering*, *2*(5), 60-71.

Al-Gubory, K. H., Fowler, P. A., & Garrel, C. (2010). The roles of cellular reactive oxygen species, oxidative stress and antioxidants in pregnancy outcomes. *The international journal of biochemistry & cell biology*, 42(10), 1634-1650.

Al Jahid, A., Essabaq, S., Elamrani, A., Blaghen, M., & Jamal Eddine, J. (2016). Chemical composition, antimicrobial and antioxidant activities of the essential oil and the hydro-alcoholic extract of *Artemisia campestris* L. leaves from southeastern Morocco. *Journal of biologically active products from nature*, *6*(5-6), 393-405.

Alomair, M. K., Alabduladheem, L. S., Almajed, M. A., Alobaid, A. A., Alkhalifah, E. A., Younis, N. S., & Mohamed, M. E. (2022). *Achillea millefolium* Essential Oil Mitigates Peptic Ulcer in Rats through Nrf2/HO-1 Pathway. *Molecules*, *27*(22), 7908.

Al-Qarawi, A. A., Abdel-Rahman, H., Ali, B. H., Mousa, H. M., & El-Mougy, S. A. (2005). The ameliorative effect of dates (*Phoenix dactylifera* L.) on ethanol-induced gastric ulcer in rats. *Journal of ethnopharmacology*, *98*(3), 313-317.

Alzand, K. I., & Mohamed, M. A. (2012). Flavonoids: Chemistry, biochemistry and antioxidant activity. *Journal of Pharmacy Research*, 5(8), 4013-4020.

Amaral, G. P., de Carvalho, N. R., Barcelos, R. P., Dobrachinski, F., de Lima Portella, R., da Silva, M. H., ... & Fachinetto, R. (2013). Protective action of ethanolic extract of *Rosmarinus*

officinalis L. in gastric ulcer prevention induced by ethanol in rats. *Food and Chemical Toxicology*, *55*, 48-55.

Amira, H., Benchikh, F., Benabdallah, H., Mamache, W., & Amira, S. (2020). Evaluation of Antioxidant activities and total phenolic content of hydro-ethanol extract from *Phlomis bovei* De Noé areal parts. *Journal of drug delivery and therapeutics*, *10*(5), 45-48.

Amira, S., Soufane, S., & Gharzouli, K. (2005). Effect of sodium fluoride on gastric emptying and intestinal transit in mice. *Experimental and Toxicologic Pathology*, *57*(1), 59-64.

Amira, S., Rotondo, A., & Mulè, F. (2008). Relaxant effects of flavonoids on the mouse isolated stomach: structure-activity relationships. *European journal of pharmacology*, *599*(1-3), 126-130.

Amira, S., Dade, M., Schinella, G. & Ríos, J.-L., (2012). Anti-inflammatory, anti-oxidant, andapoptotic activities of four plant species used in folk medicine in the Mediterranean basin. *Pakistan Journal of Pharmaceutical Sciences*, 25(1), 65-72.

Amirshahrokhi, K., & Khalili, A. R. (2015). The effect of thalidomide on ethanol-induced gastric mucosal damage in mice: involvement of inflammatory cytokines and nitric oxide. *Chemico-Biological Interactions*, *225*, 63-69.

Ansari, M. Y., Ahmad, N., & Haqqi, T. M. (2020). Oxidative stress and inflammation in osteoarthritis pathogenesis: Role of polyphenols. *Biomedicine & pharmacotherapy*, *129*, 110452.

Apak, R., Güçlü, K., Özyürek, M., & Karademir, S. E. (2004). Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. *Journal of Agricultural and Food Chemistry*, *52*(26), 7970-7981.

Asaduzzaman Khan, M., Tania, M., Zhang, D. Z., & Chen, H. C. (2010). Antioxidant enzymes and cancer. *Chinese Journal of Cancer Research*, *22*(2), 87-92.

Atere, T. G., Akinloye, O. A., Ugbaja, R. N., Ojo, D. A., & Dealtry, G. (2018). *In vitro* antioxidant capacity and free radical scavenging evaluation of standardized extract of *Costus afer* leaf. *Food Science and Human Wellness*, *7*(4), 266-272.

Aziz, R. S., Siddiqua, A., Shahzad, M., Shabbir, A., & Naseem, N. (2019). Oxyresveratrol ameliorates ethanol-induced gastric ulcer via downregulation of IL-6, TNF-α, NF-κB, and COX-2 levels, and upregulation of TFF-2 levels. *Biomedicine & Pharmacotherapy*, *110*, 554-560.

Bae, Y. S., Oh, H., Rhee, S. G., & Yoo, Y. D. (2011). Regulation of reactive oxygen species generation in cell signaling. *Molecules and Cells*, *32*(6), 491-509.

Bahorun, T., Gressier, B., Trotin, F., Brunet, C., Dine, T., Luyckx, M., ... & Pinkas, M. (1996). Oxygen species scavenging activity of phenolic extracts from hawthorn fresh plant organs and pharmaceutical preparations. *Arzneimittel-Forschung*, *46*(11), 1086-1089.

Bariş, Ö., Güllüce, M., ŞAHİN, F., Özer, H., Kiliç, H., Özkan, H., ... & Özbek, T. (2006). Biological activities of the essential oil and methanol extract of *Achillea biebersteinii Afan*.(Asteraceae). *Turkish Journal of Biology*, *30*(2), 65-73.

Bate-Smith, E. C. (1973). Haemanalysis of tannins: the concept of relative astringency. *Phytochemistry*, *12*(4), 907-912.

Belmekki, N., & Bendimerad, N. (2012). Antioxidant activity and phenolic content in methanol crude extracts from three Lamiaceae grown in southwestern Algeria. *Journal of Natural Product and Plant Resourse*, *2*(1), 175-181.

Benabdallah, H., Gharzouli, K., Khennouf, S., Amira, S., & Soufane, S. (2014). Phytochemical analysis and anti-lipid peroxidation activity of *Tamarix Africana* L. extracts. *Global Journal of Research on Medicinal Plants & Indigenous Medicine*, *3*(7), 278.

Benahmed, M., Djeddi, N., Akkal, S., & Laouar, H. (2016). *Saccocalyx satureioides* as corrosion inhibitor for carbon steel in acid solution. *International Journal of Industrial Chemistry*, *7*(2), 109-120.

Benarba, B. (2016). Medicinal plants used by traditional healers from South-West Algeria: An ethnobotanical study. *Journal of Intercultural Ethnopharmacology*, *5*(4), 320.

Benchikh F. Pharmacological effects of *myrtus communis* l. on the gastrointestinal tract of rats and mice. Ph.D. Thesis, Department of Biology and Animal Physiology, Faculté des Sciences de la Nature et de la Vie, Université Ferhat Abbas Sétif 1, Setif, Algeria, 2017.

Benchikh, F., Benabdallah, H., Dahamna, S., Khennouf, S., Flamini, G., & Amira, S. (2016). Antimotility and andidiarrhoel activity of *Myrtus communis* L. leaves essential oil in mice. *International Journal of Pharmacognosy and Phytochemical Research*, *8*(7), 1238-1244.

Benchikh, F., Amira, S., & Benabdallah, H. (2018). The evaluation of antioxidant capacity of different fractions of *Myrtus communis* L. leaves. *Annual Research & Review in Biology*, *22*(5):1-14.

Bendahou, M., Benyoucef, M., Muselli, A., Desjobert, J. M., Paolini, J., Bernardini, A. F., & Costa, J. (2008). Antimicrobial activity and chemical composition of *Saccocalyx satureioides* Coss. et Dur. essential oil and extract obtained by microwave extraction. Comparison with hydrodistillation. *Journal of Essential Oil Research*, *20*(2), 174-178.

Benelli, G., Bedini, S., Flamini, G., Cosci, F., Cioni, P. L., Amira, S., ... & Conti, B. (2015). Mediterranean essential oils as effective weapons against the West Nile vector Culex pipiens and the Echinostoma intermediate host Physella acuta: what happens around? An acute toxicity survey on non-target mayflies. *Parasitology research*, *114*(3), 1011-1021.

Bhargava, K. P., Gupta, M. B., & Tangri, K. K. (1973). Mechanism of ulcerogenic activity of indomethacin and oxyphenbutazone. *European Journal of Pharmacology*, *22*(2), 191-195.

Bhattacharyya, S. (2015). Reactive oxygen species and cellular defense system. *Free radicals in human health and disease*, 17-29.

Bi, W. P., Man, H. B., & Man, M. Q. (2014). Efficacy and safety of herbal medicines in treating gastric ulcer: a review. *World Journal of Gastroenterology: WJG*, *20*(45), 17020.

Bilić-Šobot, D., Kubale, V., Škrlep, M., Čandek-Potokar, M., Prevolnik Povše, M., Fazarinc, G., & Škorjanc, D. (2016). Effect of hydrolysable tannins on intestinal morphology, proliferation and apoptosis in entire male pigs. *Archives of animal nutrition*, *70*(5), 378-388.

Biondi, D. M., Sari, M., Ghani, Z. A., & Ruberto, G. (2006). Essential oil of Algerian *Saccocalyx satureioides* Coss. et Durieu. *Flavour and fragrance journal*, *21*(3), 546-548.

Birben, E., Sahiner, U. M., Sackesen, C., Erzurum, S., & Kalayci, O. (2012). Oxidative stress and antioxidant defense. *World allergy organization journal*, 5(1), 9-19.

Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, *181*(4617), 1199-1200.

Boeing, T., da Silva, L. M., Somensi, L. B., Cury, B. J., Costa, A. P. M., Petreanu, M., ... & de Andrade, S. F. (2016). Antiulcer mechanisms of Vernonia condensata Baker: A medicinal plant used in the treatment of gastritis and gastric ulcer. *Journal of ethnopharmacology*, 184, 196-207.

Bokoch, G. M., and Knaus, U. G. (2003). NADPH oxidases: not just for leukocytes anymore!. *Trends in biochemical sciences*, 28(9), 502-508.

Boudjada, A., Touil, A., Bensouici, C., Bendif, H., & Rhouati, S. (2019). Phenanthrene and dihydrophenanthrene derivatives from *Dioscorea communis* with anticholinesterase, and antioxidant activities. *Natural product research*, *33*(22), 3278-3282.

Boukhenouna, S., Wilson, M. A., Bahmed, K., & Kosmider, B. (2018). Reactive oxygen species in chronic obstructive pulmonary disease. *Oxidative Medicine and Cellular Longevity*, *2018*.

Campbell, J., Berry, J., & Liang, Y. (2019). Anatomy and physiology of the small intestine. In Shackelford's Surgery of the Alimentary Tract, 2 Volume Set (pp. 817-841). Elsevier.

Çekiç, S. D., Başkan, K. S., Tütem, E., & Apak, R. (2009). Modified cupric reducing antioxidant capacity (CUPRAC) assay for measuring the antioxidant capacities of thiol-containing proteins in admixture with polyphenols. *Talanta*, *79*(2), 344-351.

Cetinkaya, Y., Göçer, H., Menzek, A., & Gülçin, İ. (2012). Synthesis and antioxidant properties of (3, 4-dihydroxyphenyl)(2, 3, 4-trihydroxyphenyl) methanone and its derivatives. *Archiv der Pharmazie*, *345*(4), 323-334.

Chan, E. C., Pannangpetch, P., & Woodman, O. L. (2000). Relaxation to flavones and flavonols in rat isolated thoracic aorta: mechanism of action and structure-activity relationships. *Journal of cardiovascular pharmacology*, *35*(2), 326-333.

Chen, H., Liao, H., Liu, Y., Zheng, Y., Wu, X., Su, Z., ... & Su, Z. (2015). Protective effects of pogostone from Pogostemonis Herba against ethanol-induced gastric ulcer in rats. *Fitoterapia*, *100*, 110-117.

Cocan, I., Alexa, E., Danciu, C., Radulov, I., Galuscan, A., Obistioiu, D., ... & Dehelean, C. A. (2018). Phytochemical screening and biological activity of Lamiaceae family plant extracts. *Experimental and Therapeutic Medicine*, 15(2), 1863-1870.

Collares, E. F., Vinagre, A. M., & Collares-Buzato, C. B. (2017). Dipyrone in association with atropine inhibits the effect on gastric emptying induced by hypoglycemia in rats. *Brazilian Journal of Medical and Biological Research*, *50*.

Corne SJ, Morrissey SM, Woods RJ. 1974. A method for the quantitative estimation of gastric barrier mucus. *Jouranal of Physiology*; 242: 116-117.

Costa, E. D., Rezende, B. A., Cortes, S. F., & Lemos, V. S. (2016). Neuronal nitric oxide synthase in vascular physiology and diseases. *Frontiers in Physiology*, *7*, 206.

Csendes, A., & Braghetto, I. (2016). Changes in the anatomy and physiology of the distal esophagus and stomach after sleeve gastrectomy. *Journal of Obesity & Weight Loss Therapy*, *6*(1), 297.

Daenen, K., Andries, A., Mekahli, D., Van Schepdael, A., Jouret, F., & Bammens, B. (2019). Oxidative stress in chronic kidney disease. *Pediatric Nephrology*, *34*(6), 975-991.

Dai, J., & Mumper, R. J. (2010). Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules*, *15*(10), 7313-7352.

Daniels, I. R., & Allum, W. H. (2005). The anatomy and physiology of the stomach. In *Upper gastrointestinal surgery* (pp. 17-37). Springer, London.

Dasgupta, A., & Klein, K. (2014). Methods for measuring oxidative stress in the laboratory. *Antioxidants in Food, Vitamins and Supplements Prevention and Treatment of Disease*, *2014*, 19-40.

Decker, E. A., & Welch, B. (1990). Role of ferritin as a lipid oxidation catalyst in muscle food. *Journal of Agricultural and Food Chemistry*, *38*(3), 674-677.

De Filippis, B., Ammazzalorso, A., Fantacuzzi, M., Giampietro, L., Maccallini, C., & Amoroso, R. (2017). Anticancer activity of stilbene-based derivatives. *Chem MedChem*, *12*(8), 558-570.

Denbow, D. M. (2015). Gastrointestinal anatomy and physiology. In Sturkie's avian physiology (pp. 337-366). Academic Press.

Desai, K. M., Zembowicz, A., Sessa, W. C., & Vane, J. R. (1991). Nitroxergic nerves mediate vagally induced relaxation in the isolated stomach of the guinea pig. *Proceedings of the National Academy of Sciences*, *88*(24), 11490-11494.

De Winter, B. Y., Bredenoord, A. J., Joris, G., Moreels, T. G., Herman, A. G., & Pelckmans, P. A. (2002). Effect of inhibition of inducible nitric oxide synthase and guanylyl cyclase on endotoxin-induced delay in gastric emptying and intestinal transit in mice. *Shock*, *18*(2), 125-131.

Diaz-Vivancos, P., de Simone, A., Kiddle, G., & Foyer, C. H. (2015). Glutathione–linking cell proliferation to oxidative stress. *Free Radical Biology and Medicine*, *89*, 1154-1164.

Dikalov, S. (2011). Cross talk between mitochondria and NADPH oxidases. *Free Radical Biology and Medicine*, 51(7), 1289-1301.

Dos Santos Grasel, F., Ferrão, M. F., & Wolf, C. R. (2016). Ultraviolet spectroscopy and chemometrics for the identification of vegetable tannins. *Industrial Crops and Products*, 91, 279-285.

Eghianruwa, K. L., Ogunley, O. A., Saba, A. B., Famakind, S. A., Ola-Davies, E. O., & Abu, H. H. (2006). Influence of atropine and loperamide on reduced intestinal transit induced by *calotropis procera* latex in rats. *African Journal of Biomedical Research*, *9*(2).

El Khawand, T., Courtois, A., Valls, J., Richard, T., & Krisa, S. (2018). A review of dietary stilbenes: Sources and bioavailability. *Phytochemistry Reviews*, 17(5), 1007-1029.

Ellman, G. L. (1959). Tissue sulfhydryl groups. *Archives of biochemistry and biophysics*, *82*(1), 70-77.

Enogieru, A. B., Haylett, W., Hiss, D. C., Bardien, S., & Ekpo, O. E. (2018). Rutin as a potent antioxidant: Implications for neurodegenerative disorders. *Oxidative Medicine and Cellular Longevity*, *2018*.

Erdman Jr, J. W., Balentine, D., Arab, L., Beecher, G., Dwyer, J. T., Folts, J., ... & Burrowes, J. (2007). Flavonoids and heart health: proceedings of the ILSI North America flavonoids workshop, May 31–June 1, 2005, Washington, DC. *The Journal of Nutrition*, 137(3), 718S-737S.

Fahed, L., El Beyrouthy, M., Ouaini, N., Eparvier, V., & Stien, D. (2016). Isolation and characterization of santolinoidol, a bisabolene sesquiterpene from *Achillea santolinoides* subsp wilhelmsii (K. Koch) Greuter. *Tetrahedron Letters*, *57*(17), 1892-1894.

Fahad, D., & Mohammed, M. T. (2020). Oxidative stress: Implications on skin diseases. *Plant Archives*, *20*(2), 4150-4157.

Farag, R. S., Abdel-Latif, M. S., Abd El Baky, H. H., & Tawfeek, L. S. (2020). Phytochemical screening and antioxidant activity of some medicinal plants' crude juices. *Biotechnology Reports*, *28*, e00536.

Farhang Sardrodi, A., Soleimani, A., Kheiry, A., & Zibaseresht, R. (2017). Essential oil composition of Achillea aucheri Boiss at different growing altitudes in Damavand, Iran. *Journal of Agricultural Science and Technology*, *19*(2), 357-364.

Fatani, S. H., Babakr, A. T., NourEldin, E. M., & Almarzouki, A. A. (2016). Lipid peroxidation is associated with poor control of type-2 diabetes mellitus. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, *10*(2), S64-S67.

Gallé, Á., Czékus, Z., Bela, K., Horváth, E., Ördög, A., Csiszár, J., & Poór, P. (2019). Plant glutathione transferases and light. *Frontiers in Plant Science*, *9*, 1944.

Ganong WF, 2010. Ganong's review of medical physiology. 23rd edition. Kim Barrett, Heddwen Brooks, Scott Boitano, Susan Barman. Mc Graw Hill. New York, Chicago, San Francisco. PP: 430.

Georgieva, L., Gadjalova, A., Mihaylova, D., & Pavlov, A. (2015). *Achillea millefolium* L.-phytochemical profile and in vitro antioxidant activity. *International Food Research Journal*, *22*(4).

Gevrenova, R., Zengin, G., Sinan, K. I., Yıldıztugay, E., Zheleva-Dimitrova, D., Picot-Allain, C., ... & Dall'Acqua, S. (2021). UHPLC-MS Characterization and biological insights of different solvent extracts of two *Achillea* species (A. aleppica and *A. santolinoides*) from Turkey. *Antioxidants*, *10*(8), 1180.

Gharzouli, K., Khennouf, S., Amira, S., & Gharzouli, A. (1999). Effects of aqueous extracts from *Quercus ilex* l. root bark, P*unica granatum* l. fruit peel and *Artemisia herba-alba Asso* leaves on ethanol-induced gastric damage in rats. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *13*(1), 42-45.

Gharzouli, K., & Holzer, P. (2004). Inhibition of guinea pig intestinal peristalsis by the flavonoids quercetin, naringenin, apigenin and genistein. *Pharmacology*, *70*(1), 5-14.

Gornall, A. G., Bardawill, C. J., & David, M. M. (1949). Determination of serum proteins by means of the biuret reaction. *Journal of Biological Chemistry*, *177*(2), 751-766.

Graham, D. Y. (2014). History of Helicobacter pylori, duodenal ulcer, gastric ulcer and gastric cancer. *World Journal of Gastroenterology: WJG*, *20*(18), 5191.

Gulcin, İ. (2020). Antioxidants and antioxidant methods: An updated overview. *Archives of Toxicology*, *94*(3), 651-715.

Gupta S.K, Ghosal M, Biswas R, Saha B.K, Prasad Das A and Mandal P. (2014). Evaluation of *in vitro* antioxidant activity of methanolic extracts of some ferns from *Mawsynram* of Meghalaya, India. *International Journal Current Science*. *12*: 87-97.

Guzmán-Gómez, O., García-Rodríguez, R. V., Quevedo-Corona, L., Pérez-Pastén-Borja, R., Rivero-Ramírez, N. L., Ríos-Castro, E., ... & Chamorro-Cevallos, G. A. (2018). Amelioration of ethanol-induced gastric ulcers in rats pretreated with phycobiliproteins of *Arthrospira* (Spirulina) *Maxima*. *Nutrients*, *10*(6), 763.

Hansen, M. B. (2003). The enteric nervous system I: organisation and classification. *Pharmacology & Toxicology*, *92*(3), 105-113.

Hassanpour, S., MaheriSis, N., Eshratkhah, B., & Mehmandar, F. B (2011). Plants and secondary metabolites (Tannins). *A Review, International Journal of Forest, Soil and Erosion*, *1*(1), 47-53.

Holzer, P., Schicho, R., Holzer-Petsche, U., & Lippe, I. T. (2001). The gut as a neurological organ. *Wiener Klinische Wochenschrift*, *113*(17/18), 647-660.

Hou, W. C., Lin, R. D., Cheng, K. T., Hung, Y. T., Cho, C. H., Chen, C. H., ... & Lee, M. H. (2003). Free radical-scavenging activity of Taiwanese native plants. *Phytomedicine*, *10*(2-3), 170-175.

Hrycay, E. G., & Bandiera, S. M. (2015). Involvement of cytochrome P450 in reactive oxygen species formation and cancer. *Advances in Pharmacology*, *74*, 35-84.

Idayat, A. A., & Mubo, A. S. (2019). Use of medicinal plants for the treatment of gastric ulcer in some parts of Southwestern Nigeria. *African Journal of Pharmacy and Pharmacology*, *13*(15), 223-235.

Ighodaro, O. M., & Akinloye, O. A. (2018). First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *Alexandria Journal of Medicine*, *54*(4), 287-293.

Ito, F., Sono, Y., & Ito, T. (2019). Measurement and clinical significance of lipid peroxidation as a biomarker of oxidative stress: oxidative stress in diabetes, atherosclerosis, and chronic inflammation. *Antioxidants*, *8*(3), 72.

Izzo, A. A., Carlo, G. D., Mascolo, N., Capasso, F., & Autore, G. (1994). Antiulcer effect of flavonoids. Role of endogenous PAF. *Phytotherapy Research*, *8*(3), 179-181.

Janssen, P., Vanden Berghe, P., Verschueren, S., Lehmann, A., Depoortere, I., & Tack, J. (2011). The role of gastric motility in the control of food intake. *Alimentary Pharmacology & Therapeutics*, *33*(8), 880-894.

Jimenez, M. D., Martin, M. J., Alarcón De La Lastra, C., Bruseghini, L., Esteras, A., Herrerías, J. M., & Motilva, V. (2004). Role of L-arginine in ibuprofen-induced oxidative stress and neutrophil infiltration in gastric mucosa. *Free Radical Research*, *38*(9), 903-911.

Kaoudoune, C., Benchikh, F., Benabdallah, H., Loucif, K., Mehlous, S., & Amira, S. (2020). Gastroprotective effect and *in vitro* Antioxidant Activities of the Aqueous Extract from *Artemisia absinthium* L Aerial Parts. *Journal of Ddrug Delivery and Therapeutics*, *10*(4), 153-156.

Khanbabaee, K., & Van Ree, T. (2001). Tannins: classification and definition. *Natural Product Reports*, *18*(6), 641-649.

Kherkhache, H., Benabdelaziz, I., Silva, A. M., Lahrech, M. B., Benalia, M., & Haba, H. (2018). A new indole alkaloid, antioxidant and antibacterial activities of crude extracts from *Saccocalyx satureioides. Natural Product Research*, *34*(11), 1528-1534.

Kim, G. H., Kim, J. E., Rhie, S. J., & Yoon, S. (2015). The role of oxidative stress in neurodegenerative diseases. *Experimental Neurobiology*, *24*(4), 325.

Kimura, Y., & Taniguchi, M. (2021). Effects of Perilla frutescens var. crispa herb extract, the essential oil perillaldehyde, and the caffeetannin rosmarinic acid on gastric emptying and gastrointestinal motility in mice. *Traditional & Kampo Medicine*, *8*(3), 194-203.

Kostić, D. A., Dimitrijević, D. S., Stojanović, G. S., Palić, I. R., Đorđević, A. S., & Ickovski, J. D. (2015). Xanthine oxidase: isolation, assays of activity, and inhibition. *Journal of Chemistry*, *2015*, 1-8

Kozlowska, M., Laudy, A. E., Przybyl, J., Ziarno, M., & Majewska, E. (2015). Chemical composition and antibacterial activity of some medicinal plants from Lamiaceae family. *Acta Poloniae Pharmaceutica - Drug Research*, *72*(4), 757-67.

Kimura, Y., & Taniguchi, M. (2021). Effects of *Perilla frutescens* var. *crispa* herb extract, the essential oil perillaldehyde, and the caffeetannin rosmarinic acid on gastric emptying and gastrointestinal motility in mice. *Traditional & Kampo Medicine*, *8*(3), 194-203.

Kumar, N., & Goel, N. (2019). Phenolic acids: Natural versatile molecules with promising therapeutic applications. *Biotechnology Reports*, 24, e00370.

Kunchandy, E., & Rao, M. N. A. (1990). Oxygen radical scavenging activity of curcumin. *International journal of pharmaceutics*, *58*(3), 237-240.

Kurutas, E. B. (2015). The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. *Nutrition Journal*, *15*(1), 1-22.

Kwiecien, S., Brzozowski, T., Konturek, P. C., Pawlik, M. W., Pawlik, W. W., Kwiecien, N., & Konturek, S. J. (2004). Gastroprotection by pentoxyfiline against stress. *Journal of Physiology and Pharmacology*, *55*(2), 337-355.

Laine, L., Takeuchi, K., & Tarnawski, A. (2008). Gastric mucosal defense and cytoprotection: bench to bedside. *Gastroenterology*, *135(*1), 41-60.

Lalhminghlui, K., & Jagetia, G. C. (2018). Evaluation of the free-radical scavenging and antioxidant activities of Chilauni, Schima wallichii Korth in vitro. *Future Science OA*, *4*(2), FSO272.

Lamamra M.(2016). Activités biologiques et composition chimique des huiles essentielles d'*Ammiopsis aristidis* Coss. (Syn. *Daucus aristidis* Coss.) et d'*Achillea santolinoides* Lag. These de doctorat en sciences.Univercité Ferhat A bbas Setif 1.p 58-59

Landa, S. T., Dumon, K. R., & Dempsey, D. T. (2019). Anatomy and Physiology of the Stomach and Pylorus. In The SAGES manual of foregut surgery (pp. 49-64). Springer, Cham.

Laouer, H., Akkal, S., Debarnot, C., Canard, B., Meierhenrich, U. J., & Baldovini, N. (2006). Chemical composition and antimicrobial activity of the essential oil of *Saccocalyx satureioides* Coss. et Dur. *Natural Product Communications*, *1*(8), 1934578X0600100809.

Li, C. Y., Xu, H. D., Zhao, B. T., Chang, H. I., & Rhee, H. I. (2008). Gastroprotective effect of cyanidin 3-glucoside on ethanol-induced gastric lesions in rats. *Alcohol*, *42*(8), 683-687.

Li, H. B Cheng, K. W., Wong, C. C., Fan, K. W., Chen, F., & Jiang, Y. (2007). Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. *Food Chemistry*, *102*(3), 771-776.

Li, H., & Poulos, T. L. (2005). Structure–function studies on nitric oxide synthases. *Journal of Inorganic Biochemistry*, *99*(1), 293-305.

Li, H., Samouilov, A., Liu, X., and Zweier, J. L. (2004). Characterization of the effects of oxygen on xanthine oxidase-mediated nitric oxide formation. *Journal of Biological Chemistry*, *279*(17), 16939-16946.

Li, W., Huang, H., Niu, X., Fan, T., Mu, Q., & Li, H. (2013). Protective effect of tetrahydrocoptisine against ethanol-induced gastric ulcer in mice. *Toxicology and Applied Pharmacology*, *272*(1), 21-29.

Li, W. F., Hao, D. J., Fan, T., Huang, H. M., Yao, H., & Niu, X. F. (2014). Protective effect of chelerythrine against ethanol-induced gastric ulcer in mice. *Chemico-Biological Interactions*, *208*, 18-27.

Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., ... & Abete, P. (2018). Oxidative stress, aging, and diseases. *Clinical Interventions in Aging*, *13*, 757.

Lichtenberger, L. M., Bhattarai, D., Phan, T. M., Dial, E. J., & Uray, K. (2015). Suppression of contractile activity in the small intestine by indomethacin and omeprazole. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *308*(9), G785-G793.

Loucif, K., Benabdallah H, Benchikh F, Mehlous S, Souici C. B, Amira S. (2020) Total Phenolic Contents, DPPH Radical Scavenging and β-Carotene Bleaching Activities of Aqueous Extract from *Ammoides atlantica*, *Journal of Drug Delivery and Therapeutics*, *10*(3-s), 196-198

Madkour, L. H. (2019). Function of reactive oxygen species (ROS) inside the living organisms and sources of oxidants. *Pharmaceutical Sciences & Analytical Research Journal. 2*(2), 180023.

Mamache, W., Amira, S., Ben Souici, C., Laouer, H., & Benchikh, F. (2020). *In vitro* antioxidant, anticholinesterases, anti-α-amylase, and anti-α-glucosidase effects of Algerian *Salvia aegyptiaca* and *Salvia verbenaca*. *Journal of Food Biochemistry*, *44*(11), e1347.

Markham, K. R. (1982). *Techniques of flavonoid identification*. *Academic press*.

Martinez, V., Wang, L., & Taché, Y. (2006). Peripheral adrenomedullin inhibits gastric emptying through CGRP8–37-sensitive receptors and prostaglandins pathways in rats. *Peptides*, *27*(6), 1376-1382.

Mates, J. M. (2000). Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. *Toxicology*, *153*(1-3), 83-104.

Mayer, B., Baggio, C. H., Freitas, C. S., dos Santos, A. C., Twardowschy, A., Horst, H., ... & Marques, M. C. A. (2009). Gastroprotective constituents of *Salvia officinalis* L. *Fitoterapia*, *80*(7), 421-426.

Mazouz, W., Haouli, N. E. H., Gali, L., Vezza, T., Bensouici, C., Mebrek, S., ... & Djeddi, S. (2020). Antioxidant, anti-alzheimer, anti-diabetic, and anti-inflammatory activities of the endemic halophyte *Limonium spathulatum* (Desf.) kuntze on LPS-stimulated RAW264 macrophages. *South African Journal of Botany*, *135*, 101-108.

Mirończuk-Chodakowska, I., Witkowska, A. M., & Zujko, M. E. (2018). Endogenous non-enzymatic antioxidants in the human body. *Advances in Medical Sciences*, 63(1), 68-78.

Mohamadi, S., Zhao, M., Amrani, A., Marchioni, E., Zama, D., Benayache, F., & Benayache, S. (2015). On-line screening and identification of antioxidant phenolic compounds of *Saccocalyx satureioides* Coss. et Dur. *Industrial Crops and Products*, *76*, 910-919.

Mohammadi, T., Pirani, A., Moazzeni, H., & Vaezi, J. (2021). *Achillea eriophora* DC.: An ethnobotanical, pharmacological and phytochemical review. *Ethnobotany Research & Applications*, *21*(03).

Monnet, E. (2020). Anatomy and Physiology of the Stomach. *Gastrointestinal Surgical Techniques in Small Animals*, 129-134.

Morato, M., Sousa, T., & Albino-Teixeira, A. (2008). Purinergic receptors in the splanchnic circulation. *Purinergic signalling*, 4(3), 267-285.

Mouderas, F., Lahfa, F. B., Mezouar, D., & Benahmed, N. E. H. (2020). Valorization and identification of bioactive compounds of a spice *Ammodaucus leucotrichus*. *Advances in Traditional Medicine*, *20*(2), 159-168.

Muhammad, S., Hussain, M., Rahman, I. U., Shah, G. M., Ijaz, F., & Ullah, K. (2016). Indigenous medicinal usage of family Asteraceae in Sadda Lower Kurram Agency: A case study. *Asian Journal of Science and Technology*, *7*(12), 3998-4003.

Nakazawa, H., Ikeda, K., Shinozaki, S., Yasuhara, S., Yu, Y. M., Martyn, J. J., ... & Kaneki, M. (2019). Coenzyme Q10 protects against burn-induced mitochondrial dysfunction and impaired insulin signaling in mouse skeletal muscle. *FEBS Open Bio*, 9(2), 348-363.

Niazmand, S., Khooshnood, E., & Derakhshan, M. (2010). Effects of *Achillea wilhelmsii* on rat's gastric acid output at basal, vagotomized, and vagal-stimulated conditions. *Pharmacognosy Magazine*, *6*(24), 282.

Niedzielska, E., Smaga, I., Gawlik, M., Moniczewski, A., Stankowicz, P., Pera, J., & Filip, M. (2016). Oxidative stress in neurodegenerative diseases. *Molecular Neurobiology*, 53(6), 4094-4125.

Nishida, K., Ohta, Y., & Ishiguro, I. (1999). Modulation of stress-induced gastric mucosal lesions by exogenous L-arginine. *Journal of Laboratory and Clinical Medicine*, *133*(5), 440-450.

Noctor, G., Mhamdi, A., Chaouch, S., Han, Y. I., Neukermans, J., Marquez-Garcia, B. E. L. E. N., ... & Foyer, C. H. (2011). Glutathione in plants: an integrated overview. *Plant, cell & environment*, *35*(2), 454-484.

Nugroho, A., Kim, M.-H., Choi, J., Baek, N.-I. and Park, H.-J. (2012). *In vivo* sedative and gastroprotective activities of *Salvia plebeia* extract and its composition of polyphenols. *Archives of Pharmacal Research, 35*(8), 1403-1411.

Ode, O. J., & Asuzu, I. U. (2014). Luteolin isolate from the methanol extract identified as the single-carbon compound responsible for broad antiulcer activities of Cassia singueana leaves. *IOSR J Pharm*, *4*(10), 17-23.

Okhawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical biochemistry*, *95*(2), 351-358.

Owoade, A. O., Adetutu, A., & Olorunnisola, O. S. (2019). Free radicals as mediators of oxidative damage and disease. *IOSR Journal of Pharmacy*, *14*(2), 57-64.

Ozougwu, J. C. (2016). The role of reactive oxygen species and antioxidants in oxidative stress. *International Journal of Research*, *1*(8).

Peng, R. M., Lin, G. R., Ting, Y., & Hu, J. Y. (2018). Oral delivery system enhanced the bioavailability of stilbenes: Resveratrol and pterostilbene. *BioFactors*, *44*(1), 5-15.

Phaniendra, A., Jestadi, D. B., & Periyasamy, L. (2015). Free radicals: properties, sources, targets, and their implication in various diseases. *Indian Journal of Clinical Biochemistry*, *30*(1), 11-26.

Phillips, R. J., & Powley, T. L. (2007). Innervation of the gastrointestinal tract: patterns of aging. A*utonomic Neuroscience, 136*(1-2), 1-19.

Pilkington, L. I. (2018). Lignans: A chemometric analysis. *Molecules*, *23*(7), 1666.

Pimentel, M., Lin, H. C., Enayati, P., van den Burg, B., Lee, H. R., Chen, J. H., ... & Conklin, J. (2006). Methane, a gas produced by enteric bacteria, slows intestinal transit and augments

small intestinal contractile activity. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *290*(6), G1089-G1095.

Pisoschi, A. M., & Pop, A. (2015). The role of antioxidants in the chemistry of oxidative stress: A review. *European Journal of Medicinal Chemistry*, *97*, 55-74.

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., ... & Bitto, A. (2017). Oxidative stress: harms and benefits for human health. *Oxidative Medicine and Cellular Longevity*, 2017.

Poljšak, B., & Fink, R. (2014). The protective role of antioxidants in the defence against ROS/RNS-mediated environmental pollution. *Oxidative Medicine and Cellular Longevity*, 2014.

Potrich, F. B., Allemand, A., da Silva, L. M., Dos Santos, A. C., Baggio, C. H., Freitas, C. S., ... & Marques, M. C. A. (2010). Antiulcerogenic activity of hydroalcoholic extract of *Achillea millefolium* L.: involvement of the antioxidant system. *Journal of Ethnopharmacology*, *130*(1), 85-92.

Prasad, K. N., Yang, B., Dong, X., Jiang, G., Zhang, H., Xie, H., & Jiang, Y. (2009). Flavonoid contents and antioxidant activities from Cinnamomum species. *Innovative Food Science & Emerging Technologies*, *10*(4), 627-632.

Qazi, M. A., & Molvi, K. I. (2021). Ulcer: Pathogenesis, prevalence and management. J*ournal of Pharmacognosy and Phyto chemistry*, *10*(3), 172-175.

Rahgozar, M. A. H. D. I., Pazokitoroudi, H., Bakhtiarian, A., & Djahanguiri, B. (2001). Diazoxide, a KATP opener, accelerates restitution of ethanol or indomethacin-induced gastric ulceration in rats independent of polyamines. *Journal of Gastroenterology and Hepatology*, *16*(3), 290-296.

Rahman, M., Islam, M., Biswas, M., & Khurshid Alam, A. H. M. (2015). *In vitro* antioxidant and free radical scavenging activity of different parts of *Tabebuia pallida* growing in Bangladesh. *BMC Research Notes*, *8*(1), 1-9.

Rashmi, H. B., & Negi, P. S. (2020). Phenolic acids from vegetables: A review on processing stability and health benefits. *Food Research International*, *136*, 109298.

Ratcliffe, E. M. (2011). Molecular development of the extrinsic sensory innervation of the gastrointestinal tract. *Autonomic Neuroscience*, *161*(1-2), 1-5.

Ray, A., Henke, P. G., & Sullivan, R. M. (1990). Effects of intra-amygdalar thyrotropin releasing hormone (TRH) and its antagonism by atropine and benzodiazepines during stress ulcer formation in rats. *Pharmacology Biochemistry and Behavior*, *36*(3), 597-601.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, *26*(9-10), 1231-1237.

Reed, K. K., & Wickham, R. (2009). Review of the gastrointestinal tract: from macro to micro. *Seminars in Oncology Nursing*, *25*(1), 3-14.

Rodríguez-García, C., Sánchez-Quesada, C., Toledo, E., Delgado-Rodríguez, M., & Gaforio, J. J. (2019). Naturally lignan-rich foods: A dietary tool for health promotion?. *Molecules*, *24*(5), 917.

Rolnik, A., & Olas, B. (2021). The plants of the Asteraceae family as agents in the protection of human health. *International Journal of Molecular Sciences*, *22*(6), 3009.

Rotondo, A., Serio, R., & Mulè, F. (2009). Gastric relaxation induced by apigenin and quercetin: analysis of the mechanism of action. *Life Sciences*, *85*(1-2), 85-90.

Sánchez-Mendoza, M. E., López-Lorenzo, Y., Cruz-Antonio, L., Matus-Meza, A. S., Sánchez-Mendoza, Y., & Arrieta, J. (2019). Gastroprotection of calein D against ethanol-induced gastric lesions in mice: role of prostaglandins, nitric oxide and sulfhydryls. *Molecules*, *24*(3), 622.

Sen, S., De, B., Devanna, N., & Chakraborty, R. (2013). Total phenolic, total flavonoid content, and antioxidant capacity of the leaves of *Meyna spinosa Roxb*., an Indian medicinal plant. *Chinese Journal of Natural Medicines*, *11*(2), 149-157.

Sharifi-Rad, M., Anil Kumar, N. V., Zucca, P., Varoni, E. M., Dini, L., Panzarini, E., ... & Sharifi-Rad, J. (2020). Lifestyle, oxidative stress, and antioxidants: Back and forth in the pathophysiology of chronic diseases. *Frontiers in Physiology*, *11*, 694.

Sidahmed, H. M. A., Vadivelu, J., Loke, M. F., Arbab, I. A., Abdul, B., Sukari, M. A., & Abdelwahab, S. I. (2019). Anti-ulcerogenic activity of dentatin from *clausena excavata* Burm. f. against ethanol-induced gastric ulcer in rats: Possible role of mucus and anti-oxidant effect. *Phytomedicine*, *55*, 31-39.

Singla, R. K., Dubey, A. K., Garg, A., Sharma, R. K., Fiorino, M., Ameen, S. M., ... & Al-Hiary, M. (2019). Natural polyphenols: Chemical classification, definition of classes, subcategories, and structures. *Journal of AOAC International*, *102*(5), 1397-1400.

Sofidiya, M. O., Agunbiade, F. O., Koorbanally, N. A., Sowemimo, A., Soesan, D., & Familusi, T. (2014). Antiulcer activity of the ethanolic extract and ethyl acetate fraction of the leaves of *Markhamia tomentosa* in rats. *Journal of Ethnopharmacology*, *157*, 1-6.

Souadia, A., Gourine, N., & Yousfi, M. (2020). Variability in phytochemical composition and antioxidant activity of *Saccocalyx satureioides* essential oils due to harvest period. *Journal of Herbs, Spices & Medicinal Plants*, *26*(4), 435-446.

Sugamura, K., & Keaney Jr, J. F. (2011). Reactive oxygen species in cardiovascular disease. *Free Radical Biology and Medicine, 51*(5), 978-992.

Sun, T., Yuan, H., Cao, H., Yazdani, M., Tadmor, Y., & Li, L. (2018). Carotenoid metabolism in plants: the role of plastids. *Molecular Plant, 11*(1), 58-74.

Sverdén, E., Agréus, L., Dunn, J. M., & Lagergren, J. (2019). Peptic ulcer disease. *Bmj, 367*.

Swarnakar, S., Mishra, A., Ganguly, K., & Sharma, A. V. (2007). Matrix metalloproteinase-9 activity and expression is reduced by melatonin during prevention of ethanol-induced gastric ulcer in mice. *Journal of Pineal Research, 43*(1), 56-64.

Tack, J. (2007). Gastric motor disorders. *Best Practice & Research Clinical Gastroenterology*, *21*(4), 633-644.

Taiwo FO, Oyedeji O, Osundahunsi MT. 2019. Antimicrobial and Antioxidant Properties of kaempferol-3-O-glucoside and 1-(4-Hydroxyphenyl)-3-phenylpropan-1-one Isolated from the Leaves of *Annona muricata* (Linn.). *Journal of Pharmaceutical Research International, 26*(3): 1-13.

Tajner-Czopek, A., Gertchen, M., Rytel, E., Kita, A., Kucharska, A. Z., & Sokół-Łętowska, A. (2020). Study of Antioxidant Activity of some Medicinal Plants Having High Content of Caffeic Acid Derivatives. *Antioxidants, 9(5), 412.*

Tan, B. L., Norhaizan, M. E., Liew, W. P. P., & Sulaiman Rahman, H. (2018). Antioxidant and oxidative stress: a mutual interplay in age-related diseases. *Frontiers in Pharmacology*, 9, 1162.

Teleanu, R. I., Chircov, C., Grumezescu, A. M., Volceanov, A., & Teleanu, D. M. (2019). Antioxidant therapies for neuroprotection—A review. *Journal of Clinical Medicine*, *8*(10), 1659.

Treuting, P. M., Arends, M. J., & Dintzis, S. M. (2018). Upper gastrointestinal tract. In Comparative Anatomy and Histology. Academic Press. pp. 191-211.

Türkan, F., Atalar, M. N., Aras, A., Gülçin, İ., & Bursal, E. (2020). ICP-MS and HPLC analyses, enzyme inhibition and antioxidant potential of *Achillea schischkinii Sosn*. *Bioorganic Chemistry*, *94*, 103333.

Urbanska, A. M., Karagiannis, E. D., Au, A. S., Dai, S. Y., Mozafari, M., & Prakash, S. (2016). What's next for gastrointestinal disorders: No needles?. *Journal of Controlled Release*, *221*, 48-61.

Venditti, A., Maggi, F., Vittori, S., Papa, F., Serrilli, A. M., Di Cecco, M., ... & Bianco, A. (2015). Antioxidant and α-glucosidase inhibitory activities of *Achillea tenorii*. *Pharmaceutical Biology*, *53*(10), 1505-1510.

Vikas, B., Akhil, B. S., Remani, P., & Sujathan, K. (2017). Free radical scavenging properties of *Annonasquamosa*. *Asian Pacific Journal of Cancer Prevention*, *18*(10), 2725.

Wang, B., Sun, X., Wang, S., Guo, P., Li, S., Zhang, M., … Chen, X. (2017)a. *Comparative pharmacokinetics of (S)-MP3950, a novel 5-HT4 receptor agonist, in normal and atropine-induced gastrointestinal motility disorders rats. Xenobiotica, 48(8), 824–830.*

Wang, L., Wang, X., Zhang, S. L., Zhu, X. M., Liu, Y. Q., Song, Z. J., ... & Yuan, C. S. (2017)b. Gastroprotective effect of palmatine against acetic acid-induced gastric ulcers in rats. *Journal of Natural Medicines*, *71*(1), 257-264.

Wang, S., Yan, M., Guo, Y., Sun, R., Jin, H., & Gong, Y. (2020). *In vivo* and *in vitro* effects of *Salsola collina* on gastrointestinal motility in rats. *Iranian Journal of Basic Medical Sciences*, *23*(3), 383.

Wang, X., Zhong, Y. X., Zhang, Z. Y., Lu, J., Lan, M., Miao, J. Y., ... & Fan, D. M. (2002). Effect of L-NAME on nitric oxide and gastrointestinal motility alterations in cirrhotic rats. *World Journal of Gastroenterology*, *8*(2), 328.

Wang, X., Zhang, C., Zheng, M., Gao, F., Zhang, J., & Liu, F. (2019). Metabolomics analysis of L-arginine induced gastrointestinal motility disorder in rats using UPLC-MS after magnolol treatment. *Frontiers in Pharmacology*, *10*, 183.

Xu, D., Hu, M. J., Wang, Y. Q., & Cui, Y. L. (2019). Antioxidant activities of quercetin and its complexes for medicinal application. *Molecules*, *24*(6), 1123.

Xie, J., Wang, N., Dong, X., Wang, C., Du, Z., Mei, L., ... & Zhao, Y. (2018). Graphdiyne nanoparticles with high free radical scavenging activity for radiation protection. *ACS Applied Materials & Interfaces*, *11*(3), 2579-2590.

Yaseen, M., Ahmad, M., Wani, T. A., Ahmad, M., Gani, B. A., & Qureshi, R. (2017). Phytochemical screening and antioxidant activity of extracts of the leaf and stem of *Achillea millefolium*. *International Journal of Advanced Science and Research*, *2*(6), 55-59.

Yu, J. H., Lim, S. W., Luo, K., Cui, S., Quan, Y., Shin, Y. J., ... & Yang, C. W. (2019). Coenzyme Q10 alleviates tacrolimus-induced mitochondrial dysfunction in kidney. *The FASEB Journal*, 33(11), 12288-12298.

Yu, X., Ge, L., Niu, L., Lian, X., Ma, H., & Pang, L. (2018). The dual role of inducible nitric oxide synthase in myocardial ischemia/reperfusion injury: friend or foe?. *Oxidative Medicine and Cellular Longevity*, 2018.

Zhao, G. R., Xiang, Z. J., Ye, T. X., Yuan, Y. J., & Guo, Z. X. (2006). Antioxidant activities of *Salvia miltiorrhiza* and *Panax notoginseng*. *Food Chemistry*, *99*(4), 767-774. B
Zhao, J., Liao, D., Chen, P., Kunwald, P., & Gregersen, H. (2008)a. Stomach stress and strain depend on location, direction and the layered structure. *Journal of Biomechanics*, *41*(16), 3441-3447.

Zhao, H., Fan, W., Dong, J., Lu, J., Chen, J., Shan, L., ... & Kong, W. (2008)b. Evaluation of antioxidant activities and total phenolic contents of typical malting barley varieties. *Food Chemistry*, *107*(1), 296-304.

Zheng, H., Chen, Y., Zhang, J., Wang, L., Jin, Z., Huang, H., ... & Gao, W. (2016). Evaluation of protective effects of costunolide and dehydrocostuslactone on ethanol-induced gastric ulcer in mice based on multi-pathway regulation. *Chemico-Biological Interactions*, *250*, 68-77.

Ziani, B. E., Barros, L., Boumehira, A. Z., Bachari, K., Heleno, S. A., Alves, M. J., & Ferreira, I. C. (2018). Profiling polyphenol composition by HPLC-DAD-ESI/MSn and the antibacterial activity of infusion preparations obtained from four medicinal plants. *Food & Function*, *9*(1), 149-159.

Zulfiqar, F., & Ashraf, M. (2021). Bioregulators: unlocking their potential role in regulation of the plant oxidative defense system. *Plant Molecular Biology*, 105(1), 11-41.1.

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Evaluation of Antioxidant Activity and Polyphenols Content of the Hydro-methanolic Extract from *Saccocalyx satureioides* Coss and Dur

Soulaf MEHLOUS[1]*, Fatima BENCHIKH[1], Hassiba BENABDALLAH[1], Karima LOUCIF[1], Chahrazed KAOUDOUNE[1, 2], Hocine LAOUER[3] and Smain AMIRA[1]

1- Laboratory of Phytotherapy Applied to Chronic Diseases, Department of Animal Biology and Physiology, Faculty of Nature and Life Sciences, University Ferhat Abbas, Setif-1, 19000, Algeria

2- Laboratory of Environmental Biosurveillance, Faculty of Sciences, Department of Biology, BadjiMokhtar University, 23000-Annaba, Algeria

3- Laboratory of Valorisation of Natural Biological Resources, Department of Biology, University of Setif1, Algeria

ABSTRACT

This study aimed to estimate the total phenolic and flavonoid contents and to evaluate the antioxidant activity of the Hydro-methanolic from *Saccocalyx satureioides* Coss and Dur aerial part. Total polyphenols contents were determined using Folin-Ciocalteu's reagent. The flavonoids were estimated using the method of Aluminum chloride ($AlCl_3$). The antioxidant capacity was evaluated using two *in vitro* models (the 1,1-diphenyl-2-picrylhydrazyl (DPPH) and reducing power). Total phenolic and flavonoid content in the extract were 171.34 ±1.43 mg Gallic acid equivalent/g of dry extract (GAE/g) and 18.6 ± 0.46 mg Quercetin equivalent / g of dry extract (QE/g), respectively. The methanol extract has an important capacity to scavenge the free radical DPPH with an IC_{50} of 0.03 ± 0. 0024 mg/ml. In addition, the plant extract exhibited high reducing power with an IC_{50} of 0.54 ± 0.00625 mg/ml. The results of the present study may prove that the medicinal plants are a good resource of natural antioxidants.

Keywords: Antioxidant activity, *Saccocalyx satureioides,* polyphenols, flavonoids.

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***Address for Correspondence:**

Soulaf MEHLOUSLaborat, ory of Phytotherapy Applied to Chronic Diseases, Department of Animal Biology and Physiology, Faculty of Nature and Life Sciences, University Ferhat Abbas, Setif-1, 19000, Algeria

INTRODUCTION

Active oxygen and free radicals exist in human body in the form of superoxide anion (O2.-) hydrogen peroxideH_2O_2) and hydroxyl radical (OH.) and so on. As normal metabolic action going on in human body, active oxygen and free radicals are constantly formed. If they reach high levels, oxidative stress in human body would be created, which leads to a variety of biochemical and physiological lesions and often results in metabolic impairment and cell death [1].

Antioxidants are compounds that can delay or inhibit the oxidation of lipids and other molecules and by doing so inhibit the initiation and propagation of oxidative chain reactions. They act by one or more of the following mechanisms: reducing activity, free radical scavenging, potential complexation of pro-oxidant metals and quenching of singlet oxygen [2]. Plants develop different kind of antioxidants that aid in antioxidant defense system, protecting plants against damage caused by active O2 formed due to exposure to ultraviolet radiation. In the group of anti-oxidant and free radical scavenging agents, plants synthesize different compounds, principally phenolic derivatives, such as flavonoids, phenyl-propanoids, stilbenes and other [3]. The use of medicinal plants in health recovery has evolved over the times from the simplest forms of local treatment and up techno-logically sophisticated forms of industrial manufacturing currently used [4].

Saccocalyx satureioides Coss. &Du., an endemic species of Algeria, is a small aromatic shrub growing in Sahara septentrional and belonging to the Lamiaceae family [5]. This plant is 20–100 cm high. Its flowers can be white, pink or crimson [6]. It has attracted a great attention due to its traditional medicinal usage for gastric disorders and spasms [7]. The aim of the present study is to determine the polyphenolic content of the hydro-methanolic extract from *Saccocalyx satureioides* and to evaluate its antioxidant capacity using DPPH radical scavenging and reducing power assays.

MATHERIALS AND METHODS

Collection of plant and preparation of hydro-methanolic extract

The plant *Saccocalyx satureioides* Cossa nd Dur was harvested in June 2015, from Djelfa, located at an elevation of 3,734 1,138 m in the Ouled Naïl Range of North-Central Algeria. The plant was identified and authenticated by Prof. Laouer H., a botanist at the Department of Biology and Vegetal Ecology, University of Sétif, Algeria.

The collected plant was parched in dimness at room temperature. After drying, plant material was ground to a fine powder using electric grinder.100 grams of the plant powder was mixed with 1000 ml of methanol (85%) at room temperature for 3 days. The resultant suspension was filtered and concentered using evaporation at 45 C°, the extract was stored at 4 C° until use.

Determination of polyphenols and flavonoids content

Total phenolic determined using the Folin-Ciocalteu reagent [8]. A volume of 100 µl of each extract (or Gallic acid) was added to 500 µl of Folin-Ciocalteu reagent (diluted 10 times).After 4 min,400 µl of Na_2CO_3 (7,5%) solution was added. Then the final mixture was shaken and incubated in dark at room temperature for 90 min. The absorbance of all samples was measured at 760 nm. Total phenolics content of the plant extract was estimated using the calibration curve of Gallic acid. The results were expressed as mg of Gallic acid equivalent (GAE) per gram of dried plant extract.

Total flavonoids content was estimated using aluminum chloride assay [9]. 1ml of the extract or standard (Quercetin) was mixed with 1ml of solution (2%) of $AlCl_3$.The mixture was incubated for 10 min in dark at room temperature, and then the absorbance was read at 430nm against the blank. The flavonoid content was expressed as Quercetin equivalent per gram of dry plant extract weight (mg QE/g) using the calibration curve of Quercetin.

Estimation of *in vitro* antioxidant activities

DPPH radical scavenging assay

The DPPH assay was based on the measurement of altering the purple color of DPPH radical to yellow at 517 nm after reaction with antioxidant compound. The effect of antioxidants on DPPH radical was thought to be due to their hydrogen donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [10].

The free radical-scavenging activity of the extract was estimated using the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) by measuring the decrease of DPPH maximum absorbance at 517 nm [11].

In this test, 50 µl of different concentrations of the plant extract or standard was added to 1250 µl of DPPH (0.004% in methanol). The mixture was incubated at room temperature for 30 min, and then the absorbance was read at 517 nm. Vitamin C was used as standard.

% of Inhibition of free radical DPPH was calculated in the following way:

%inhibition = [(Absorbance of control-Absorbance of sample)/Absorbance of control] ×100

Where A control is the absorbance of the blank solution (containing all reagents except the test compound), and A sample is the absorbance in the presence of the test compound.

Reducing power assay

The reducing power of the Hydro-methanolic extract of the plant was estimated and the BHT was used as standard [12]. A volume of 0.1 ml of different concentrations of the plant extract or BHT was mixed with 0.1phosphate buffer (0.2 M, PH 6. 6) and potassium ferricyanide (1%). The mixture was incubated at 50 Cº for 20 min to reduce ferricyanide into ferrocyanide. After that, 0.25 ml of trichloroacetic acid (1%)was added to stop the reaction and the mixture was centrifuged at 3000 rpm for 10 min. A volume of 0.25 ml of the supernatant was mixed with 0.25 ml of distilled water and 0.5 ml FeCl3 (0.1%), then the absorbance was measured at 700 nm. BHT was used as a standard.

Statistical Analysis

The results were represented as the means ±standard deviation (SD) (n=3). All measurements were conducted in three determinations (n=3). The statistical interpretation was done by the help of Student's t-test for significance with the aid of Graph Pad Prism 7.00. Differences were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Total polyphenols and flavonoids content

Total phenolics and flavonoids contents the plant extract were 171.34± 1.43 mg Gallic acid equivalent/g of dry extract (GAE/g) and 18.6 ± 0.46mg Quercetin equivalent / g of dry extract (QE/g), respectively. The results are represented in table 1.

Table 1: Total polyphenols and flavonoids contents of *Saccocalyx satureioides* Coss and Dur Hydro-methanolic extract.

Extract	Total phenolics (mg GAE/g Dw)	Total flavonoids (mg QE/g DW)
HME	171.37 ± 1.43	18.6 ± 0.46

Results were presented as means ± standard deviation (SD), (n=3), HME: Hydro-methanolic extract.

Antioxidant activity estimation

DPPH radical scavenging activity

The results of DPPH radical scavenging activity of the plant extract are presented in table 2. Hydro-methanolic extract showed high scavenging activity against DPPH (IC_{50}= 0.03003 ± 0.0024 mg/ml). This activity remains lower than Vitamin C as positive standard(IC_{50}= 0.002663 ± 0.0002 mg/ml).

Polyphenols possess ideal structural chemistry for free radical scavenging activity, and they have

Antioxidant properties of polyphenols arise from their high reactivity as hydrogen or electron donors, and from the ability of the polyphenol derived radical to stabilize and delocalize the unpaired electron [13]. The strong activity of the plant extract to scavenge the radical DPPH could be attributed to its richness in polyphenols (171.34± 1.43 mg Gallic acid equivalent/g of dry extract).

Table2: DPPH radical scavenging activity of *Saccocalyx satureioides* Hydro-methanolic extract.

Extract	HME(mg/ml)	Vit C (mg/ml)
IC_{50}	0.03003 ± 0.0024****	0.002663 ± 0.000209

Data were presented as means ± standard deviation (SD) of IC_{50}, (n=3), HME: Hydro-methanolic extract. **** **p**< 0.0001 compared to Vit C as standard.

Reducing power activity

The ferric reducing assay measures the ability of an antioxidant to reduce a reactive oxygen species. The results are represented in table 3. From these results, the reducing power (the effective concentration at which the absorbance was 0.5) of the plant extract (EC_{50} = 0.5435 ± 0.00625 mg/ml) was promising. These values are comparable to that of BHT as positive standard (0.3276 ± 0.00496 mg/ml).

Table 3: reducing power activity of *Saccocalyx satureioides* Hydro-methanolic extract.

Extract/standard	HME (mg/ml)	BHT (mg/ml)
IC_{50}	0.5435 ± 0.00625****	0.3276 ± 0.00496

Data were presented as means ± standard deviation (SD) of IC_{50}, (n=3), HME: Hydro-methanolic extract. **** p< 0.0001 compared to BHT standard.

CONCLUSION

The results of this study showed that the hydro-methanolic extract from *Saccocalyx satureioides* aerial parts is rich in polyphenols and has strong antioxidant capacity to scavenge free radicals. These results lead to think about the use of medicinal plants and by the correct way, especially in the field of medicine.

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REFERENCES

1. Belmeki N, Bendimerad N. Antioxidant activity and phenolic content in methanol crudeextracts from three Lamiaceae grown in southwesternAlgeria. J Nat Prod Plant Resour 2012, 2 (1):175-181.

2. Dian M S, Trilianty L, Fransisca D L, Helena J, Iskandar T. Antioxidant and Anti-Glycation Activity of Ethanol Lemongrass (*Cymbopogon citratus*) Leaves Extract. International Journal of Pharmacognosy and Phytochemical Research, 2017; 9(5):710-715.

3. Amira S, Dade M, Schinella G, Rios JL. Anti-inflammatory, antioxidant and apoptotic activities of four plant species used in folk medicine in the Mediterraneanbasin. Pak J Pharmaceut Sci. 2012, 25:65-72.

4. Clarice DCV, Mayara B D, Marianne D OM, Marcelo H D S, Alexandre G-P, Fabiana C V. Evaluation of anxiolytic-like effect of 7-epiclusianone isolated from *Garcinia brasiliensis* in mice. Revista Brasileira de Farmacognosia, 2018;28:378–381.

5. Quezel P, Santa S. Nouvelle flore de l'Algérie et des régions désertiques méridionales. Centre National de la Recherche Scientifique, Paris. 1963.

6. Kherkhache H, Benabdelaziz I, Silva A M S, Lahrech M B, BenaliaM, Haba H. A new indole alkaloid, antioxidant and antibacterial activities of crude extracts from *Saccocalyx satureioides*. Natural Product Research, 2018; 34(11):1528-1534.

7. Benahmed M, Djeddi N, Akkal S, Laouar H. *Saccocalyx satureioides* as corrosion inhibitor for carbon steel in acid solution. Int J Ind Chem, 2016; 7: 109–120.

8. Li HB, Cheng KW, Wong CC, Fan KW, Chen F, Jiang Y. Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. Food Chem, 2007; 102:771-776.

9. Bahorun T, Gressier B, Trotin F, Brunete C, Dine T, Vasseur J, Luyckx M, Cazin M, Pinkas M. Oxygen species scavenging activity of phenolic extract from *Hawthorn* fresh plant organs and pharmaceutical preparations. Arzneim Forsch/Drug Res, 1996; 46: 1086-1089.

10. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature, 1958; 181:1199-1200.

11. Burits M, Bucar F. Antioxidant activity of *Nigella sativa* essential oil. Phytother Res, 2000; 14:323-28.

12. Chung YC, Chen SJ, Hsu CK, Chang CT, Chou ST. Studies on the antioxidative activity of *Graptopetalum paraguayense*. Food Chemistry, 2005; 91:419-424.

13. Benchikh F, Amira S, Benabdallah, H. The evaluation of antioxidant capacity of different fractions of *Myrtus communis* L. leaves. Annual Research & Review in Biology, 2018; 22(5):1-14.

ملخص

تعتبر كلا من *Achillea santolinoides* L. (*A. santolinoides* L.) و *Saccocalyx satureioides* Coss and Dur (S. *Satureioides*) نبتتان مهمتان من النباتات الجزائرية. كان الهدف من الدراسة الحالية تقييم محتوى عديدات الفينول، الفلافونويدات والدباغ ودراسة النشاطية المضادة للأكسدة في المختبر للمستخلص المائي، الميثانولي والهيدروميثانولي لكل من *S. satureioides* (SME، SAE، SHME) و*A. santolinoides* L. (AAE،AME ، AHM) ودراسة التحليل الكيميائي لمستخلصات SAE، SME، AAE وAME، بالإضافة إلى الإفراغ المعدي، العبور المعوي والتأثير الوقائي المعدي للمستخلصين SAE وAAE. أظهر التحليل الكيميائي النباتي لـ *S. Satureioides* أن SAE و SME يحتويان على أحماض فينولية مشتركة بين المستخلصين (lutéoline-7-O-rutinoside, lutéoline-O-diglucuronide et lutéoline-7-O-glucuronide)، في حين كان SME أكثر احتواء على المركبات الكيمائية مقارنة ب SAE. يكشف التحليل الكيميائي النباتي لنبات *A. santalinoides* L. أن AME أغنى من AAE من حيث المركبات الفينولية. يحتوي AAE على apigening (-' 2-apigenin O-pentosyl-8-C-glucoside وapigenin-*O*-glucuronide) وluteolin-7-*O*-rutinoside. يحتوي AME على apigenin أيضًا (apigenin-2 " -O-pentosyl-8-C-glucoside وapigenin-*O*-glucuronide)، و حمض Dicaffeoylquinic acid (Dicaffeoylquinicacid-3،4 و 3,5-Dicaffeoylquinicacid). تنسب أعلى كمية من عديدات الفينول لكل من SME وAME، كما أنه في معظم الاختبارات، أظهرت النشاطية المضادة للأكسدة أفضل نشاط لكلا المستخلصين. تم تقييم تأثير مستخلصات SAE وAAE على الإفراغ المعدي والعبور المعوي لدى الفئران. وجد أن كلا المستخلصين أظهر تأثيرا معنويا في إفراغ المعدة وتأخر العبور المعوي. كان تأثير كلا المستخلصين عبر مسار NO وانزيمات الأكسدة الحلقية بالإضافة إلى تدخل المستقبلات المسكارينية بالنسبة لمستخلص AAE. في حين لم تتدخل المستقبلات المسكارينية في تأثير SAE لإفراغ المعدة. أعطى كل منAAE و SAEعند الجرعات المختبرة (100، 200 و400 مجم / كجم) حماية جيدة للغشاء المخاطي ضد تقرح المعدة المحدث بالإيثانول في الجرذان ويتدخل المسار المسكاريني في التأثير المعدي لكلا المستخلصين.

الكلمات المفتاحية: *S. satureioides*، *A. santolinoides* L.، مضادات الأكسدة، الدراسة الكيميائية، الإفراغ المعدي، العبورالمعوي، قرحة المعدة.

Résumé

Saccocalyx satureioides Coss and Dur (*S. satureioides*) et *Achillea santolinoides* L. (*A. santolinoides* L.) sont deux plantes importantes de la flore Algérienne. Le but de la présente étude était d'évaluer la teneur totale en phénols, flavonoïdes et tannins et l'activité antioxydante *in vitro* des extraits aqueux, méthanoliques et hydrométhanoliques de *S. satureioides* (SAE, SME et SHME) et d'*Achillea santolinoides* L. (AAE, AME et AHME), et d'établir le profil chimique des extraits SAE, SME, AAE et AME. En plus, la vidange gastrique, le transit intestinal et l'effet gastro-protecteur des extrais SAE et AAE ont été étudiés. L'analyse phytochimique de *S. satureioides* a révélé que SAE et SME contenaient plusieurs acides phénoliques communs aux deux extraits (lutéoline-7-*O*-rutinoside, lutéoline-*O*-diglucuronide et lutéoline-7-*O*-glucuronide). L'extrait SME était plus riche en composés phénoliques que le SAE. L'analyse phytochimique d'*A. santalinoides* L. révèle que l'AME est plus riche en composés phénoliques que l'AAE. L'AME contient de l'apigénine (apigénine-2"-*O*-pentosyl-8-C-glucoside et apigénine-*O*-glucuronide) et de lutéoline-7-*O*-rutinoside. L'AME contient aussi de l'apigénine (apigenin-2"-O-pentosyl-8-C-glucoside et apigenin-*O*-glucuronide) et l'acide dicaffeoylquinic (acide 3,5-dicaffeoylquinic et acide 3,4-dicaffeoylquinic). SME et AME présentaient la plus grande quantité de polyphénols. Dans la majorité des tests, la meilleure activité antioxydante a été montrée avec ces extraits. L'effet des extraits SAE et AAE sur la vidange gastrique et le transit intestinal a été étudié chez les souris. Il a été constaté que les deux extraits présentaient un effet significatif sur la vidange gastrique et le retard du transit intestinal. L'effet des deux extraits se fait *via* les voies NO et cyclooxygénase, en plus, les récepteurs muscariniques sont impliqués avec l'extrait AAE, sauf que les récepteurs muscariniques ne sont pas impliqués en présence de l'extrait SAE. SAE et AAE aux doses testées (100, 200 et 400 mg/kg) ont offert une grande protection de la muqueuse contre l'ulcération gastrique induite par l'éthanol chez le rat. La voie muscarinique est impliquée dans l'effet gastroprotecteur des deux extraits.

Mots clés: *S. satureioides, A. santolinoides* L., activité antioxydant, vidange gastrique, transit intestinal, ulcère gastrique.

Abstract

Saccocalyx satureioides Coss and Dur (*S. satureioides*) and *Achillea santolinoides* L. (*A. antolinoides* L.) are two important plants of Algerian flora. The aim of the present study is to assess total phenolic, flavonoids and tannins content and the *in vitro* antioxidant activity of the aqueous, methanol and hydromethnol extracts of *S. satureioides* (SAE, SME and SHME) and *A. santolinoides* L. (AAE, AME and AHME), and to establish the chemical profile of SAE, SME, AAE and AME. In addition, to the gastric emptying, intestinal transit and the gastro-protective effect of SAE and AAE extracts were studied. The phytochemical analysis of *S. satureioides* revealed that SAE and SME contained common phenolic acids between the two extracts (luteolin-7-*O*-rutinoside, luteolin-*O*-diglucuronide and luteolin-7-*O*-glucuronide). SME extract was richer in phenolic compounds than SAE. The phytochemical analysis of *A. santalinoides* L. revealed that AME is richer in compounds than AAE. AAE contains apigenin (apigenin-2''-*O*-pentosyl-8-C-glucoside and apigenin-*O*-glucuronide) and luteolin-7-*O*-rutinoside. AAE Also contains apigenin (apigenin-2''-O-pentosyl-8-C-glucoside and apigenin-*O*-glucuronide) and of dicaffeoylquinic acid (3,5-dicaffeoylquinic acid and 3,4-dicaffeoylquinic acid). SME and AME had the highest amount of polyphenols. In the majority of the used tests, the extracts showed the best antioxidant activity. The effect of SAE and AAE on gastric emptying and intestinal transit were studied in mice. It was found that both extracts induced a significant delay in gastric emptying and in intestinal transit. The effect of both extracts are *via* the NO and the cyclooxygenase pathways, in addition to the involvement of muscarinic receptors for the effect of AAE. In contrary, the muscarinic receptors are not involved in gastric emptying in the presence of SAE. SAE and AAE at the tested doses (100, 200 and 400 mg/kg) offered a significant protection of the mucosa against ethanol-induced gastric ulceration in rats. Muscarinic pathway is involved in the gastroprotective effect of both extracts.

Keywords: *S. satureioides*, *A. santolinoides* L., antioxidants, phytochemical analysis, gastric emptying, intestinal transit, gastric ulcer.