الجمهورية الجزائرية الديمقراطية الشعبية
وزارة التعليم العالي والبحث العلمي

Université Ferhat Abbas Sétif 1
Faculté des Sciences de la
Nature et de la Vie

جامعة فرحات عباس، سطيف 1
كلية علوم الطبيعة والحياة

DÉPARTEMENT OF BIOCHEMISTRY

N°...../SNV/**2020**

THESIS

Presented by

KADDOUR Sabrina Manel

For the fulfillment of the requirements for the degree of

DOCTORAT 3 CYCLE

BIOLOGY

Special filed: BIOCHEMISTRY

TOPIC

Phytochemical, Antioxidant and Anti-inflammatory Effects of *Medicinal Plants Extracts*

Presented publically in: 24 / 12 / 2020

MEMBERS OF JURY

President:	**KHENNOUF Seddik**	Prof. UFA Setif 1
Supervisor:	**BAGHIANI Abderrahmane**	Prof. UFA Setif 1
Examiners:	**BOURAS Mourad**	Prof. Univ. Batna
	DJARMOUNI Meriam	MCA UFA Setif 1
	BOUAZIZ Amel	MCA UFA Setif 1

Laboratory of applied biochemistry

ACKNOWLEDGEMENTS

First and foremost, praises and thanks to ALLAH subhana-Wa-Taala, the Almighty, for providing me the blessings throughout my research work to complete the research successfully.

I wish to express my sincere gratitude to my supervisor Pr. Baghiani Abderrahmane, for his valuable advices and guidance, timely suggestions and whole-hearted supports. His continuous interest and great generosity helped my work to be accomplished and also supported me during a course of this study.

I would like to thank my Committee members, Pr. Khennouf Seddik University Ferhat Abbas Setif 1, Pr. Bouras mourad University of Batna, Dr. Djarmouni Meriam University Ferhat Abbas Setif 1 and Dr. Bouaziz Amel University Ferhat Abbas Setif 1, for critical reading of the thesis, and for their valuable comments, discussions and suggestions.

I want to offer my special thanks to Pr. Arrar Lekhmissi and Dr. Guemaz Thoraya for their valuable advices, continuous support, assistance, and to all other technical supports. I am also thankful to Pr. Charef Noureddine, Pr. Khennouf Seddik, Pr. Dahamna Saliha for help and provision of research facilities in their laboratories and Dr. Safsaf University Hospital of Sétif for providing all the facilities to realise histological slides and interpreting them.

Many thanks send to all my colleagues in laboratory of applied biochemistry, who provided the most amicable and lively working environment.

This work carried out in laboratory of applied biochemistry and laboratory of phytotherapy applied to chronic diseases.

DEDICATE

I would like to thank my family, especially my mother and my husband and everyone who stood by me who gave me strength to go on all the way of getting to the end. For all their selfless encouragements and sacrifices, I am ever grateful.

ملخص

تهدف هذه الدراسة الى تقييم النشاطية المضادة للأكسدة و المضادة للالتهاب بالاضافة الى القدرة على حماية الكبد لمختلف المستخلصات المحضرة من الجزء الهوائي للنباتين *Halogeton sativus*و *Arthrophytum scoparium*.

تم تقدير عديدات الفينول الكلية و الفلافونويدات و الدباغ في كل من المستخلص الميثانولي الخام للنبتتين والأطوار الناتجة منها كالمستخلص الكلوروفورمي و مستخلص أسيتات الاثيل و المستخلص المائي بالاضافة الى تقدير هذه المركباتى النباتين المذكورين. بينت النتائج أن مغلى *A. Scoparium* يحتوي على كمية عالية من عديدات الفينول, الفلافونويدات و الدباغ بينما احتوى مستخلص الايثيل اسيتات لنبتة *H.sativus* على أعلى القيم بالنسبة للمركبات المذكورة.

أظهر المستخلص الخام لنبتة *A. Scoparium* و مستخلص الاثيل أسيتات لنبات *H.sativus* قدرة عالية لازاحة جذورABTS, DPPH و الهيدروكسيل بالاضافة الى القدرة الارجاغية و القدرة المخلبية للأيونات, بينما أظهر كلا من مغلى نبتة *A.scoparium* و المستخلص الخام لنيتة *H.sativus* القدرة الأعظمية لازاحة بيروكسيد الهيدروجين.

عمل مستخلص الاثيل أسيتات لنبتة *H.Sativus* والمستخلص الخام لنبتة *A.scoparium* على تثبيط أكسدة البيتاكاروتين بنسبة 99.12 ± 0.41% و 69.35 ± 0.32 % على الترتيب.عمل كل من المغلى و المستخلص الخام لكلتا النبتتين على تثبيط انحلال كريات الدم الحمراء المحدث بواسطة AAPH. وكان لكل من المستخلص الخام لنبتة *A.Scoparium* و مستخلص الاثيل اسيتات لنبتة *H.sativus* تأثيرا تثبيطيا ضد انحلال كريات الدم الحمراء المحدث بواسطة $FeSO_4$.

ولدراسة النشاطية الواقية للكبد للمستخلص الخام لكلا النبتتين تمت معاملة الجزذان بجرعتين من هذه المستخلصات(250و500مغ/كغ) لمدة 7أيام متتالية وبعد 24ساعة من المعاملة تم حقن مركب CCl_4.أدت المعاملة بمركب CCl_4 الى ارتفاع محسوس في كل من مستويات اليوريا و الكرياتنين وALT وAST ,بينما ارتفعت بعض مؤشرات الاجهاد التأكسدي مثل GSH ونشاطية انزيمCAT لدى الجرذان المعاملة بكل من المستخلصين المذكورين,بينما لوحظ انخفاظ في مستوى MDA لدى هذه الجرذان وهذه المؤشرات تغيرت حسب الجرعة المعطاة.أكدت الدراسات النسيجية الدور الواقي من التسمم الكبدي لكلا المستخلصين الخامين للنبتتين.

أدت معاملة الفئران بكل من 200,100,و 400مغ/كغ للمستخلصين الخامين للنبتتين على تثبيط وذمة الأذن المحدثة بواسطة xylene وكان هذا التأثير مرتبطا بالجرعة.

تمّ اختبار قدرة المستخلصين بتركيز 300مغ/كغ على حماية المعدة و تركيبها لدى الجرذان و يستخلص ممّا سبق أنّ نبتتي *H.Sativus* و *A.scoparium* لها القدرة المضادة للأكسدة في كل من الحي و الزجاج لذا يمكن أن تستعمل هذه النباتات كعوامل مضادة للأكسدة و مضادة للالتهاب و واقية للكبد من التسممات, وهذه النتائج قد تدعم الاستعمال الشعبي لهاتين النبتتين في علاج العديد من الأمراض.

الكلمات المفتاحية: *Halogeton sativus*, *Arthrophytum scoparium*,عديدات الفينول, الفلافونويدات,النشاطية المضادة للأكسدة ,التسمم الكبدي.

ABSTRACT

The aim of this study is to evaluate, *in vitro* and *in vivo*, the antioxidant, anti-inflammatory and hepatoprotective activities of different extracts prepared from the aerial parts of *Arthrophytum scoparium* and *Halogeton sativus*. The contents of polyphenols, flavonoids and tannins of the crude methanolic extract (CrE) and its fractions: extract of chloroform (ChE), ethyl acetate (EAE), aqueous (EQA) and decoction (DEC) have been determined. The results showed that the DEC extract of *A. scoparium* contains the largest amount of phenolic compounds, flavonoids and tannins, whereas for *H. sativus* EAE contained the largest amount of polyphenols, flavonoids and tannins. CrE extracts from *A. scoparium* and EAE from *H. sativus* showed the highest scavenger (DPPH, ABTS and hydroxyl radicals), reductive and ion chelating activities. DECs of *A. scoparium* and *H. sativus* CrE exhibited the greatest activity of eliminating hydrogen peroxide. EAE extracts from *H. sativus* and CrE from *A. scoparium* were found to be the most potent inhibitors of β-carotene oxidation with 99.12 ± 0.41% and 69.35 ± 0.32%, respectively. The DECs and CrEs of both plants showed the highest anti-hemolytic effect against AAPH-induced hemolysis, with significant HT_{50} values. In addition, *A. Scoparium* CrE and *H. sativus* EAE have satisfactory inhibitory properties against $FeSO_4$-induced hemolysis. In a hepatoprotective study, ASE (*A.scoparium* crude extract) and HSE (*H.sativus* crude extract) were administered orally (250 and 500 mg / kg) for 7 consecutive days and then, after 24 hours, CCl_4 was administered. The treatment of rats with $CC1_4$ resulted in a significant increase in serum levels of ALT, AST, urea and creatinine. The analysis of the antioxidant potential parameters showed a significant increase in the GSH level and CAT activity of the groups treated with ASE and HSE. On the other hand, a significant decrease in MDA levels has been recorded. These parameters were significantly altered in a dose depending manner. *In vivo*, ASE and HSE showed a potent entrapment effect on DPPH and a reducing power. A histological study confirmed the antihepatotoxic potential of the extracts of both plants. Anti-inflammatory activities showed that the administration of 100, 200 and 400 mg / kg of ASE and HSE inhibited xylene-induced ear edema in a dose-dependent manner. The gastroprotective effect of ASE and HSE at a dose of 300 mg / kg was evaluated by restoring the architecture of the rats' stomachs. In conclusion, *A. scoparium* and *H. sativus* exert potent antioxidant activity both *in vitro* and *in vivo*. The selected plants can therefore be used as antioxidant, anti-inflammatory and hepatoprotective agents. The results of this study support the traditional use of these plants to treat many diseases.

Key words: *Arthrophytum scoparium*, *Halogeton sativus*, polyphenols, Flavonoids, Antioxidant activity, hepatotoxicity.

RESUME

Le but de cette étude est d'évaluer l'activité antioxydante, anti-inflammatoires et hépato protectrices *in vitro* et *in vivo* de différents extraits préparés à partir des parties aériennes d'*Arthrophytum scoparium* et de *Halogeton sativus*. La quantification des polyphénols, flavonoïdes et tanins d'extrait méthanolique brut (CrE) et de ses fractions: extraits de chloroforme (ChE), d'acétate d'éthyle (EAE), aqueux (AQE) et de décoction (DEC) a été déterminée. Les résultats ont montré que *A. scoparium* DEC contient la plus grande quantité de composés phénoliques, de flavonoïdes et de tanins. Considérant que *H.sativus* a montré que l'EAE contenait la plus grande quantité de phénol, de flavonoïdes et de tanins. *A.scoparium* CrE et *H.sativus* EAE ont montré l'activité la plus élevée de piégeage des radicaux de DPPH, d'ABTS, de pouvoir réducteur, de radicaux hydroxyles et d'activités de chélation d'ions. *A. scoparium* DEC et *H.sativus* CrE présentaient respectivement l'activité de piégeage la plus élevée du peroxyde d'hydrogène. *H. Sativus* EAE et *A. scoparium* CrE se sont révélés être les inhibiteurs les plus puissants de l'oxydation du β-carotène avec 99,12 ± 0,41% et 69,35 ± 0,32%, respectivement. Les DEC et CrE des plantes étudiées ont montré l'effet antihémolytique le plus élevé contre l'hémolyse induite par l'AAPH et ont révélé des valeurs importantes de HT_{50} dans la gamme de concentration utilisée (0,125 à 1 mg / ml). De plus, *A.scoparium* CrE et *H.sativus* EAE présentaient des propriétés inhibitrices satisfaisantes contre l'hémolyse induite par $FeSO_4$. Les doses administrées (2000 mg / kg et 5000 mg / kg) n'ont pas subi de modifications du comportement général, de la toxicité ou de la mortalité des rats testés.

Dans une étude hepatoprotective, l'ASE et l'HSE ont été administrés par voie orale à des doses de 250 et 500 mg / kg pendant 7 jours consécutifs. Après 24h, l'administration de CCl_4 (2 ml / kg, i.p) a été réalisée. L'analyse des paramètres du potentiel antioxydant a montré une augmentation significative du taux de GSH et de l'activité CAT des groupes traités avec l'ASE et l'HSE. En outre, une diminution significative des niveaux de MDA a été enregistrée. Le traitement des rats au $CC1_4$ a provoqué une augmentation significative des taux sériques d'ALT, d'AST, d'urée et de créatinine. Ces paramètres ont été réduits de manière significative en fonction de la dose lors du traitement par ASE et HSE. *In vivo*, l'ASE et l'HSE ont montré un puissant effet de piégeage du DPPH et un pouvoir réducteur. Une étude histologique a confirmé le potentiel antihépatotoxique d'extraits de plantes sélectionnées. Les activités anti-inflammatoires ont montré que l'administration de 100, 200 et 400 mg / kg d'ASE et d'HSE inhibait l'augmentation d'œdème de l'oreille induite par xylène d'une manière dépendante de la dose. L'effet gastroprotecteur de l'ASE et de l'HSE à une dose de 300 mg / kg est confirmé par la restauration de l'architecture normale de l'estomac de rat. Donc, On peut conclure qu'*A.scoparium* et *H.sativus* exercent une puissante activité antioxydante à la fois *in vitro* et *in vivo*. Les résultats indiquent clairement que les plantes sélectionnées peuvent être utilisées comme agent hépatoprotecteur et soutiennent l'utilisation traditionnelle de ces plantes pour traiter de nombreuses maladies à médiation radicalaire

Mots-clés: *Arthrophytum scoparium*, *Halogeton sativus*, polyphénols, flavonoïdes, activité antioxydante, hépatotoxicité.

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

•LOO−	Lipid peroxyl
•NO−	Nitric oxide
•OH	Hydroxyl radical
$^1O_2$	Singlet oxygen
4-HNA	4-hydroxyl nonenal
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AQE	Aqueous extract
AST	Aspartate aminotransferase
CAMs	Cell adhesion molecules
CAT	Catalase
cGMP	Cyclic guanosine monophosphate
ChE	Chloroform extract
COX	Cyclooxygenase
CrE	Crude extract
CVD	Cardiovascular disease
DEC	Decoction
DEN	Diethylnitrosamine
EAE	Ethyle acetate extract
EC_{50}	Effective concentration of 50%
eNOS	Endothelial nitric oxide synthase
ER	Endoplasmic reticulum
GAE	Gallic acid equivalent
GPx	Glutathione peroxidase
GSH	Reduced glutathione
HLA	Human Leukocyte antigen
HNO_2^-	Nitrous acid
HT_{50}	Half-time of 50% hemolysis
IC_{50}	Inhibitory concentration at 50%
L•	Carbon centered lipid radical
LD_{50}	Lethal dose 50
LOOH	Lipid peroxide
MDA	Malondialdehyde
NAFLD	Non – alcoholic Fatty liver Diseases
NASH	Non-alcoholic steatohepatitis
NK	Natural killer
NSAIDs	Nonsteroidal anti-inflammatory drugs
$O2^{\bullet-}$	Superoxide
OECD	Organisation for economic co-operation and development
$ONOO^-$	Peroxynitrite
OS	Oxidative stress
PCM	Paracetamol
PUDs	Peptic ulcer disease
PUFA	Polyunsaturated fatty acids
QE	Quercetin equivalent
RBCs	Red blood cells
RNS	Reactive nitrogen species.
ROS	Reactive oxygen species.
SOD	Superoxide dismutase
TAE	Tannic acid equivalent
XO	Xanthine oxidase

LISTE OF SCIENTIFIC PRODUCTIONS

ARTICLES

Kaddour Sabrina Manel, Arrar Lekhmeci, Baghiani Abderrahmane.(2020). Anti-Inflammatory Potential Evaluation (In-Vitro and In Vivo) of Arthrophytum scoparium Aerial Part, Journal of Drug Delivery and Therapeutics, 10(5):213-218.

Kaddour Sabrina Manel, Zerargui Fatima, Arrar Lekhmeci and Baghiani Abderrahmane. (2019). Acute, sub-acute and antioxidant activities of *Arthrophytum scoparium* aerial parts. IJPSR, 10(9): 4167-4175.

Kaddour Sabrina Manel, Guemmaz thoraya, Arrar Lekhmeci and Baghiani Abderrahmane. (2016). Hydroxyl radical scavenging and chelating activity effects of *Haloxylon salicornicum* extracts. Revue des Régions Arides n°43 (3/2017) – Numéro spécial – Actes du 5ème Meeting International sur l'Arido-culture et les Cultures Oasiennes : Biotechnologie végétale en zones arides et oasiennes.

COMMUNICATIONS

Kaddour Sabrina Manel, Arrar Lekhmeci et Baghiani Abderrahmane. (2016).*In vitro* antioxidant effect of *Haloxylon salicornicum* extracts. doctoriales de l'UFAS1 sur l'innovation le 19 mai 2016, école doctorales el bez sétif, Algérie.

Kaddour Sabrina Manel, Arrar Lekhmeci et Baghiani Abderrahmane. (2016).*In vitro* antioxidant effect of *Haloxylon salicornicum* extracts. doctoriales de faculté des sciences de la nature et de la vie le 31 mai 2016 sétif, Algérie.

Kaddour Sabrina Manel, Arrar Lekhmeci et Baghiani Abderrahmane. (2016).l'effet de *Nigella sativa* sur la production de xanthine oxydoréductase chez les rats traités par l'éthanol. 1er séminaire national de biologie, santé et stress oxydant le 9et 10 novembre 2017 Tébessa, Algérie.

Kaddour Sabrina Manel, Guemmaz thoraya, Arrar Lekhmeci and Baghiani Abderrahmane (2016).Hydroxyl radical scavenging and chelating activity effects of *Haloxylon salicornicum* extracts**.** V meeting international sur la biotechnologie végétale en zones arides et oasiennes, 19au21 décembre 2016, Zarzis, Tunisie.

Kaddour Sabrina Manel, Arrar Lekhmeci and Baghiani Abderrahmane (2017).Radical scavenging, lipid peroxidation inhibition and chelatins properties of extracts from *Arthrophytum scoparium* aerial parts extracts, congrès international de phytothérapie du 29 Avril au 1 mai 2017, Monastir, Tunisie.

Kaddour Sabrina Manel, Arrar Lekhmeci and Baghiani Abderrahmane (2017). Antioxidant proprieties of *Haloxylon salicornicum* aerial parts extracts ,8 journées scientifiques internationales sur la valorisation des bioressources 5,6 et 7 mai 2017 à Monastir, Tunisie.

Kaddour Sabrina Manel and Sobhi Widad. (2017). Effet hépatoprotecteur de Nigella sativa chez les rats traités par l'éthanol. The first international congres on biotechnologies for sustainable development CIBSDD ,24-25 october , Boumerdes, Algeria.

Kaddour Sabrina Manel, Arrar Lekhmeci and Baghiani Abderrahmane. (2018).Analgesic and radical scavenging effect against hydrogen peroxyde and hydroxyl radical of *Arthrophytum scoparium* extracts.VI congres international de biotecghologies et valorisation des bio-ressources,organisé par l'ATBVBR du 20-23Mars 2018 à Tabarka, Tunisie.

Kaddour Sabrina Manel, Arrar Lekhmeci and Baghiani Abderrahmane. (2018). Polyphenols content, DPPH and ABTS radical scavenging activities of Halogeton sativus extracts. UFAS1, 25 Avril 2018, Setif, Algeria.

Kaddour Sabrina Manel, Arrar Lekhmeci and Baghiani Abderrahmane. (2018).Anti-inflammatoiry activity of *Arthrophytum scoparium* extracts.9 journées scientifiques internationales sur la valorisation des bioressources du 3-6 mai 2018, Monastir, Tunisie.

Kaddour Sabrina Manel, Arrar Lekhmeci and Baghiani Abderrahmane. (2018). Antihemolytic and antioxidant effect of *Arthrophytum scoparium*. Congres international ; les rencontre de l'agriculture et de la biologie du 05-07 mai 2018 Constantine, Algérie.

Kaddour Sabrina Manel, Arrar Lekhmeci and Baghiani Abderrahmane. (2018). Antioxidant properties of *Halogeton sativus* aerial parts extracts. Auditorium M. K. Nait Belkacem, UFAS,23 juin 2018, Setif, Algeria.

Kaddour Sabrina Manel, Arrar Lekhmeci and Baghiani Abderrahmane. (2018).Total phenolic content, ion chelating and reducing power properties of *Halogeton sativus* extracts. The 2nd international conference on bioanalysis :food and healthHigher institute of applied sciences and technology ,December 15th, Mahdia, Tunisia.

Kaddour Sabrina Manel, Arrar Lekhmeci and Baghiani Abderrahmane. (2019). The preventive effect of *arthrophyrum scoparium* against gastric ulcer induced with ethanol. VII congres international de biotecghologies et valorisation des bio-ressources,organisé par l'ATBVBR du 20-23Mars 2019 à Tabarka, Tunisie.

CONTENTS

Introduction

Medicinal plants have been playing an essential role in the development of human culture as a source of medicines. They have always been at forefront virtually all cultures of civilizations. Medicinal plantes are regarded as rich sources of traditional medicines and from these plants many of the modern medicines are produced. For thousands of years, medicinal plants have been used to treat health disorders, to add flavor, concerve food and to prevent epidemic diseases. The present study was undertaken to valorize *A.scoparium* and *H.sativus* extracts as antioxidant and anti-inflammatory agents.

Phenolic compounds and flavonoids are present in nutrients and herbal medicines, both flavonoids and many other phenolic compounds are effective as antioxidants, antibacterial, cardioprotective agents, anti-inflammatory agents, immune system promoting, skin protecting compounds, skin protection from UV radiations, and interesting candidate for pharmaceutical and medicinal use.

WHO (World Health Organization) estimated that 80% of people worldwide rely on herbal medicines for some aspect of their primary health care needs. According to WHO, around 21.000 plant spices have the potential for being used as medicinal plants.

Reactive oxygen species (ROS), highly reactive molecules, are produced by living organisms as a result of normal cellular metabolism and environmental factors, and can damage nucleic acids and proteins, there by altering their functions. The human body has several mechanisms to counter act oxidative stress by producing antioxidants. A shift in the balance between oxidants and antioxidants in favor of oxidants is termed as “oxidative stress”.Oxidative stress results in macromolecular damage and is implicated in various disease states such as atherosclerosis, diabetes, cancer, neurodegeneration, and aging. Naturally occurring phytochemical antioxidants have occupied a prominent position as effective antioxidants for the prevention and/or treatment of several disorders and diseases. The premise for this has been the antioxidant actions of the phytochemicals as free-radical scavengers, oxidative stress relievers, and lipoperoxidation inhibitors. In recent years, plant polyphenols have been reported as effective antioxidants in the prevention and treatment of several diseases, including CVDs, cerebrovascular diseases, Alzheimer’s disease, resperatory system disease, and cancer (Liu *et al.,* 2018).

Arthrophytum scoparium and *Halogeton sativus* belongs to the family of chenopodiaceae. They are worldwide distributed, especially in desert and semi desert areas. *A.scoparium* is a

local medicinal plant, which has been used extensively in the southwestern part of Algeria. It is used to treat numerous human diseases especially infectious (skin infections, urinary and genital infections), Rheumatism, diabetes, cancer, infertility, hair problems and stomach disorder (Allaoui *et al.,* 2016, fatehi *et al*., 2017),however, Many studies reported the traditional use of Halogeton species in the treatment of hypertension and demonstrated the hypoglycaemic effects (TUNDIS *et al*., 2008).

In the present study, the following objectives are formulated:

- Evaluation of the *in vitro* antioxidant activity of plant extracts using different methods.
- Estimation of the LD_{50} of the *Arthrophytum scoparium* and *Halogeton sativus* of crude methanolic extracts of aerial parts extracts.
- Evaluation of the acute toxicity of *Arthrophytum scoparium* and *Halogeton sativus*.
- Evaluation of the protective effect of crude extracts of selected plants against CCl_4-induced liver injury.
- Evaluation of plasma antioxidant capacity using DPPH radical scavenging test and reducing power method.
- Evaluation of the *in vivo* antioxiadant activity of crude extract of selected plants by assessing the MDA and GSH levels, and catalase activity in the liver of rats.
- Evaluation of the antiinflammatory effect of plant extracts in mice against xylene-induced ear odema.
- Evaluation of the protective effect of crude extracts of selected plants against ethanol induced gastric ulcer.

1. Oxidant and antioxidant system

1 .1.Oxidative stress

The term “stress” was first used in the biomedical literature as a description of hyperactivity in the hormone system, in particular concerning the corticosteroids of the adrenal cortex .The scientist summarized some 20 years later how the idea of stress, stress response, and homeostasis as a dynamic equilibrium gradually developed into a highly useful idea in general physiology and the study of diseases. According the literature, it’s reported that “stress” primarily as a factor causing disease, and even today, as exemplified by this thematic issue, modern stress research is still largely concerned with path mechanisms of human disease. (Breitenbach and Eckl, 2015). Oxidative stress (OS) is conceptually defined as the imbalance between generations and clearances of oxidants and it has increasingly become a major interested point of basic science and clinical research.

1.2. Oxidants

1.2.1. Endogenous Sources of ROS

The term reactive oxygen species (ROS) includes the reduced form of oxygen and their reaction products with other molecules. ROS can be divided into two groups: free radicals and nonradical. Reactive Nitrogen species (RNS) are special forms of ROS that contain nitrogen. Similar to ROS, RNS can also include radicals and non-radicals form (Mugoni *et al*., 2013; Collin, 2019). Molecules containing one or more unpaired electrons and thus giving reactivity to the molecule are called free radicals. When two free radicals share their unpaired electrons, nonradical forms are created. ROS/RNS are summarized in **Table 1**.

Table 1. Radical and non-radical ROS and RNS **(**Phaniendra *et al*., 2015).

	ROS		**RNS**	
Free radicals	Hydroxyl radical	(•OH)	Nitric oxide	(•NO−)
	Superoxide anion	($•O_2^-$)	Nitrous acid	(HNO_2 −)
	Lipid peroxyl	($•LOO^-$)		
	Thiyl	(•RS)		
Non-radicals	Hydrogen peroxide	(H_2O_2)	Nitrogen dioxide	NO
	Singlet oxygen	($^1O_2$)		
	Ozone	(O_3)	Dinitrogen	N_2O_4
	Lipid peroxide	(LOOH)	tetraoxide	
	Peroxynitrite	(ONOO−)		

In the living organisms, ROS are generated in several cellular Systems localized on the plasma membrane, in the cytosol, in the peroxisomes, and on membranes of mitochondria and endoplasmic reticulum (**Fig.1**). Several soluble cell components, including thiols, hydroquinones, catecholamines, and flavins, can contribute to intracellular ROS production as they are able to undergone redox reactions (Matsubara *et al.,* 2015). Moreover, several cytosolic enzymes produce ROS during their catalytic activity. Probably the most studied ROS producing enzyme is xanthine oxidase (XO) (Rác *et al.,* 2015). It has now been about 50 years since mitochondrial H_2O_2 production in the presence of respiratory substrates was first recorded (Treberg *et al.,* 2019), followed shortly after by the detection of mitochondrial generation of superoxide radical anion (Dudylina *et al.,* 2019). The discovery that electron-transfer along the inner mitochondrial membrane carriers is associated with formation of ROS suggested the mitochondrial involvement in degenerative processes linked to several diseases and aging (Kausar *et al.,* 2018). Although peroxisomes have long been known as organelles involved in cellular metabolism of H_2O_2, it is now clear that they are involved in several metabolic pathways (Pascual-Ahuir *et al.,* 2017). Important functions performed by peroxisomes include fatty acid β- and α-oxidation, amino acid and glyoxylate metabolism,

and synthesis of lipidic compounds, and most enzymes catalyzing these processes produce ROS during their activity (Antonenkov *et al.,* 2010; Wanders *et al.,* 2016). The endoplasmic reticulum (ER) is involved in multiple functions, such as synthesis, folding, and transport of Golgi, lysosomal, secretory, and cell-surface proteins , calcium storage , lipid metabolism, and, in some cell types, drug detoxification (Palipoch, 2013; Schwarz and Blower, 2016). Smooth endoplasmic reticulum presents a chain of electron transport, constituted by two systems devoted to xenobiotic metabolism and introduction of double bonds in fatty acids, which are also able to produce ROS (Di Meo *et al.,* 2016).

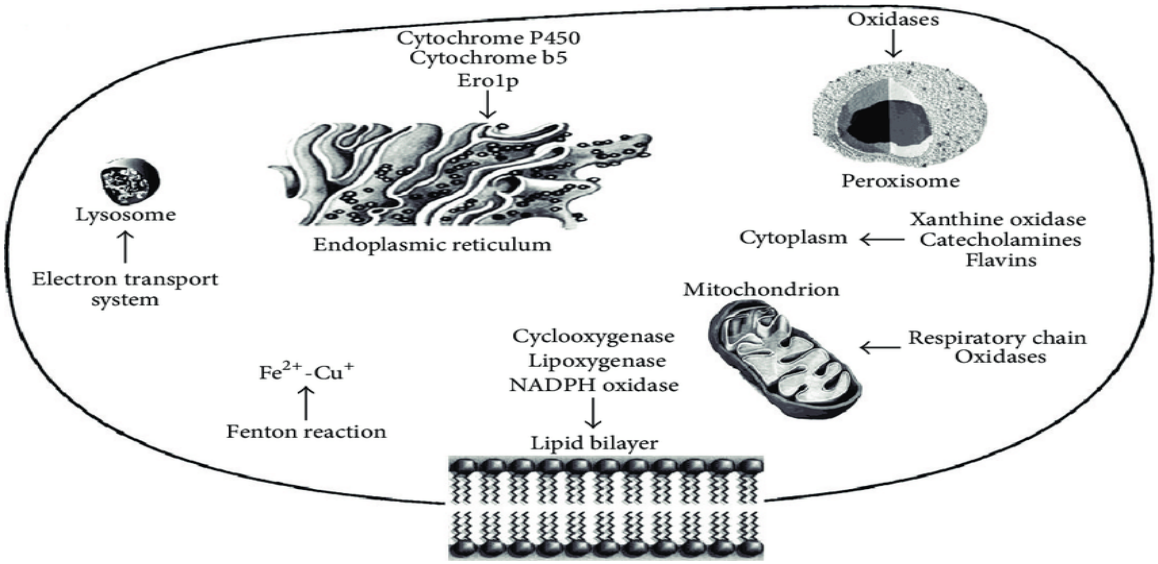


Figure 1. Cellular sources of ROS production. Subcellular organelles and structural and soluble cell components all contribute to production of a wide variety of reactive species (Venditti *et al.,* 2015).

1.2.2. Exogenous Source of ROS

a. Cigarette Smoke

In the last two decades, the central role of free radical mechanisms in tobacco smoke carcinogenesis and oxidative stress has been established by a series of studies. Cigarette smoke contains many oxidants and free radicals and organic compounds, such as superoxide anion ($O_2^{\bullet-}$) and subsequently H_2O_2 and the reactive hydroxyl radical (HO•), which cause oxidative damage to cellular membrane lipids, proteins, enzymes and DNA (Tian *et al*., 2017). ROS in the cigarette gas-phase promote the destruction of endogenous

antioxidants (vitamins and enzymatic antioxidants) reducing the vital role of cellular antioxidant defenses (Forouzandeh *et al*., 2017). Several studies show that antioxidant vitamins are lower in smokers resulting in systemic oxidative stress, whereas dietary antioxidant supplements provide only limited protection to smokers (Kim *et al*., 2017).

b. Hyperoxia

Hyperoxia refers to conditions of higher oxygen levels than normal partial pressure of oxygen in the lungs or other body tissues. It leads to greater production of reactive oxygen and nitrogen species (Ozougwu, 2016).

c. 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, such as Fe and Cu, via Fenton reactions and thus induce oxidative stress (Kashmiri *et al*., 2014; Collin, 2019).

d. Heavy Metal Ions

Heavy metal ions, such as iron, copper, cadmium, mercury, nickel, lead, and arsenic, can induce generation of reactive radicals and cause cellular damage via depletion of enzyme activities through lipid peroxidation and reaction with nuclear proteins and DNA (Ozougwu, 2016).

1.3. Effect of oxidative stress

Oxidative stress occurs when the balance between antioxidants and ROS are disrupted because of either depletion of antioxidants or accumulation of ROS. Higher production of ROS in body may change DNA structure, result in modification of proteins and lipids, activation of several stress-induced transcription factors, and production of proinflammatory and anti-inflammatory cytokines.

a. Effects of oxidative stress on DNA

Both ROS/RNS can oxidatively damage the nucleic acids. The mitochondrial DNA is more vulnerable to the ROS attack than the nuclear DNA, because it is located in close proximity to the ROS generated place. ROS, most importantly, the OH• radical directly reacts with all components of DNA such as purine and pyrimidine bases, deoxyribose sugar backbone (Phaniendra *et al*., 2015) and causes a number of alternations including single and double stranded breaks in DNA. A number of modified purine (e.g. 8-hydroxydeoxy guanosine, 2,6-diamino-4-hydroxy-5-formamidopyrimidine) and pyrimidine (e.g. thymine glycol, uracil glycol, 5-hydroxydeoxyuridine) base produced by attack of OH• radical and DNA- protein cross links (**Fig.2**). On the other hand, the RNS, most importantly, peroxynitrite ($OONO^-$) interacts with guanine to produce nitrative and oxidative DNA lesions such as 8-nitroguanine and 8-oxodeoxyguanosine respectively (Kawanishi *et al*., 2016).

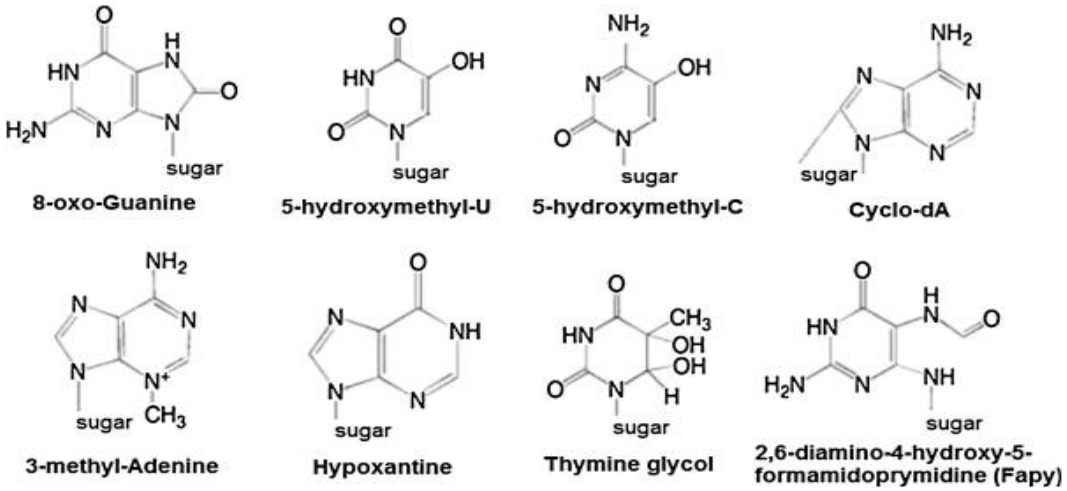


Figure 2. Base modifications introduced by ROS (Birben *et al.,* 2012).

b. Effects of oxidative stress on lipids

The membrane lipids, especially the polyunsaturated fatty acid residues of phospholipids are more susceptible to oxidation by free radicals. The lipid peroxidation results in the loss of membrane functioning, for example, decreased fluidity, inactivation of membrane bound enzymes and receptors (Phaniendra *et al*., 2015). The lipid peroxidation is initiated, when any

free radical attacks and abstracts hydrogen from a methylene groups (CH_2) in a fatty acid (LH) which results in the formation of a carbon centered lipid radical (L•). The lipid radical can react with molecular oxygen to form a lipid peroxyl radical (LOO•). The resultant lipid peroxyl radical (LOO•) undergo rearrangement via a cyclisation reaction to form endoperoxides, which finally form malondialdehyde (MDA) and 4-hydroxyl nonenal(4-HNA),the toxic end products of lipid peroxidation that cause damage to the DNA and proteins (Hashemi, 2019). These lipid peroxyl radicals can further propagate the peroxidation process by abstracting hydrogen atoms from the other lipid molecules. Isoprostanes constitute the important product of lipid peroxidation of arachidonic acid and are considered as the makers of the oxidative lipid damage (Ayala *et al*., 2014; Taso *et al.,* 2019).

c. Effects of oxidative stress on proteins

Many radical species ($O_2^{\bullet-}$, $OH^{\bullet}$, peroxyl, alkoxyl, hydroperoxyl) and non radical species (H_2O_2, O_3, HOCl, singlet oxygen, OONO-) can be induced the protein oxidation (Ferreira *et al.,* 2018). ROS oxidize different amino acids present in the proteins, causing formation of protein– protein cross linkages, results in the denaturing and loss of functioning of proteins, loss of enzyme activity, loss of function of receptors and transport proteins (Nita and Grzybowski, 2016). The methionine and cysteine are more susceptible to oxidation by ROS because these amino acids contained sulphur and are converted to disulphides and methionine sulphoxide, respectively. However in biological systems, only these two oxidized forms of proteins can be converted back to their native form by two different enzymes namely disulfide reductases and methionine sulfoxide reductases respectively (Phaniendra *et al*., 2015; dos Santos *et al*., 2018). The ROS induced oxidative damage of amino acid residues such as lysine, proline, threonine and arginine yields carbonyl derivatives. The presence of carbonyl groups in proteins has been considered as the marker of ROS mediated protein oxidation. The

other specific markers of protein oxidation are O-tyrosine (a marker for hydroxyl radical) and 3-nitrotyrosine (a marker for RNS) (Davies, 2016).

d. Effects of oxidative stress on signal transduction

ROS can induce expression of several genes involved in signal transduction.(Dudziak *et al*.,2019) Disruption of the ratio GSH/GSSG causes activation of redox sensitive transcription factors, such as NF-κB, AP-1, nuclear factor of activated T cells and hypoxia-inducible factor 1 , that are involved in the inflammatory response (Ren *et al*., 2017). Activation of transcription factors via ROS is achieved by signal transduction cascades that transmit the information from outside to the inside of cell (Nguyen *et al*., 2017). Tyrosine kinase receptors, most of the growth factor receptors (epidermal growth factor receptor, vascular endothelial growth factor receptor, receptor for platelet-derived growth factor, protein tyrosine phosphatases, and serine/threonine kinases) are targets of ROS (Alexander *et al*., 2017).

1.4. ROS in normal physiological processes

It has been widely documented that ROS/RNS act as intracellular signaling molecules to exert their physiological function.ROS are involved in signaling through chemical reactions with specific atoms of target proteins that lead to covalent protein modification (Griendling *et al*., 2016). Thus, ROS molecular recognition occurs at the atomic and not at the macromolecular level. Moreover, ROS exert their particular signaling properties by specifically targeting elements in protein. For example, $O_2^{\cdot-}$ preferably reacts with iron-sulfur (Fe-S) clusters. Both cysteine residues and methionine residues are the major targets of H_2O_2 (Mettert and Kiley, 2015). However,$^{\cdot}$OH has no preferable reactivity toward biological molecules. Furthermore, selenocysteine is more reactive with H_2O_2 than cysteine, and may operate in H_2O_2 based redox regulation (Benchoam *et al*., 2019). H_2O_2 can act as direct modifier of thiol containing

proteins, to produce a redox state of signaling proteins, to produce a redox dependent modification of enzyme. For the same reason not all ROS are aqually suitable for signal transduction (Song and zou, 2015; Rampon *et al*., 2018).

Many of low grade ROS-mediated acute responses actually protect the cell against oxidative damage and re-establish or maintain the intracellular redox homeostasis (Marengo *et al*., 2016). ROS are involved in important physiological responses/events, such as defense against envirommental pathogens, responce to growth factors, cellular proliferation, differentiation, apoptosis, and autophagy. Additionally, duox expression and H_2O_2 formation are the rate-limiting processes for the synthesis of thyroid hormones (Di Marzo *et al*., 2018).

1.5. Antioxidants

Just like free radicals, antioxidants can be endogenously produced (e.g. superoxide dismutase (SOD) and reduced glutathione (GSH)) and can also be introduced to the biological system exogenously, usually through diet (e,g. vitamin c, caratenoids and vitamin E) (Amber *et al*., 2013; Ighodaro and Akinloye, 2017). Antioxidants primarily function to balance out free radicals generated during metabolic processes including during mechanisms involved in protecting the gut from inflammation and injury (Nwosu *et al*., 2016). Antioxidants can be said to go about its defence system in three main ways: a: by proteins sequestering with transition metals preventing their availability for reaction with free radicals.b: inhibiting their deleterious effect, making available small molecules that have the capability of scavenging free radicals.c: specific mechanisms for the correction of ROS-induced DNA damage (Ofor *et al*., 2016).

1.5.1. Enzymatic Antioxidants

a. Superoxide dismutase

SODs are a class of closely related enzymes that catalyse the breakdown of the superoxide anion into oxygen and H_2O_2 present in almost all aerobic cells and in extracellular fluids (He *et al*., 2017). It comprises of three families depending on the metal co-factor; Cu/Zn, Fe and Mn and the Ni type

which binds nickel. Mn-SOD has been found mostly in mitochondria and peroxisomes, Fe-SOD has been found in peroxisomes and Cu-Zn-SOD in peroxisomes and cytosol (Ighodaro and Akinloye, 2017). Superoxide dismutase's converts superoxide to hydrogen peroxide: $2O_2^{\cdot-} + 2H = H_2O_2 + O_2^{\cdot}$

b. Catalase

This is predominant in cells exposed to oxygen and is frequently used to catalyse the decomposition of H_2O_2 to oxygen and water (Webb *et al*., 2017). Catalase has one of the highest turnover rates for all enzymes; with one molecule of catalase being able to convert approximately 6million molecules of H_2O_2 to water and oxygen each minute (Obeagu, 2018).

c. Glutathione system

The Glutathione system comprises of three main classes: Glutathione reductase, Glutathione peroxidise (GPx) (competes with catalase for H_2O_2 and is the major source of protection against low levels of oxidative stress) and Glutathione S transferase (Kivrak *et al*., 2017).

1.5.2. Nonenzymatic Antioxidants

Nonenzymatic antioxidants include low-molecular-weight compounds but are not limited to; Glutathione (GSH), ascorbic acid (Vitamin C), tocopherols and tocotrienols (Vitamin E) , uric acid, melatonin, alpha lipoic acid (Tan *et al*., 2018).

a. Glutathione

They are synthesized from constituent amino acids in cells. It is a cysteine containing peptide. In cells it is maintained as Glutathione reductase which subsequently reduces other metabolites and enzyme systems while still capable of reacting with oxidants (Lian *et al*., 2018).

b. Vitamin C (Ascorbic Acid)

Vitamin C is usually maintained in the reduced form in the body by its reaction with glutathione which can be catalysed by protein. Vitamin C is able to prevent formation of nitrosamines, neutralise ROS such as H_2O_2, thus boosting immunological response (Pehlivan, 2017).

c. Vitamin E (α-Tocopherol)

They are fat soluble vitamins that exist in eight different forms and possess antioxidative properties. The main function of vitamin E is protecting the cell from lipid peroxidation. In the human biological system, α-tocopherol is the most studied and is said to have the highest bioavailability with the body preferentially absorbing and metabolizing this most active form (Obeagu, 2018).

d. Melatonin

This is a powerful antioxidant that has the ability to easily cross cell membranes and blood-brain barrier. Unlike other antioxidants melatonin does not undergo redox recycling (repeated reduction and oxidation), once oxidized cannot be reduced to previous state because it forms several stable end products when it reacts with free radicals. Thus has been termed terminal (or suicidal) antioxidant (Galano *et al*., 2018; Debnath *et al*., 2019).

e. Polyphenol and flavonoid compounds

Plant metabolism is mainly classified as primary or secondary. Compounds produced through primary metabolism, which are generally referred to as primary metabolites; include sugars, fatty acids, amino acids and nucleic acids (Singh *et al*., 2017). Primary metabolites are required for maintenance of plant cells while secondary metabolites are essential to the normal growth, development and defense of plants (Wang *et al*., 2019). To date, thousands of different types of secondary metabolites have been identified in plants chemically, these compounds are either nitrogen-containing (alkaloids) or nitrogen-deficient (terpenoids and

phenolics) (Patra *et al*., 2013; Deepak *et al*., 2015).).Plant phenolics are mainly classified into five major groups, phenolic acids, flavonoids, lignans, stilbenes and tannins (Myburgh, 2014). Phenolic compounds generally possess one or more aromatic rings with one or more hydroxyl groups (Tanase *et al*., 2019). It has commonly been assumed that the antioxidant capacity of phenolics will increase with the number of free hydroxyls and conjugation of side chains to the aromatic rings Flavonoids and phenylopropanoids are also oxidized by peroxidase, and act as H_2O_2 scavengers (Lee *et al*., 2017).

The basic structure of a phenolic compound comprises of an aromatic ring with one or more –OH groups. However, phenolic compound found in Nature are structurally diverse from simple phenolic molecules to complex polymerized compound (Bhuyan and A Basu, 2017). Phenolics found in food material can be divided into three major groups: simple phenol and phenolic acids, hydroxycinnamic acid derivatives and flavonoids. Phenolic acids, flavonoids and tannins are considered as the main dietary phenolics (Ștefănescu *et al*. 2019). Flavonoids constitute the largest group of low-molecular-weight plant phenolics and have been studied most extensively. They are also the most important plant pigments. Over 4,000 different types of flavonoids are found in Nature (Panche *et al*., 2016). Flavonoids usually occur bound to sugar molecules and consist mainly of catechins, proanthocyanins, anthocyanidins, flavons and flavonols and their glycosides (Talapatra and Talapatra, 2015). Phenolic acids are divided into two subgroups: hydroxybenzoic and hydroxycinnamic acids (Bhuyan *et al*. 2015). Phenolic acids are significant components of fruit and vegetables. These compounds play an important role in color stability, aroma profile and antioxidant activity. They act as acids because of their carboxylic group (Baião *et al*. 2017). Tannins are the third important group of polyphenolics which can further be divided into two subcategories: condensed and hydrolysable tannins (Bhuyan and Basu, 2017). These are high-molecular-weight polymers. Fruits, grains and legumes consist of condensed tannins which are mainly polymers of

catechins or epicatechins, whereas hydrolysable tannins are polymers of gallic or ellagic acid and found in berries and nuts (Smeriglio *et al*. 2017).

Numerous studies have reported the potential health benefits of plant polyphenolics in particular. Due to their potent antioxidant properties, plant phenolics have scientifically proven to prevent various oxidative stress-related as well as chronic diseases, such as cancer, cardiovascular and neurodegenerative diseases (Koch, 2019).

Oxidative stress plays an important role in carcinogenesis. Several mechanisms contribute to the overall formation of tumours from oxidative damage. (Lee *et al*. 2014). Therefore, phenolics with antioxidant properties have been found to be beneficial in preventing or treating the oxidative damage that can induce cancer (Liu *et al*. 2018). Polyphenols may affect the molecular events in the initiation, promotion and progression stages of carcinogenesis and isoflavones and lignans may affect the estrogen-related activities related to tumour formation (Hsu *et al*. 2015 ; Moein, 2015). Flavonoids have been reported to modulate key enzymes and receptors involved in signal transduction pathways of cellular proliferation, differentiation, apoptosis, inflammation, angiogenesis, metastasis and reversal of multidrug resistance (Baião *et al*. 2017).

Cardiovascular disease (CVD) is one the major killers in all developed countries with a rise in prevalence (Tome-Carneiro and Visioli 2016). Several epidemiologic studies and intervention trials suggest that polyphenols present in fruits and vegetables are associated with decreased risk of cardiovascular diseases (Rangel-Huerta *et al*. 2015). As polyphenols are known for their antioxidant activities, increased intake of dietary antioxidants may protect against the development of CVD. In addition, recent evidence suggests that polyphenols have immunomodulatory and vasodilatory properties which may also contribute in reducing the risk of CVD (Mahmoud *et al*., 2019).

2. The inflammation

Inflammation is biological response of the immune system's that can be triggered by a variety of factors, such as pathogens, damaged cells, toxic compounds, or irradiation. These factors may induce acute and/or chronic inflammatory responses in the heart, pancreas, liver, kidney, lung, brain, intestinal tract and reproductive system, potentially leading to tissue damage or disease. (Chen *et al.,* 2018; Liu *et al*., 2019), and acts by removing injurious stimuli and initiating the healing process. Inflammation is therefore a defense mechanism that is vital to health (Zhou *et al*., 2016). Usually, during acute inflammatory responses, cellular and molecular events and interactions efficiently minimize impending injury or infection. This mitigation process contributes to restoration of tissue homeostasis and resolution of the acute inflammation. However, uncontrolled acute inflammation may become chronic, contributing to a variety of chronic inflammatory diseases (Chen *et al*., 2018; Donald *et al*., 2018). At the tissue level, inflammation is characterized by redness, swelling, heat, pain, and loss of tissue function, which result from local immune, vascular and inflammatory cell responses to infection or injury (Gallo *et al*., 2017). Important microcirculatory events that occur during the inflammatory process include vascular permeability changes, leukocyte recruitment and accumulation, and inflammatory mediator release (Chertov *et al*., 2000; Chen *et al*., 2018).

Mechanism of inflammation represents a chain of organized, dynamic responses including both cellular and vascular events with specific humoral secretions. These pathways involve changing physical location of white blood cells (monocytes, basophils, eosinophils, and neutrophils), plasma, and fluids at inflamed site (Huether and McCance, 2015). A group of secreted mediators and other signaling molecules (e.g., histamine, prostaglandins, leukotrienes, oxygen- and nitrogen-derived free radicals, and serotonin) are released by immune defense cells principally in the mechanism which can contribute in the event of inflammation (Abdulkhaleq *et al*., 2018). Whatever, the inflammatory response is triggered

through two phases: (a) acute and (b) chronic, and each is apparently mediated by a different mechanism (Serhan *et al*., 2015). These immune responses which involved in acute inflammation can be divided into vascular and cellular (Nguyen, 2012).

2.1. Phases of inflammation

The responses which occur in microvasculature normally appear in few minutes following tissue injury or microbial infection in the presence of other inflammatory stimuli named vascular events (Boff *et al*., 2018). The occurrence of these processes is rapid and eventually will lead to vasodilatation and subsequently makes the vessels become more permeable. This process will result in entry of inflammatory mediators and produces interstitial oedema (Porter, 2013; Simon *et al*., 2018). Infiltration of white blood cells from circulatory system is essential during inflammatory responses (Goljan, 2014; Kumar *et al*., 2013). A group of chemotactic agents such as microbial endotoxins holding amino terminal N-formyl methionyl groups, C5a complement fragment, and interleukins along with the secretions of basophils such as platelets activating factor, histamine, and leukotriene B can stimulate intense leukocytes infiltration within few minutes (Bitencourt *et al*., 2013; Abdulkhaleq *et al*., 2018). Among the leukocytes, neutrophils are the first inflammatory cells that are recruited at the acute inflammation site (Curcic *et al*., 2015). Infiltration of immune cells triggered via a complicated mechanism in which white blood cells work together with endothelium in postcapillary venules (Filippi, 2016). Cellular events encompass the successive capture, trundling, and firming an adhesion to the microvascular endothelium (Leick *et al*., 2014). These events in the mobilization pathway are arranged by cell adhesion molecules (CAMs). Following a period of stationary adhesion, the white blood cells may leave the postcapillary venules extending pseudopodia between endothelial cells and reach into the subendothelial space. This complex event is often referred as white blood cell extravasations and transendothelial migration (Abdulkhaleq *et al*., 2018; Muro, 2018). The inflammations of

chronic events are distinguished by mononuclear cell infiltration (e.g., monocyte and lymphocytes), fibroblasts proliferation, collagen fibers, and connective tissue formation (Shahid *et al*., 2018). With chronic inflammation, the tissue degeneration is normally mediated by nitrogen species, proteases, and other reactive oxygen species released from infiltrated inflammatory cells (Murakami, 2012).

2.2. Anti-inflammatory drugs

The main anti-inflammatory drugs are either steroidal (e.g., betamethasone, prednisolone, and dexamethasone) or nonsteroidal (Juthani *et al*., 2017) (e.g. aspirin, diclofenac, ibuprofen and indomethacin.) used to treat both acute inflammatory condition and chronic inflammatory diseases such as osteoarthritis and rheumatoid arthritis (Abdulkhaleq *et al*., 2018). However, their prolonged use is associated with various side effects; for example, steroidal drug causes adrenal atrophy osteoporosis, suppression of response to infection or injury, euphoria. Cataracts, glaucoma (Phalitakul *et al.*, 2011; Juthani *et al*., 2017), and non-steroidal drug cause peptic ulcers and bronchospasm due to blockade of both the physiological and inflammatory prostaglandins and concurrent production of leukotrienes (Gunaydin and Bilge, 2018). Thus taking into account the adverse effects and high cost of synthetic conventionally available steroidal or non-steroidal drugs, the search for new anti-inflammatory agents from herbal sources is getting popular with the objective to obtain greater safety, better efficacy, and a more economical way to treat inflammation (Arulselvan *et al*., 2016).

2.3. Natural products in anti-inflammation

Medicinal plants continues to be an accepted form of treatment in the Orient, and plant drugs based on traditional practice represent a huge portion of the pharmaceutical products in modern western countries (Levin and Laufer, 2012; Yuan *et al*., 2016). First, concerns have been raised that modern pharmaceutical practice too often involves costly drugs that produce

unacceptable side effects (Lemonnier *et al*., 2017); second, the experience shows that natural substances can apparently address several modern health concerns with fewer side effects (Shao *et al*, 2017); and third, experience shows that modern medicine and traditional herbal medicine can be combined (Yuan *et al*., 2016).

3. The hepatoprotective effect

The liver is the largest solid organ, the largest gland and one of the most vital organs that functions as a centre for metabolism of nutrients and excretion of waste metabolites (Ozougwu and Eyo, 2014). Its primary function is to control the flow and safety of substances absorbed from the digestive system before distribution of these substances to the systemic circulatory system (Ozougwu, 2017).

3.1. General description of the liver

The liver weighs approximately 1500g and accounts for approximately 2.5% of adult body weight. The liver lies mainly in the right upper quadrant of the abdomen where it is hidden and protected by the thoracic cage and diaphragm (Mubbunu *et al*., 2018). The liver is divided into 4 lobes: right, left, caudate, and quadrate. The right and left lobes are the largest, while the caudate and quadrate are smaller and located posteriorly (Kumon, 2017). Adjacent to the caudate lobe is the sulcus for the inferior vena cava. Just inferior to the caudate lobe is the porta hepatis, where the hepatic artery and hepatic portal vein enter the liver (Lowe and D'Angelica, 2016). The portal vein carries nutrient laden blood from the digestive system. Inferior to the porta hepatis is the bile duct which leads back to the gallbladder, also the hepatic vein, where post-processed blood leaves the liver, is found inferior and adjacent to the sulcus for the inferior vena cava (Singh and Rabi, 2019).

3.2. Histology of the liver

The basic functional unit of the liver is the liver lobule. A single lobule is about the size of a sesame seed and is roughly hexagonal in shape (Sadri *et al*., 2015). The primary structures found in a liver lobule include:

- Plates of hepatocytes which forms the bulk of the lobule.
- Portal triads at each corner of hexagon.
- Central vein.
- Liver sinusoids that run from the central vein to the portal triads.
- Hepatic macrophages (Kupffer cells).
- Bile canaliculi (―little canals‖) – formed between walls of adjacent hepatocytes.
- Space of Disse – a small space between the sinusoids and the hepatocytes.

The liver is the first site of passage for venous blood arriving from the intestines via vena porta. The areas around the influx blood vessels are named periportal. The areas surrounding efflux blood vessels are the perivenous. The periportal area is highly complex and consists of a dense matrix containing collagen where afferent blood vessels are found, together with bile ducts, nerve and lymph (Butura, 2008; Kietzmann, 2017). Spaces within the matrix contain a variable cell population, such as fibroblasts, hematopoietic cells and inflammatory cells. Also found here are epithelial cells of the bile ducts, endothelial cells of the blood vessels, and smooth muscles of arteries and veins (Ozougwu, 2017; Selene *et al*., 2018). The liver lobule consists mainly of plates of hepatocytes and sinusoids, with a light matrix of collagen to form a network between the two. Kupffer cells reside mainly in the tissue space between the hepatocyte and the sinusoids. Terminal bile ductules connect here to the bile cannaliculi between hepatocystic plates (Marrone *et al*., 2016). The walls of the hepatic sinusoid are lined by three different cell types: the sinusoidal endothelial cells, kupffer cells and stellate cells.

Additionally, pit cells, the liver specific natural killer (NK) T cells are often present in the sinusoidal lumen (Freitas-Lopes *et al*., 2017). The liver is made up of four main different cell types.

a. Hepatocytes

Hepatocytes represent 60 % of the liver‘s cells and about 80 % of the liver‘s total cell mass. Most of the liver‘s synthetic and metabolic capabilities stem from the work of hepatocytes (Ozougwu, 2017). Hepatocytes are large and rich in organelles such as endoplasmic reticulum and Golgi apparatuses. They contain many and large mitochondria, as well as lysosomes and peroxisomes (Krishna *et al.,* 2018). The main function of hepatocytes is to participate in lipid, carbohydrate and protein metabolism. They also produce serum proteins such as albumin and coagulation factors. Furthermore, hepatocytes produce and secrete bile as well as detoxify and excrete cholesterol, steroid hormones and xenobiotic drugs (Ozougwu and Eyo, 2014).

b. Endothelial Cells

The sinusoidal endothelial cells line the walls of the hepatic sinusoid and perform a function of filtration due to the presence of fenestrae (Zhao *et al*., 2015). They may also function as antigen presenting cells and secrete certain cytokines and eicosanoids (Marrone *et al*., 2016).

c. Kupffer Cells

Kupffer cells are located within the sinusoid and are in constant contact with gut-derived particles that lead to low but constant amount of activation of these monocyte derived cells. Upon activation they are able to secrete a vast range of inflammatory mediators such as cytokines, reactive oxygen species, eicosanoids and nitric oxide (Triantafyllou *et al*., 2018). Kupffer cells have receptors that enable them to bind cells covered with immunoglobulins or bind to complement receptors and subsequently phagocytose cell (Dixon *et al*., 2013).

d. Stellate Cells

The liver plays a central role in uptake and storage of vitamins A (Retinol) and stores about 95 % of retinoids found in the body where the stellate cells are the main vitamin A storing cells (Chelstowska *et al*., 2016). Additionally, they function to control the turnover of extracellular matrix and regulate sinusoid contractility. The stellate cells may become activated under stressful conditions and transformed into myofibroblast – like cells which play a key role in inflammatory fibrotic response (Kobayashi, 2019).

3.3. Hepatotoxic agents used in animal models

In-vitro and *in vivo* models are routinely used for studying hepatotoxicity. These models provide the information of drug or describe their potency in prevention and cure of various hepatic disorders (induced by hepatotoxic agents) (Singh *et al*., 2016). Various chemical agents normally used for inducing animal hepatotoxicity include carbon tetrachloride, paracetamol, acryl amide, adriamycin, alcohol, antitubercular drugs etc (Kuna *et al*., 2018). These hapatotoxins changes into toxic metabolites and interfere with macromolecules like protein, nucleic acid and lipids. These interferences induce lipid dysfunction, DNA damage and oxidative stress leading to hepatocytes disruption (Iqubal *et al*., 2016).

In *in-vitro* models hepatic cell lines are treated with hepatotoxin and the effect of the test drugs on the same is evaluated. Depending on the models, the method of induction can be divided as follow.

a.Non-invasive method

Chemical-induced hepatotoxicity, drug-induced hepatotoxicity, metal-induced hepatotoxicity, phytotoxin induced hepatotoxicity, radiation-induced hepatotoxicity and diet-induced hepatotoxicity (Jing *et al*., 2018).

b.Invasive method

Invasive methods generally used are Bile duct ligation and Portal vein ligation (Rhu *et al*., 2019). Since long time, animal model has been used as a tool for biomedical research. An ideal animal model should depict the same mechanism as human do (Perlman *et al*., 2016). Animals such as rat, pig, mice, rabbit, guinea pig, cattle, ship and monkey are reliable for conducting hepatotoxic studies (Martinez, 2011).

3.3.1. Chemical-induced hepatotoxicity

There are several chemicals which cause hepatotoxicity like carbon tetrachloride (CCl_4), thioacetamide (TTA), diethylnitrosamine (DEN) (Bhakuni *et al*., 2015). The metabolites of these chemicals lead to various pathological and biological changes. It has been found that glutathione and neutrophils play a critical role in chemically induced hepatotoxicity (Bloomer and Brown 2019). Activation and inhibition of signaling kinase, transcription factors, and gene expression profiles may have effects on organelles like mitochondria, cytoskeleton, endoplasmic reticulum, microtubules and nucleus (Nair *et al*., 2019). The resultant deleterious effect may lead to cell death caused by either cell shrinkage, nuclear apoptosis or swelling and necrosis (Iqubal *et al*., 2016).

a. CCl_4

Carbon tetrachloride is one of the most common chemical agents used in the laboratory for the study of various liver disorders at acute and chronic condition (Rane *et al*., 2016). CCl_4 alters the plasma membrane, lysosomal membrane and mitochondrial membrane (Fortea *et al*., 2018). A metabolite of CCl_4, called trichloromethyl (CCl_3) produced by CYP2E1 isozymes, combines with cellular lipids and proteins to form trichloromethyl peroxy radical which attacks lipids on the membrane of endoplasmic reticulum faster than trichloromethyl free radical that causes lipid peroxidation and lobular necrosis (Al-Jawad *et al*., 2019). A single dose of CCl_4 reaches to its peak plasma concentration within 3 hours and within 24 hours of administration, it causes a change in the histological and biochemical makeup of

hepatocytes (Velmurugan and Arunachalam, 2014). Repeated dose of CCl_4 can induce fibrosis and necrosis (Wu *et al*., 2018).

b. Thioacetamide (TAA)

Thioacetamide is a white crystalline solid often used to induce fibrosis (Starkel and Leclercq, 2011). Thioacetamide as such is not toxic to the liver but its metabolite, thioacetamide S-oxide (ROS) is heapato-toxic and reduces the number of hepatocytes and oxygen consumption, thus making the hepatocytes oxygen deficient (Amin *et al*., 2012; Schyman *et al*., 2018). Thioacetamide also reduces the movement of bile salt and induces cholestasis (Wei *et al*., 2018). Metabolite increases the intracellular concentration of Ca^{2+} in nuclear volume that obstructs the activities of mitochondria, causing necrosis and death of hepatocyte in zone 1 and zone 3 (El-Agamy *et al*., 2018).

c. Diethyl-nitrosamine (DEN)

DEN is well known carcinogenic chemical. In the liver DEN is biotransformed by CYP2E1 (hydroxylation) into ethyldiazonium ion which acts as alkylating agent and reacts with DNA and induce cancer (Yalcin *et al*., 2016). DEN induces hepatotoxicity when animals are treated at a concentration of 50–200 mg/kg, i.p. for 4–12 weeks (Rezaie *et al*., 2013).

3.3.2. Drug-induced hepatotoxicity

a. NSAIDs

It is one of the most frequent prescribed drugs in fever, all grades of pain, inflammation and arthritis (Wongrakpanich *et al*., 2018). NSAIDs impart toxicity to liver and kidney. Among all NSAIDs used clinically, paracetamol was found to be the most hepatotoxic and thus it is used in the laboratory to induce hepatotoxicity so that mechanism of hepatocellular injury can be better understood (Meek *et al*., 2010; Sriuttha *et al*., 2018).

b. Paracetamol

PCM is a widely prescribed analgesic and antipyretic. When consumed, it gets metabolized into N- acetylP- benzoquinone imine and this metabolite is detoxified by GSH (Ghanem *et al*., 2016). When an excess of the metabolite is produced in the body, the level of endogenous GSH depletes. This toxic metabolite binds with nucleophilic macromolecule of hepatocyte and causes necrosis (More *et al*., 2017). PCM, when given at a dose of 500 mg/kg, p.o, for 14 days, induces hepatotoxicity (Caparrotta *et al*., 2018).

c. Anticancer drugs

Cancer is a complex disease, causes abnormal cell growth (Adjiri, 2017). With advancement in understanding the pathophysiology of cancer, newer drug is being developed with least side effects (Watts and Nimer, 2018). Almost all the anticancer drugs cause hepatotoxicity; hence anticancer drugs are used in the laboratory for inducing hepatic injury and understanding its action (Kuna *et al*., 2018).

3.3.3. Metal- induced hepatotoxicity

a. Mercury

Mercury is used randomly in industries and is one of the common causes of water pollution and health hazard. Mercury is a transition metal which promotes the formation of ROSs like H_2O_2 and induces lipid peroxidation, mitochondrial damage and hepatocellular deterioration (Rice *et al*., 2014). Mercury also diminishes the activities of natural antioxidants like GSH, SOD and Catalase. Mercury, when given at a dose of 5 mg/kg, i.p, for 20 days and 3 mg/kg for 30 days, induces hepatotoxicity (Ekambaram *et al*., 2014).

b. Cadmium (Cd)

It is another metallic toxin of global concern due to its capacity to impart lipid peroxidation, mitochondrial injury and hepatocellular damage. Cd promotes the formation of ROSs like superoxide and hydroxyl radicals that induces hepatotoxicity (Ghasemi *et al*., 2014; Nita *et al*., 2016). Cd induces inflammation and congestion in bile canaliculi and sinusoids and

elevates the level of biological markers like ALT, AST and GGT, when given at the dose of 1 mg/kg p.o for 15 days, induces marked hepatotoxicity (Singh *et al*., 2014).

c. Alcohol

Alcohol is well known for causing hepatotoxicity (ALD, fatty infiltration, hepatitis & cirrhosis) (Ohashi *et al*., 2018). Alcohol induced fatty infiltration is reversible and subsides when consumption is withdrawn (Lamas-Paz *et al*., 2018). Alcohol causes hepatitis and cirrhosis by inducing lipid peroxidation. Excessive lipid peroxidation results in altered lipid imbalance in the cell membrane (made up of lipid bilayer) that causes loss of integrity of cell membrane and finally there is an elevation in glutamyl transpeptidase (Fung and Pyrsopoulos, 2017; Teschke, 2018).

3. 4.Free radicals and liver diseases

ROS and RNS are produced by metabolism of normal cells (Kuchakulla *et al*., 2018). However, in liver diseases, redox is increased thereby damaging the hepatic tissue; the capability of ethanol to increase both ROS/RNS and peroxidation of lipids, DNA, and proteins was demonstrated in a variety of systems, cells, and species, including humans (Phaniendra *et al*., 2015). ROS/RNS can activate hepatic stellate cells, which are characterized by the enhanced production of extracellular matrix and accelerated proliferation (Lackner and Tiniakos, 2019). Cross-talk between parenchymal and nonparenchymal cells is one of the most important events in liver injury and fibrogenesis; ROS play an important role in fibrogenesis throughout increasing platelet derived growth factor (Peters *et al*., 2018; Gracia-Sancho *et al*., 2019). Most hepatocellular carcinomas occur in cirrhotic livers, and the common mechanism for hepatocarcinogenesis is chronic inflammation associated with severe oxidative stress; other risk factors are dietary aflatoxin B1 consumption, cigarette smoking, and heavy drinking (Nevola *et al*., 2018). Ischemia–reperfusion injury affects directly on hepatocyte viability, particularly during transplantation and hepatic surgery; ischemia

activates Kupffer cells which are the main source of ROS during the reperfusion period (Chun *et al*., 2018). The toxic action mechanism of paracetamol is focused on metabolic activation of the drug, depletion of glutathione, and covalent binding of the reactive metabolite N-acetyl-pbenzoquinone imine to cellular proteins as the main cause of hepatic cell death; intracellular steps critical for cell death include mitochondrial dysfunction and, importantly, the formation of ROS and peroxynitrite. Infection with hepatitis C is associated with increased levels of ROS/RNS and decreased antioxidant levels (Yan *et al*., 2018; Emon and Zesmin, 2018). As a consequence, antioxidants have been proposed as an adjunct therapy for various liver diseases (Obeagu *et al*., 2018).

4. Gastric ulcer

Peptic ulcer is a break (like a sore) in the lining of the stomach or the upper part of the small intestine, with a diameter of at least 0.5 cm penetrating through the muscularis mucosa (Sayehmiri *et al*., 2018). It is typically a non-fatal disease that majorly represented by symptoms of epigastric pain typically relieved by food or alkali, often exhibit periodicity (Sharifi-Rad *et al*., 2018). Peptic ulcers or PUDs are generally categorized based on their anatomical origin as gastric or duodenal. (Kulshreshtha *et al*., 2017). Gastric ulcers are found along the lesser curvature of the stomach, and duodenal ulcers usually occur in the duodenal bulb, the area most exposed to gastric acid (Habeeb *et al*., 2016). Helicobacter pylori had been thought as the main etiological factor for 90% duodenal and 80% gastric ulcers. With recent decline in prevalence in *H. pylori* in western countries, PUDs, especially gastric ulcers induced with nonsteroidal anti-Inflammatory drugs (NSAIDs) and acetylsalicyclic acid (ASA) (Graham, 2014 ; Dong *et al*., 2018).

4.1. Etiology

Till the last decade it has been estimated that 80% of gastric ulcer is attributed due to *H. pylori* (Mohsina *et al*., 2016). About 14%–25% of gastric and duodenal ulcers are found to be associated with NSAID use. Interaction data and randomized trial with NSAIDs and *H. pylori* eradication therapy revealed that the ulcer-inducing effects of both risk factors are cumulative (Drini *et al*., 2017). However, their potential interaction in the induction of ulcer disease remains unidentified. Eradication of H pylori did not reduce the rate of ulcer relapse in existing long term NSAID users (Sostres *et al*., 2014). PUDs posse a multifactorial disease pathway majorly governed by acid disbalance and low mucosal defense leading to inflammation. This is represented by hypersection of hydrochloric acid and pepsin (Sayehmiri *et al*., 2018). This causes an imbalance between gastric luminal factors and degradation in the defensive function of the gastric mucosal barrier such as mucus, secretion of bicarbonate, mucosal blood flow, and epithelial cell defense (Khoder *et al*., 2016). On invasion of acid and pepsin through a weakened area of the mucosal barrier leads to release of histamine. Histamine stimulates parietal cells to secrete more acid (Hunt *et al*., 2015). With the continuation of this vicious cycle resulting in erosions to form the ulcer (Habeeb *et al*., 2016).

4.2. Various ulcer models

a. Ethanol-induced ulcers

Administration of 1ml of ethanol leads to the ulceration via penetrating in the gastric mucosa. It has been documented that ethanol increases the vascular permeability and causes the release of vasoactive products (Mahmood *et al*., 2016). Further, Ethanol causes severe damage to the gastrointestinal mucosa with micro vascular injury results in increase vascular permeability (Yazan *et al*., 2018). Moreover, ethanol decreases the secretion of bicarbonate (HCO_3^-) and mucus damage (Choi *et al*., 2016). Various evidences indicates that during ethanol-induced ulceration there is increase neutrophils infiltration in the gastric mucosa that results in increase oxidative stress with increased generation of free radicals, that is reported as major

hallmark in the pathogenesis of ulceration (kaur *et al*., 2012; Guzmán-Gómez *et al*., 2018).

b. Indomethacin-induced gastric ulcer

Indomethacin belongs to the category of the NSAIDS plays an important role in the progression of the gastric ulcers. Ulcer formation induced by indomethacin is known to be related the inhibition of the cyclooxygenase (COX), that in turn inhibit the release of the mucus (Drini *et al*., 2017; Gunaydin and Bilge, 2018). It has been reported that indomethacin also accounts for the activation of the neutrophil, generation of the free radicals in response to the oxidative stress, which than contribute to the lipid peroxidation. Indomethacin induced ulcers. Indomethacin exerts its anti-inflammatory effects by the suppression of PG synthesis. Suppression of PG have a negative impact upon the secretion of the mucus and bicarbonate by gastric acid (kaur *et al*, 2012; Mahmood *et al*., 2016).

c. HCl-induced ulcers

Oral administration of 1.5 ml of HCl contributes to the significant increase in the number of alterations in the gastric mucosa, volume of gastric juice. Oxidative stress also play pivotal role in the progression of the ulcers induced by HCl. Further, this oxidative stress leads to the generation of the free radicals and lipid peroxidation. The administration of the HCl leads to the sodium (Na) and potassium (k) flux into the lumen and increase the release of histamine and pepsin (Saini *et al*., 2012).

5. Selected Plants

1. The family of chenopodiaceae

1.1. General description

Herbs annual, subshrubs, or shrubs, rarely perennial herbs or small trees. Stems and branches sometimes jointed (articulate); indumentum of vesicular hairs (furfuraceous or farinose), ramified (dendroid), stellate, rarely of glandular hairs, or plants glabrous. Leaves alternate or

opposite, exstipulate, petiolate or sessile; leaf blade flattened, terete, semiterete, or in some species reduced to scales. Stamens shorter than or equaling perianth segments and arranged opposite them; filaments subulate or linear, united at base and usually forming a hypogynous disk, sometimes with interstaminal lobes; anthers dorsifixed, incumbent in bud, 2-locular, extrorse, or dehiscent by lateral, longitudinal slits, obtuse or appendaged at apex. Probably about 100 genera and 1400 species: mainly in arid areas, deserts, and coastal and saline habitats of N and S Africa, Asia, Australia, Europe, and North and South America. Many species of Chenopodiaceae are adapted to, and are major components of, arid or ruderal environments. (Kung *et al*., 1979) They are often intimately involved with the daily life of people. For example, *Beta vulgaris* is one of the most important sources for sugar, *Chenopodium quinoa* is a new high-protein crop, *Spinacia oleracea* and *Beta vulgaris* are excellent vegetables; *Dysphania ambrosioides* and *Salsola collina* are used medicinally, seeds of *Agriophyllum squarrosum* are called ìsand-riceî locally and are edible, seeds of *Corispermum declinatum* are used for making gin, the ash of *Halogeton arachnoideus* and some species of Salsola contains soda which is used in noodle-making and *Anabasis aphylla* can be used as an insecticide. Many species are important as animal forage in desert, semidesert, and steppe regions, and some species make good windbreaks and soil binders. *Haloxylon ammodendron* has been used extensively in biological reconditioning of the desert. (Li *et al*., 2003).

1.2. Classification of the chenopodiaceae

- **Kingdom:** Plantae
- **Phylum:** Tracheophyta
- **Class:** Magnoliopsida
- **Order:** Caryophyllales
- **Family:** Chenopodiaceae

1.3. Economic importance of the chenopodiaceae

It is a family which provides food plants. Chenopodiaceae cultivated are rather resistant to salt, and generally require a region rich in nitrogen. Most known in our areas are the various varieties of Swiss chards and beets (*Beta vulgaris*), spinach (*Spinacia oleracea*), orache of the gardens (*Atriplex hortensis*), wild spinach (Chenopodium no-claims bonus-henricus), which can be consumed like spinaches, or the quinoa (*Chenopodium quinoa*), cultivated in South America like cereal. Kochia scoparia, nameed cypress of summer, is a decorative species of the hedges.

2. *Arthrophytum scoparium*

2.1. Botanical description

Arthrophytum scoparium belongs to the family chenopodiaceae, which has 120 genera and more than 1700 species (Wen *et al*., 2010 ; Ibrahim *et al*., 2007) . They are worldwide distributed especially in desert and semi desert areas in soils containing much salt. The plants are herbs, shrubs, subshrubs and rarely small trees (Mabberley, 1997). Subshrubs or shrubs, glabrous or with papillate processes (**Fig.3**). Annual branches terete or obtusely 4-angled, jointed, slightly fleshy. Leaves opposite, sessile, linear, semiterete or clavate, fleshy, rarely subulate or scale-like, base slightly expanded, apex obtuse or with a short, acicular awn; leaf axil usually cottony. Flowers solitary in leaf axils, bisexual, with 2 bractlets. Perianth subglobose; five segments, orbicular to broadly elliptic, herbaceous, abaxially somewhat thickened, bearing a transverse, winglike process a little below apex in fruit, adaxially convex, margin membranous, apex usually recurved (Miguel *et al*., 2014). The plant is a small, highly-branched halophytic shrub distributed in south–east Spain, North Africa and parts of Iran, Turkey, Iraq and Syria (Irano-Turanian region) (Salah *et al*., 2002). Its flowers are discrete but at the end of the autumn, when humidity is sufficient, the end of its branches

covers fruits surrounded by a crown of brilliant membranous and highly colored of pink or red. In Algeria, it is commonly known as «Remth» (Taïr *et al*., 2016).

2.2. Taxonomy

The *A.scoparium* is called: Remth (local name), *Saligne à balai* (French). It has many synonyms : *Hammada articulata* (Moq.) O. Bolòs & Vigo (1974), *Haloxylon articulatum* (Moq.) Bunge (1851), *Hammada scoparia* (Pomel) Iljin, *Haloxylon scoparium* Pomel (1875) and *Salsola articulata.*

- **Kingdom :** Plantae
- **Subkingdom :** Tracheobionta
- **Division :** Magnoliophyta
- **Class :** Magnoliopsida
- **Order :** Caryophyllales
- **Family :** Chenopodiaceae
- **Genus :** Arthrophytum
- **Specie:** *Arthrophytum scoparium* Cronquist (1981).

Figure 3.*Arthrophytum scoparium* pictures (www.botany.cz, 2008).

2.3. Traditionaland medicinal uses of *A.scoparium*

Algeria's distinctive morphology, varied topography and under changing climatic conditions permits the grow of more than 3000 plants species (Benamar *et al*., 2010 ; Cheriti *et al*., 2006).The south Algerian flora is very rich and a great number of species has been used traditionally for the treatment of several diseases without any scientific background (Melekmi *et al*., 2006). Of the shrub and tree species encountered in the desert of southwest Algeria., *A.scoparium* is a local medicinal plant, which has been used extensively in the southwestern part of Algeria. It was subjected to an ethnopharmacological survey (Naama, Tindouf, Adrar and in Bechar), phytochemical screening and anti nosocomial activity. The ethnopharmacological survey revealed that the *A.scoparium* used to treat numerous human diseases especially the infectious one such as skin infections, urinary and genital infections, Rheumatism, diabetes, cancer, eczema, infertility, disorders of pregnancy, hair problems, stomach, wounds and sepsis, itching, burns, antitoxin, snake bite and scorpion sting (Fatehi *et al*., 2017), and is reportedly used for diabetes, with antiseptic and anti-inflammatory characteristics (Chaouche *et al*.,2014). In Tunisia and Morocco *A.scoparium* is used to treat eye disorders also to cure stomach, scorpion bites, wounds infertility and bone pain (Allaoui *et al., 2016*). *Arthrophytum scoparium* is known in Morocco as “âkenoud”, “Remth” and “âssây”. In this region, H. scoparium (whole plant) can be used as cicatrizing agent to treat headaches, for antidiabetic medications, or in the treatment of hypertension (Miguel *et al*.,2014), In Oman the stems of this species are used as a mordant for dyeing wool in traditional weaving (Lamchouri *et al*.,2012).

2.4. Chemical composition

Few species of the genus Haloxylon have been chemically investigated, which resulted in the isolation of the several alkaloids belonging to mainly seven classes of alkaloids. These classes are: aliphatic quaternary alkaloids, pyridine alkaloids, indole alkaloids, isoquinoline alkaloids,

isoquinolone alkaloids, β-carboline alkaloids and phenylethylamine alkaloids (Lamchouri *et al*., 2012). *Haloxylon scoparium* (Hammada) from Algeria has been reported to contain the alkaloids carnegine and Methylisosalsoline as major tetrahydroisoquinoline alkaloids in addition to isosalsoline, salsolidine, dehydrosalsolidine, is salsolidine, N-methylcorydaldine, tryptamine and N-methyltryptamine as minor alkaloids (El-Shazlyb and Winka,2003).

3. *Halogeton sativus*

3.1. Botanical description

Halogeton is a plant genus of the family chenopodiaceae. The genus name, Halogeton, derives from the Greek words for "salt" and for "neighbor." (Akhani *et al*., 2007).The genus Halogeton includes both annual and perennial species, 20-70 cm tall. Stem branched from base; branches alternate or lower ones opposite, spreading, light green, sometimes whitish, not straight, glabrous The leaves are fleshy cylindrical, terminating in a persistent or caducous bristle (**Fig.4**). There are three to several flowers in the axil of each floral leaf. The perianth segments are membranous. The stamens are fixed on a papillose staminodial disk. In fruit, the tepals develop five wings. The annual species grow in temperate salines and ruderal places, while the perennials are found in warm and hot deserts. They are tolerant of fairly saline soils (Li *et al*., 2003 ; Holmgren, 2004).

Figure 4. *Halogeton sativus* pictures.

The plant is a small, highly-branched halophytic shrub distributed in Saline lake shores, meadows with saline soils in north Africa, centrer and south west of Asia, south Europe; locally naturalized in North America (California) and South America (Argentina) (**Fig.5**). In Algeria *H.sativus* distributed in the pre-saharan region: Beni-ounif, Biskra, Laghouat et Msila (Ozenda, 1983).

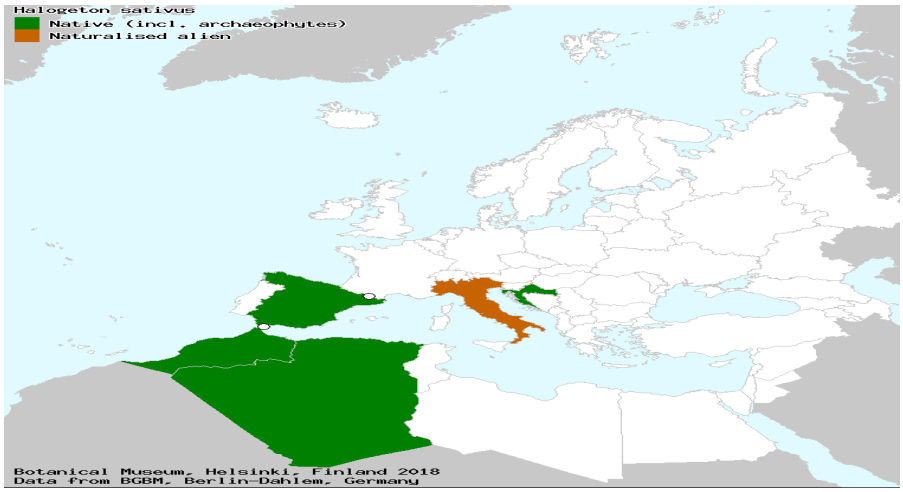


Figure 5. Geografical distribution of *Halogeton sativus*.

3.2. Taxonomy

The *H.sativus* is called: Remth (local name). It has many synonyms: *Salsola sativa* (LINNAEUS, 1762) and *Salsola polyclonos* (LINNAEUS, 1767)

- **Kingdom :** Plantae
- **Subkingdom :** Tracheobionta
- **Division :** Magnoliophyta
- **Class :** Magnoliopsida
- **Order :** Caryophyllales
- **Family :** Chenopodiaceae
- **Genus:**Halogeton
- **Specie:** *Halogeton sativus (*KÜhn *et al*. 1993).

Salsola species are well known in folk medicine of Russia, China and Bahrain where there used such as diuretic, anti-hypertensive, anti-cancer, purgative, emollient, anti-ulcerandanti-inflammatory (TUNDIS *et al*., 2008).Some Salsola species, such as *S. kali* and *S. soda*, were used as food. Halogeton sativus was cultivated for the enormous 18th Century barilla industry in Spain that produced soda ash. The species was considered to be a "saltwort" plant (Akhani *et al*., 2007). Many studies supported scientifically the traditional use of Salsola species in the treatment of hypertension and demonstrated the hypoglycaemic effects of *S. soda*, *S. tragus* and *S. oppositifolia* extracts by the inhibition of a-amylase enzyme (Loizzo *et al*., 2007 ; TUNDIS *et al*.,2007). Phytochemical evaluations of Salsola species resulted in the isolation of flavonoids, acetophenones, coumarines and sterols. Simple tetrahydroisoquinoline alkaloids are also common in the Chenopodiaceae family and were isolated in different Salsola species (TUNDIS *et al*.,2008).

1. Materials

1.1. Plant Material

Vegetal material was collected in April 2016 from ouled djellal, Biskra, and Ain safra, Naama, Algeria. The plants specimens were identified by Pr. Oujhih B. Institut of nutrition and agronomy, university of Batna (Algeria) a voucher specimen were kept under the number 227/ISVSA/ DA/UHLB1/17 and 226/ISVSA/ DA/UHLB1/17. *A. scoparium and H. sativus* Aerial parts were washed, cut, dried for two weeks in free air in dark, and then powdered using electrical grinder. The powder was conserved in glass bottle at ambient temperature until use.

1.2. Experimental animals

Healthy female adult wistar rats and female NMRI mice (nulliparous and non-pregnant) weighing from 200 to 300 g and 30 to 35 g, respectively were used. They purchased from Pasteur institute, Algiers, Algeria. The animals were acclimatized for one week, prior to experiments. They were fed with standard diet and water and kept in a standard with natural light and dark cycle (OECD, 2008).

1.3. Chemicals and reagents

Linoleic acid, β-carotene, butylated hydroxytoluene (BHT) were purchased from Fluka Chemical Co. (Buchs, Switzerland).salicylate sodium, 2, 2-diphenyl-1-picryl- hydrazyl (DPPH), ethylenediamine tetraacetic acid (EDTA), gallic acid, quercitin, 2.2′-Azino-bis (3-ethylbenzenothiazoline 6- sulfonic acid) (ABTS), Folin-Ciocalteu reagent, Potassium persulphate, potassium ferricyanide (K_3FeCN_6), trichloroacetic acid (TCA), thiobarbituric acid (TBA), Ferrozine, .ferrous and ferric chloride, Furosemide, DTNB(5-5'-dithiobis2-nitrobenzoïc acid), H_2O_2, n-butanol ,methanol. These chemicals were obtained from Sigma Chemical Co. (St. Louis, MO), Fluka and Pro-lab Company.

2. Methods

2.1. Preparation of plants extracts

Extraction of phenolic compounds was conducted as reported by Madoui et *al*. (2018). Aerial parts of plants (100 g) were powdered, mixed with one liter of methanol-water solution (85: 15 v/v) and kept under agitation overnight at room temperature. The extract was filtered on filter paper then on sintered glass to obtain the first filtrate. This procedure was repeated on the residue using 50% aqueous–methanol under agitation for 4 hours to obtain the last filtrate. The first and the last filtrates were combined then the crude extract (CrE) was further concentrated by allowing it to stand in an oven at 40°C. The CrE was successively fractionated with different solvents of increasing polarity; with hexane several times until a clear upper layer of hexane (hexane extract, HxE). The lower layer was then extracted successively with chloroform and ethyl acetate to obtain 3 fractions; chloroform (ChE), ethyl acetate (EAE) and aqueous extracts (AQE) (**Fig.6**). All extracts were stored at -20ºC until use. Decoction (DEC) was carried out according to the protocol described by Ferreira et *al.* (2013), with slight modifications. Briefly, the dried plants were sprayed using a grinder, then 50 g of the plant powder are added to 500 ml of extraction solvent (water). The mixture was boiled continuous stirring for 20 minutes. Then, the water extract was filtered through Whatman No. 4 filter paper and centrifuged at 3000 rpm for 15 min. Finally, the supernatant obtained was dehydrated until a brown powder formed, which was stored in opaque bottles until used.

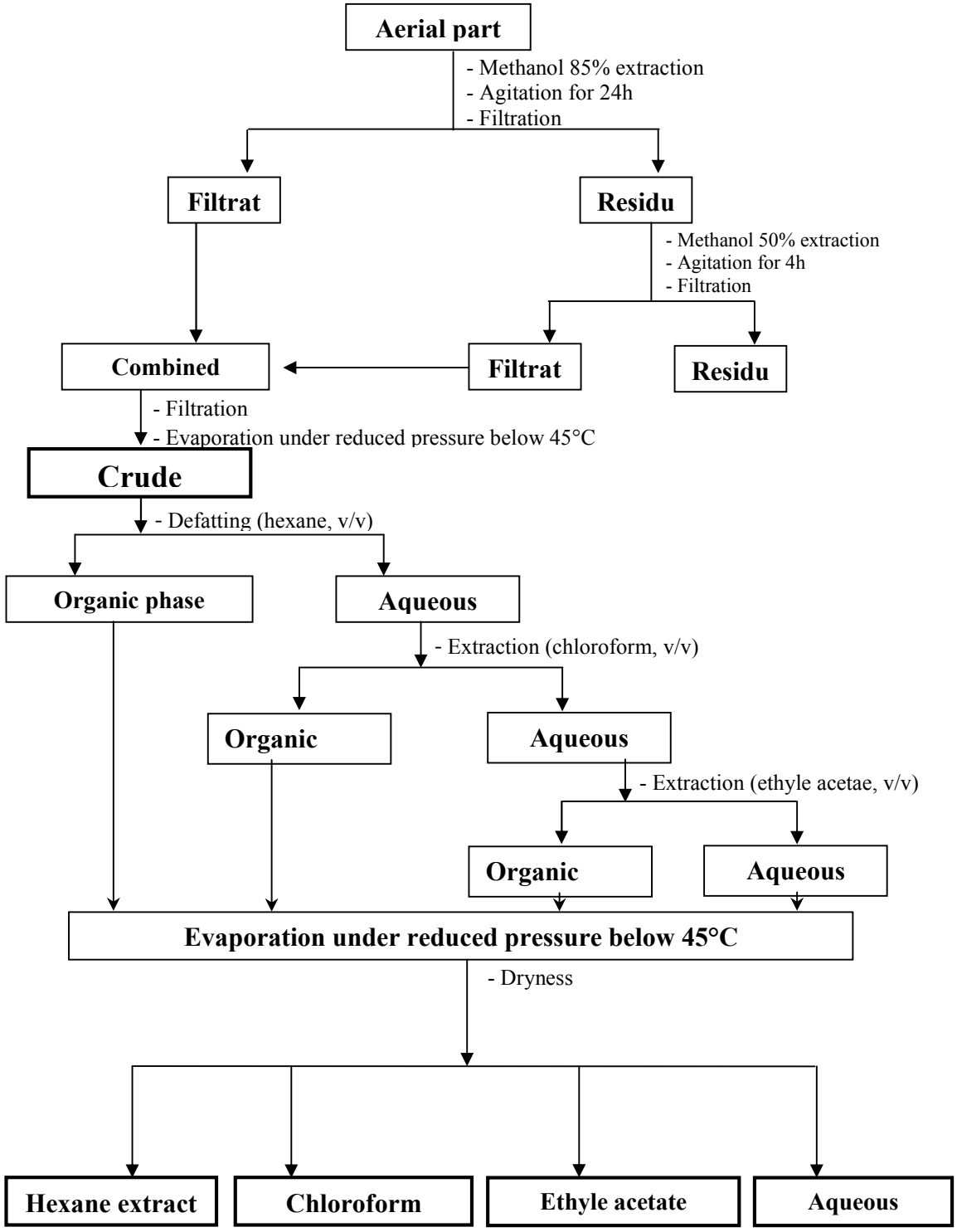


Figure 6. Diagram represents the process of extraction of phenolic compounds from *A.scoparium* and *H.sativus* aerial parts.

2.2.Determination of polyphenols,flavonoids and tannins content

2.2.1. Determination of total phenolic content

The Folin-Ciocalteu reagent is a mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolibic ($H_3PMO_{12}O_{40}$), it is reduced by phenols to a mixture of blue oxides of tungsten (W_8O_{23}) and molybdenum (Mo_8O_{23}), (Agbor *et al*., 2014). The intensity of blue color is proportional to the amount of phenolic compounds in the plant extracts. Total phenolic compounds in *A.scoparium* / *H.sativus* extracts, were determined following Boussoualim *et al*. (2016), with slight modifications. 0.1 ml of samples and standard was mixed with 0.5 ml of Folin-Ciocalteu reagent (diluted 10 folds). After incubation for 4 min, 0.4 ml of sodium carbonate (Na_2CO_3) solution (7.5%) was added. After 1 h and 30 min of incubation in the dark at room temperature, the measurement of the absorbance was affected at 760 nm using a spectrophotometer thermo spectronic and the results are expressed as mg gallic acid equivalent (GAE)/g of extract (**Fig.7**).

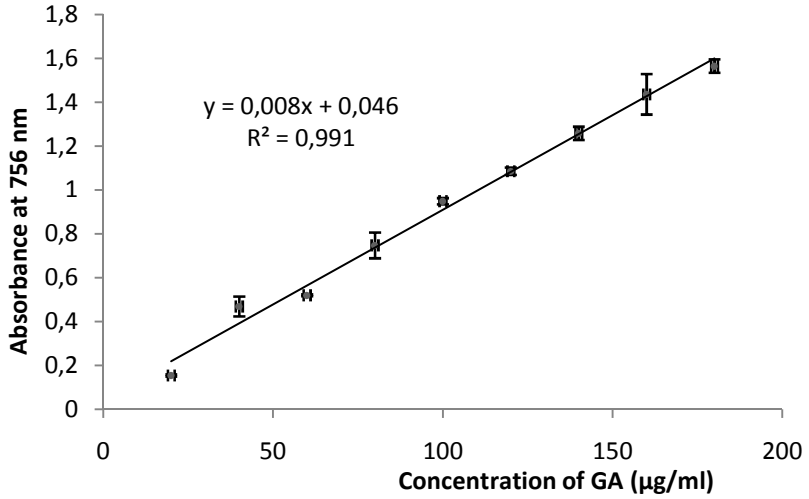


Figure 7. Standard curve of gallic acid for the determination of total phenolic compounds in various plant extracts. Each value represent mean ± SD (n = 3).

2.2.2. Determination of flavonoids Content

Total flavonoids content in each extract was estimated using the aluminum chloride colorimetric method as described by Guemmaz *et al*. (2018). Flavonoids have a free hydroxyl (OH) group at position 5, which react with the CO group to produce a colored complex with chloride aluminum. The flavonoids form yellow complexes by chelating metals (iron and aluminum) (Rani *et al.,* 2018). This reaction based to the release of two electrons of the metal (Al) to react with two oxygen atoms of the phenolic molecules acting as an electron donor (Petrou *et al.,* 2018). Briefly, 1ml of diluted sample was mixed with 1 ml of 2% aluminum chloride in methanol. After incubation at room temperature for 10 min, the absorbance was measured at 430 nm and the flavonoids content was expressed in mg quercitin equivalent per gram of dry weight (QE) (**Fig.8**).

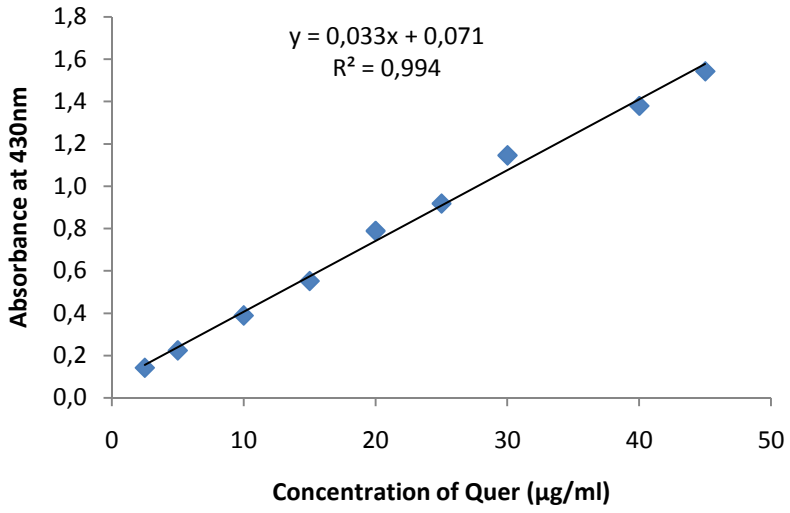


Figure 8. Standard curve of quercetin for the determination of flavonoids in various plant extracts. Each value represent mean ± SD (n=3).

2.2.3. Determination of tannin content

The capacity to precipitate hemoglobin was determined using bovine fresh blood according to Bouaziz *et al.* (2014). Briefly, an equal volumes of each extract and hemolysed bovine blood (absorbance = 1.6) were mixed. After 20 min, the mixture was centrifuged at 4000 rpm for 10

min, and the absorbance of the supernatant was measured at 756 nm. The results were expressed as mg equivalent tannic acid per gram dried weight (mg TAE/g DW) (**Fig.9**).

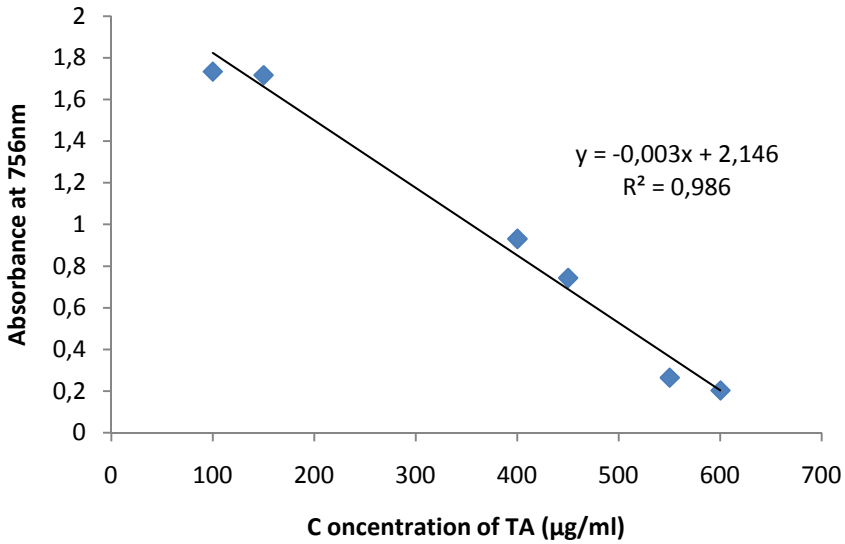


Figure 9. Standard curve of tannic acid for the determination of tannins in various plant extracts. Each value represent mean ± SD (n=3).

2.3. The evaluation of antioxidant activity *in Vitro*

2.3.1. DPPH radical scavenging activity

Quantitative measurement of DPPH is widely used due to relatively short time required for the analysis. The DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical is very stable, reacts with compounds that can donate hydrogen atoms, and has a maximum absorbance at 517 nm. The method is based on the scavenging of DPPH by antioxidants, which upon a reduction reaction decolorizes the DPPH methanol solution (Morabbi Najafabad and Jamei, 2014) (**Fig.10**).

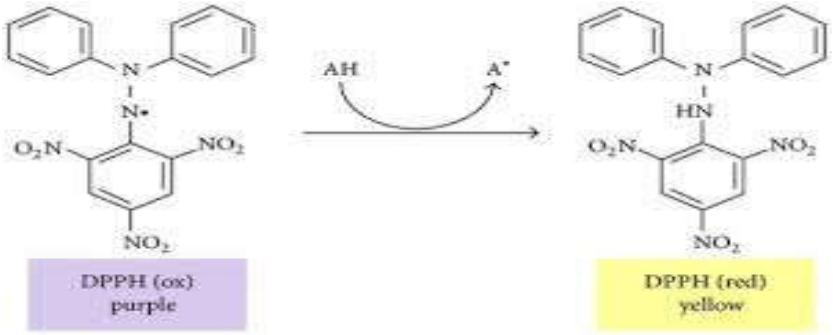


Figure 10. Radical and non-radical forms of DPPH (Morandi Vuolo *et al.,* 2019)

The assay measures the reducing ability of antioxidants toward the DPPH radical. The method was carried out as described by Aouachria *et al.* (2017), with slight modifications. In this assay, the purple radical (picrylhydrazyl) is reduced by antioxidant compounds to the corresponding pale yellow hydrazine (picrylhydrazine). 1250 µl DPPH (0.4 mM in methanol) was mixed with 50 µl of extract. The reaction mixture was vortexed and left in the dark at room temperature for 30 min. the absorbance was read at 517 nm. DPPH radical's concentration was calculated using the following equation:

DPPH radical scavenging activity (%) = $(A_C - A_S / A_C) \times 100$

Where $\mathbf{A_C}$: is the absorbance of the control.

$\mathbf{A_S}$: is the absorbance in the presence of the sample/ standard.

2.3.2. ABTS radical scavenging activity

The spectrophotometric measurement of $ABTS^+$ (2 2'-azino-bis3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging activity was determined according to Bouaziz *et al.* (2014). This method is based on the ability of antioxidants to quench the long-lived ABTS radical cation, a blue/green chromophore is formed with characteristic absorption at 734nm (**Fig.11**).

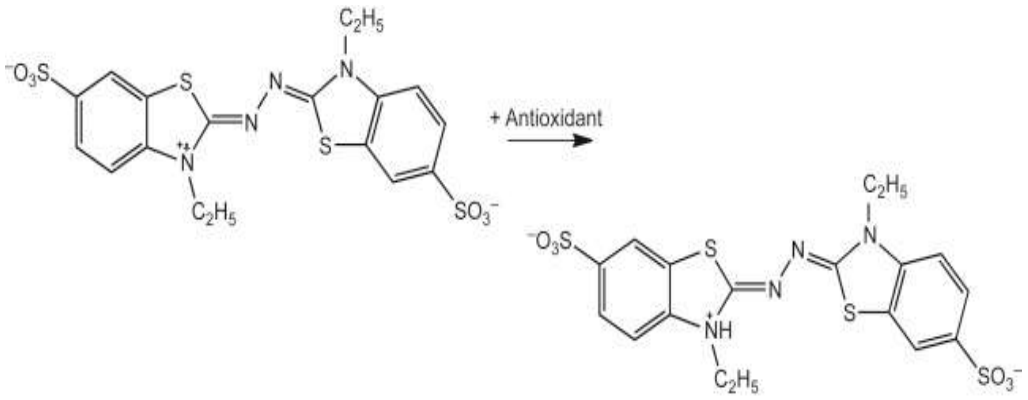


Figure 11. ABTS chemical reaction with antioxidant compound (Morandi Vuolo *et al*., 2019)

In this assay, the radical cation was produced by reacting 2 mM ABTS in H_2O with 2.45 mM potassium persulfate ($K_2S_2O_8$), stored in the dark at room temperature for 16 h. Before use, the $ABTS^+$ solution was diluted with sodium phosphate buffer (0.1 M, pH 7.4) to get an absorbance of 0.750 ± 0.025 at 734 nm. Then, 2 ml of fresh $ABTS^+$ solution was added to 20 µL of extract solution in methanol at different concentrations. The percentage of inhibition at 734 nm was calculated for each concentration relative to a blank absorbance after 7 min. The extent of decolorization is calculated as percentage reduction of the absorbance.

$ABTS^+$ scavenging effect (%) $= (A_c - A_s)/ A_c * 100$

Where $\mathbf{A_C}$: is the absorbance of the control (ABTS);

$\mathbf{A_S}$: is the absorbance in the presence of the sample/ standard.

2.3.3.β-Carotene/linoleic acid assay

Linoleic acid incorporated into β-carotene-linoleic acid bleaching acid is an unsaturated fatty acid that is prone to oxidation and thus forms ROS or lipid radicals (Akanbi and Barrow, 2018). Formation of ROS will eventually trigger oxidation of β-carotene, which is a yellowish color. In the presence of an antioxidant, the oxidation of β-carotene is inhibited and the yellowish-orange coloration is retained (λmax = 470 nm) (Veronezi and Jorge, 2018). The capacity of plant extracts to anticipate bleaching of β -carotene was evaluated as described by Guemmaz *et al*., (2018). The antioxidant capacity is determined by measuring the inhibition

of the conjugated diene hydroperoxides arising from linoleic acid oxidation. The solution mixture of β-carotene-linoleic acid was prepared as follows: 0.5 mg β-carotene was dissolved in 1 ml of chloroform and 25 µl linoleic acid and 200 mg Tween 40. Chloroform was completely evaporated, and then, 100 ml distilled water saturated with oxygen were added. Into test tubes, 2.5 ml of reaction mixture were disbursed and 350 µl of the various extracts with a same concentrations (2 mg/ml), were added and the emulsion was incubated at room temperature for 48 h. The same procedure was repeated with synthetic antioxidant BHT as positive control, and blanks (MeOH and H_2O). The measurement of absorbance of the reaction mixture was effected after; 0, 1, 2, 4, 6, 24h at 490 nm. The antioxidant activity (AA) was measured in terms of successful bleaching of β -carotene by using the following equation:

AA% = A sample / A BHT × 100

A sample: Absorbance in the presence of the extract.

A BHT: Absorbance in the presence of positive control BHT.

2.3.4. Reducing power assay

The ferric-reducing antioxidant power assay evaluates the reducing potency of the antioxidant to react on ferric tripyridyltriazine (Fe^{3+}–TPTZ) complex (Wang *et al.,* 2019). When antioxidant donates a hydrogen atom to ferric complex, the radical chain reaction breaks and produces blue-color ferrous λmax = 593 nm (Anand *et al*., 2018). The ability to reduce ferric ions was measured according to the method described by Bencheikh *et al*. (2016). Briefly, 0.1 ml of each plant extract with different concentrations was mixed with an equal volume of phosphate buffer (0.2 M, pH 6.6) and 1% potassium ferricyanide. After incubation for 20 min at 50 ºC, 0.25 ml of 10% trichloroacetic acid was added to the mixture and centrifuged at 3000 rpm for 10 min. Then 0.25 ml of the supernatant was added to 0.25 ml distilled water

and 0.5 ml of ferric chloride (0.1%). The absorbance was measured to determine the amount of ferric ferrocyanide (Prussian blue) formed at 700 nm against a blank.

2.3.5. Ion chelating assay

Ferrozine can quantitatively chelate with Fe^{2+} and form a red colored complex. This reaction is limited in the presence of other chelating agents and results in a decrease of the red color of the ferrozine-Fe^{2+} complex. Measurement of the color reduction estimates the chelating activity to compete with ferrozine for the ferrous ions (Gayathri *et al*., 2017). The measurement of the inhibition of the formation of Fe^{2+} -ferrozine complex after the treatment of various concentrations of plant fractions with Fe^{2+}, was performed according to Aouachria *et al*. (2017). 0.25 ml aliquot of dissolved extract was added to 0.05 ml (0.6 mM) aqueous $FeCl_2$ - $4H_2O$ and 0.45ml Methanol. After 5min, the reaction was initiated by the addition of 0.05 ml (5 mM) ferrozine solution. After 10 min, the absorbance at 562 nm was recorded. The control contained all the reagents except the extract or positive control. EDTA was used as a positive control. The inhibition percentage of ferrozine-Fe^{2+} complex formation was calculated using the formula:

Chelating activity% = ((A control – A sample)/A control)*100

EC_{50} values were calculated by linear regression analysis; linearity range between antioxidant concentration and chelating activity.

2.3.6. Hydroxyl radical scavenging assay

Radical scavenging ability was assessed following the procedure described by khiter *et al.* (2018). Hydroxyl radical is one of the potent reactive oxygen species in the biological system. It reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and causes damage to cell (Collin, 2019). The Principe of this method is based on the production of OH• in the reaction medium through the reaction of Fenton, then the OH product reacted with

sodium salicylate to produce the hydroxyl salicylate complex. The reaction mixture (3 ml) contained 1 ml of $FeSO_4$ (1.5 mM), 0.7 ml of H_2O_2 (6 mM), 0.3 ml of sodium salicylate (20 mM) and varying concentrations of plant extracts and standard. This mixture was incubated at 37°C for 1h, after which the absorbance of the hydroxylated salicylate complex was measured at 562 nm. The percentage scavenging effect was calculated as follows:

Scavenging rate %= $[1-(A_1-A_2)/A_0]*100$

Where, A_0 was the absorbance of the control (without sample) and A_1 was the absorbance in the presence of the sample; A_2 was the absorbance without sodium salicylate.

2.3.7. Hydrogen peroxide scavenging activity

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups (Banothu *et al.,* 2016). Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H_2O_2 can probably react with Fe^{2+}, and possibly Cu^{2+} ions to form hydroxyl radical (Smirnoff and Arnaud, 2019). The hydrogen peroxide scavenging assay was carried out following the procedure mentioned by Khither *et al.* (2018). For this aim, a solution of H_2O_2 (43 mM) was prepared in phosphate buffer (0.1 M, pH 7.4). The plant extracts at different concentrations in 3.4 ml phosphate buffer was added to 0.6 ml of H_2O_2 solution (43 mM). The absorbance value of the reaction mixture was recorded at 230 nm. Blank solution was containing the sodium phosphate buffer without H_2O_2. The percentage of H_2O_2 scavenging activity of samples and standard compounds was calculated using the following equation:

H_2O_2 scavenging effect % = $(A_c - A_s)/ A_c * 100$

Where, A_C is the absorbance of the control and A_S is the absorbance in the presence of the sample or standards.

2.3.8. Anti-hemolytic assay

a. The AAPH-induced hemolysis

Mice erythrocytes hemolysis inhibition was assessed according to the method described by Guemmaz *et al*. (2018). The addition of AAPH (peroxyl radical initiator) to the mice erythrocyte suspension induces the oxidation of cell membrane lipids and proteins, resulting in hemolysis. Mice erythrocytes were isolated by centrifugation at 3000 rpm for 15 min and washed three times with phosphate buffer (10 mM, pH 7.4) until the supernatant became colorless. The erythrocytes were then diluted with phosphate buffer to give 2% (v/v) suspension. Briefly, 80 µl of 2% erythrocytes suspension was added to 20 µl of extract (0.1 mg/ml). the mixture was treated then by 136 µl of AAPH (300 mM). The mixtures were incubated in microplate with a 96-well at 37ºC. The kinetics of the gradual disappearance of erythrocytes was followed by the measurement of the decrease in absorbance at 630 nm. Results were expressed as the time corresponding to 50% of maximal hemolysis (half-hemolysis time, HT_{50} in min). Ascorbic acid was used as standard.

b. ferrous ion-induced hemolysis

The effect of extracts on ferrous ion-induced hemolysis was evaluated according to Thephinlap *et al*. (2013). Blood was obtained from mice collected in heparinized tubes and centrifuged at 3500 rpm for 15 min. The Red blood cells (RBCs) were suspended in 10 volumes of NaCl (0.9%) and centrifuged at 2500 rpm for 10 min. RBC suspension was incubated with 5 µM ferrous sulfate and various concentrations of the extracts at 37°C for 30 min. Hemolysis was determined by measuring the optic density (OD) of the supernatant at 540 nm. The reaction without extract was used as control sample. Percentage of anti-hemolysis was calculated from following equation:

Inhibition % = (Ac-Ae)/Ac*100

2.4. In vivo study

2.4.1. Acute Toxicity

Acute toxicity study was conducted according to guidelines 425 (OECD, 2008). Two groups of five rats were used to determine the doses which will be used *in vivo* study. Crude extract of *A.scoparium* (ASE) / crude extract of *H.sativus* (HSE) were administrated orally, the first dose was 2000 mg/Kg, animals were observed if any sign of toxicity appears for 14 days. If no mortality is observed at this dose, the same procedure will be repeated for the dose of 5000 mg/Kg. The results of this study permit to determine the doses which will be used in the *in vivo* studies.

2.4.1.1. Observation

Animals were observed for 14 days after the administration of HSE. All mortalities, clinical signs, time of onset, duration, and reversibility of toxicity were recorded. Body weight of each animal was carefully recorded at the begging of the study, then once after every seven days and on the day of sacrifice.

2.4.1.2. Plasma preparation and biochemical analysis

After 14 days, all surviving animals were fasted overnight and anesthetized. Blood was collected in heparinized tubes and centrifuged at 3000 rpm for 15 min. The serum was separated and analyzed for various biochemical parameters such as creatinine, urea, cholesterol, HDL, LDL, triglyceride, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatases (ALP) using commercial kits.

2.4.1.3. Organs weight

Organs; liver, spleen, kidneys, heart, lungs and stomach were quickly removed and weighed. The relative organ weight of each animal was then calculated relating the absolute organ weight and body weight of the animal on the day of sacrifice.

2.4.1.4. Histological analysis

Liver and kidneys were fixed in 10% formol and embedded in parrafin. Sections at 5 µm were stained with hematoxylin and eosin and examined under light microscopy. The analyses of the different organs (liver, kidneys) were performed using an Optika B500T i-5 microscope (10 and 40×) in order to verify alterations in the tissues. Digital images were obtained using the Optika camera associated to the microscope, and all the images were analyzed using the image processing software, Optika Vision Pro.

2.4.2. Hepatoprotective assay

According to the method described by Aichour *et al.* (2018), the *in vivo* hepatoprotective action of HSE was determined using CCl_4-induced hepatic injury. Groups of rats received the respective dose of extract orally once day for 7 consecutive days. After 24 h, the administration of CCl_4 (2 ml/kg, intraperetentially) was performed one hour after the last dose on day 7. Twenty four hours after the hepatic lesion induction, the animals were anesthetized using diethyl ether and blood samples were collected for biochemical parameter determination.

2.4.2.1. Histopathological studies

The liver tissue was removed and immediately fixed in formalin (10%) for 24 h, dehydrated using a graded ethanol, cleared with xylene, and then embedded in paraffin. The paraffin sections were cut into 5-µm thick slices using a microtome (Leica RM2125RT), and stained

with hematoxylin and eosin. In the final step, the sections were observed under an optical microscope (Leica DM LB 2) and then photographed**.**

2.4.2.2. DPPH radical-scavenging activity of plasma

The capacity of plasma to trap the DPPH radical was evaluated according to Guemmaz *et al*., (2018), while being based on the same principle as that of the test of DPPH carried out previously *in vitro*. Briefly, a volume of plasma was added to DPPH methanolic solution (0.004%). After 30 min of incubation in the darkness followed by a centrifugation, the absorbance of the supernatant was measured at 517 nm.

2.4.2.3. Reducing power assay

The reducing power was determined according to Bencheich *et al*. (2016). Briefly, 125 µl of sodium phosphate buffer (0.2 M and pH 6.6) and 125 µl of potassium ferricyanide (1%) were added to an equal volume of plasma. Mixtures were incubated at 50°C for 20 min, and then 125 µl TCA (10%) was added, the mixtures were centrifuged (3000 rpm for 10 min). The supernatant solution (100 µl) was mixed with 100 µl of distilled water and 20 µl of 1% ferric chloride to react for 10 min. Subsequently, the absorbance was measured at 700 nm

2.4.2.4. Preparation of tissue homogenates

Livers were washed and homogenized in ice-cold KCl (1.15%) with a ratio of 1/10, using electric homogenizer. Homogenates were centrifuged at 5000 rpm for 15 min at 4°C; the resulting supernatant was used for the determination of catalase activity, malondialdehyde (MDA) and glutathione contents in liver.

2.4.2.5. Determination of Catalase activity

Catalase (CAT, E.C.1.11.1.6) activity was measured according to Aouachria *et al*. (2017). The elimination of hydrogen peroxide in the presence of catalase was followed by the decrease in absorption of peroxide solution in the ultraviolet (UV) region. Briefly, 0.1 ml of

supernatant was added to 1.9 ml of 50 mM phosphate buffer (pH 7.0). The reaction was started by the addition of 1.0 ml of freshly prepared H_2O_2 (30 mM). The rate of decomposition of H_2O_2 was measured at 240 nm in an interval of 30 seconds at room temperature. Catalase activity was expressed as UI/mg protein.

2.4.2.6. Estimation of glutathione content

The estimation of reduced glutathione (GSH) was carried out as described by Bentahar *et al*. (2016) with slight modifications. The mixture of the homogenate and TBA (20%) with equal volume was centrifugation for 10 min at 2000 rpm. The supernatant (200 µl) was added to 1.8 ml of the Ellman's reagent (5, 5'-dithio bis-2-nitrobenzoic acid) (0.1mM), which was prepared in 0.3M. The absorption of solutions was measured at 412 nm against blank. Absorbance values were compared with a standard curve from known GSH (0.4-20 µmol/ml) .Reduced glutathione was expressed as µmol/ g tissue.

2.4.2.7. Estimation of MDA

The Lipid peroxidation in liver homogenate was determined by measuring the amounts of malondialdehyde (MDA) produced, according to the method described by Zerargui *et al.* (2015). Tissue homogenate (125 µl) was mixed with TCA (125 µl, 20%) and TBA (250 µl, 0.67%) and then heated in a water bath at 100°C for 15 min. After incubation, the tubes were cooled by raining the cold water, and MDA-TBA complex was extracted with 1 ml of butanol and centrifuged at 3000 rpm for 15 min. The upper organic layer was taken and its absorbance was measured at 532 nm against an appropriate blank.

2.4.3. Xylene induced ear edema

NMRI mice were divided into four groups of six animals each. Animals were treated orally with the extract (100, 200 and 400 mg/kg), Aspirin (1 mg/kg) and distilled water (3 ml/kg).one hour and thirty minutes later, edema was induced in each mouse group by applying a drop of xylene to the inner surface of the right ear. After 15 min, the right ears were sized. The anti inflammatory activity was expressed as the percentage inhibition (Ma *et al.,* 2017) of edema in the treated mice in comparison with the control mice.

2.4.4. Ethanol- induced ulcer Gastric

Ulceration was induced in 24 hours starved rats using the method described by Almasaudi *et al.* (2016). The rats were given the plant extract at the doses of 50,150 and 300 mg/kg orally; the positive controls received ranitidine at dose of 50 mg/kg, while negative control received distilled water. One hour 30 minutes after drug treatment, 1 ml of ethanol at 75% was given orally to each rat. Rats were killed 1 hour later using an over dose of ether, and the stomach was removed, washed and elongated on a cork board and observed for ulcers in the glandular region. The surface area of each lesion was measured and scored as described by Sistani Karampour *et al.* (2019). The ulcer index for each rat was taken as the mean ulcer score. The percentage ulcerated surface was calculated as the total area covered by all lesions expressed as a proportion of the total corpus mucosal surface area. The percentage of inhibition of ulcer (IU %) was calculated using the following formula:

$$IU\% = (USc - USt) * 100 / USc$$

Where, USc = ulcer surface area of control and USt = ulcer surface area of test animal.

2.5. Statistic Analysis

Results were expressed as mean ± standard deviation. Statistical analysis for *in vitro* results was undertaken using student test and results were expressed as mean ± standard deviation

(SD). The differences between groups were determined by analysis of variance one-way ANOVA followed by Dunnett's test. The pharmacological results were presented as mean ± standard error of mean (SEM). All results were analyzed using GraphPad Prism version 5.00. Differences were considered significant at $p < 0.05$.

1. Extraction yield

The maceration of 100 g of *Arthrophytum scoparium* and *Halogeton sativus* aerial part powder in 1L of hydromethanolic mixture (85/15 v/v) was carried out in two steps according to Madoui *et al.* (2018). The first one is to give the crude extract (total polyphenols). The second step is used to separate the crude extract compounds according to their solubility in solvents and their glycosylation degree. For this the crude extract was displayed to solvents with increasing polarity. Five extracts were obtained: crude extract (CrE), hexane extract (HxE; contains mainly lipids), chloroform extract (ChE; contain mainly aglycone flavonoids) ethyl acetate (EAE; glycoside flavonoids) and aqueous extract (AQE; contain proteins and other substances) (Markham, 1982). Extraction yield is shown in **Table 2**, for aqueous decoction of *A.scoparium* and *H.sativus* were found to be in order of 19.15 % and 6.71% (w/w) in term of dry weight.

Table 2. Extraction yields of Aerial parts of plants extracts.

Extract	***A. scoparium***	***H. sativus***
CrE	2.06%	15.47%
ChE	0.25%	1.17%
EAE	3.16%	4.91%
AQE	4.9%	4.85%
DEC	19.15%	6.71%

An extraction technique is that which is able to obtain extracts with high yield and with minimal changes to the functional properties of the extract. Many researchs have demonstrate variations in the biological activities of extracts required using different extraction techniques. The extract ability of solvents depends mainly on the solubility of the compound in the solvent (Dhanani *et al.,* 2017). The major factors affecting the choice of solvent are quantity and quality of phytochemicals to be extracted, the rapid physiologic absorption of the extract, and toxicity of the solvent in the bioassay process (Velavan, 2015). The efficiency of aqueous

extraction increases with temperature because the high-temperature water causes disruption of cells which promote penetration and solubilization of the solvent molecules (Guettaf *et al.,* 2016). Higher extraction yield of water might be due to the fact that water has the ability to dissolve polar as well as nonpolar molecules and some impurities (like carbohydrate and protein) (Mujtaba *et al.,* 2016).

2. Total phenolics, flavonoids and tannins content in plants extracts

Secondary plant metabolites represent major groups of plant constituents that work predominantly as antioxidants or scavenger of free radicals. In this study, the amounts of total phenolics, flavonoids and tannins in *A.scoparium* and *H.sativus* extracts were carried out. The total phenolics in extracts were determined according to Folin-Ciocalteu method using gallic acid as a standard. Whereas, the flavonoids content was estimated by the $AlCl_3$ method and expressed as milligram of qurcetin equivalents per gram of extracts. The results in **Table 3** showed that *A. scoparium* DEC contain the highest amount of phenolic compounds (23.29 ± 1.01 mg GAE/g E), flavonoids (99.77 ± 0.18 mg QE/g E) and tannins amount (113.33 ± 0.08. mg TAE/g E). Whereas, in *H.sativus* it was found that EAE contained the biggest amount of phenolic, flavonoids and tannis compounds with (257.58 ± 0.52 mg GAE/g E),(149.55 ± 1.67 mg QE/g E) and (126.41 ± 0.46mg TAE/g E), respectively.

Table 3. Phenolic, flavonoid and tannins content of plants extracts.

	A.scoparium			*H.sativus*		
Extract	Polyphenols mg GAE/g DW	Flavonoids mg QE/g DW	Tannins mg TAE/g DW	Polyphenols mg GAE/g DW	Flavonoids mg QE/g DW	Tannins mg TAE/g DW
CrE	18 ± 1.6	83.11 ± 0.8	93.63 ± 0.6	193.75 ± 0.25	78 ± 0.66	109.5 ± 0.16
ChE	21.30 ± 0.4	95.94 ± 0.8	68.33 ± 0.9	147.57 ± 0.52	63.55 ± 1.01	117.91 ± 0.28
EAE	3.83 ± 0.36	7.88 ± 0.42	51.77 ± 0.4	257.58 ± 0.52	149.55 ± 1.67	126.41 ± 0.46
AQE	20.74 ± 0.3	19.16 ± 0.1	24.97 ± 0.8	41.91 ± 0.62	76.22 ± 1.01	97.05 ± 0.12
DEC	23.29 ± 1.01	99.77 ± 0.1	113.33 ± 0.08	229.83 ± 0.94	61.55 ±1.38	123.83 ± 1.69

Each value represents the mean ± SD (n = 3). GAE: gallic acid equivalent. QE: quercetin equivalent. TAE: tannic acid equivalent. CrE: crude extract. ChE: chloroform Extract. EAE: ethyl acetate extract. AQE: aqueous extract. DEC: decoction.

The comparison between the amount of phenolic compounds in *A.scoparium*, *H.sativus*, *Atriplex halimus* and *Anabasis articulata* showed that the content found for the two plant extracts in our study were higher than those of *Atriplex halimus* and *Anabasis articulata* (Belyagoubi-Benhammou *et al*., 2014). The differences in total phenolic contents could be due to geographical and environmental variations (climate, location, temperature…), processing methods and other intrinsic and extrinsic factors (genetic, extracting solvent, development stage) (Aryal *et al.,* 2019). The Phenolic compound are more soluble in polar organic solvents due to the presence of a hydroxyl group, morever the genetic diversity, biological and environmental factors could be affected the amount of flavonoids of plants (Kumar and Roy, 2018).

3. Antioxidant activity of *A.scoparium* and *H.sativus* extracts

3.1. *In Vitro* Antioxidant Activity

3.1.1. DPPH-scavenging assay

1, 1 Diphenyl 2- Picryl Hydrazyl is a stable free radical with purple color which turns yellow when scavenged. Antioxidants react with DPPH and reduce it to DPPH-H and as consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant extracts or compounds in terms of hydrogen donating ability. The scavenging ability of the extracts was expressed as IC_{50} value, where the DPPH scavenging activity of the extracts is presented in **Fig.12**.

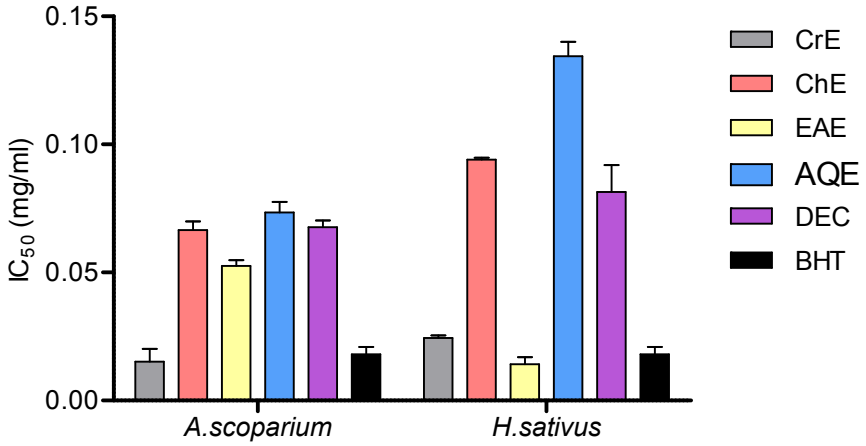


Figure 12.IC_{50} values of *A.scoparium* and *H.sativus* extracts in DPPH free radical scavenging activity. Data were presented as means ± SD (n = 3) compared to BHT as Standard.

Results showed that all extracts exhibited an important activity in scavenging DPPH radicals (**Fig.12**). For *A.scoparium*, the CrE exhibited the highest radical scavenging activity with IC_{50} = 0.015 ± 0.005 mg/ml. whereas, the highest radical scavenging activity for *H.sativus* was exhibited by EAE with IC_{50} = 0.014 ± 0.0027 mg/ml. These results were comparable to BHT (0.018 ± 0.002 mg/ml) used as standard. The results reported by Belyagoubi-Benhammou *et*

al., (2008) showed that ethyl acetate fraction of *A. halimus* pocess a radical scavenging effect against DPPH (IC_{50} = 2.04 mg/ml). These capacities could be attributed to the abundance of flavonols (kaempferol, quercrtin) which constitute the main class of *Atriplex* species (Bylka *et al.,* 2001). Because of the difference in amounts of total phenolic and flavonoids contents, antioxidant activities are more variable between species of plants (inter-species) than within the same species (intra-species) (Podio *et al.,* 2015). The antioxidant effect of plant extract is likely related to the amount of polyphenols present, and may also differ depending on the quality of polyphenols and flavonoids (Podio *et al.,* 2017). The mechanism of the reaction between the antioxidants and DPPH depends on the structural conformation of the antioxidant. DPPH radical can be influenced by the structural characteristics of the antioxidant molecule wich leading to the reduction of DPPH number equal to that of the hydroxyl groups present in the antioxidant compound (Baroni *et al.,* 2018).

3.1.2. ABTS radical cation decolorization assay

ABTS assay is a decolorizing assay, which involves the direct radical into monocation, which has a long wavelength absorption spectrum without involvement of any intermediary radical (Chaput *et al.,* 2016). The low IC_{50} value indicates strong ability of the extract to act as ABTS scavenger. The results shown in **Fig.13** revelated that all plant extracts are effective as ABTS radical scavenger. Similar to DPPH assay, the CrE and EAE exhibited the highest radical scavenging activity with IC_{50} of 0.0066 ± 0.0003 mg/ml and 0.0042 ± 0.0004 mg/ml for *A.scoparium* and *H.sativus*, respectively.

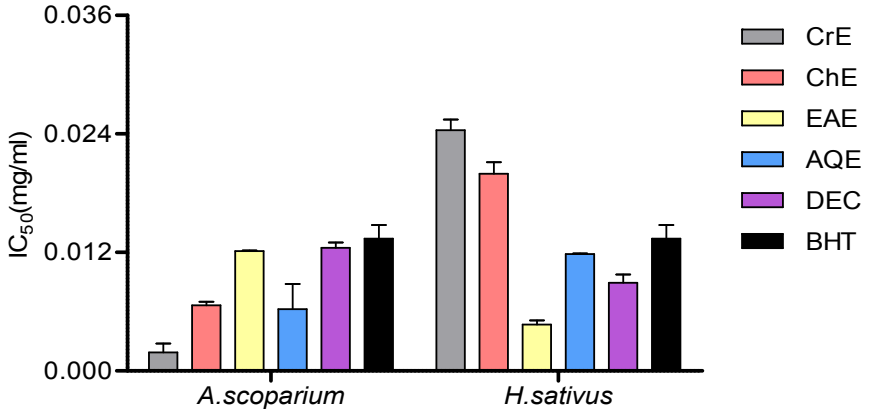


Figure 13.IC_{50} values of *A.scoparium* and *H.sativus* extracts in ABTS radical scavenging activity. Data were presented as means ± SD (n = 3) compared to BHT as Standard.

The results obtained from the present study revealed that CrE and EAE for *A.scoparium* and *H.sativus* exhibited the strongest antioxidant activity in ABTS assay superior to the results found for *Atriplex hamilus* extracts (Belyagoubi-Benhammou *et al*., 2014). The high radical scavenging activity of polyphenols can be attributed to their high degree of hydroxylation of aromatic rings, the arrangement of the hydroxyl group, as well as the number of galloyl group and ortho-hydroxyl groups, on benzene nucleus structure (Ghedadba *et al.,* 2015).

3.1.3. β-Carotene / Linoleic Acid Assay

The test of β-carotene bleaching consists on inhibition of lipid peroxidation by donating a hydrogen atom. The occurrence of biomolecules with antioxidant potential can prevent β-carotene bleaching by neutralizing the linoleate free radicals (Guettaf *et al.,* 2016). *H. sativus* extracts exihibited a significant antioxidant activity as represented in **Fig.14** and **Fig.16**. The order of decreasing of antioxidant activity was EAE (99.12 ±0.41%) followed by CrE (91.73 ± 0.9%), DEC (89.54± 1.2%) and ChE (83.89 ± 1.2%), respectively. As shown in **Fig.14** and **Fig.15**, all *A. scoparium* extracts inhibit the oxidation of β-carotene in important level compared to BHT (97.35%). CrE is the best inhibitor of β-carotene oxidation within 24 hours

(69.35 ± 0.32 %), followed by DEC, EAE and ChE; 67.12 ± 0.37 %, 60.58 ± 0.18% and 56.96 ± 0.11%), respectively.

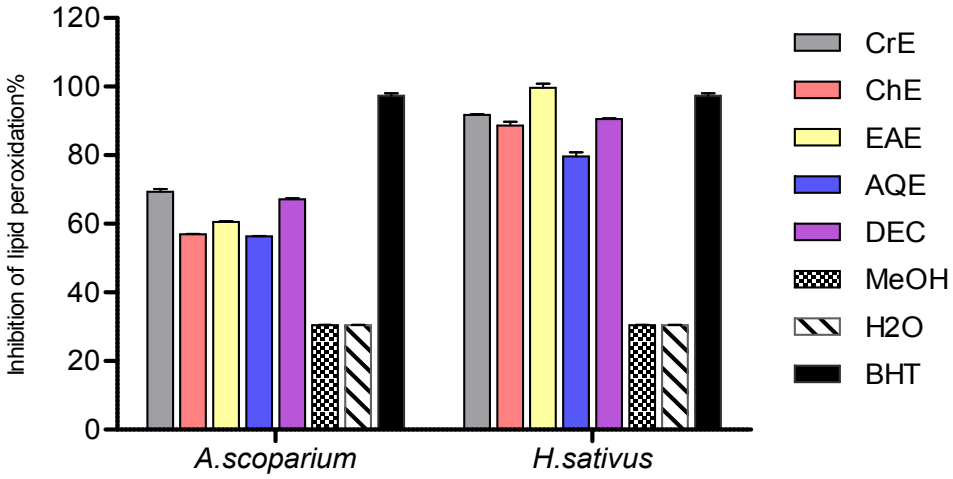


Figure 14.The changes in the percentage of the inhibition of linoleic acid oxidation using *A.scoparium* and *H.sativus* extracts. BHT was used as reference antioxidant. Values are means ± SD (n = 3) compared to BHT as standard.

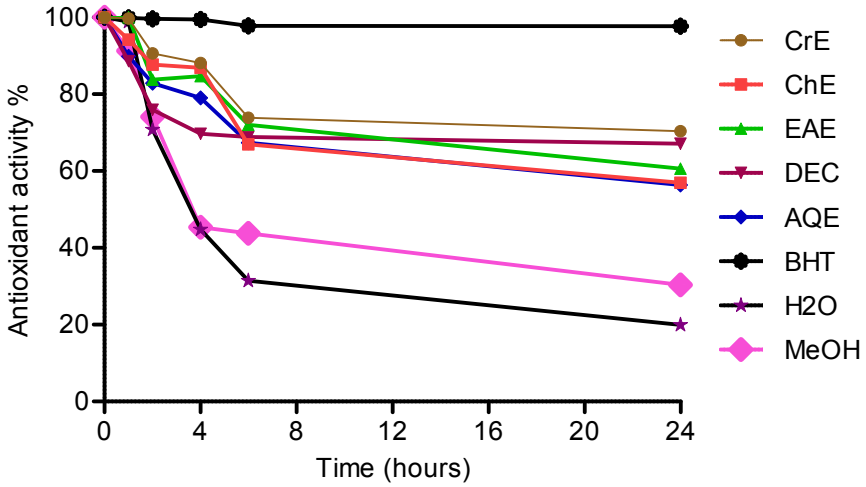


Figure 15.Antioxidant activities of *A.scoparium* extracts measured by β-carotene bleaching method, compared to BHT as a positive control during 24 h. CrE: crude extract, ChE: chloroform extract, EAE: ethyl acetate extract. AQE: aqueous extract, DEC: decoction.

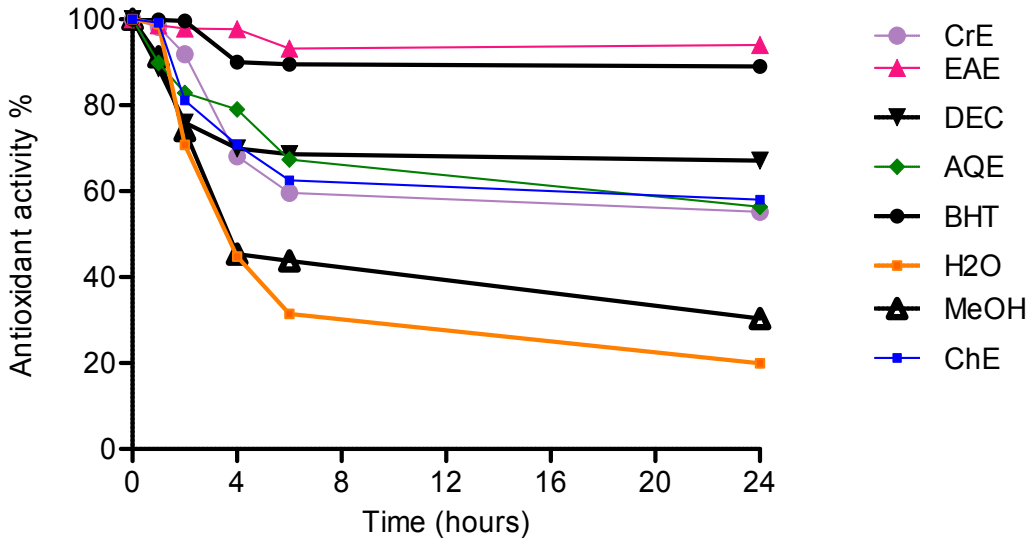


Figure 16.Antioxidant activities of *H.sativus* extracts measured by β-carotene bleaching method, compared to BHT as a positive control during 24 h. CrE: crude extract, ChE: chloroform extract, EAE: ethyl acetate extract. AQE: aqueous extract, DEC: decoction.

The bleaching kinetics of β-carotene is due to the radical scavenging of hydroperoxides formed during the peroxidation of linoleic acid and the inhibition of linoleic acid peroxidation (Soccio *et al.,* 2018). The inhibition of lipid peroxidation is due to a strong occurrence of phenolic compounds such as, phenols, saponins, terpenoids and specially flavonoids and tannins. In fact, flavonoids are accounted for its free radical as well as antioxidant activity (Malki *et al.,* 2017).

3.1.4. Reducing Power

The transformation of ferric iron to ferrous iron was determined as reducing capacity. The reducing capacity of polyphenols may represent an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action, and can be strongly correlated with other antioxidant properties (Benchikh *et al.,* 2018; Cianciosi *et al.,* 2018). As shown in **Fig.17**, For *A. scoparium*, CrE exhibited the best reducing power with EC_{50} value of

0.43 ± 0.003 mg/ml. While, for the second plant the EAE was the best for reducing power potent with EC_{50} = 0.092 ± 0.006 mg/ml, even beter than BHT (EC_{50} = 0.18 ± 0.020 mg/ml) as standard. The results obtained by Benhammou *et al*., (2014) showed that the ethyl acetate extract of *A. halimus* exhibited an interesting antioxidant activity to reduce iron.

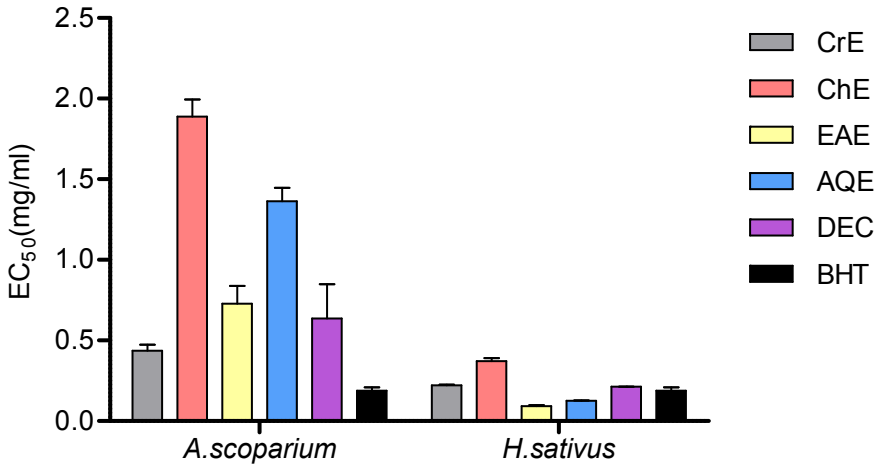


Figure 17. EC_{50} values of *A.scoparium* and *H.sativus* extracts in reducing power assay. Data were presented as EC50 means ± SD (n = 3) compared to vitamin C as standard. CrE: crude extract, ChE: chloroform extract, EAE: ethyl acetate extract, AqE: aqueous extract, DEC: decoction.

The presence of phenolic compounds in these extracts may be explained there reducing power. Relations between Fe^{3+} reducing activity and total phenol content have been reported in the literature (Madoui *et al*., 2018). There might be possibility of synergism with other active constituents, which are nonphenolic in nature. Moreover, the lack of correlation between EC_{50} and flavonoids content may be linked to the assumption that most of the flavonoids were in their glycoside forms and thus less effective compared to the aglycone forms (Anwar *et al.,* 2013).

3.1.5. Ferrous ion chelating activity

The metal chelating capacity is important, since it reduces concentration of the catalyzing transition metal in lipid peroxidation to generate the first few radicals and initiate the radical mediated oxidative chain reactions in biological systems. It has been reported that the

chelating agents form bonds with a metal are effective as secondary antioxidants because they reduce the redox potential (Apak *et al.,* 2016). The present study revealed that all extracts from *A.scoparium* and *H.sativus* showed chelating activity as demonstrated by their effect to inhibit the formation of Fe^{2+} ferrozine complex. As shown in **Fig.18**, *A.scoparium* extracts showed chelating activity in the following decreasing order; CrE (IC_{50} = 0.16 ± 0.04 mg/ml) > DEC (IC_{50} = 0.24 ± 0.003 mg/ml) > EAE (IC_{50} = 0.31 ± 0.13) > AQE (IC_{50} = 0.34 ± 0.001 mg/ml) > ChE (IC_{50} = 1.25 ± 0.04).whereas, the EAE from *H.sativus* showed an excellent chelating activity on ferrous ions with IC_{50} = 0.05± 0.017 mg/ml. From these results, we can deduce that CrE and EAE of the two plants had an important activity even far superior to that of EDTA (IC_{50} = 0.49 ± 0.002 mg/ml).

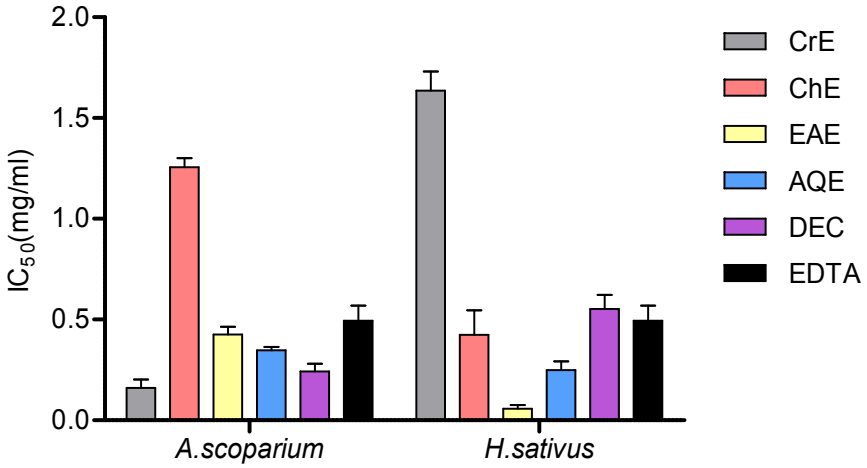


Figure 18. The IC_{50} values of ferrous iron chelating activity of *A.scoparium* and *H.sativus* and EDTA. Values were expressed as mean ± SD (n = 3). CrE: crude extract, ChE: chloroform extract, EAE: ethyl acetate extract, AQE: aqueous extract, DEC: decoction, EDTA: ethylenediamine tetraacetic acid.

According to the literature, several studies demonstrated that the polyphenols and flavonoids can be excellent chelators for iron and copper with a certain structure and functional groups (Papuc *et al.,* 2017). Catechol moieties and combinations of hydroxyl and carbonyl groups present in polyphenols with high affinity for metal ions; however, large differences have been observed in the metal-chelating capacity of different polyphenols (Halake *et al.,* 2018).

Furthermore, it was reported that the compounds with structures containing two or more of the following functional groups: – OH, –SH, –COOH, $–PO_3H_2$, –C=O, $–NR_2$, –S– and –O– have a pronounced metal chelating activity (Freyssin *et al*., 2018).

3.1.6. Hydroxyl Radical-scavenging Assay

Hydroxyl radical is one of the potent reactive oxygen species. In the biological system, It reacts with polyunsaturated fatty acid of cell membrane and causes damage to cell. In the biological systems, It is central to the toxicity produced by ROS as it reacts and inactivates or disrupts proteins, lipids, DNA, and RNA (Wojnárovits *et al.,* 2018). The antioxidants act as a major defence against radical mediated toxicity by protecting the damages caused by free radicals. They mainly function as free radical scavengers, chain breaking antioxidants, metal chelators, reducing agents, oxidative enzyme inhibitors and quenchers of singlet oxygen. (Tijani *et al.,* 2018). The studied extracts showed an important anti-radical effect which is positively related to their amount of total phenolic and flavonoids contents and this might be due to their active hydrogen donor ability of hydroxyl substitution (Lupu and Cremer, 2018). The sequence for *A. scoparium* for this radical scavenging activity, as shown in **Fig. 19,** was as follows in a decreasing order: CrE (IC_{50} = 0.022 ± 0.013 mg/ml) > AQE (IC_{50} = 0.046 ± 0.004mg/ml) >ChE (IC_{50} = 0.051 ± 0.002 mg/ml). For *H. sativus*, it was found that the EAE recorded the highest hydroxyl radical scavenging effect with IC_{50} = 0.026 ± 0.001mg/ml.

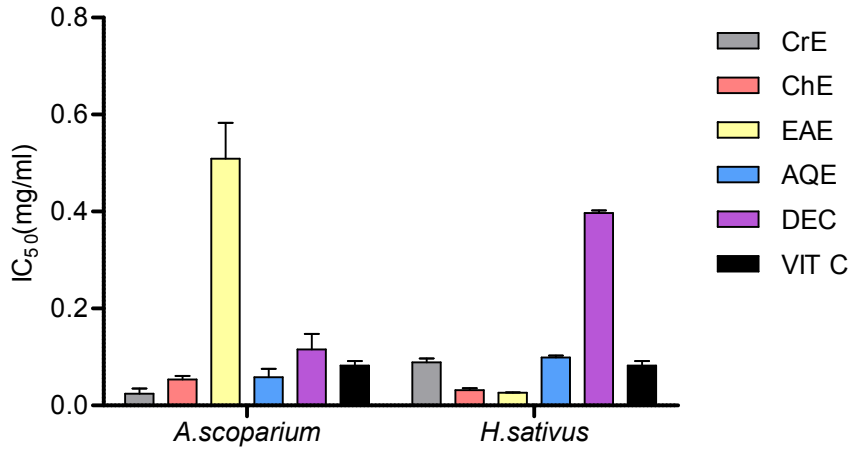


Figure 19. IC_{50} values of different plants extracts in hydroxyl radical scavenging activity. CrE: crude extract, ChE: chloroform extract, EAE: ethyl acetate extract, AQE: aqueous extract, DEC: decoction. Data were presented as IC_{50} means ± SD (n = 3) compared to vitamin C as standard.

Polyphenols and flavonoids may act as antioxidants with two mechanisms: prevention of initiation of oxidation, or as chain breaking antioxidants. Prevention of initiation of oxidation occurs by inhibiting superoxide anion production, degrading hydrogen peroxide and chelating or reducing metal ions, while chain breaking antioxidants act by scavenging radicals, mostly hydroxyl radicals, thereby inhibiting the chain of oxidative events that leads to damage of lipid membranes, proteins and DNA (Singh *et al.,* 2014; Santos-Sánchez *et al.,* 2019).

3.1.7. Hydrogen peroxide scavenging activity

Hydrogen peroxide itself is not very reactive, but it can sometimes be toxic. Hydrogen peroxide is a weak oxidizing agent that inactivates a few enzymes directly, usually by oxidation of essential thiol-groups (- SH). It can cross cell membranes rapidly; once inside the cell, it can probably react with Fe^{2+} and possibly with Cu^{2+} ions to form hydroxyl radicals and this may be the origin of many of its toxic effects (Ngonda *et al*., 2013; Mailloux, 2018).AS represented in **Fig.20**, results showed that the strongest scavenging effects were registered

with DEC extract and CrE of *A. scoparium* and *H. sativus* with IC_{50}=0.29 ± 0.002mg/ml and IC_{50}=0.19 ± 0.03mg/ml, respectively.

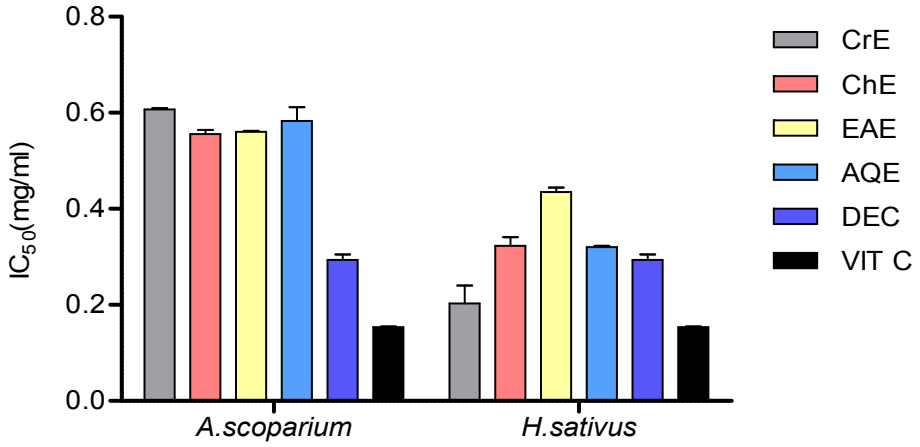


Figure 20. The IC_{50} values of H_2O_2 scavenging activity of *A.scoparium* and *H.sativus*. Values were expressed as mean ± SD (n = 3). Crude extract, ChE: chloroform extract, EAE: ethyl acetate extract, AQE: aqueous extract, DEC: decoction.

According to literature, many studies demonstrate the positive relation between total phenols and H_2O_2 scavenging activity appears to be the trend in many plant species (Ruskin *et al.,* 2017), the present study revealed a significant correlation between both total phenols and flavonoids contents and IC_{50} of H_2O_2 scavenging activity suggested that phenolics compounds were responsible for this antioxidant activity.

3.1.8. Anti-hemolytic assay

a. AAPH induced hemolysis

Erythrocytes are considered as major target for the free radicals owing to the presence of both high membrane concentration of polyunsaturated fatty acids (PUFA) and the oxygen transport associated with redox active hemoglobin molecules, which are potent promoters of activated oxygen species (Belcher *et al.,* 2016 ; Kuhn *et al.,* 2017). AAPH is a peroxyl radical initiator

that generates free radicals by its thermal decomposition and will attack erythrocytes, to induce the chain oxidation of lipid and protein, disturbing the membrane organization and eventually leading to hemolysis (Ramchoun *et al.,* 2015). In the present study, all extracts of *A. scoparium* and *H. sativus* exhibited potent anti-hemolytic activity and revealed important values of HT_{50}, among which DEC and CrE of studied plants displayed the highest anti-hemolytic effect within the concentration range used (0.125 to 1 mg/ml) and they were significantly superior than aspirin (HT_{50} = 118.32 ± 1.13 min) as positive standard (**Tables 4** and **Table 5**).

Table 4. The HT_{50} values of *A. scoparium* anti-hemolytic activity of, control and aspirin.

	A. scoparium HT_{50} (min)			
C (mg/ml)	1	0.5	0.25	0.125
Aspirin	118.32 ± 1.13	/	/	/
Control	85.40 ±1.44	/	/	/
CrE	136.6 ± 0.96 ***	130.91 ± 1.11***	112.80 ± 1.05***	109.20 ± 0.25***
ChE	122.93 ± 0.96***	112.54 ±1.24***	99.53 ± 0.51***	98.50 ± 0.43***
EAE	118.45 ± 0.73 ns	115.63 ± 0.96 ns	114.10 ± 0.16 **	108.01 ± 0.81***
AQE	135.21 ± 1.25***	129.25 ± 1.52***	118.91 ± 0.22 ns	102.35 ± 1.57***
DEC	164.18 ± 1.20***	132.18 ± 1.15***	130.95 ± 1.44***	118.23 ± 0.2 ns

Values were expressed as mean ± SD (n = 3). (**p < 0.001, ***p < 0.0001, ns : non significant). CrE: crude extract, ChE: chloroform extract, EAE: ethyl acetate extract, AQE: aqueous extract, DEC: decoction.

Table 5. The HT_{50} values of *H. sativus* anti-hemolytic activity, control and aspirin.

	H.sativus HT_{50} (min)			
C (mg/ml)	1	0.5	0.25	0.125
Aspirin	118.32 ± 1.13	/	/	/
Control	85.40 ±1.44	/	/	/
CrE	147.03 ± 1.62 ***	134.35 ± 1.5***	125.42 ± 0.28***	116.56 ± 1.2***
ChE	127.65 ± 1.04***	118.36 ±1.11***	115.33 ± 0.61***	114.99 ± 1.17***
EAE	133.16 ± 1.4 ns	118.03 ± 1.28 ns	117.04 ± 0.58 **	105.641 ± 0.21***
AQE	123.75 ± 1.5***	117.16 ± 0.81***	109.32 ± 1.22 ns	101.35 ± 0.67***
DEC	121.24 ± 0.81***	118.13 ± 1.11***	117.62 ± 0.82***	104.56 ± 0.79 ns

Values were expressed as mean ± SD (n = 3). (**p < 0.001, ***p < 0.0001, ns : non significant). CrE: crude extract, ChE: chloroform extract, EAE: ethyl acetate extract, AQE: aqueous extract, DEC: decoction.

Our results indicated that there is a significant positive correlation between total phenolics content and HT_{50} of anti-hemolytic activity. Peroxyl radicals attack erythrocyte membrane components, such as proteins and lipids, causing changes in their structure and function, which may result in hemolysis. Recent study showed that antioxidant compound can protect both hemoglobin degradation and erythrocyte membrane during AAPH-induced hemolysis. The flavonoids and the widely distributed subgroup of polyphenols had beneficial effect on the erythrocyte membrane stability, where its can be incorporated into the erythrocyte membranes. (Ramchoun *et al*., 2015; Lins *et al.,* 2018).

b. FeSO4-induced hemolysis

Inhibitory effect of extracts on ferrous ion induced hemolysis was illustrated in **Fig.21**. The present study tested the effect of the extracts on human red blood cells (RBCs). All extracts exhibited satisfactory inhibitory properties against hemolysis at low concentration. The *A. scoparium* CrE inhibited hemolysis with 88.57 ± 0.099% followed by ChE and EAE.

Whereas, the maximum anti-hemolytic activities of *H. sativus* was registered with EAE (69.55 ± 0.34%) followed by DEC.

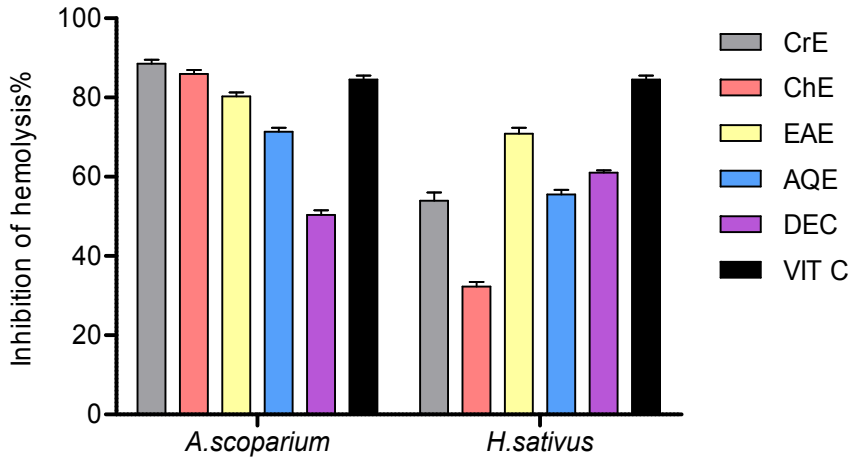


Figure 21. Inhibition percentage values of *A.scoparium* and *H.sativus* in antihemolytic activity using $FeSO_4$. Data were presented as means ± SD (n = 3) compared to ViT C as standard.

The Erythrocyte contains a conjugate protein in the form of hemoglobin wich it main function is the binding and releasing oxygen and carbon dioxide. The membrane of RBC helps in maintenance of structure of erythrocytes. RBCs as oxygen carriers are continuously exposed to high oxygen tension. Oxidative stress, which decrease the antioxidant capacity, irreversibly damages erythrocytes, resulting in their eventual damage by hemolysis and their removal by circulation (Nematbakhsh *et al.,* 2013; Maurya *et al.,* 2015), The red blood cell membrane contains high amount of polyunsaturated fatty acids, they are vulnerable to oxidative stress. The erythrocyte membrane is rich in phosphatides containing proteins present outside the membrane. The proteins present in the outer membrane are easy target for free radicals which results in the formation of MDA. Increased MDA level results in alterations of the cell membrane polarity, charge sharing across lipid phase surface and oligomer formation (Taher *et al.,* 2013; Rocha *et al.,* 2015). The abilities of extracts to scavenge free radicals and bind iron were further confirmed by inhibition of ferrous ion-

induced hemolysis (Mladenov *et al.,* 2015). The binding of polyphenols to RBC membrane matrix, in close proximity to tryptophan residues, results in inhibition of lipid peroxidation and subsequent antihemolytic activity (Krishna *et al.,* 2018).

3.2. In vivo study

3.2.1. Acute Toxicity

According to OECD guideline 425, the present study was performed. The observations of animals after treatment with the extract revealed that the ASE and HSE did not cause any changes in animal behavior during the study period. Moreover, no mortality was registered during 14 days of treatment. This means that the LD_{50} is higher than 5000 mg/kg BW. The body weight increased gradually throughout the study period. Statistical analysis of body weight did not reveal any significant difference between treated and untreated animals (**Fig. 22**).

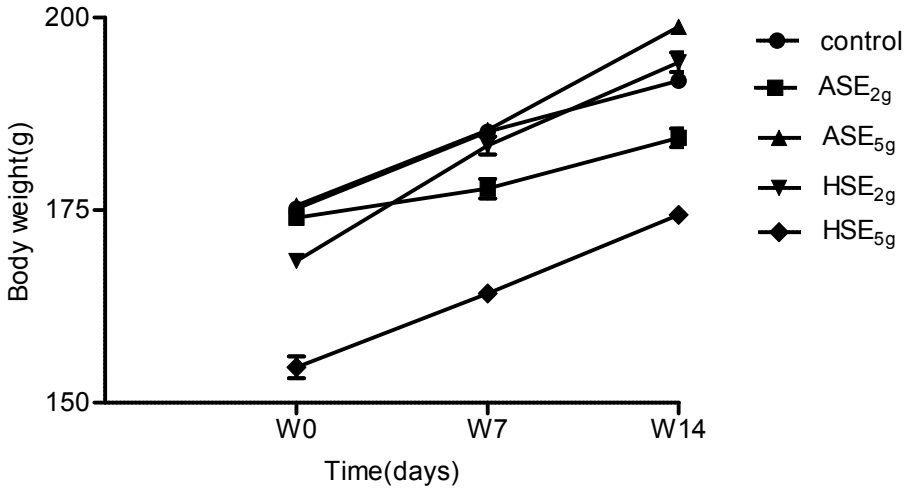


Figure 22. Increase in Body weight of rats treated orally with *A.scoparium* and *H.sativus*. Values were expressed as mean ± SD, n= 5 animals/group.

3.2.1.1. The effect of treatment with ASE and HSE on relative organ weights of rats

The treatment of animals with pharmacological doses of ASE and HSE did not produce any significant changes in relative weight of the different organs: heart, lungs, stomach, liver, kidneys and spleen (**Table 6**).

Table 6. Relative organs weight of rats treated with ASE and HSE.

Groups	Control	ASE 2g/kg BW	ASE 5g/kg BW	HSE 5g/kg BW	HSE 5g/kg BW
Heart	2.76 ± 0.07	3.40 ± 0.42 ^{ns}	3.30 ± 0.089 ^{ns}	3.47 ± 0.06 ^{ns}	3.68 ± 0.098 ^{ns}
Lung	6.75 ± 0.89	9.67 ± 0.65 ^{ns}	8.39 ± 1.26 ^{ns}	7.16 ± 0.24 ^{ns}	8.91 ± 0.97 ^{ns}
Stomach	6.72 ± 0.22	7.54 ± 1.44 ^{ns}	6.84 ± 0.41 ^{ns}	6.86 ± 0.47 ^{ns}	7.81 ± 1.53 ^{ns}
Liver	37.07± 2.62	38.25 ± 4.47 ^{ns}	34.03 ± 2.43 ^{ns}	35.95 ± 2.75 ^{ns}	40.10 ± 4.76 ^{ns}
Kidney	5.31 ± 0.33	7.10 ± 0.76 ^{ns}	6.38 ± 0.45 ^{ns}	6.3 ± 0.32 ^{ns}	7.43 ± 0.39 ^{ns}
Spleen	3.19 ± 0.22	4.75 ± 2.48 ^{ns}	3.45 ± 0.33 ^{ns}	4.45 ± 2.47 ^{ns}	3.63 ± 1.22 ^{ns}

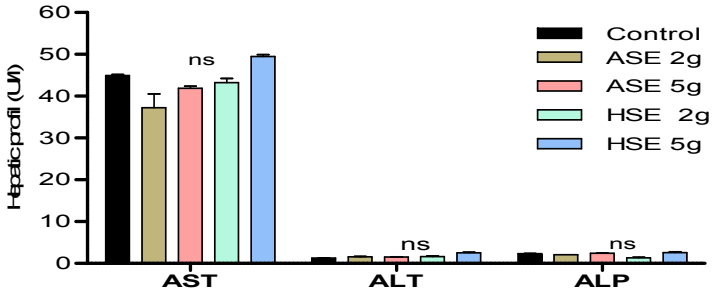
Values expressed as mean±SD (n=5). ns: non significant compared to control group values.

3.2.1.2. Effect of plant extract on some biochemical parameters.

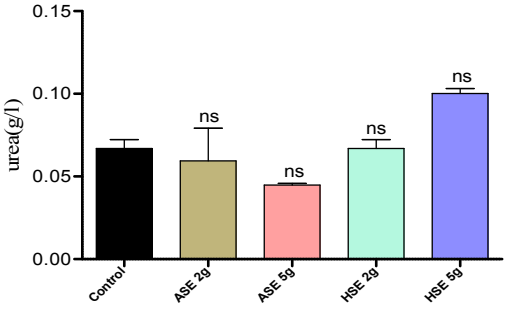
The evaluation of some biochemical parameters; AST, ALT, ALP, urea, creatinine, total cholesterol, HDL, LDL and Triglycerides indicates that there were no significant differences between treated and untreated animals (**Fig. 23**).

The most frequently and specific indicators of hepatic damage are the activity of amino-transferase, which represent a markers of hepatocellular necrosis. Moreover the general increase in these enzymes in the plasma is linked to pathologic changes in cell membrane permeability or hepatic cell interruption (Orji *et al*., 2016). ALP plays two major roles: in phosphate metabolism and in the prevention of the external membrane from damages (Toshimi and Keiichi, 2019).In the present study (**Fig. 23**), no changes in ALT, AST and ALP activities suggest that the oral administration of ASE and HSE extracts did not alter the hepatocytes function.

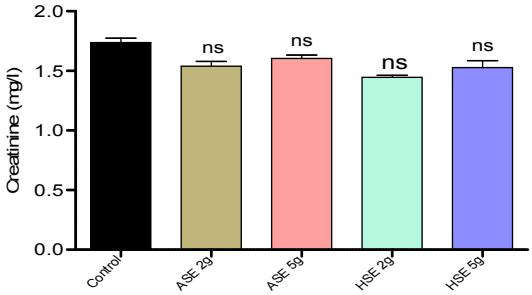
(A)



(B)



(C)



(D)

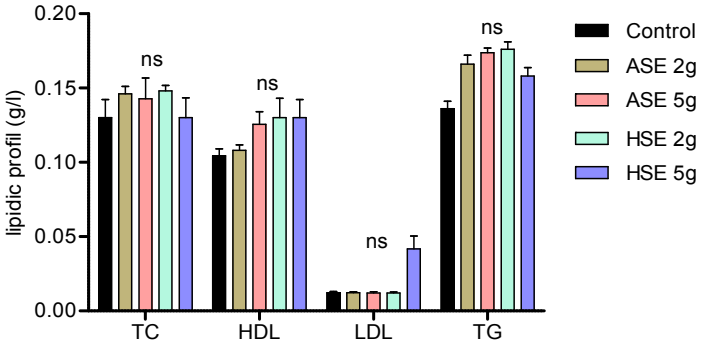
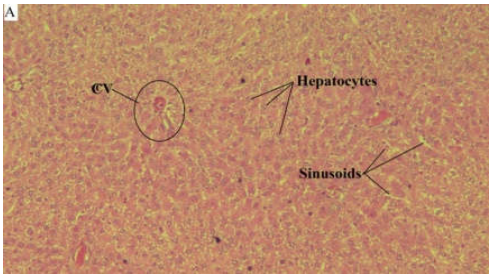


Figure 23. Biochemical parameters of control and rats treated with ASE and HSE measured during the acute toxicity. (A): hepatic profil, (B)urea, (C)creatinine, (D) lipidic profil. Values were expressed as mean ± SEM (n = 5). (ns: no significant). ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatases, TC: total cholesterol, TG: triglyceride.

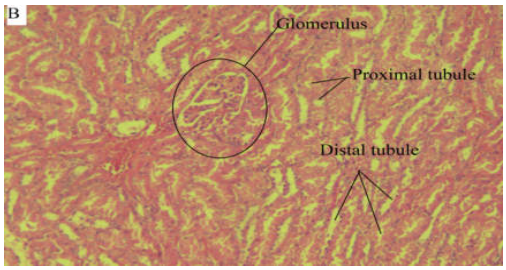
The liver is the site of cholesterol degradation and its major site of synthesis (Afonso *et al.,* 2018). In the same perspective, the liver also controls glucose synthesis and generates free glucose from hepatic glycogen stores (Petersen *et al.,* 2017). As no significant changes were observed in lipidic profil in this study, this suggests that ASE and HSE extracts have no

effects on the lipid metabolism of the rats treated. There was also no significant change in urea and creatinine, between the treated groups and the control group. Creatinine is nitrogenous end products of metabolism removed from the blood by the kidneys (Seki *et al.,* 2019). It is the most commonly used serum biomarkers of renal damage (den Bakker *et al.,* 2018).

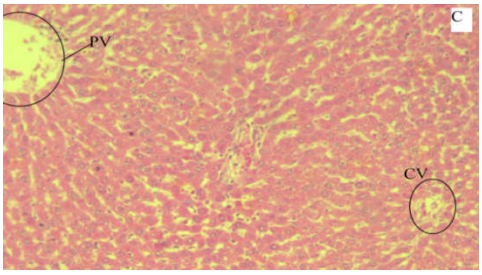
3.2.1.3. Effect of traetment with ASE and HSE on hepatic and renal tissue



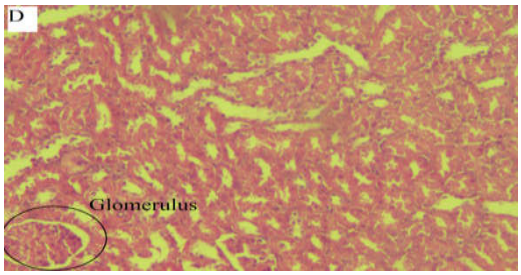
(A) Liver section of rats treated with ASE 2g (x100)



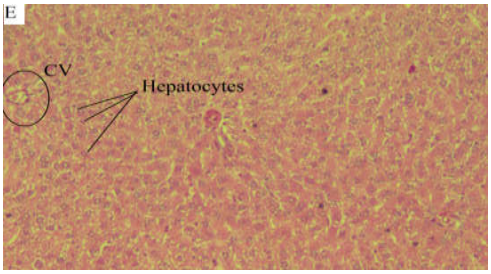
(B) Kidney section of rats treated with ASE 2g (x100).



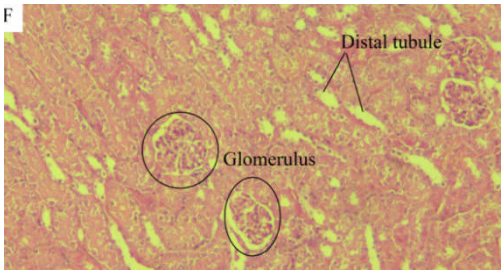
(C) Liver section of rats treated with ASE 5g (x100)



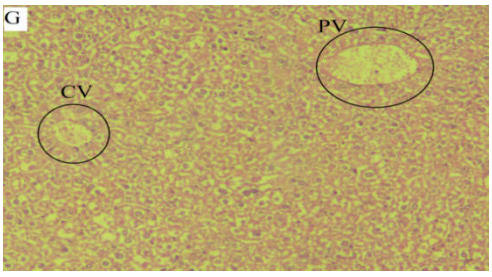
(D) Kidney section of rats treated with ASE 5g (x100)



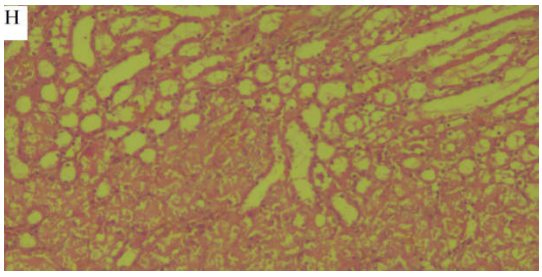
(E) Liver section of rats treated with HSE 2g (x100)



(F) Kidney section of rats treated with HSE 2g (x100).



(G) Liver section of rats treated with HSE 5g (x100)



(H) Kidney section of rats treated with HSE 5g (x100).

Figure 24. Sections of liver and Kidney tissues of rats treated with ASE and HSE. CV: central vein. PV: portal vein.

The observation of histological sections of the liver and kidneys of treated rats with plants extracts showed a preservation of the cellular architecture of the two organs.

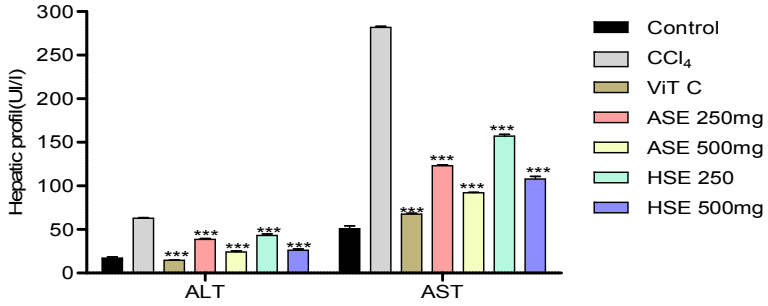
3.2.2. Hepatoprotective assay

3.2.2.1. Effect of ASE and HSE on biochemical parameters in CCl_4-induced hepatotoxicity

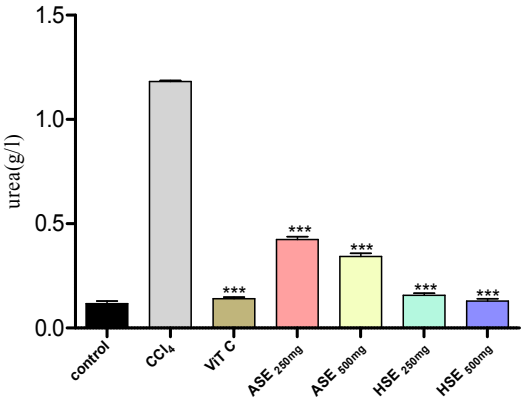
The evaluation of urea and creatinine levels indicates that there were a significant difference ($p<0.0001$) between treated animals with ASE/HSE, ViT C and treated rats with CCl_4. There was also a significant decrease regarding AST and ALT levels for treated animals compared to group treated with CCl_4 (**Fig.25**).

Some enzymes such as ALT,AST and ALP can be used as sensitive indicator of hepatocellular activity (Ezeja *et al.,* 2014).**Fig.25** revelated that a significant diminution dose-dependent ($p<0.0001$) was registred in the level of plasmatic AST ,ALT, urea and creatinine in the groups of rats treated with combination of ASE+CCl_4 and HSE+CCl_4 at doses of 250, 500 mg/kg and ViT C+CCl_4 at dose of 200 mg / kg compared to the group treated with only CCl_4. The increase in plasma cellular enzymes indicates a sign of injury in hepatic tissue (Mohamadi Yarijani *et al*., 2019). The assessments of ALT, AST are used as diagnostic marker to indicate liver damages induced by hapatotoxins (Sriuttha *et al*., 2018). Moreover, many studies evaluated the scientific basis of herbs and fruits rich in natural antioxidants on the hepatoprotective activity associated to decrease the accumulation of fat, lipid peroxidation, and free radicals scavenging effects of these plants (Gangwar *et al.,* 2014; Ahangarpour *et al*., 2016).The CCl_4 is distributed and deposited to organs such as the liver, brain, kidney, lung and heart (Mohamed *et al.,* 2014). It has also been reported that the systemically administration of CCl_4 in rats was distributed at higher concentrations in the liver than in the kidney (Sahreen et a, 2015). Since the kidney has high affinity for CCl_4 and contains cytochrome P450 predominantly in the cortex, CCl_4 is extensively metabolized in the kidney generating more reactive metabolites (Abd El-mohsen Ali *et al.,*2014).

(A)



(B)



(C)

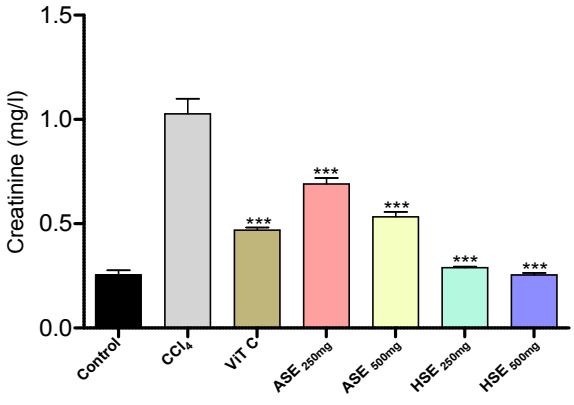


Figure 25. Effects of treatment with ASE and HSE on some biochemical parameters compared to group treated with CCl_4. (A) hepatic profil, (B) urea, (C) creatinine. Values were expressed as mean ± SEM ($p < 0.0001$). ALT: alanine aminotransferase, AST: aspartate aminotransferase.

3.2.2.2. Effect of ASE/HSE on plasma antioxidant capacity

a. DPPH radical scavenging

The DPPH method is largely used to evaluate the antioxidant activity of plant extracts and food. It is also used to evaluate the plasmatic antioxidant capacity which is expressed as a percentage inhibition. The present study aim to evaluate the effectiveness of an antioxidant treatment, the scavenger effect against DPPH radical was assessed; the obtained results (**Fig.26)** showed that oral administration of ASE, HSE and ViT C leads to significant increase in plasma antioxidant capacity compared to group treated with CCl_4 20.46 ± 4,19%.

b. reducing power

In the reducing power assay, the presence of antioxidants in the sample would result in the reducing of Fe^{3+}–Fe^{2+} by donating an electron. The results obtained in present study (**Fig.26**)

showed that the oral administration of ASE and HSE increased plasma antioxidant capacity. This increase is statistically significant compared with CCl_4 group.

The results indicate that the treatment with selected plants extracts improve free radical scavenging activity and reducing capacity regarding the importance of these activities of the ASE and HSE determined *in vitro* and these effects may be primarily attributed to the polyphenolic contents. According to the litterature, many researchs demonstrate the significant correlation between the measured plasma antioxidant capacity and the total phenol content of plant extracts (Boumerfeg *et al.,* 2012; Vicente *et al.,* 2014; Avila-Nava *et al.,* 2014). Whereas, *in vivo* these activities are limited by several factors such as low bioavaibility and metabolic conversion of the absorbed polyphenols (plasma concentration rarely exceeds 1µM) (Veskoukis *et al.,* 2012).

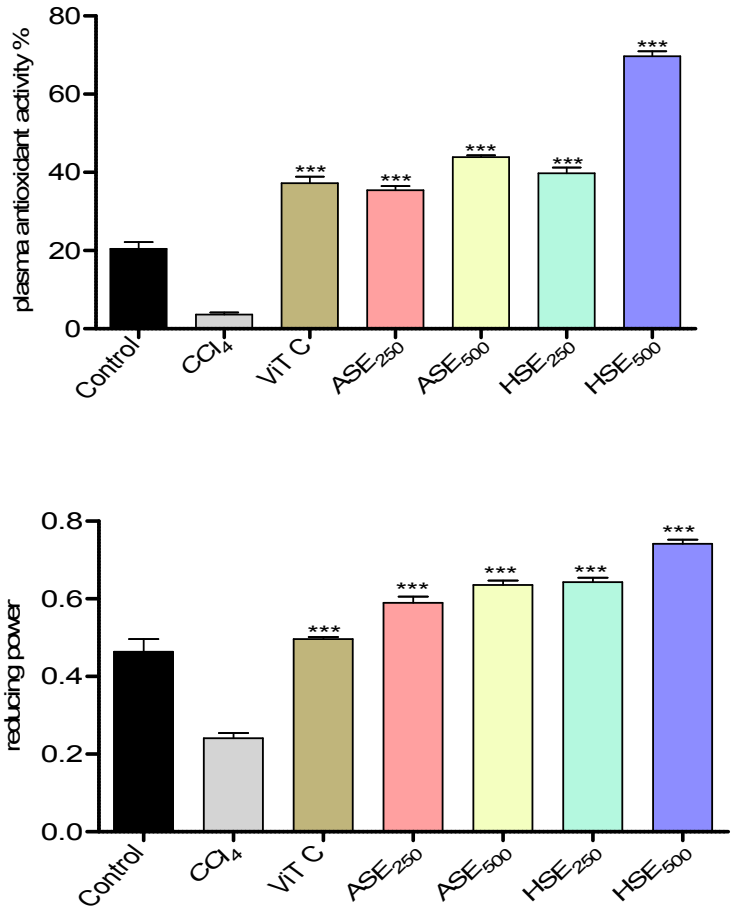


Figure 26. Effect of different concentrations of ASE, HSE and ViT C on DPPH radical scavenging and reducing power activities. Data were presented as means ± SEM (n = 6). (*** $p < 0.0001$) compared to group treated with CCl_4 alone.

3.2.2.3. Effect of treatment with ASE /HSE on reduced Glutathione (GSH)

GSH level of plant extract on the experimental groups are shown in **Fig.27**. GSH levels in the group treated with only CCl_4 (12.98 ± 2.82µmol/g of tissue) was significantly lower than contol group and pretreated group with ViT C (positive normal) (19.65 ± 1.99 µmol/g of tissue). The pretreated rats with ASE (500mg/kg) and HSE (500 mg/kg) recorded a high GSH levels comparable to GSH levels for CCl_4 treated group (negative control) with 35.49 ± 2.74, and 44.68 ± 4.3 µmol/g of tissue, respectivelly. GSH antioxidant system plays central role in cellular defense against reactive free radicals especially in redox status balance. Morever, it can serve as a marker of decrease in antioxidant defense, susceptibility, early and reversible tissue damage (Jain and Singhai, 2012). Furthermore, it was reported that the increase in the levels of GSH may possibly be due to de novo synthesis and/or GSH regeneration (Nasir *et al.,* 2013) via glutathione reductase activation and inhibiting free radical formation.

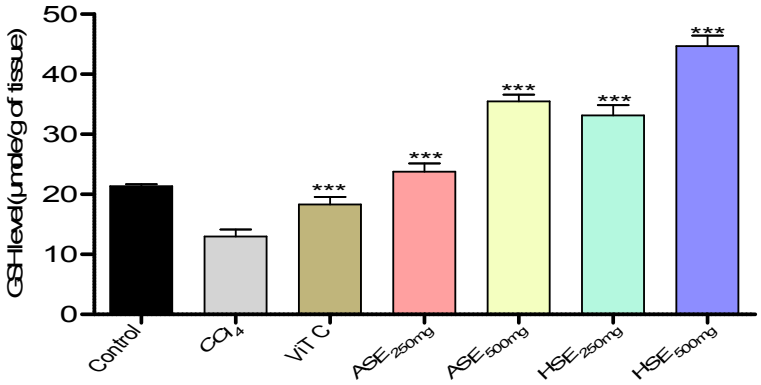


Figure 27. Effect of treatment with ASE, HSE and vitamin C on reduced glutathione level in liver of rats. Values are given as means ± SEM (n=6). (*** $p < 0.0001$) compared to group treated with CCl_4 alone.

3.2.2.4. Effect of treatment with ASE and HSE on Catalase (CAT)

Changes in the activity of CAT in livers of rats was investigated in the present study (**Fig.28**). The CAT activity has undertaken a significant changes dose-dependent during treatment with ASE and HSE at doses (250 and 500 mg/kg). Morever, no change was noticed in positive control group (7.53 ± 1.73 U/g of tissue) comppared to negative control (6.28 ± 0.86 U/g of tissue). Catalase activity varies greatly from tissue to tissue, the highest activity is found in liver and kidney, whereas the lowest activity is seen in the connective tissue (Mitjavila and Moreno, 2012). Inhibition of this enzyme may enhance sensitivity to free radical-induced cellular damage. Therefore reduction in the activity of CAT may leads to deleterious effects as a result of superoxide and hydrogen peroxide assimilation (Merghem *et al.,* 2019). The mechanisms underlying the activation of antioxidant enzymes by polyphenols are not fully understood but a number of studies indicated also an association between polyphenols and antioxidant enzyme expression via nuclear factor-erythroid-2–related factor 2 (Nrf 2) activation in vivo and in various cultured cell lines (Oliveras-López *et al.,* 2014; Nakayama *et al.,* 2015).

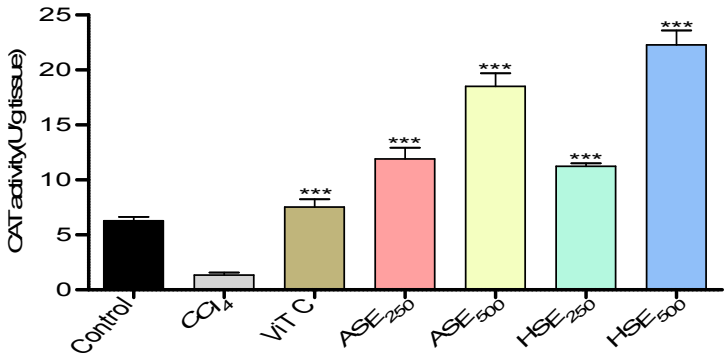


Figure 28. Effect of treatment with ASE, HSE and vitamin C on catalase activity in liver of rats. Values are given as means ± SEM (n=6). (*** $p < 0.0001$) compared to group treated with CCl_4 alone.

3.2.2.5. Effect of treatment with ASE and HSE on MDA

Lipid peroxidation is an auto-catalytic, free-radical mediated, destructive process, where by polyunsaturated fatty acids in cell membranes undergone degradation to form lipid hydroperoxides (Sardar et al, 2019). The present study demonstrated that the level of liver

MDA in CCl_4 treated group (263.69 ± 8.70nmole/ g of tissue) was significantly higher than the control group ((24.76 ± 2.65nmole/ g of tissue). According to the results represented in **Fig.29**, a significant decrease of MDA level was observed in livers of rats treated with Vit C (200 mg/kg), ASE and HSE at doses of 250 and 500 mg/kg. In CCl_4 treated group, the increase in MDA level suggests enhanced peroxidation leading to tissue damage and failure of the antioxidant mechanisms to prevent the production of excessive free radicals. Our result came in agreement with other reporters who demonstrated that CCl_4 significantly increased the hepatic MDA (Liguori *et al.,* 2018; Kawamura *et al.,* 2018). Our findings also suggest the protective effects of plants extracts against CCl_4-induced oxidative stress, could be attributed mainly to the presence of high content of phenolics and flavenoids which have profound antioxidant activity (Mazani *et al.,*2018). These compounds could scavenge the free radicals of CCl_4 generated through cytochrome P450 enzyme system thereby diminished the oxidative injuries (Kasote *et al.,* 2015). Histopathological examinations are in agreement with biochemical analysis.

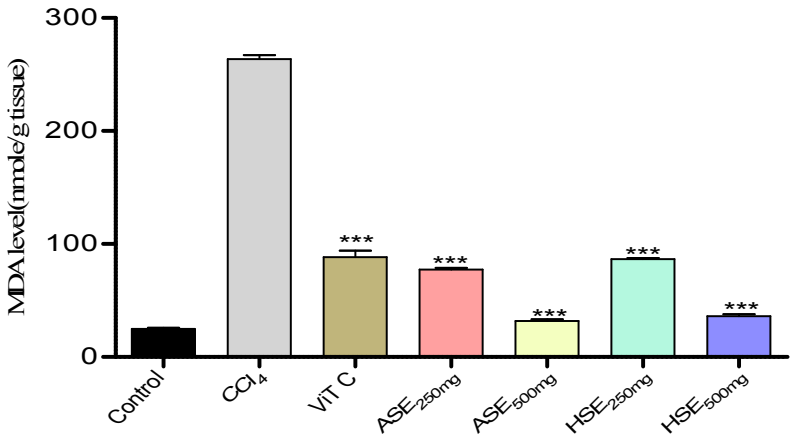
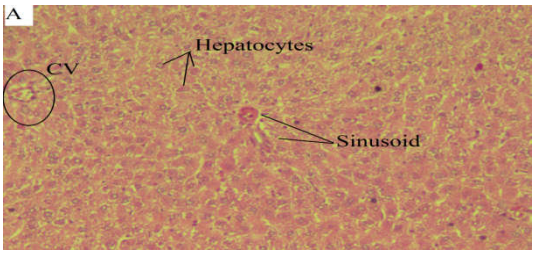
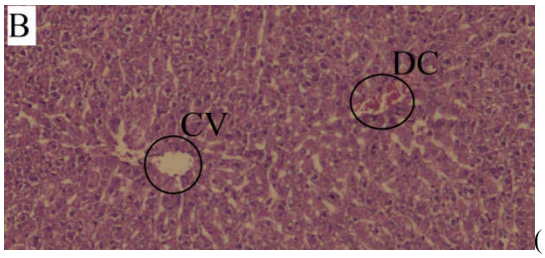


Figure 29. Effect of treatment with ASE, HSE and vitamin C on MDA level in liver of rats. Values are given as means ± SEM (n=6). (*** $p < 0.0001$) compared to group treated with CCl_4 alone.

3.2.2.6. Effect of treatment with ASE/HSE on hepatic tissue

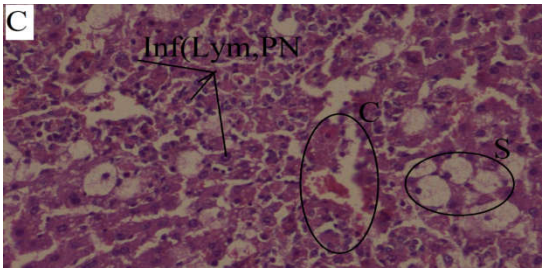
Normal liver cellular architecture was showed in the histological observation of the native control. Histological changes and severe liver damages, infiltration of inflammatory cells, necrosis and congestion, caused by CCl_4 treatment was noticed compared to tissue of treated groups with ASE and HSE at two doses and vitamin C, where alterations were minimized and the liver injuries were improved.

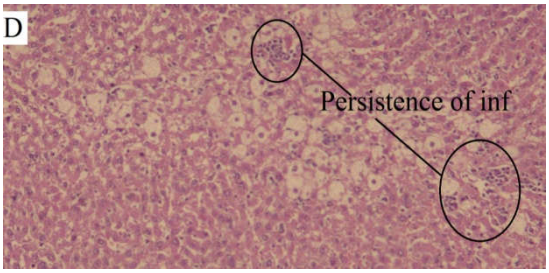




A) Liver section of Control animal (x100)

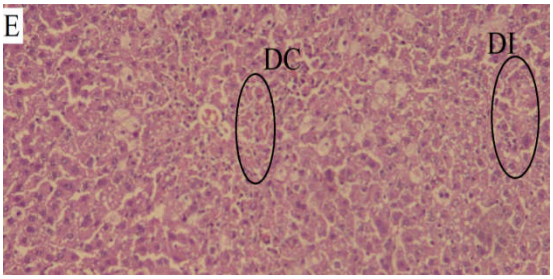
(B) Liver section of rats treated with ViT C (x100)

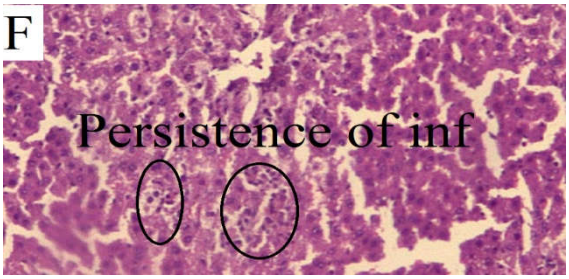




(C) Liver section of rats treated with CCl_4 (x100).

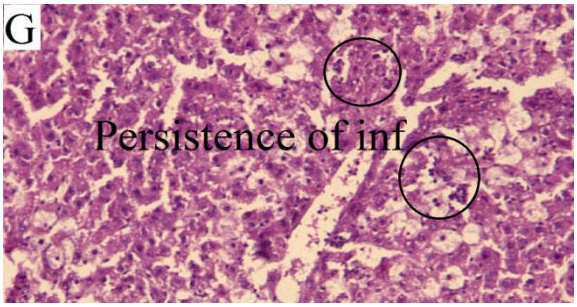
(D) Liver section of rats treated with CCl_4+ASE (250mg/kg) (x100).





(E) Liver section of rats treated with CCl_4+ASE (500mg/kg) (x100).

(F) Liver section of rats treated with CCl_4+HSE (250mg/kg) (x200).



(G) Liver section of rats treated with CCl_4+HSE (500mg/kg) (x200).

Figure 30. Sections of liver tissue of rats treated with ASE, HSE and ViT C compared to liver section of rats treated with CCl_4 alone. DI: discrete inflammation, CV: central vein. DC: discrete congestion, C: congestion, S:steatos, Inf (Lym,PN): inflammation (lymphocytes and polynuclear).

4. Xylene induced ear oedema

Xylene-induced mice ear oedema reflects the oedematization occurred during the early stages of acute inflammation, which is probably related with the release of inflammation mediators (Torres-Rêgo *et al.,* 2016).The results obtained are summarized in **Fig.31**. As shown, ASE and HSE at different doses reduced the pourcentage of the ear oedema in a dose related manner compared to control (16.60 ± 1.69%).

The xylene ear edema model shows the evaluation of anti inflammatory steroids and is less sensitive to non-steroidal anti inflammatory agents (Yasmen *et al.,* 2018). Severe vasodilation, edema changes of skin and infiltration of inflammatory cells are detected as signs of acute inflammation after topical application of xylene (Vetriselvan *et al.,* 2013) . The increased thickness of ear tissues is caused by these histopathological changes .In the present investigation, the plant extract significantly inhibited the xylene – induced increases in ear oedema in a dose dependent manner. This inhibition capacity of the plant extract can be regarded as the evidence of anti-inflammatory efficacy through reducing vasodilation.

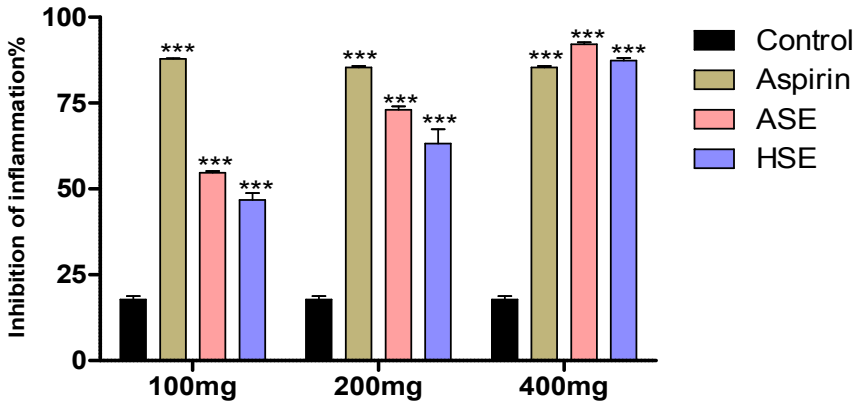


Figure 31. Effect of treatment with ASE and HSE on xylene-induced ear oedema. Values are given as means ± SEM (n=5). (*** $p < 0.0001$) compared to control group.

5. Ethanol- induced ulcer Gastric

Gastric ulceration is a benign lesion of the mucosal epithelium upon exposure of the stomach to excess acid and aggressive pepsin activity (Richard *et al.,* 2017). Ethanol-induced gastric ulcer is a usual, convenient animal model for the investigation of gastroprotective drugs (Escobedo-Hinojosa *et al.,* 2018). The role of ethanol is explained by its ulcerogenic effect. Ethanol progresses disarrangement in mucosal microcirculation and ischemia, produces free radicals, and releases endothelium (Fahmi *et al.,* 2019). the significant increase in ulcer index following oral administration of ethanol in the ulcerated rats may be attributed to either free radicals formation or inhibition of prostaglandin synthesis (Sharifi-Rad *et al.,* 2018). Decreased prostaglandin level has been attributed to impaired gastro protection and increased gastric secretion which are important events in the etiology of mucosal ulceration (Adefisayo *et al.,* 2018). In the present study, the oral administration of ASE and HSE displayed protective effects against ethanol induced gastric lesions. Flavonoids are found to increase mucosal Prostaglandin content. Decrease histidine decabonxylases, and are free radical Scavengers. Tannins are known to tan the outer layer of the mucosa and render it less permeable and more resistant to chemical and mechanical injury or irritation (Richard *et al.,* 2017).

Table 7.Effect of ASE and HSE on Ethanol Induced Gastric Ulcer in Rats.

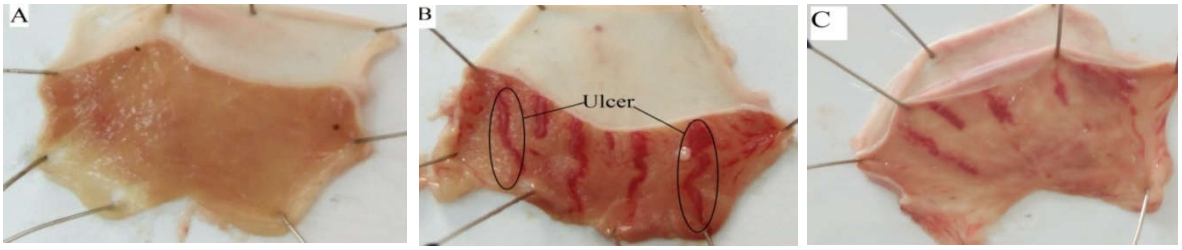
Treatment	Mean ulcer index	Preventive index %
EtOH (1ml)	5.57 ± 0.20	/
Ranitidine (50 mg/kg)	1.14± 0.20***	79.48
ASE (50mg/kg)	3.57 ±0.99***	35.89
ASE (150 mg/kg)	1.71± 0.80***	69.23
ASE (300 mg/kg)	1.57 ± 0.26***	71.79
HSE (50mg/kg)	4.71 ± 0.28***	15.38
HSE (150mg/kg)	2.85 ± 0.91***	47.71
HSE (300 mg/kg)	2.14 ± 0.40***	61.53

Values are given as means ± SEM (n=7). (*** $p < 0.0001$) compared to group treated with EtOH alone.

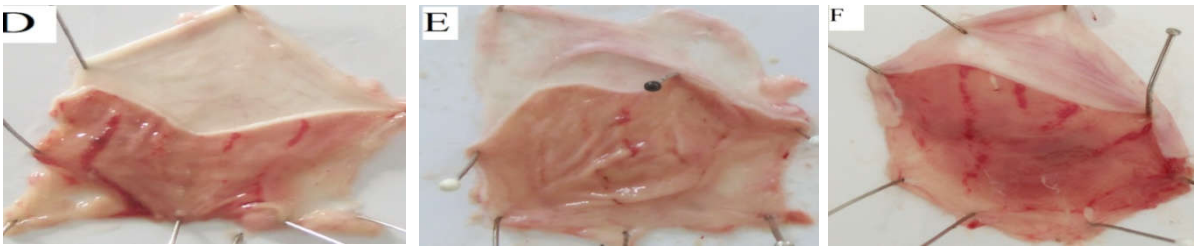
Table 8. Percentage protection of ASE and HSE treated rats against ethanol induced gastric ulcer.

Treatment	Stomach total surface area (cm^2)	Ulcerated surface area (cm^2)	Percentage of protection %
Negative control	177,77 ± 8,12	/	100
EtOH	64,25 ± 10,63	29,67 ± 2,23	0
Ranitidine	172,27 ± 4,61	12,52 ± 3,32	62.3
ASE (50mg/kg)	79,47 ± 3,98	5,2 ± 3,11	28.45
ASE (150mg/kg)	132,92 ± 2,14	9,25 ± 2,51	72.86
ASE (300mg/kg)	85,45 ± 1,16	3,3 ± 1,29	88.19
HSE (50mg/kg)	132,17 ± 6,88	15,8 ± 5,19	11.29
HSE (150mg/kg)	98,55 ± 5,63	10,8 ± 4,18	46.10
HSE (300mg/kg)	139,95 ± 5,67	6,9 ± 1,68	82.47

Values are given as means ± SEM (n=7).



(A) Untreated group (B) Group treated with EtOH 75%
(C) Stoamach of rat treated with EtOH + ASE (50mg/kg)



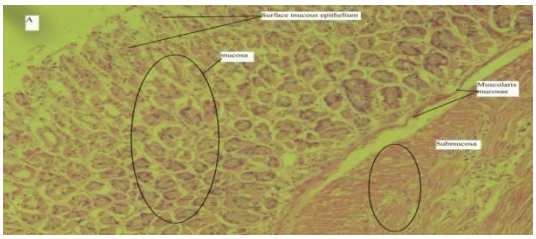
(D) Stoamach of rat treated with EtOH + ASE (150mg/kg) (E) Stoamac treated with EtOH + ASE (300 mg/kg)
(F) Stoamach of rat treated with EtOH + HSE (50mg/kg)

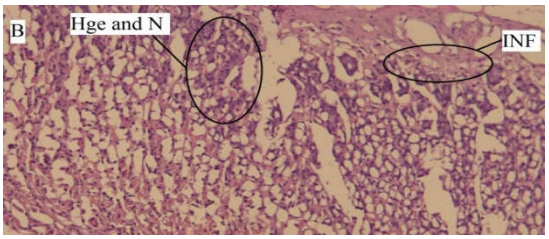
(G) Stoamach of rat treated with EtOH + HSE (150mg/kg) (H) Stoamach treated with EtOH + HSE (300mg/kg)

(I) Stoamach of rat treated with ranitidine (50 mg/kg)

Figure 32. Macroscopic observation of glandular regiond of treated and untreated groups.

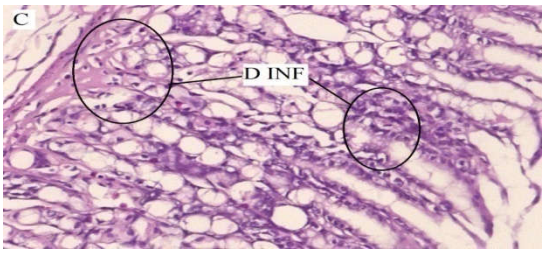
5.1. Effect of treatment with ASE/HSE on stomach tissue

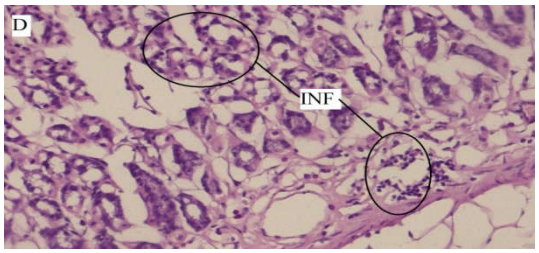




(A) Section of rat stomach treated with distilled water only (control animal) (x200).

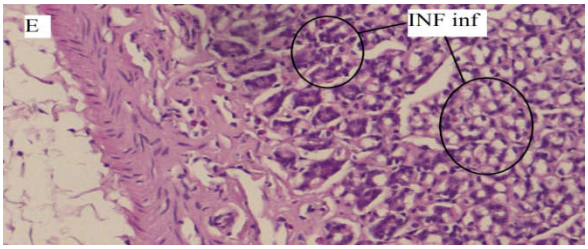
(B) Section of rat stomach treated with EtOH (x200); Hge: Haemorrhage, N: necrosis, INF: inflammation.

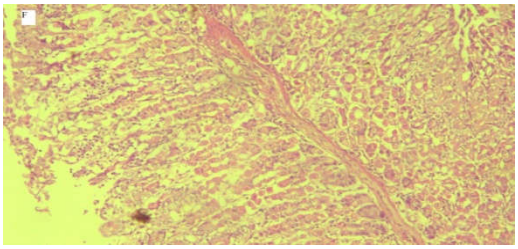




(C) Section of rat stomach treated with ranitidine (50 mg/kg) (x400): D INF: discret inflammation.

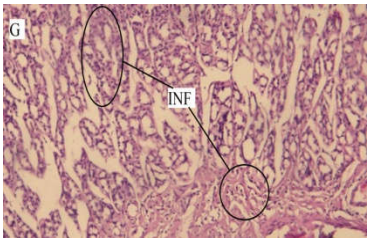
(D) Section of rat stomach treated with ASE (50 mg/kg) (x400).

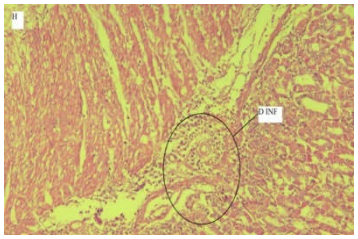


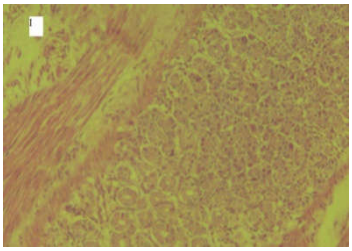


(E) Section of rat stomach treated with ASE (150 mg/kg) (x400); INF inf: inflammation infiltrate.

(F) Section of rat stomach treated with ASE 300 mg, normal tissue, no alteration (x200).







(G) Section of rat stomach treated with HSE (50 mg/kg) (x200); INF: inflammation.

(H) Section of rat stomach treated with HSE (150 mg/kg) (x200); D INF: discrete inflammation.

(I) Section of rat stomach treated with HSE (300 mg/kg) (x200); normal tissue, no alteration.

Figure 33. Histological Sections of stomach in normal and treated animals**.**

Conclusion

Results obtained in the present study revealed that the level of phenolic and flavonoids compounds in various extracts of *A.scoparium* were considerably higher in DEC than that in other extracts. Whereas, *H.sativus* EAE contained the higher amount of phenolic and flavonoids compounds. This could be due to the nature and structure of compounds present in these plant extracts, and to the degree of polarity of the solvents used for the extraction.

Antioxidant capacity is related to compounds capable of protecting biological system against the potentially harmful effect of processes or reactions involving reactive oxygen and nitrogen species (ROS and RNS). Many methods, which differ from each other in terms of reaction mechanisms, oxidant and target/probe species, reaction conditions, and expression of results have been developed and tested. For this reason, the extracts of selected plants seem to be of potential interest due to their antioxidant activities.

It can be noted that different extracts from *A.scoparium* and *H.sativus* exhibited different effective degrees *in vitro* antioxidant activity using different methods. Among all *A.scoparium* and *H.sativus* extracts, crude and ethyl acetate extract were shown to possess a significant antioxidant activity by scavenging DPPH, ABTS and hydroxyl radicals, inhibiting β-Carotene oxidation, reducing power and ferrous ion chelating properties. For hydrogen peroxyde radical scavenging activity, *A.scoparium* and *H.sativus* decoction and crude extract exhibited the strongest effect.

The antihemolytic property of *A.scoparium* and *H.sativus* was studied against AAPH and ferrous ion-induced oxidative hemolysis. In the present study, all extracts of *A.scoparium* and *H.sativus* exhibited potent antihemolytic activity and revealed an important values of HT_{50}, among which DEC and CrE of studied plants displayed significantly the highest antihemolytic effect, at all tested concentrations. For ferrous ion-induced hemolysis, all extracts exhibited inhibitory properties against hemolysis. But *A.scoparium* CrE and *H.sativus* EAE registered the maximum anti-hemolytic effect.

The toxicity study revealed that crude extracts of the two plants did not cause any mortality or other toxicity signs. It is concluded that the CrE of selected plants are safe up to 5 g /Kg.

In vivo antioxidant properties. Crude extract of *A.scoparium* and *H.sativus* were investigated. The present study evaluated the protective effect of selected plants against CCl_4-induced liver injury. The results showed that oral administration of crude extract of selected plants for 7 days at a daily dose of 250 and 500 mg/kg BW resulted in an increase in total antioxidant

capacity of plasma. It was found that 500 mg/kg of *A.scoparium* and *H.sativus* extracts increased significantly total plasma antioxidant capacity using DPPH radical scavenging assay. The higher electron donation capacity was noticed for *A.scoparium* and *H.sativus* at the dose of 500 mg/kg. It was found that *A.scoparium* and *H.sativus* extracts were more effective in increasing catalase activity and GSH level, and decreasing the MDA level.

The anti-inflammatory activity of plants extract was evaluated using two methods. The oral administration of *A.scoparium* and *H.sativus* crude extracts exhibited an important anti-inflammatory effect against xylene-induced ear oedema at dose of 400 mg/kg BW of mice. However, the crude extract of two selected plants exerted an interesting protective effect on the gastric mucosa against ethanol-induced gastric ulcer at dose of 300 mg/kg.

Further study is needed to clarify the antioxidant mechanism of these extracts and to evaluate other biological properties of these two plants. Some further investigations for the future work are:

- Identify, isolate and characterize the active constituents responsible for the observed antioxidant activity in order to determine the exact mechanism of the antioxidant and antiflammatory activities of extracts.
- Evaluate the bioactive molecules in therapeutic significance in the prevention of diseases induced by oxidative stress as well as the enzymes involved in the production of the reactive oxygen species.
- Study the Chronic toxicity of the plant with other in vivo tests of antioxidant assays.

References

Abd El-mohsen Ali S., Hussein Abdelhafiz Abdelaziz D. (2014).The Protective Effect of Date Seeds on Nephrotoxicity Induced by Carbon Tetrachloride in Rats. Int J Pharm Sci Rev Res.26(2).

Abdulkhaleq L.A., Assi M.A., Abdullah R., Zamri-Saad M., Taufiq-Yap Y.H., Hezmee M.N.M. (2018). The crucial roles of inflammatory mediators in inflammation: A review. Vet World. 11(5).

Adefisayo M.A., Akomolafe R.O., Akinsomisoye O.S., *et al*. (2018). Protective Effects of Methanol Extract of *Vernonia amygdalina* (*del*.) Leaf on Aspirin-Induced Gastric Ulceration and Oxidative Mucosal Damage in a Rat Model of Gastric Injury. Dose Response.16(3).

Adjiri A. (2017). DNA Mutations May Not Be the Cause of Cancer. Oncol Ther. 5(1).

Afonso MS, Machado RM, Lavrador MS, Quintao ECR, Moore KJ, Lottenberg AM. Molecular Pathways Underlying Cholesterol Homeostasis. Nutrients.10 (6).

Agbor G.A., Vinson J.A. and. Donnelly P.E. (2014). Folin-Ciocalteau Reagent for Polyphenolic Assay. Int J Food Sci Nutr Diet. 3(8).

Ahangarpour A., Ramazani Ali-Akbari F., Mohaghegh S.M., Asadinia E. (2016). Effects of Arctium lappa aqueous extract on lipid profile and hepatic enzyme levels of sucrose-induced metabolic syndrome in female rats. Braz J Pharm Sci.52.

Aichour R., Benzidane N., Arrar L., Charef N. and Baghiani A. (2018). Hepatoprotective and Anti-inflammatory Activities of Algerian *Capparis spinosa. L*. Annu Res Rev Biol. 25(3).

Akanbi T.O. and Barrow C.J. (2018). Lipase-Produced Hydroxytyrosyl Eicosapentaenoate is an Excellent Antioxidant for the Stabilization of Omega-3 Bulk Oils, Emulsions and Microcapsules. Molecules. 23(2).

Akhani H., Edwards G., Roalson E.H. (2007). Diversification Of The Old World Salsoleae S.L. (Chenopodiaceae): Molecular Phylogenetic Analysis Of Nuclear And Chloroplast Data Sets And A Revised Classification In: Int J Plant Sci. 168(6).

Alexander S.P., Fabbro D., Kelly E., et al. (2017). The concise guide to pharmacology 2017/18: Catalytic receptors. Br J Pharmacol. 174(1).

Al-Jawad F.H., Al-Attar Z., Abbood M.S. (2019). The Protective Effect of Nitroglycerin, N-Acetyl Cysteine and Metoprolol in CCL4 Induced Animal Model of Acute Liver Injury. Open Access Maced J Med Sci. 7(11).

Allaoui M., Cheriti A., Chebouat E., Dadamoussa B. and Gherraf N. (2016). Comparative Study of the antioxidant activity andpheols and flavonoids contents of the ethyl acetate extracts from two saharan chenopodacea: Haloxylon scoparium AND Traganum nudatum. Algerian journal of arid environment .6(1).

Almasaudi S.B., El-Shitany N.A., Abbas A.T., Abdel-Dayem U.A., Ali S.S., Al Jaouni S.K., Harakeh S. (2016). Antioxidant, Anti-Inflammatory, and Antiulcer Potential of Manuka Honey against Gastric Ulcer in Rats. Oxid. Med. Cell. Longev.

Amber K., Shiman M., and Badiavas E. (2013). 'The Use of Antioxidants in RadiotherapyInduced Skin Toxicity', Integr Cancer Ther.13(1).

Amin Z.A., Bilgen M., Alshawsh M.A., Ali H.M., Hadi A.H., Abdulla M.A. (2012). Protective role of Phyllanthus niruri extract against thioacetamide-induced liver cirrhosis in rat model. Evid Based Complement Alternat Med. 20.

Anand S.P., Deborah S., Velmurugan G. (2018). Evaluation of antioxidant activity of some wild edible fruits collected from Boda and Kolli hills. J Phytopharmacol. 7(2).

Antonenkov V. D., Grunau S., Ohlmeier S., and Hiltunen J. K. (2010). "Peroxisomes are oxidative organelles". Antioxid Redox Signal. 13(4).

Anwar F., Shaheen N., Shabir G., Ashraf M., Alkharfy K.M. and Gilani A.H. (2013). Variation in antioxidant activity and phenolic and flavonoid contents in the flowers and leaves of Ghaneri (Lantana camara L.) as affected by different extraction solvents. Int J Pharmacol. 9.

Aouachria S., Boumerfeg S., Benslama A., Benbacha F., Guemmez T., Khennouf ., Arrar L., Baghiani A. (2017). Acute, sub-acute toxicity and antioxidant activities (*in vitro* and *in vivo*) of Reichardia picroide crude extract. J. Ethnopharmacol.

Apak R., Özyürek M., Güçlü K. and Çapanoğlu E. (2016). Antioxidant activity/capacity measurement. 1. Classification, physicochemical principles, mechanisms, and electron transfer (et)-based assays. J Agric Food Chem. 64.

Arulselvan P., Fard M.T., Tan W.S. *et al*. (2016). Role of Antioxidants and Natural Products in Inflammation. Oxid Med Cell Longev.

Aryal S., Kumar M. B., Danekhu K., Kunwar P., Gurung R and Koirala N. (2019). Total Phenolic Content, Flavonoid Content and Antioxidant Potential of Wild Vegetables from Western Nepal. Plants. 8(96).

Avila-Nava A., Calderón-Oliver M., Medina-Campos O.N., Zou T., Gu L., Torres N., Tovar A.R. *et al*. (2014). Extract of cactus (Opuntiaficus indica) cladodes scavenges reactive oxygen species in vitro and enhances plasma antioxidant capacity in humans. J functi foods.10.

Ayala A., Muñoz M. F., and Argüelles S. (2014). Lipid Peroxidation: Production, Metabolism, and Signaling Mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal. Oxid Med Cell Longev.

Baião D. D. S., De Freitas C. S., Gomes L.P, Silva D.D., Correa A C N. T. F., Pereira P. R., Del Aguila E. M. and Paschoalin V. M. F. (2017). Polyphenols from Root, Tubercles and Grains Cropped in Brazil: Chemical and Nutritional Characterization and Their Effects on Human Health and Diseases. Nutrients. 9 (9).

Banothu V., Neelagiri C., Adepally U., Lingam J. & Bommareddy K. (2016). Phytochemical screening and evaluation of in vitro antioxidant and antimicrobial activities of the indigenous medicinal plant Albizia odoratissima. Pharm Biol. 55 (1).

Baroni M.V., Gastaminza J., Podio N.S., Lingua M.S, Wunderlin D.A, Rovasio J. L., Dotti R., Carlos Rosso J , Ghione S., and Ribotta P.D.(2018). Changes in the Antioxidant Properties of Quince Fruit (Cydonia oblonga Miller) during Jam Production at Industrial Scale. J. Food Qual.9.

Belcher J.D., Chen C., Nguyen J., Zhang P., Abdulla F.., Nguyen P., Killeen T., Xu P., O'Sullivan G., Nath K.A. and Vercellotti G.M. (2016).Control of oxidative stress and inflammation in sickle cell disease with the Nrf2 activator dimethyl fumarate. Antioxid Redox Signal.

Belyagoubi-Benhammou N. *et al*., (2014).Phenolic content and antioxidant activities in vitro of some selected Algerians plants. J Med Plant Res.8(40).

Benamar H., Rached W., Debour A., & Marouf A. (2010). Screening of Algerian medicinal plants for acetylcholinesterase inhibitory activity. J Biol Sci.10(1).

Bencheich D., Khennouf S., Bouaziz A., Baghiani A., Dahamna S., Amira S., Arrar L. (2016). Antioxidant ans antidiabetic activities of the methanolic extract of Olea europae L. leaves in streptozotocin induced diabetes in rats.International. J Pharmacogn Phytochem. 8(8).

Benchikh F., Amira S. and Benabdallah H. (2018).The Evaluation of Antioxidant Capacity of Different Fractions of Myrtus communis L. Leaves. Annu Res Rev Biol. 22(5).

Benchoam D., Cuevasanta E ., Möller M.N. and Alvarez B. (2019). Hydrogen Sulfide and Persulfides Oxidation by Biologically Relevant Oxidizing Species. Antioxidants. 8(2).

Benhammou N., Atik Bekkara F., Kadifkova Panovska T. (2008). Antioxidant and antimicrobial activities of the Pistacia lentiscus and Pistacia atlantica extracts. Afric J Pharm Pharmacol. 2(2).

Bentahar A., Khennouf S., Bouaziz A., Baghiani A., Dahamna S., Amira S., Arrar L. (2016). Polyphenols content and antioxidant activities of selected Algerian plants used for gastro-duodenal ulcers. Der Pharma Chemica. 8(12).

Bhakuni G.S., Bedi O., Bariwal J., Deshmukh R., Kumar P. (2015). Animal models of hepatotoxicity. Inflamm Res.

Bhuyan D. J. and Basu A. (2017). Phenolic Compounds Potential Health Benefits and Toxicity. CHAPTER 2, Utilisation of Bioactive Compounds from Agricultural and Food Waste.

Bhuyan D. J., Vuong Q. V., Chalmers A. C., van Altena I. A., Bowyer M. C., and Scarlett C. J. (2015). Microwave-assisted extraction of Eucalyptus robusta leaf for the optimal yield of total phenolic compound. Ind Crops Prod. 69.

Bitencourt C.S, Bessi V.L, Huynh D.N, Ménard L., Lefebvre J.S., Lévesque T. and Marleau S. (2013). Cooperative role of endogenous leucotrienes and platelet-activating factor in ischaemia–reperfusion-mediated tissue injury. J Cell Mol Med.17.

Birben E., Murat Sahiner U., Sackesen C., Erzurum S and Kalayci O. (2012). Oxidative Stress and Antioxidant Defense. WAO Journal. 5.

Bloomer S. A. and Brown K. E. (2019).Iron-Induced Liver Injury: A Critical Reappraisal. Int J Mol Sci. 20.

Boff D., Crijns H., Teixeira M. M., Amaral F. A. and Proost P. (2018). Neutrophils: Beneficial and Harmful Cells in Septic Arthritis. Int J Mol Sci.19(2).

Bouaziz A., Khennouf S., Abdalla S., Djidel S., Abu Zarga M ., Bentahar A., Dahamna S., Baghiani A., Amira S. (2014).Phytochemical Analysis, Antioxidant Activity and Hypotensive Effect of Algerian Azarole (Crataegus azarolus L.) Leaves Extracts. Res J Pharm Biol Chem Sci. 8(6).

Boumerfeg S. *et al*. (2012). Inhibitory Activity on Xanthine Oxidase and Antioxidant Properties of Teucrium polium L. Extracts. *Chin Med.* 3.

Boussoualim N., Krache I., Baghiani A., Trabssa H., Aouchria S., Arrar L. (2016). Human xanthine oxidase inhibitory effect, antioxidant in vivo of Algerian extracts (Globularia alypum L.).J Pharmacogn Phytochem. 8(4).

Breitenbach M. and Eckl P. (2015). Introduction to Oxidative Stress in Biomedical and Biological Research. Biomolecules. 5.

Butura A. (2008). Drug and Alcohol Induced Hepatotoxicity. Ph. D Thesis Department of Physiology and Pharmacology Karolinska Institutet, Stockholom, Sweden.

Bylka W., Stobiecki M., Frahski R. (2001).Sulphated flavonoid glycosides from leaves of Atriplex hortensis. Acta Physiol plant.23.

Caparrotta T.M., Antoine D.J., Dear J.W. (2018). Are some people at increased risk of paracetamol-induced liver injury? A critical review of the literature. Eur J Clin Pharmacol.74 (2).

Chaouche T.M., Haddouchi F., Ksouri R., Atik-Bekkara F. (2014). Evaluation of antioxidant activity of hydromethanolic extracts of some medicinal species from south Algeria. J Chin Med Assoc.77.

Chaput J.P., Gray C.E., Poitras V.J., Carson V., Gruber R., Olds T., *et al.* (2016). Systematic review of the relationships between sleep duration and health indicators in school-aged children and youth. Appl Physiol Nutr Metab. 41(3).

Chelstowska S., Widjaja-Adhi M.A., Silvaroli J.A., Golczak M. (2016). Molecular Basis for Vitamin A Uptake and Storage in Vertebrates. Nutrients. 8(11).

Chen L., Deng H., Cui H., Fang J., Zuo Z., Deng J., Li Y., Xun W. and Ling Z. (2018).Inflammatory responses and inflammation-associated diseases in organs. Oncotarget. 9(6).

Cheriti A., Belboukhari N., Sekkoum S., & Hacini S. (2006). Plants of Algerian semi-arid regions used for the treatment of gastro-intestinal disorders. Journal Algerien des regions arides.5.

Chertov O., Yang D., Howard O., Oppenheim J.J. (2000). Leukocyte granule proteins mobilize innate host defenses and adaptive immune responses. Immunol Rev.177.

Choi Y.J., Kim N., Lee J.Y., et al. (2016).Gastroprotective Effects of PMK-S005 against Ethanol-Induced Acute Gastric Damage in Rats. Gut Liver. 10(3).

Chun S.K., Lee S., Flores-Toro J., *et al.* (2018). Loss of sirtuin 1 and mitofusin 2 contributes to enhanced ischemia/reperfusion injury in aged livers. Aging Cell. 17(4).

Cianciosi D., Forbes-Hernández T.Y., Afrin S., *et al*. (2018).Phenolic Compounds in Honey and Their Associated Health Benefits: A Review. Molecules. 23(9).

Collin F. (2019). Chemical Basis of Reactive Oxygen Species Reactivity and Involvement in Neurodegenerative Diseases. Int J Mol Sci.20.

Curcic S., Holzer M., Frei R., Pasterk L., Schicho R., Heinemann A. and Marsche G. (2015). Neutrophil effector responses are suppressed by secretory phospholipase A 2 modified HDL. Biochim Biophys Acta (BBA) Mol Cell Biol Lipids.

Davies M. J. (2016). Protein oxidation and peroxidation. Biochem J.1 (473).

Debnath B., Islam W., Li M., Sun Y., Lu X., Mitra S., Hussain M.,Liu S. and Qiu D.(2019). Melatonin Mediates Enhancement of Stress Tolerance in Plants. Int J Mol Sci.20(5).

Deepak M., Kasote l., Surendra S., Mahabaleshwar V., Hegde Hanhong Bae. (2015). Significance of Antioxidant Potential of Plants and its Relevance to Therapeutic Applications. Int J Biol Sci.11(8).

Den Bakker E., Reinoud Gemke J. B. J. and Bökenkamp A. (2018). Endogenous markers for kidney function in children: a review. Crit Rev Cl Lab Sci.55 (3).

Dhanani T., Shah S., Gajbhiye N.A., Kumar S. (2017). Effect of extraction methods on yield,

phytochemical constituents and antioxidant activity of Withania somnifera. Arab. J. Chem. 10.

Di Marzo N., Chisci E. and Giovannon R. (2018).The Role of Hydrogen Peroxide in Redox-Dependent Signaling: Homeostatic and Pathological Responses in Mammalian Cells. Cells.7(156).

Di Meo S., Reed T.T., Venditti P.and Victor V.M. (2016).Role of ROS and RNS Sources in Physiological and Pathological Conditions. Oxid Med Cell Longev.

Dixon L.J., Barnes M., Tang H., Pritchard M.T., Nagy L.E. (2013). Kupffer Cells in the Liver. Compr Physiol. 3(2).

Dizdaroglu M. and JarugaP. (2012). Mechanisms of free radical-induced damage to DNA. Free Radic Res.46(4).

Donald K. K. B., Geneviève I-N. A., Etienne E. K., Landry K. S., djadji A. T. L., Fabrice T. A., Felix Y. H. (2018).Evaluation of Anti-inflammatory Activity of Crinum scillifolium Extracts in Wistar Rats. Int J Biochem Biophys. 6(4).

Dong S.X.M., Chang C.C.Y., Rowe J. K. (2018). A collection of the etiological theories, characteristics, and observations/phenomena of peptic ulcers in existing data. Data Brief. 19.

Dos Santos S. L., Petropoulos I., and Friguet B. (2018). The Oxidized Protein Repair Enzymes Methionine Sulfoxide Reductases and Their Roles in Protecting against Oxidative Stress, in Ageing and in Regulating Protein Function. Antioxidants (Basel). 7(12).

Drini M. (2017).Peptic ulcer disease and non-steroidal anti-inflammatory drugs. Aust Prescr. 40(3).

Dudylina A.L., Ivanova M.V., Kalatanova A.V., Kalenikova E.I., Makarov V.G., Makarova M.N., Shumaev K.B., Ruuge E.K. (2019). The Generation of Superoxide Radicals by Cardiac Mitochondria and the Antioxidant Effect of the Water-Soluble Form of Ubiquinol-10. Biophysics.64 (2).

Dudziak K., Zapalska M., Börner A., Szczerba H., Kowalczyk K., Nowak M. (2019). Analysis of wheat gene expression related to the oxidative stress response and signal transduction underg short-term osmotic stress. Sci Rep. 9(1).

Ekambaram M., Ramalingam K., Balasubramanian A. (2014). Effect of Portulaca quadrifida Linn on Mercury-Induced Hepatotoxicity in Swiss Albino Mice. Res Rev J Pharmacol Toxicol Stud. 2.

El-Agamy D.S., Almaramhy H.H., Ahmed N., Bojan B., Alrohily W.D., Elkablawy M., A. (2018). Anti-Inflammatory Effects of Vardenafil Against Cholestatic Liver Damage in Mice: a Mechanistic Study. Cell Physiol Biochem. 47.

El-Shazlyb A. and Wink M. (2003). Tetrahydroisoquinoline and -Carboline Alkaloids from Haloxylon articulatum (Cav.) Bunge (Chenopodiaceae). Z. Naturforsch. 58c.

Emon R and Zesmin F.D. (2018). Effect of n-hexane extract of Tinospora crispa on reduced glutathione and malondialdehyde level in paracetamol induced hepatotoxicity in rats. Update Dent Coll J. 8(2).

Escobedo-Hinojosa W.I., Gomez-Chang E., García-Martínez K., Guerrero Alquicira R., Cardoso-Taketa A., Romero I. (2018). Gastroprotective Mechanism and Ulcer Resolution Effect of Cyrtocarpa procera Methanolic Extract on Ethanol-Induced Gastric Injury. Evid Based Complement Alternat Med.

Ezeja I.M., Anaga A.O. and Asuzu I.U. (2014). Acute and sub-chronic toxicity profile of methanol leaf extract of Gouania longipetala in rats. J Ethnopharmacol. 151.

Fahmi A.A., Abdur-Rahman M., Aboul Naser A.F., *et al.* (2019).Chemical composition and protective role of Pulicaria undulata (L.) C.A. Mey. subsp. undulata against gastric ulcer induced by ethanol in rats. Heliyon.5(3).

Fatehi N., Allaoui M., Berbaoui H., Cheriti A., Boulenouar N., Belboukhari N. (2017). Haloxylon Scoparium: An Ethnopharmacological Survey, Phytochemical Screening and Antibacterial Activity against Human Pathogens Causing Nosocomial Infection. PhytoChem & BioSub Journal. 11(2).

Ferreira C.A., Ni D., Rosenkrans Z.T. et al. (2018). Scavenging of reactive oxygen and nitrogen species with nanomaterials. Nano Res.11.

Filippi M.D. (2016). Mechanism of Diapedesis: Importance of the Transcellular Route. Adv Immunol.129.

Forouzandeh H., Kalantari H., Saki N., Foruozandeh Z., Arefian E., Farahani A., Hassani G., Bazrafshan M. R., Rasouli S. (2017). Role of oxidative stress in liver cancer. Clin Cancer Investig J. 6.

Fortea J. I., Fernández-Mena C., Puerto M., Ripoll C., Almagro J., Bañares J., Bellón J.M. , Bañares R. & Vaquero J.(2018). Comparison of Two Protocols of Carbon Tetrachloride-Induced Cirrhosis in Rats – Improving Yield and Reproducibility. Sci. Rep. 8.

Freitas-Lopes M. A., Mafra K., David A. B., Carvalho-Gontijo R. and Menezes G. B. (2017). Differential Location and Distribution of Hepatic Immune Cells. Cells. 6(4).

Freyssin A., Page G., Fauconneau B. and Rioux Bilan A. (2018). Natural polyphenols effects on protein aggregates in Alzheimer's and Parkinson's prion-like diseases. Neural Regen Res.13(6).

Fung P. and Pyrsopoulos N. (2017).Emerging concepts in alcoholic hepatitis. World J Hepatol. 9(12).

Galano A., Tan D. X. and Reiter R. J. (2018).Melatonin: A Versatile Protector against Oxidative DNA Damage. Molecules. 23(3).

Gallo J., Raska M., Kriegova E., .Goodman S B. (2018). Inflammation and its resolution and the musculoskeletal system. J Orthop Transl. 10.

Gangwar M., Gautam M.K., Sharma A.K., Tripathi Y.B., Goel R.K., Nath G. (2014). Antioxidant capacity and radical scavenging effect of polyphenol rich Mallotus philippenensis fruit extract on human erythrocytes: an in vitro study. Sci World J.

Gayathri M., Ramasamy M., Santhiya N.and Dineshkumar G. (2017).In vitro antioxidant properties from tissue extract of Gastropods around Lower and Grand Anicut Reservoir, Tamil Nadu. J Mar Biol. 3(1).

Ghanem C.I., Pérez M.J., Manautou J.E., Mottino A.D. (2016). Acetaminophen from liver to brain: New insights into drug pharmacological action and toxicity. Pharmacol Res. 109.

Ghasemi H., Rostampour F., Ranjbar A. (2014). The Role of Oxidative Stress in Metals Toxicity/ Mitochondrial Dysfunction as a Key Player. 3(14).

Ghedadba N., Hambaba L., Ayachi M. C., Aberkane H., Bousselsela S. M. (2015). Polyphénols totaux, activités antioxydant et antimicrobienne des feuilles de Marrubiumdeserti de Noé. Phytothérapie. 13(2).

Goljan E.F. (2014). Rapid Review Pathology: With Student Consult Online Access. Elsevier Health Sciences, Philadelphia, PA.

Gracia-Sancho J., Marrone G. & Fernández-Iglesias A. (2019). Hepatic microcirculation and mechanisms of portal hypertension. Nat Rev Gastroenterol Hepatol. 16.

Graham D.Y. (2014). History of Helicobacter pylori, duodenal ulcer, gastric ulcer and gastric cancer. World J Gastroenterol. 20.

Griendling K. K., Touyz R. M.., Zweier J. L, Dikalov S., Chilian W., Chen Y-R., Harrison D.G., Bhatnagar A. (2016). Measurement of Reactive Oxygen Species, Reactive Nitrogen Species, and Redox-Dependent Signaling in the Cardiovascular System: A Scientific Statement From the American Heart Association. Circ Res.119(5).

Guemmaz T., Zerrargui F., Boumerfeg S., Arrar L., Aoucharia S., Khennouf S., Charef N., Baghiani A. (2018). Anti-hemolytic, Anti-lipid Peroxidation, Antioxidant Properties and Toxicity of Xanthium strumarium Leaves Extracts. Annu Res Rev Biol. 24 (3).

Guettaf S., Abidli N., Kariche S., Bellebcir L. and Bouriche H. (2016). Phytochemical screening and antioxidant activity of aqueous extract of Genista Saharae (Coss. & Dur.). J Der Pharm Lett. (8).

Gunaydin C. and Bilge S.S. (2018). Effects of Nonsteroidal Anti-Inflammatory Drugs at the Molecular Level. Eurasian J Med. 50(2).

Guzmán-Gómez O., García-Rodríguez R.V., Quevedo-Corona L., et al. (2018). Amelioration of Ethanol-Induced Gastric Ulcers in Rats Pretreated with Phycobiliproteins of Arthrospira (Spirulina) Maxima. Nutrients. 10(6).

Habeeb A., Tiwari S.K., Bardia A., Khan S., Vishwakarma S.K., Habeeb S. and Aleem Khan A.(2016). Peptic Ulcer Disease: Descriptive Epidemiology, Risk Factors, Management and Prevention.India.

Halake K., Cho S., Kim J, Lee T., Cho Y., Chi S., Park M., Kim K., Lee D., Ju H., Cho Y., Jang M., Choe G., Lee J. (2018). Applications Using the Metal Affinity of Polyphenols with MusselInspired Chemistry. Macromol. Res. 26(2).

Hashemi S. (2019). Effect of Nanoparticles on Lipid Peroxidation in Plant.

He L., He T., Farrar S., Ji L., Liu T., Ma X. (2017). Antioxidants Maintain Cellular Redox Homeostasis by Elimination of Reactive Oxygen Species. Cell Physiol Biochem. 44.

Holmgren Ned A. (2004). "Halogeton C. A. Meyer," in Flora of North America: North of Mexico Volume 4: Magnoliophyta: Caryophyllidae, part 1, Editorial Committee of the Flora of North America (Oxford University Press, 2004). ISBN 978-0-19-517389-5.

Hsu Y. L., Hung J. Y., Tsai Y. M., Tsai E. M., Huang M. S., Hou M. F., and Kuo P. L. (2015). 6-shogaol, an active constituent of dietary ginger, impairs cancer development and lung metastasis by inhibiting the secretion of CC-chemokine ligand 2 (CCL_2) in tumor-associated dendritic cells. J Agric Food Chem.63.

Huether S.E. and McCance K. L. (2015). Understanding Pathophysiology. Elsevier Health Sciences, Förlag.

Hunt R.H., Camilleri M., Crowe S.E., *et al.* (2015). The stomach in health and disease. Gut. 64(10).

Ibrahim L. F., et al. (2007). A comparative study of the flavonoids and some biological activities of

two Chenopodium species. Chem Nat Compd. 43(1).

Ighodaro O.M. and Akinloye O.A. (2017). First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alex J Med.

Iqubal A., Iqubal M.K. and Haquea S.E. (2016). Experimental hepatotoxicity Inducing agents: A Review. Int J Pharm Res.10.

Jain N K and Singhai A K: Hepatoprotective activity of Chenopodium album Linn: in vitro and in vivo studies. J Exp Integr Med. 2(4).

Jing J., Teschke R.(2018). Traditional Chinese Medicine and Herb-induced Liver Injury: Comparison with Drug-induced Liver Injury. J Clin Transl Hepatol. 6(1).

Juthani V.V., Clearfield E., Chuck R.S.(2017). Non-steroidal anti-inflammatory drugs versus corticosteroids for controlling inflammation after uncomplicated cataract surgery. Cochrane Database Syst Rev. 7(7).

Kashmiri Z. N. and Manka S. A. (2014). Free radicals and oxidative stress in bacteria. Int.J.Curr.Microbiol.App.Sci. 3(9).

Kasote D.M., Katyare S.S., Hegde M.V., Bae H. (2015).Significance of antioxidant potential of plants and its relevance to therapeutic applications. Int J Biol Sci. 11(8):982–991.

Kaur A., Singh R., Sharma R., Kumar S. (2012).Peptic ulcer: A review on etiology and pathogenesis. Int. Res. J. Pharm.3(6).

Kausar S., Wang F.and Cui H. (2018).The Role of Mitochondria in Reactive Oxygen Species Generation and Its Implications for Neurodegenerative Diseases. Cells. 7(12).

Kawamura T. and Muraoka I. (2018). Exercise-Induced Oxidative Stress and the Effects of Antioxidant Intake from a Physiological Viewpoint. Antioxidants (Basel). 7(9).

Kawanishi S., Ohnishi S., Ma N., Hiraku Y., Oikawa S. and Murata M. (2016). Nitrative and oxidative DNA damage in infection-related carcinogenesis in relation to cancer stem cells. Genes Environ.38.

Khither H., Sobhi W., Khenchouche A., Mosbah A. and Benboubetra M. (2018). In-vitro Antioxidant Effect of Thymoquinone. Annu Res Rev Biol. 25(5).

Khoder G., Al‐Menhali A.A., Al‐Yassir F. and Karam S.M. (2016). Potential role of probiotics in the management of gastric ulcer (Review). Exp Ther Med. 12.

Kietzmann T. (2017). Metabolic zonation of the liver: The oxygen gradient revisited. Redox Biol. 11.

Kim S., Song Y., Lee J.E., Jun S., Shin S., Wie G., Cho Y.H., Joung H. (2017). Total Antioxidant Capacity from Dietary Supplement Decreases the Likelihood of Having Metabolic Syndrome in Korean Adults. Nutrients.9(10).

Kivrak E.G., Yurt K.K., Kaplan A.A., Alkan I., Altun G. (2017). Effects of electromagnetic fields exposure on the antioxidant defense system. J Microsc Ultrastruct. 5.

Kobayashi H. (2019). Somatic driver mutations in endometriosis as possible regulators of fibrogenesis (Review). World Appl. Sci. J. 12.

Koch W. (2019). Dietary Polyphenols—Important Non-Nutrients in the Prevention of Chronic Noncommunicable Diseases. A Systematic Review. Nutrients. 11(5).

Krishna P.G.A., Sivakumar T.R., Jin C., Li S-H., Weng Y-J., Yin J., Jia J-Q., Wang C-Y. and Gui Z-Z. (2018). Antioxidant and Hemolysis Protective Effects of Polyphenol-Rich Extract from Mulberry Fruits. Pharmacogn Mag.14(53).

Kuchakulla M., Masterson T., Arora H., Kulandavelu S., Ramasamy R. (2018). Effect of nitroso-redox imbalance on male reproduction. Transl Androl Urol.7(6).

Kuhn V., Diederich L., T.C. Stevenson Keller I.V., Kramer C.M., Wiebke Lu¨ cksta¨ dt., Panknin C., Suvorava T., . Isakson B. E., Kelm M. and M Miriam. (2017). Red Blood Cell Function and Dysfunction: Redox Regulation, Nitric Oxide Metabolism, Anemia. Antioxid Redox Signal. 26 (13).

Kulshreshtha M., Srivastava G., Singh M.P. (2017).Pathophysiological status and nutritional therapy of peptic ulcer: An update. Environ Dis. 2.

Kumar V., Abbas A.K and Aster J.C. (2013). Robbins Basic Pathology. Elsevier Health Sciences.

Kumar V., Roy B.K. (2018).Population authentication of the traditional medicinal plant Cassia tora L. based on ISSR markers and FTIR analysis. Sci. Rep. 8.

Kumon M. (2017).Anatomical Study of the Caudate Lobe with Special Reference to Portal Venous and Biliary Branches Using Corrosion Liver Casts and Clinical Application. Liver Cancer. 6(2).

Kuna L., Bozic I., Kizivat T.*et al.* (2018). Models of Drug Induced Liver Injury (DILI) - Current Issues and Future Perspectives. Curr Drug Metab. 19(10).

Kung Hsien-wu., Chu Ge-lin., Tsien Cho-po C. P., Ma Cheng-gung & Li An-jen. (1979). Chenopodiaceae. In: Kung Hsien-wu & C. P. Tsien Chopo, eds., Fl. Reipubl. Popularis Sin. 25(2).

Lackner C., Tiniakos D. (2019). Fibrosis and alcohol-related liver disease. *J. Hepatol*.70 : 294–304.

Lamas-Paz A., Hao F., Nelson L.J. et al. (2018). Alcoholic liver disease: Utility of animal models. World J Gastroenterol. 24(45).

Lamchouri F., Benali T., Bennani B., Toufik H., Ibn Majdoub Hassania L., Bouachrine M., Lyoussi B. (2012). Preliminary phytochemical and antimicrobial investigations of extracts of Haloxylon scoparium. J Mater Environ Sci. 3 (4).

Lee D. H., Kim D. W., Jung C. H., Lee Y. J., and Park D. (2014). Gingerol sensitizes TRAIL-induced apoptotic cell death of glioblastoma cells. Toxicol Appl Pharmacol. 279.

Lee M. T., Lin W. C., Yu B., Lee T. T. (2017). Antioxidant capacity of phytochemicals and their potential effects on oxidative status in animals — A review. Asian-Australas J Anim Sci. 30(3).

Leick M., Azcutia V., Newton G., Luscinskas F.W. (2014).Leukocyte recruitment in inflammation: basic concepts and new mechanistic insights based on new models and microscopic imaging technologies. Cell Tissue Res.355(3).

Lemonnier N., Zhou G.B. Prasher B., Mukerji M., Chen Z., Brahmachari S. K., Noble, D., Auffray C., Sagner M. (2017). Traditional Knowledge-based Medicine: A Review of History, Principles, and Relevance in the Present Context of P4 Systems Medicine. Progprev Med.2 (7).

Levin J.I. and Laufer S. (2012). Anti-inflammatory Drug Discovery (No. 26). Royal Society of Chemistry, London.

li k., Gelin Z., Sergei Mosyakin L., Steven Clemants E. (2003). CHENOPODIACEAE. Flora of China 5.

Lian G., GnanaprakasamJ. N. R., Wang T., Wu R., Chen X., Liu Y, Shen Y., Yang Y., Yang J., Chen Y., Vasiliou V., Cassel T. A., Green D. R., Liu Y., Fan T. W. M., Wang R. (2018). Glutathione de novo synthesis but not recycling process coordinates with glutamine catabolism to control redox homeostasis and directs murine T cell differentiation.7.

Liguori I., Russo G., Curcio F., *et al*. (2018). Oxidative stress, aging, and diseases. Clin Interv Aging.13.

Lins P.G., Pugine S.M.P., Scatolini A.M., de Melo M.P. (2018). *In vitro* antioxidant activity of olive leaf extract (*Olea europaea* L.) and its protective effect on oxidative damage in human erythrocytes. J heliyon. 4(9).

Liu Y., Kim S., Kim Y.J., Perumalsamy H., Lee S., Hwang E., Yi T-H. (2019). Green synthesis of gold nanoparticles using Euphrasia officinalis leaf extract to inhibit lipopolysaccharide-induced inflammation through NF-κB and JAK/STAT pathways in RAW 264.7 macrophages. Int J Nanomedicine. 14.

Liu Z., Ren Z., Zhang J., Chuang C-C., Kandaswamy E., Zhou T., Zuo L. (2018). Role of ROS and Nutritional Antioxidants in Human Diseases. Front Physiol.9.

Liu Z., Ren Z., Zhang J., et al. (2018). Role of ROS and Nutritional Antioxidants in Human Diseases. Front Physiol. 9.

Loizzo M.R., Tundis R., Statti G.A., Passalacqua N., Peruzzi L., Menichini F. (2007).Nat Prod Res. 21.

Lowe M.C., D'Angelica M.I. (2016). Anatomy of Hepatic Resectional Surgery. Surg Clin North Am. 96(2).

Lupu A. R. and Cremer L. (2018). HYDROXYL RADICAL SCAVENGER ACTIVITY OF NATURAL SOD. Roum Arch Microbiol Immunol. 77(1).

Ma H., Wang F., Jiang J., Cheng L., Zhang H. and Zhang G. (2017). In vivo anti-inflammatory activity of Liquidambar formosana Hance infructescence extract. Trop J Pharm Res. 16 (10).

Mabberley D. J., (1997). The Plant-Book, A portable dictionary of the vascular plants. Cambridge University Press, UK.

Madoui S., Charef N., Arrar L., Baghianni A. and Khennouf S. (2018).In vitro Antioxidant Activities of Various Extracts from Flowers-Leaves Mixture of Algerian Cytisus triflorus. Annu Res Rev Biol. 26(3).

Mahmood A.A., Al-Bayaty F.H., Salmah I., Nor Syuhada A.B., Harita H. and Mughrabi F. (2016). F3Enhacement of gastric ulcer of gastric ulcer by areca catechu nut in ethanol induced gastric mucosal injuries in rats. J. Med. Plant Res. 5(12).

Mahmoud A.M., Bautista R. J. H., Sandhu M. A. and Hussein O. E. (2019). Beneficial Effects of Citrus Flavonoids on Cardiovascular and Metabolic Health. Oxid Med Cell Longev.

Mailloux R.J. (2018).Mitochondrial Antioxidants and the Maintenance of Cellular Hydrogen Peroxide Levels. Oxid Med Cell Longev.

Malki F., Touati A. and Moulay S. (2017). Comparative study of antioxidant activity of some amides. J Anal Pharm Res. 5(3).

Marengo B., Nitti M., Furfaro A. L., Colla R., De Ciucis C., Marinari U. M., Pronzato M. A., Traverso N., Domenicotti C. (2016). Redox Homeostasis and Cellular Antioxidant Systems: Crucial Players in Cancer Growth and Therapy. Oxid Med Cell Longev.

Markham K.R. (1982). Techniques of flavonoid identification (Chapter 1 and 2). First edition, Academic Press : London.1-113.

Marrone G., Shah V.H., Gracia-Sancho J. (2016). Sinusoidal communication in liver fibrosis and regeneration. J Hepatol. 65(3).

Martinez M.N. (2011). Factors influencing the use and interpretation of animal models in the development of parenteral drug delivery systems. AAPS PharmSciTech. 13(4).

Matsubara K., Higaki T., Matsubara Y., & Nawa A. (2015).Nitric oxide and reactive oxygen species in the pathogenesis of preeclampsia. Int J Mol Sci. 16(3).

Maurya P.K., Kumar P., Chandra P. (2015). Biomarkers of oxidative stress in erythrocytes as a function of human age. World J Methodol. 5(4).

Mazani M., Mahmoodzadeh Y., Chinifroush Asl M.M., Banaei S., Rezagholizadeh L., Mohammadnia A. (2018). Renoprotective effects of the methanolic extract of Tanacetum parthenium against carbon tetrachloride-induced renal injury in rats. Avicenna J Phytomed. 8 (4).

Meek I.L., Van de Laar M.A.F.J., Vonkeman H.E. (2010). Non-steroidal anti-inflammatory drugs: an overview of cardiovascular risks. Pharmaceuticals. 3.

Melekmi N., Saad A., Belboukhari N., & Cheriti A. (2006). Antimicrobial activity of the essential oil of Bubonium graveolens.Annales de l'Université de Bechar, 2.

Merghem M., Dahamna S., Khennouf S. (2019). In Vivo Antioxidant Activity of Ruta montana L. Extracts. J Mater Environ Sci.10 (5).

Mettert E.L. and Kiley P.J. (2015). Fe-S proteins that regulate gene expression. Biochim Biophys Acta.1853(6).

Miguel M., Bouchmaaa N., Aazza S., Gaamoussi F. and Lyoussi B. (2014). Antioxidant,anti-inflammatory and anti-inflammatory and anti-acetylcholinesterase activities of eleven extracts of moroccan plants. Fresenius Environ Bull. 23(6).

Mitjavila M. T. and Moreno J. J. (2012). The effects of polyphenols on oxidative stress and the arachidonic acid cascade. Implications for the prevention/treatment of high prevalence diseases. Biochem Pharmacol. 84.

Mladenov M., Gokik M., Hadzi-Petrushev N., Gjorgoski I., Jankulovski N. (2015).The relationship between antioxidant enzymes and lipid peroxidation in senescent rat erythrocytes. Physiol Res. 64(6).

Moein S. (2015). Polyphenols and cancer: A review. Mol. Med. 1(1).

Mohamadi Yarijani Z., Najafi H., Shackebaei D., Hamid Madani S., Modarresi M.,Vahid Jassemi S. (2019). Amelioration of renal and hepatic function, oxidative stress, inflammation and histopathologic damages by Malva sylvestrisextract in gentamicin induced renal toxicity. Biomed. Pharmacother.112.

Mohamed N.Z., Abd-Alla H.I., Aly H.F., Mantawy M., Ibrahim N., Hassan S.A. (2014). CCl_4 - induced hepatonephrotoxicity: protective effect of nutraceuticals on inflammatory factors and antioxidative status in rat. J. Appl. Pharm. Sci. 4 (2).

Mohsina S., Muthusami A., Shankar G., Sureshkumar S., Kate V. (2016).Helicobacter pylori eradication in complicated peptic ulcer: Beneficial in most? Int J Adv Med Health Res. 3.

Morabbi Najafabad A. and Jamei R. (2014). Free radical scavenging capacity and antioxidant activity of methanolic and ethanolic extracts of plum (Prunus domestica L.) in both fresh and dried samples. Avicenna J Phytomed. 4(5).

Morandi Vuolo M., SilvaLima V., Maróstica Junior M.R. (2019). Chapter 2 - Phenolic Compounds: Structure, Classification, and Antioxidant Power. Bioactive Compounds. Health Benefits and Potential Applications.

More S. S., Nugent J., Vartak A. P., Nye S. M., Vince R. (2017). Hepatoprotective Effect of ψ-Glutathione in a Murine Model of Acetaminophen-Induced Liver Toxicity. Chem. Res. Toxicol. 30 (3).

Mubbunu L., Bowa K., Petrenko V., Silitongo M. (2018). Correlation of Internal Organ Weights with Body Weight and Body Height in Normal Adult Zambians: A Case Study of Ndola Teaching Hospital. Anat. Res. Int.5.

Mugoni V., Santoro M.M. (2013). Manipulating redox signaling to block tumor angiogenesis, research directions in tumor angiogenesis, Dr. Jianyuan Chai (Ed.).

Mujtaba A., Masud T., Ahmad A., Naqvi S. M. S., Qazalbash M. A. and Levin R. E. (2016). Effect of Solvents on Extraction Yield, Total Flavonoid, Total Phenolic Contents, DPPH Scavenging Activity and Antibacterial Potential of Three Apricot Cultivars. Transylv. Rev. XXIV(10).

Murakami M. (2012). The molecular mechanism of chronic inflammation development. Frontiers E-Books, Tokyo.

Muro S. (2018). Alterations in Cellular Processes Involving Vesicular Trafficking and Implications in Drug Delivery. Biomimetics. 3 (3).

Myburgh K.H. (2014). Polyphenol supplementation: benefits for exercise performance or oxidative stress? Sports Med. 1.

Nair A., Chauhan P, Saha B. and. Kubatzky K. F. (2019).Conceptual Evolution of Cell Signaling. Int J Mol Sci. 20.

Nakayama S., Kishimoto Y., Saita Y., Sugihara N., Toyozaki M., Taguchi C., Tani M., Kamiya T., Kondo K. (2015). Pine bark extract prevents low-density lipoprotein oxidation and regulates monocytic expression of antioxidant enzymes. Nutr Res. 35(1).

Nasir A., Abubakar M.G., Shehu R. A., Aliyu U., Toge B.K. (2013). Hepatoprotective effect of the aqueous leaf extract of Andrographis paniculata Nees against carbon tetrachloride – induced hepatotoxicity in rats. Nig J Basic Appl Sci. 21(1).

Nematbakhsh M., Pezeshki Z., Moaeidi B.A., Eshraghi-Jazi F., Talebi A., Nasri H., Baradaran S., Gharagozloo M., Safari T., Haghighi M. (2013). Protective Role of Silymarin and Deferoxamine Against Iron Dextraninduced Renal Iron Deposition in Male Rats. Int J Prev Med. 4.

Nevola R., Rinaldi L., Giordano M., Marrone A., Adinolfi L. E. (2018). Mechanisms and clinical behavior of hepatocellular carcinoma in HBV and HCV infection and alcoholic and non-alcoholic fatty liver disease. Hepatoma Res.4(55).

Ngonda F. (2013). In- vitro Anti-oxidant Activity and Free Radical Scavenging Potential of roots of Malawian Trichodesma zeylanicumm (burm. f.). Asian j biomed pharm. Sci. 3(20).

Nguyen G.T., Green E.R., Mecsas J. (2017). Neutrophils to the ROScue: Mechanisms of NADPH Oxidase Activation and Bacterial Resistance. Front Cell Infect Microbiol. 7.

Nguyen T.T. (2012). Systems Biology Approaches to Corticosteroid Pharmacogenomics and Systemic Inflammation (Doctoral dissertation, Rutgers UniversityGraduate School-New Brunswick).

Nita M. and Grzybowski A. (2016). The Role of the Reactive Oxygen Species and Oxidative Stress in the Pathomechanism of the Age-Related Ocular Diseases and Other Pathologies of the Anterior and Posterior Eye Segments in Adults. Oxid Med Cell Longev.

Nita M., Grzybowski A. (2016). The Role of the Reactive Oxygen Species and Oxidative Stress in the Pathomechanism of the Age-Related Ocular Diseases and Other Pathologies of the Anterior and Posterior Eye Segments in Adults. Oxid Med Cell Longev.

Nwosu D. C., Obeagu E. I., Ezenwuba C., Agu G.C., Amah H., Ozims S. J., Nwanjo H.U., Edward A., Izuchukwu I. F., Amadike J. N., Nwagwu A. J., and Emesowum A. C.(2016). Antioxidant status of children with Plasmodium falciparum malaria in Owerri municipal Council of Imo state. Int J Curr Res Chem Pharm. sci. 3(8).

Obeagu E. I. (2018). A Review on Free Radicals and Antioxidants. Int J Res Med Sci. 4(2).

Obeagu E.I., Ochei K.C., Obeagu G.U., Ezeh-Ifeanyi G.N., Ekelozie I.S. and Onyenweaku F. (2018). A REVIEW ON HEPATITIS AND HAEMOSTASIS. Int J *Compr Res Biol Sci. 5(2).*

OCDE: Organisation for Economic Co-operation and Development. (2008). Guideline for testing of chemicals, 425, Acute oral toxicity-fixed dose method. Paris.1-27.

Ofor I. B., Obeagu E. I., Ochei K.C., and Odo M. (2016). Evaluation of Superoxide Dismutase, Glutathione, Vitamins C, E and Trace Element status in Prostate Cancer Patients in Orlu Teaching Hospital, Imo State. Int J Curr Res Chem Pharm sci. 3(2).

Ohashi K., Pimienta M., Seki E. (2018). Alcoholic liver disease: A current molecular and clinical perspective. Liver Res. 2(4).

Oliveras-López M.G., Berná G., Jurado-Ruiz E., López-García de la Serrana H., Martín F. (2014). Consumption of extra-virgin olive oil rich in phenolic compounds has beneficial antioxidant effects in healthy human adults. J Funct Foods. 10.

Orji O.U., Ibiam U.A., Aloke C., Awoke J., Aja P.M., Ugwu Okechukwu P.C., Ezeani N. and Nzubechukwu E. (2016). Biochemical Assessment of Ethanol Leaf Extract of Cnidoscolus aconitifolius on Liver Integrity of Albino Rat Treated with Lead. Glob. J. Pharmacol. 10(4).

Ozenda. (1983). Flore du sahara.deuxieme edition,editions du centre national de la recherché scientifique 15, quai Anatole-France-75700 paris.

Ozougwu J.C., Eyo J.E. (2014). Hepatoprotective effects of Allium cepa extracts on paracetamol-induced liver damage in rat. Afr. J. Biotechnol. 13(26).

Ozougwu Jevas C. (2016). The Role of Reactive Oxygen Species and Antioxidants in Oxidative Stress. Int. J. Pharm. Biol. Sci. 3(6).

Ozougwu Jevas C. Ph.D. (2017). Physiology of the liver. Int J Res Pharm Biosc. 4(8): 13-24.

Palipoch S. (2013). A review of oxidative stress in acute kidney injury: Protective role of medicinal plants-derived antioxidants. Afr J Tradit Complement Altern Med. 10(4).

Panche A.N., Diwan A.D., Chandra S.R. (2016). Flavonoids: an overview. J Nutr Sci. 5.

Papuc G., Goran G.V., Predescu C.N., Nicorescu V. and Stefan G. (2017). Plant polyphenols as antioxidant and antibacterial agents for shelf-life extension of meat and meat products: classification, structures, sources, and action mechanisms. Comp Rev Food Sci Food Safety. 16.

Pascual-Ahuir A., Manzanares-Estreder S. and Proft M. (2017). Pro- and Antioxidant Functions of the Peroxisome-Mitochondria Connection and Its Impact on Aging and Disease. Oxid Med Cell Longev.

Patra B., Schluttenhofer C., Wu Y., Pattanaik S., Yuan L. (2013). Transcriptional regulation of secondary metabolite biosynthesis in plants. Biochim Biophys Acta. 1829.

Pehlivan F. E. (2017). Vitamin C: An Antioxidant Agent..

Perlman R.L. (2016). Mouse models of human disease: An evolutionary perspective. Evol Med Public Health.1.

Peters K.M., Wilson R.B., Borradaile N.M. (2018). Non-parenchymal hepatic cell lipotoxicity and the coordinated progression of non-alcoholic fatty liver disease and atherosclerosis. Curr Opin Lipidol. 29(5).

Petersen M.C., Vatner D.F., Shulman G.I. (2017). Regulation of hepatic glucose metabolism in health and disease. Nat Rev Endocrinol.13(10).

Petrou A.L., Petrou P.L., Ntanos T., Liapis A. (2018). A Possible Role for Singlet Oxygen in the Degradation of Various Antioxidants. A Meta-Analysis and Review of Literature Data. Antioxidants (Basel). 7(3).

Phalitakul S., Okada M., Hara Y. and Yamawaki H. (2011). Vaspin prevents TNF-α-induced intracellular adhesion molecule-1 via inhibiting reactive oxygen species-dependent NF-κB and PKCθ activation in cultured rat vascular smooth muscle cells. Pharmacol. Res. 64.

Phaniendra A., Jestadi D. B., Periyasamy L. (2015). Free Radicals: Properties, Sources, Targets, and Their Implication in Various Diseases. Ind J Clin Biochem. 30(1).

Podio N. S., Baroni M. V. and Wunderlin D. A. (2017). "Relation between polyphenol profile and antioxidant capacity of different Argentinean wheat varieties. A Boosted Regression Trees study," Food Chem. 232.

Podio N. S., López-Froilán R., Ramirez-Moreno E. et al. (2015). "Matching in vitro bioaccessibility of polyphenols and antioxidant capacity of soluble coffee by boosted regression trees," J. Agric. Food Chem. 63(43).

Porter S. (2013). Tidy's Physiotherapy. Elsevier Health Sciences, Amsterdam.

Rampon C., Volovitch M., Joliot A. and Vriz S. (2018). Hydrogen Peroxide and Redox Regulation of Development. Antioxidants. 7.

Ramchoun M., Sellam K., Harnafi, Alem C., Benlyas M., Khallouki F. and Amrani S. (2015). Investigation of antioxidant and antihemolytic properties of Thymus satureioides collected from Tafilalet Region, south-east of Morocco. Asian Pac J Trop Biomed. 5.

Rane J., Jadhao R., Bakal R. L. (2016). Liver diseases and herbal drugs:-A review. J. innov. Pharm.3(2).

Rezaie A., Fazlara A., Karamolah M.H., Shahriari A., Zadeh H.N., Pashmforosh M.(2013). Effects of Echinacea purpurea on Hepatic and Renal Toxicity Induced by Diethylnitrosamine in Rats. Jundishapur J Nat Pharm Prod. 8.

Rhu J., Choi G.S., Kim J.M., Kwon C.H.D., Joh J.W. (2019). Intraoperative Ultrasonography as a Guidance for Dividing Bile Duct During Laparoscopic Living Donor Hepatectomy. Ann Transplant. 24.

Rice K.M., Walker E.M., Wu M., Gillette C., Blough E.R. (2014). Environmental mercury and its toxic effects. J Prev Med Public Health. 47(2).

Richard D.K., Jauro A., Nvau J.B. and Dabun L.J. (2017). Ethanol-induced gastric ulceration in rats: Protective roles of methanol and water extracts of Cyathula prostrata Linn Blume. *J* Pharmacogn Phytochem. 6(5).

Rocha S., Gomes D., Lima M., Bronze-da-Rocha E., Santos-Silva A. (2015). Peroxiredoxin 2, glutathione peroxidase, and catalase in the cytosol and membrane of erythrocytes under H_2O_2-induced oxidative stress. Free Radic Res.49.

Ruskin R., Vasanthakumari B. and Citarasu T. (2017). In vitro antioxidant activity of various leaf extracts of canthium coromandelicum (Burm.f.) Alston. Asian J Pharm Clin Res. 10.

Rác M., Křupka M., Binder S., Sedlářová M., Matušková Z., Raška M., & Pospíšil P.(2015). Oxidative damage of U937 human leukemic cells caused by hydroxyl radical results in singlet oxygen formation. PLoS ONE. 10(3).

Ren X., Zou L., Zhang X., et al. (2017). Redox Signaling Mediated by Thioredoxin and Glutathione Systems in the Central Nervous System. Antioxid Redox Signal. 27(13).

Rangel-Huerta O. D., Pastor-Villaescusa B., Aguilera C. M., and Gil A. (2015). A systematic review of the efficacy of bioactive compounds in cardiovascular disease: Phenolic compounds. Nutrients. 7.

Rani R., Arora S., Kaur J. and Kumari Manhas R. (2018). Phenolic compounds as antioxidants and chemopreventive drugs from Streptomyces cellulosae strain TES17 isolated from rhizosphere of Camellia sinensis. BMC Compl Alt Med. 18

Sadri A.R., Jeschke M.G., Amini-Nik S. (2015). Cellular and molecular cascades during liver regeneration. Surg Res Open J. 2(2).

Sahreen S., Rashid Khan M., Ali Khan R. and Mohammad Alkreathy H. (2015). Protective effects of Carissa opaca fruits against CCl4-induced oxidative kidney lipid peroxidation and trauma in rat. Food Nutr Res.59.

Saini A., Sharma N., Sharma R. (2012). ANIMAL MODELS TO EVALUATE THE CAUSE BEHIND GASTRIC ULCERS. Int. Res. J. Pharm.3(8).

Salah H.B., Jarraya R., Martin M.T., Veitch N.C., Grayer R. J., Simmonds M.S.J. and Damak M. (2002). Flavonol triglycosides from the leaves of Hammada scoparia (Pomel) Iljun. Chem. Pharm. Bull. 50.

Santos-Sánchez N.F., Salas-Coronado R., Villanueva-Cañongo C. and Hernández-Carlos B. (2019). Antioxidant Compounds and Their Antioxidant Mechanism.

Sardar M.M.R., MOI A., Pervin F., Yeasmin F., Ahmed N., SA L. (2019). A study on gender related differences in oxidative stress status. Mediscope.6(1).

Sayehmiri K., Abangah G., Kalvandi G., Tavan H., Aazami S. (2018).Prevalence of peptic ulcer in Iran: Systematic review and meta-analysis methods. J Res Med Sci. 23(8).

Schwarz D.S. and Blower M.D. (2016).The endoplasmic reticulum: structure, function and response to cellular signaling. Cell Mol Life Sci. 73.

Schyman P., Printz R.L., Estes S. K., Boyd K. L., Shiota M. and Wallqvist A. (2018). Identification of the Toxicity Pathways Associated With Thioacetamide-Induced Injuries in Rat Liver and Kidney. Front. Pharmacol.

Seki M., Nakayama M., Sakoh T., Yoshitomi R, Fukui A., E., Tsuda S., Nakano T., K and Kitazono T. (2019).Blood urea nitrogen is independently associated with renal outcomes in Japanese patients with stage 3–5 chronic kidney disease: a prospective observational study. BMC Nephrology. 20(115).

Selene E. Carlo D., Peduto L. (2018). The perivascular origin of pathological fibroblasts. J Clin Invest. 128(1).

Serhan C. N., Dalli J., Colas R.A., Winkler J Wand Chiang N. (2015). Protectins and maresins: New pro-resolving families of mediators in acute inflammation and resolution bioactive metabolome. Biochim. Biophys. Acta (BBA) Mol. Cell Biol. Lipids. 1851.

Shahid F., Lip G Y.H. and Shantsila E. (2018). Role of Monocytes in Heart Failure and Atrial Fibrillation. Am Heart J. 7(3).

Shao A., Drewnowski A., Willcox D.C., *et al.* (2017). Optimal nutrition and the ever-changing dietary landscape: a conference report. Eur J Nutr. 56(1).

Sharifi-Rad M., Tsouh Fokou P.V.,Sharopov F. *et al.* (2018). Antiulcer Agents: From Plant Extracts to Phytochemicals in Healing Promotion. Molecules. 23(7).

Simon F., Oberhuber A., Floros N. *et al.* (2018). Acute Limb Ischemia-Much More Than Just a Lack of Oxygen. Int J Mol Sci.19(2).

Singh D, Cho WC, Upadhyay G. (2016). Drug-Induced Liver Toxicity and Prevention by Herbal Antioxidants: An Overview. Front Physiol.6.

Singh H. R., Rabi S. (2019).Study of morphological variations of liver in human.Transl. Res. Anat.14.

Singh M., Kaur M., Silakari O. (2014). Flavones: An important scaffold for medicinal chemistry. Eur J Med Chem. 84.

Singh R., Kumar M., Mittal A., Mehta P. K. (2017). Microbial metabolites in nutrition, healthcare and agriculture. 3 Biotech. 7(1).

Sistani Karampour N, Arzi A, Rezaie A, Pashmforoosh M, Kordi F. (2019).Gastroprotective Effect of Zingerone on Ethanol-Induced Gastric Ulcers in Rats. Medicina (Kaunas).55(3).

Smeriglio A., Barreca D., Bellocco E., Trombetta D. (2017). Proanthocyanidins and hydrolysable tannins: occurrence, dietary intake and pharmacological effects. Br J Pharmacol. 174(11).

Smirnoff N. and Arnaud D. (2019). Hydrogen peroxide metabolism and functions in plants. New Phytol. 221.

Soccio M., Laus M.N., Flagella Z., Pastore D. (2018). Assessment of Antioxidant Capacity and Putative Healthy Effects of Natural Plant Products Using Soybean Lipoxygenase-Based Methods. An Overview. Molecules. 23(12).

Song P., Zou M.H. (2015). Roles of reactive oxygen species in physiology and pathology.Atherosclerosis : risk, mechanisms, and therapies.First edition.edited by hong wang and cam patterson.

Sostres C., Gargallo C.J., Lanas A. (2014).Interaction between Helicobacter pylori infection, nonsteroidal anti-inflammatory drugs and/or low-dose aspirin use: old question new insights. World J Gastroenterol. 20(28).

Sriuttha P., Sirichanchuen B., Permsuwan U. (2018).Hepatotoxicity of Nonsteroidal Anti-Inflammatory Drugs: A Systematic Review of Randomized Controlled Trials. Int J Hepatol.

Starkel P. and Leclercq I.A. (2011). Animal models for the study of hepatic fibrosis. Best Pract Res Clin Gastroenterol. 25.

Ştefănescu B.E., Szabo K., Mocan A., Crişan G. (2019). Phenolic Compounds from Five Ericaceae Species Leaves and Their Related Bioavailability and Health Benefits. Molecules. 24(11).

Taher A.T., Viprakasit V., Musallam K.M., Cappellini M.D. (2013). Treating iron overload in patients with non-transfusion-dependent thalassemia. Am. J. Hematol. 88.

Talapatra S. K., and Talapatra B. (2015). Shikimic acid pathway. pp. 625–678. Chemistry of Plant Natural Products: Stereochemistry, Conformation, Synthesis, Biology and Medicine. Springer Berlin Heidelberg, Berlin, Heidelberg.

Tan B. L., Norhaizan M. E., Liew W. P. P., Rahman H. S. (2018).Antioxidant and Oxidative Stress: A Mutual Interplay in Age-Related Diseases. Front Pharmacol. 9.

Tanase C., Cosarcă S. and Muntean D. L. (2019). A Critical Review of Phenolic Compounds Extracted from the Bark of Woody Vascular Plants and Their Potential Biological Activity. Molecules. 24.

Taso O.V., Philippou A., Moustogiannis A., Zevolis E., Koutsilieris M. (2019). Lipid peroxidation products and their role in neurodegenerative diseases. Annal for research hospitals.

Taïr K., Kharoubi O., Taïr O.A., Hellal N., Benyettou I., Aoues A. (2016).Aliminium –induced acute neurotoxicity in rats: traetment with aqueous extract of *Arthrophytum* (*Hammada scoparia*). J Acute Dis. 5(6).

Teschke R. (2018). Alcoholic Liver Disease: Alcohol Metabolism, Cascade of Molecular Mechanisms, Cellular Targets, and Clinical Aspects. Biomedicines. 6(4).

Thephinlap C., Pangjit K., Suttajit M. and Srichairatanakool S. (2013). Anti-oxidant properties and anti- hemolytic activity of Psidiumguajava, Pandanousodorus and Rhinacanthusnasutus. J Med Plant Res. 7(27).

Tian T., Wang Z. and Zhang J. (2017). Pathomechanisms of Oxidative Stress in Inflammatory Bowel Disease and Potential Antioxidant Therapies. Oxid Med Cell Longev.

Tijani A.A., Adekomi D.A., Adewole S.O.(2018). In vitro and in vivo Determination of Hydroxyl Radical Scavenging Activity (HRSA) of Fractions of Aqueous Extract of Moringa Oleifera Leaves (AEMOL). Eurasian J Med Oncol.2(4).

Tome-Carneiro J. and Visioli F. (2016). Polyphenol-based nutraceuticals for the prevention and treatment of cardiovascular disease: Review of human evidence. Phytomedicine.23.

Torres-Rêgo M., Furtado A.A., Bitencourt M.A., et al. (2016). Anti-inflammatory activity of aqueous extract and bioactive compounds identified from the fruits of Hancornia speciosa Gomes (Apocynaceae). BMC Complement Altern Med.16.

Toshimi M. and Keiichi O. (2019). Roles of Phosphate in Skeleton. Front Endocrinol. 10.

Treberg J. R., Braun K., Selseleh P. (2019). Mitochondria can act as energy-sensing regulators of hydrogen peroxide availability . Redox Biol. 20.

Triantafyllou E., Woollard K.J., McPhail M. J. W., Antoniades C.G. and Possamai L. A. (2018).The Role of Monocytes and Macrophages in Acute and Acute-on-Chronic Liver Failure. Front Immunol.

Tundis R., Loizzo M.R., Statti G.A., Menichini F. (2007).Pharmazie. 62.

Velavan S. (2015). PHYTOCHEMICAL TECHNIQUES - A REVIEW. World *J Sci Res.1(2).*

Velmurugan V., Arunachalam G. (2014). Hepatoprotective activity of methanol extract of stem bark of Prosopis cineraria Linn against carbon tetrachloride induced hepatotoxicity. Int J Pharm Sci.6.

Velavan S. (2015). Phytochemical techniques –a review. World J Sci Res. 1(2).

Venditti A., Maggi F., Vittori S., Papa F., Serrilli A.M., Di Cecco M., Ciaschetti G., Mandrone M., Poli F and Bianco A.(2015). Antioxidant and α-glucosidase inhibitory activities of Achillea tenorii. J Pharm. Biol. 53(10).

Veronezi C.M. and Jorge N. (2018). Effect of Carica papaya and Cucumis melo seed oils on the soybean oil stability. Food Sci Biotechnol. 27(4).

Veskoukis A.S., Kyparos A., Nikolaidis M.G., Stagos D., Aligiannis N., Halabalaki M. *et al.* (2012). The antioxidant effects of a polyphenol-rich grape pomace extract in vitro do not correspondin vivo using exercise as an oxidant stimulus. Oxid Med Cell Longev.

Vetriselvan S., Subasini U., Velmurugan C., Muthuramu T., Shankar Jothi, Revathy. (2013). Anti-inflammatory activity of cucumis sativus seed in carrageenan and xylene induced edema model using albino wistars rats. Int J Biopharmaceutics. 4(1).

Vicente S.J., Ishimoto E.Y., Torres E.A.F.S. (2014). Coffee modulates the transcription factor Nrf2 and highly increases the activity of antioxidant enzymes in rats. J Agr Food Chem.12.

Wan T.C., Chen C.M., Lin L.C. (2013). Hepatoprotective effects of natural Calculus Bovis against diethylnitrosamine induced hepatic injury in rats. J Pharmacogn Phytother. 5.

Wanders R.J.A., Waterham H.R. and Ferdinandusse S. (2016). Metabolic Interplay between Peroxisomes and Other Subcellular Organelles Including Mitochondria and the Endoplasmic Reticulum. Front Cell Dev Biol.28.

Wang S., Alseekh S., Fernie A. R., Luo J. (2019). The Structure and Function of Major Plant Metabolite Modifications. Mol Plant. 12 (7).

Wang W., Fan J., Wang X., Zhang H., Li J., Zhang X. (2019). Phenolic compounds and bioactivity evaluation of aqueous and methanol extracts of Allium mongolicum Regel. Food Sci Nutr. 7.

Watts J., Nimer S. (2018).Recent advances in the understanding and treatment of acute myeloid leukemia. F1000Res. 7:F1000 Faculty Rev-1196.

Webb R., Hughes M.G., Thomas A.W., Morris K. (2017). The Ability of Exercise-Associated Oxidative Stress to Trigger Redox-Sensitive Signalling Responses. Antioxidants. 6(63).

Wei D.D., Wang J.S., Duan J.A., Kong L.Y. (2018). Metabolomic Assessment of Acute Cholestatic Injuries Induced by Thioacetamide and by Bile Duct Ligation, and the Protective Effects of Huang-Lian-Jie-Du-Decoction. Front Pharmacol.9.

Wen Z. B., Zhang M. L., Zhu G. L., & Sanderson S. C. (2010). Phylogeny of Salsoleae (Chenopodiaceae) based on DNA sequence data from ITS, psbB–psbH, and rbcL, with emphasis on taxa of northwestern China. Plant System Evolution. 288(1).

Wojnárovits L., Tóth T. and Takács E. (2018) Critical evaluation of rate coefficients for hydroxyl radical reactions with antibiotics.Crit Rev Env Sci Tec. 48(6)0

Wongrakpanich S., Wongrakpanich A., Melhado K., Rangaswami J. (2018).A Comprehensive Review of Non-Steroidal Anti-Inflammatory Drug Use in The Elderly. Aging Dis.9(1).

Wu T., Zhang Q., Song H. (2018). Swertiamarin attenuates carbon tetrachloride (CCl4)-induced liver injury and inflammation in rats by regulating the TLR4 signaling pathway. Braz J Pharm Sci.54 (4).

Yalcin E., de la Monte S. (2016).Tobacco nitrosamines as culprits in disease: mechanisms reviewed. J Physiol Biochem. 72(1).

Yan M., Huo Y., Yin S., Hu H. (2018). Mechanisms of acetaminophen-induced liver injury and its implications for therapeutic interventions. Redox Biol. 17.

Yasmen N., Aziz Md. A., Tajmim A., Akter Mst. I., Hazra A. K. and Rahman S. M. M. (2018). Analgesic and Anti-Inflammatory Activities of Diethyl Ether and n-Hexane Extract of Polyalthia suberosa Leaves. Evid-Based Compl Alt.

Yazan L.S., Zainal N. A., Ali R.M., Zali M. F. S. M., Sze O. Y., Sim T.Y., Gopalsamy B., Ling V. F., Sapuan S., Esa N., Haron A. S., Zakaria F. H. (2018). Antiulcer Properties of Kelulut Honey against Ethanol-Induced Gastric Ulcer. Pertanika J Sci & Technol. 26 (1).

Yuan H., Ma Q., Ye L., Piao G. (2016). The Traditional Medicine and Modern Medicine from Natural Products. Molecules.21(5).

Zerargui F., Boumerfeg S., Charef N., Baghiani A., Djarmouni M., Khennouf S., Arrar L., Abu Zaga M., Mubarak M. (2015). Antioxidant potential and xanthine oxidase inhibitory effect of two furanocoumarins isolated from Tamus communis L. Med Chem.11.

Zhao X., Zhao Q., Luo Z., Yu Y., Xiao N., Sun X., Cheng L. (2015). Spontaneous immortalization of mouse liver sinusoidal endothelial cells. Indian J Med Microbiol. 35(3).

Zhou Y., Hong Y., Huang H. (2016). Triptolide Attenuates Inflammatory Response in Membranous GlomeruloNephritis Rat via Downregulation of NF-κB Signaling Pathway. Kidney Blood Press Res. 41.