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Abstract

The present study aimed to analyze chemically and evaluate the hypotensive, diuretic and the antioxidant activities of *Crataegus azarolus* (CA), *Myrtus communis* (MC) and *Origanum glandulosum* (OG) which are widely used as natural remedy for hypertension in the Algerian folk medicine. *In vitro* antioxidant potential of methanolic extract (ME) and its three fractions: Chloroform (CHE), ethyl acetate (EAE) and water (AqE) extracts has been investigated using several assays. Chemical analysis of EAE of selected plants revealed the isolation of five compounds; two flavonoids((+) - catechin and hyperoside) were isolated from CA, one flavonol glycoside (myricetin -3-O-rhamnoside) was isolated from MC, one flavanone (katuranin), and one monoterpene (5-isopropyl-3-methylphenol) were isolated from OG. The quantification of polyphenols and flavonoids showed that EAE from the three studied plants contained the highest amount of polyphenols. However, CHE-MC, CHE-OG and EAE-CA contained the highest amount of flavonoids. EAE from CA, MC and OG exhibited the highest activity in scavenging DPPH, ABTS and hydroxyl radicals and reducing power test; whereas, ME and AqE exhibited higher chelating activity than CHE and EAE. Inhibition of lipid peroxidation was performed using the β -carotene bleaching assay, ferric thiocyanate method and thiobarburic acid method. CHE-MC, CHE-OG and EAE-CA were found to be the strongest inhibitors of lipid peroxidation in the three methods. Intravenous administration of ME and EAE decreased the mean arterial blood pressure in anesthetized rats dose-dependently, at the dose range of 0.4 to 12 mg/kg, and the strongest hypotensive effect was noticed for CA. The diuretic activity of ME (100, 300 and 600 mg/kg) was assessed orally in rats using furosemide as a standard drug. The results showed that only CA and OG produced an increase in urine output and the urinary excretion of Na^+ and Cl^- . The present study also assessed the effect of administration of ME (100 and 200 mg/kg) in rats for 14 days on plasma antioxidant capacity (PAC) and oxidative stress markers in liver and kidney. It was found that ME-CA (100 and 200 mg/Kg) and ME-OG (200 mg/kg) caused significant elevation of PAC using DPPH radical whereas only ME-CA at a dose of 200 mg/kg increased significantly the plasma reducing power. The administration of the same doses of ME of the three studied plants increased proteins level in liver. The results showed that ME-CA was more effective in enhancing antioxidant status in liver and kidney than other plant extracts by reducing MDA level and increasing catalase activity and GSH level. These results clearly indicate that these plants can be used as a hypotensive agent and as a protective shield against numerous free-radical mediated diseases.

Key words: *Crataegus azarolus*, *Myrtus communis*, *Origanum glandulosum*, phytochemistry, polyphenols, antioxidant, hypertension.

Résumé

L'objectif de cette étude est l'analyse phytochimique, l'évaluation des effets hypotensifs, diurétiques et l'activité antioxydante de *Crataegus azarolus* (CA), *Myrtus communis* (MC) et *Origanum glandulosum* (OG) qui sont largement utilisées en médecine traditionnelle algérienne dans le traitement de l'hypertension. Le potentiel antioxydant de l'extrait méthanolique (EM) et ses trois fractions; l'extrait de chloroforme (ECH), l'extrait d'acétate d'éthyle (EAE) et l'extrait aqueux (EAq) a été étudié *in vitro* en utilisant plusieurs techniques. L'analyse phytochimique d'EAE a permis d'identifier 5 molécules; deux flavonoïdes ont été isolés du CA ((+) - catéchine et l'hypéroside), un flavonole glycosylé (myricétine -3-O-rhamnoside) a été isolé du MC, un flavanone (katuranine), et un monoterpène (5-isopropyl-3-méthylphénol) ont été isolés d'OG. La quantification des polyphénols et des flavonoïdes a montré que l'extrait EAE de trois plantes étudiées est le plus riche en polyphénols. Cependant, les extraits ECH-MC, ECH-OG et EAE-CA ont présenté le contenu le plus élevé en flavonoïdes. L'EAE présente une activité antioxydante très importante en utilisant le test de piégeage des radicaux de DPPH, d'ABTS et d'hydroxyle, et le test du pouvoir réducteur. Cependant, l'EM et l'EAq présentent une activité chélatrice plus élevée que celle de l'ECH et l'EAE. L'inhibition de la peroxydation lipidique a été évaluée par les tests du blanchissement de β -carotène, de thiocyanate ferrique et d'acide thiobarburique. Les extraits ECH-MC, ECH-OG et EAE-CA ont révélé l'inhibition la plus puissante dans ces trois méthodes. L'administration intraveineuse de l'EM et l'EAE (0.4 à 12 mg/kg) a diminué la pression artérielle de manière dose dépendante chez les rats anesthésiés, mais l'effet hypotensif le plus fort a été noté pour la plante CA. L'activité diurétique de l'EM (100, 300 et 600 mg/kg) évaluée chez les rats a prouvé que seulement CA et OG qui ont produit une augmentation du volume d'urine et de l'excrétion urinaire de Na^+ et Cl^- . En outre, l'activité antioxydante des plantes a été évaluée *in vivo* par l'administration de l'EM (100 et 200mg/kg) chez les rats pendant 14 jours. L'EM-CA (100 et 200 mg/Kg) et l'EM-OG(200mg/kg) ont entraîné une augmentation significative de la capacité antioxydante plasmatique en utilisant le radical DPPH tandis que seulement l'EM-CA (200 mg/kg) a augmenté significativement le pouvoir réducteur du plasma. Les mêmes doses administrées ont augmenté significativement le taux des protéines dans le foie. Les résultats ont montré aussi que l'EM-CA était le plus efficace dans l'amélioration du statut antioxydant dans le foie et le rein par rapport aux autres extraits en réduisant le niveau du MDA, et en augmentant l'activité du catalase et le niveau de GSH. Ces résultats fournissent une base scientifique pour l'usage de ces plantes comme un agent hypotensif et protecteur contre de nombreuses maladies associées au stress oxydant.

Mots clés : *Crataegus azarolus*, *Myrtus communis*, *Origanum glandulosum*, phytochimie, polyphénols, antioxydant, hypertension.

ملخص

تهدف هذه الدراسة إلى التحليل الكيميائي وتقييم التأثير المخفض للضغط الدموي والمدر للبول والنشاطية المضادة للأوكسدة لكل من مستخلصات *Crataegus azarolus* (CA)، *Myrtus communis* (MC)، و *glandulosum* (OG) و *Origanum* التي تستخدم على نطاق واسع في الطب الشعبي الجزائري لعلاج ارتفاع ضغط الدم. أسفر التحليل الكيميائي لمستخلص حالات الإيثيل لهذه النباتات إلى التعرف على خمسة مركبات. فقد تم عزل فلافونويدين (catechin - (+) و hyperoside) من CA و فلافونول سكري (myricetin -3-O-rhamnoside) من MC و فلافانون (katuranin) و تاريخين أحادي (5-isopropyl-3-methylphenol) من OG. تم تقدير النشاطية المضادة للأوكسدة مخبريا (*in vitro*) لكل من المستخلص الميثانولي (ME) والكلوروفورمي (CHE) ومستخلص حالات الإيثيل (EAE) والمستخلص المائي (AqE) باستخدام عدة تقنيات. أظهر التقدير الكمي لعديدات الفينول والفلافونويدات أن مستخلص EAE للنباتات الثلاثة يحتوي على أكبر كمية من عديدةات الفينول بينما يحتوي كل من CHE-MC و CHE-OG و EAE-CA على أكبر كمية من الفلافونويدات. أظهر مستخلص EAE نشاطية مضادة للأوكسدة معتبرة جدا باستخدام التأثير الإزاحي اتجاه كل من جذر DPPH و ABTS والهيدروكسيل وتقنية القدرة الارجاعية، بينما تبين أن كل من ME و AqE يملكان قدرة عالية في استخلاص المعادن مقارنة بمستخلصي CHE و EAE. تم تقييم تثبيط فوق أكسدة الليبيدات عن طريق اختبار تبييض β - كاروتين، وثيوسيانات الحديد وحامض ثيوباربريك. وقد تبين أن كل من المستخلصات EAE-CA و CHE-MC و CHE-OG تملك قدرة تثبيطية عالية. أدت معالجة جردان مخدرة بجرعات من 0.4 إلى 12 مغ/كغ للمستخلصات ME و EAE محقونة وريديا إلى تخفيض الضغط الشرياني بشكل يتناسب مع الزيادة في التركيز، وقد سجل التأثير الأقوى عند حقن مستخلصات CA. كما أدى تقييم النشاط المدر للبول لجرعات (100 و 300 و 600 مغ/كغ) من المستخلص ME المعطى شفويا لدى الجرذان، أن المعاملة بنباتي CA و OG أدت إلى زيادة معنوية في حجم البول وإطراح الصوديوم و الكلور. درست النشاطية المضادة للأوكسدة للمستخلصات حيويا (*in vivo*) بجرعات 100 و 200 مغ/كغ من المستخلص الميثانولي عند الجرذان لمدة 14 يوما. حيث أدت المعاملة بكل من ME-CA (100 و 200 ملغ / كغ) و OG-EM (200 مغ / كغ) إلى زيادة معتبرة في القدرة المضادة للأوكسدة للبلازما باستخدام جذر DPPH، في حين كانت المعاملة بجرعة EM-CA (200 مغ / كغ) هي الوحيدة التي أدت إلى زيادة معنوية في القدرة الارجاعية للبلازما. كما أدت المعاملة بكل الجرعات إلى زيادة كبيرة في مستويات البروتينات في الكبد. بالإضافة إلى ذلك، أظهرت النتائج أن مستخلص ME-CA كان الأكثر فعالية في تحسين النشاطية المضادة للأوكسدة للكبد والكلية بالمقارنة مع المستخلصين الآخرين، حيث عمل هذا الأخير على خفض مستوى MDA وزيادة نشاط الكاتالاز ومستوى GSH. يمكن لهذه النتائج أن توفر أساسا علميا في استخدام هذه النباتات كعامل خافض للضغط وواقى ضد العديد من الأمراض المرتبطة بالإجهاد التأكسدي.

الكلمات المفاتيح: *Crataegus azarolus*، *Myrtus communis*، *Origanum glandulosum*، التحليل

الكيميائي، عديدةات الفينول، ارتفاع الضغط الشرياني.

List of abbreviations

Ap -1: Activator protein 1

APCI -MS: Atmospheric pressure chemical ionization-mass spectrometry

br. s : broad singlet

BH4 : Tetrahydrobiopterin

cAMP : Cyclic adenosine monophosphate

cGMP: Cyclic guanine monophosphate

cGKI : cGMP-dependent protein kinase

CDCl₃ : Chloroform deuterated

COSY : Correlated Spectroscopy

d: doublet

dd: double of doublets

DBP : Diastolic Blood Pressure

DEPT : Distortionless Enhancement by Polarization Transfer

DMSO : Dimethylsulfoxide

DNA: Deoxyribonucleic acid

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

ELISA: enzyme-linked immunosorbent assay

EtOH: Ethanol

Fe²⁺ : Ferrous iron

Fe³⁺ : Ferric iron

HMBC : Heteronuclear Multiple Bond Correlation

HMQC: Heteronuclear Multiple Quantum Coherence

HNE: 4-hydroxy-2-nonenal

HPLC: high performance liquid chromatography

HSQC : Heteronuclear Single Quantum Coherence

Hz : Hertz

I%: Inhibition percentage.

IC₅₀%: Inhibitory concentration for 50% of activity

IP₃ : inositol 1,4,5-trisphosphate

J : Coupling constant

LDL : Low density lipoprotein

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

m: multiplet

MeOH: Methanol

MLCP: myosin light chain phosphatase

NF- κ B :Nuclear factor kappa B

PDE : Phosphodiesterase

s: singulet

SBP: Systolic Blood Pressure

SD: Standard deviation

SEM: Standard error of the mean

Sp-1: Specific protein 1

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Introduction

Presently, hypertension is one of the most important risk factors for cardiovascular diseases and is associated with coronary heart, cerebrovascular and renal diseases, therefore is a major cause of morbidity and mortality (Katoch *et al.*, 2013).

Oxidative stress is described as a condition in which cellular antioxidant defenses are inadequate to completely inactivate reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Aquil *et al.*, 2013). This oxidative stress is considered to be a major etiological or pathogenic agent of many diseases including hypertension, hypercholesterolemia, diabetes, and heart failure (Gönenç *et al.*, 2013).

In human essential hypertension, ROS especially superoxide radical ($O_2^{\bullet-}$), accelerate the breakdown of nitric oxide (NO) by binding it to form peroxynitrite ($ONOO^-$). NO is the main vasodilator factor that maintains vascular homeostasis, normal vasomotor tone and inhibits renal tubular sodium reabsorption. Thus, reduction of bioavailability of NO leads to hypertension through increasing peripheral vasoconstriction and sodium retention (Pendyala *et al.*, 2009; Frombaum *et al.*, 2012).

Antioxidants act as radical- antioxidants scavengers, and inhibit lipid peroxidation and other antioxidant processes: therefore, they are able to protect the human body from several diseases related to oxidative stress. Use of synthetic antioxidant in the prevention of this free radical damage had many toxicological side effects including carcinogenicity, making attractive the search for antioxidant and scavenger natural products (Yadegarinia *et al.*, 2006). A great deal of attention has been focused on a variety of non-vitamin antioxidants such as phenolic compounds which can be found in many plant species and may also contribute to the cellular antioxidative defense mechanisms (Rah *et al.*, 2005).

Furthermore, a large number of flavonoids and phenolic compounds have been isolated from crude plant extracts with proven vasodilatory, hypotensive (Akhlaghi and Bandy, 2009) and diuretic potentials (Arafat *et al.*, 2008) are used as therapeutics for hypertension.

In Algeria, as in many countries, medicinal plants are widely used in the folk medicinal system to treat diseases including arterial hypertension. Ethnopharmacological survey carried out by our lab team in Setif region (Eastern Algeria) indicated that *Crataegus azarolus*, *Myrtus communis* and *Origanum glandulosum* are extensively used in folk medicine for the treatment of hypertension (Bouaziz *et al.*, 2012). However, so far, there are little studies on their medicinal use as hypotensive and diuretic agents. Indeed, the valorization of these hypotensive plants of the national flora will be of a great contribution for the Algerian pharmaceutical industry. Thus, the present study was undertaken to evaluate the hypotensive and diuretic effects on *in vivo* and the antioxidant properties of various extracts from these selected plants; *Crataegus azarolus*, *Myrtus communis* and *Origanum glandulosum*. It also aimed to establish a correlation between the antioxidant activity and total phenolic content in the extracts.

In the present study, the following objectives are formulated:

- Phytochemical analysis of the ethyl acetate fraction of *Crataegus azarolus*, *Myrtus communis* and *Origanum glandulosum* in order to identify some new phenolic compounds present in these plants.
- Determination of polyphenols and flavonoids contents in different plant extracts.
- Evaluation of the *in vitro* antioxidant activity of plant extracts using different methods.
- Evaluation of plasma antioxidant capacity using DPPH radical scavenging test and reducing power method.

- Evaluation of the *in vivo* antioxidant activity of methanolic extract of selected plants by assessing the MDA and GSH levels, and catalase activity in the liver and kidney of rats.
- Evaluation of the hypotensive effect of plant extracts in anaesthetized rats using the invasive method of blood pressure recording.
- Evaluation of the diuretic effect and the urine electrolytes level of methanolic extract administration of studied plants in rats.

Review of literature

1. Oxidative stress

1.1. Definition

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

1.2. Free radicals

Free radicals are defined as molecules or molecular fragments containing one or more unpaired electrons in the outer orbit. This unpaired electron(s) is unstable and usually gives a significant degree of reactivity to the free radical (Valko *et al.*, 2007; Birben *et al.*, 2012). ROS and RNS include radical and non-radical species.

Oxygen free radicals include superoxide ($O_2^{\bullet-}$), hydroxyl ($\bullet OH$), peroxy ($ROO\bullet$), lipid peroxy ($LOO\bullet$), alkoxy ($RO\bullet$) radicals. Nitrogen free radicals include nitric oxide ($NO\bullet$) and nitrogen dioxide ($NO_2\bullet$). Oxygen and nitrogen free radicals can be readily converted to other

non-radical reactive species which are harmful for health. Hydrogen peroxide (H_2O_2), ozone (O_3), singlet oxygen ($^1\text{O}_2$), hypochlorous acid (HOCl), nitrous acid (HNO_2), peroxynitrite (ONOO^-), dinitrogen trioxide (N_2O_3), lipid peroxide (LOOH) are not free radicals and generally named oxidants and can easily lead to free radical reactions in living organisms (Valko *et al.*, 2007; Sen and Chakraborty, 2011; Nunes *et al.*, 2012).

1.3. Sources of ROS and RNS

These reactive species are produced in animals and humans under physiologic and pathologic conditions. Free radicals can be produced from both endogenous and exogenous substances and their production in the body is continuous and a normal part of human physiology.

1.3.1. 1. Exogenous source of free radicals

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

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

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

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

1.3.2. Endogenous sources of free radicals

Major sources of ROS and RNS include, but are not limited to, NADPH oxidases, xanthine oxidase, NO synthases, mitochondrial respiratory chain enzymes (Figure. 1).

1.3.2.1. NADPH oxidases (Nox) (EC 1.6.3.1):

They are enzyme complexes that catalyze the reduction of molecular oxygen using NADPH as an electron donor. Generally, the product of the electron transfer reaction is $O_2^{\bullet -}$.



The Nox family comprises seven members (Nox1-5 and Duox1-2) with distinct tissue distribution and functions (Bedard *et al.*, 2007). So far, only Nox1, Nox2 and Nox4 have been shown to play relevant roles in hypertension pathophysiology (Datla and Griendling, 2010). These isoforms are localized in major sites of blood pressure control. For example, Nox1, Nox2 and Nox4 are expressed in the central nervous system where they regulate sympathetic nerve activity. Nox2 and Nox4 participate in the regulation of renal functions and contribute to end-organ damage associated with hypertension (Nistala *et al.*, 2008). In the vasculature, Nox1 controls smooth muscle cell growth and migration, Nox2 contributes to endothelial dysfunction and Nox4 controls vascular smooth muscle cell differentiation and improves endothelial-dependent vasodilatation (Sousa *et al.*, 2012).

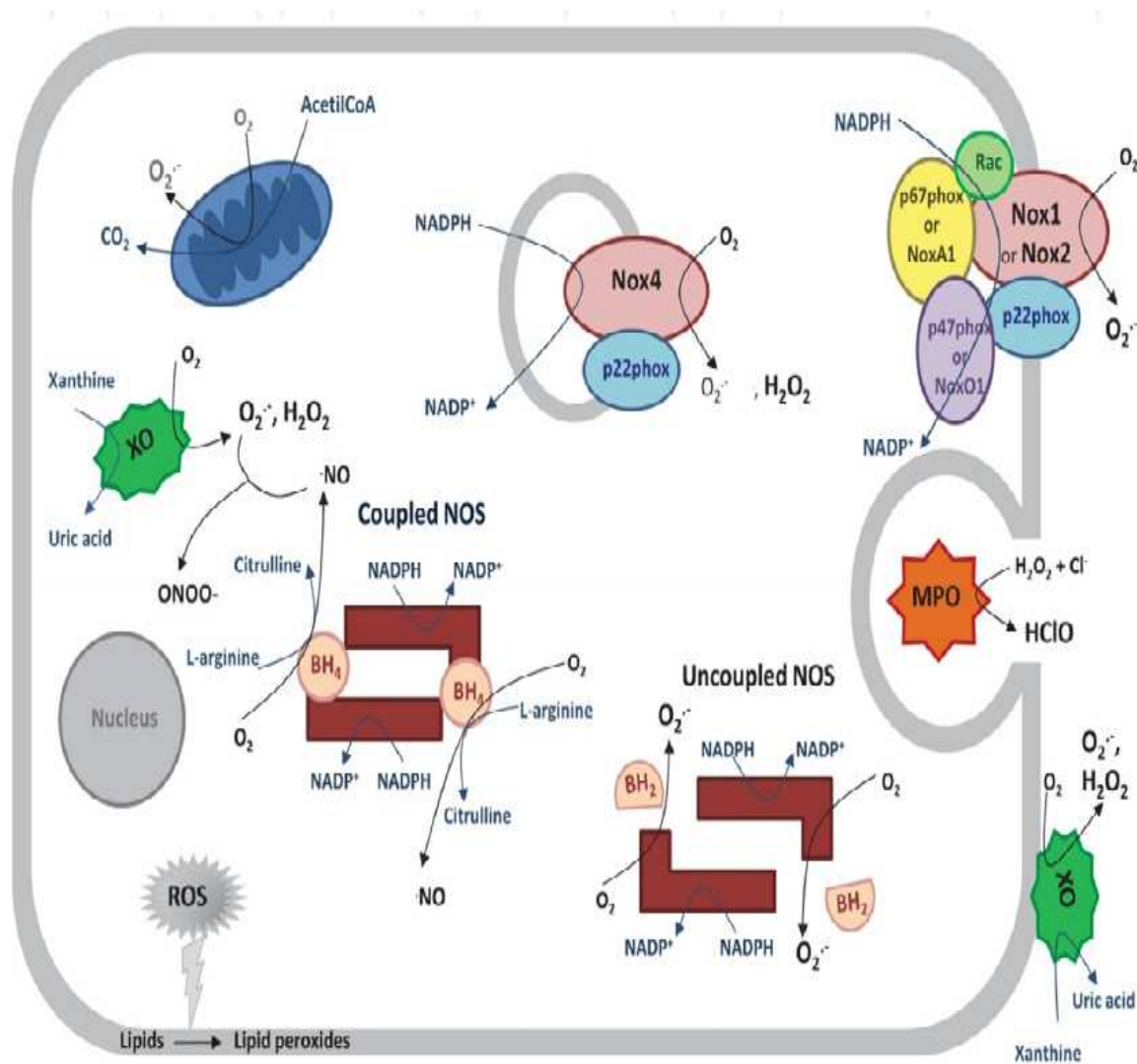
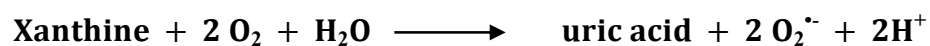


Figure. 1: Endogenous sources of ROS and/or RNS generation (Sousa *et al.*, 2012).

1.3.2.1. Xanthine oxidoreductase (EC 1.17.3.2)

Xanthine oxidoreductase has two inter-convertible forms, xanthine dehydrogenase (XDH) and xanthine oxidase (XO), that participate in purines metabolism catalyzing the conversion of hypoxanthine to xanthine and xanthine to uric acid. XDH preferentially uses NAD^+ as an electron acceptor while the oxidase reduces molecular oxygen in a reaction that generates $\text{O}_2^{\bullet-}$ and H_2O_2 (Sousa *et al.*, 2012).



The XO form predominates in oxidative stress conditions and may contribute to endothelium dysfunction due to its localization in the luminal surface of vascular endothelium (Sousa *et al.*, 2012). Besides the production of ROS by XO, both XDH and XO generate uric acid which possesses antioxidant properties, such as scavenging of ONOO⁻ and HO[•], prevention of oxidative inactivation of endothelium enzymes and stabilization of vitamin. On the other hand, uric acid may also have pro-oxidant and pro-inflammatory effects. Indeed, high systemic levels of uric acid are associated with increased cardiovascular disease and poor outcome but it is not clear whether these effects reflect deleterious actions of uric acid or the oxidative damage caused by XO-derived ROS (Li *et al.*, 2013).

1.3.2.3. Mitochondrial respiratory chain enzymes

They are primary intracellular sources of ROS. More than 90 % of the total oxygen consumed by aerobic organisms is utilized by mitochondrial oxidases which produce ATP in a process coupled to the reduction of cellular oxygen to water. ROS is produced in complex I during reverse electron transport, where electrons enter complex I through coenzyme Q binding. However, mitochondrial complex III catalyzes the electron transfer from ubiquinol to ferric cytochrome c, which is coupled to proton translocation for ATP synthesis (Ye *et al.*, 2014).

Mitochondrial membrane potentials and enhanced proton-motive forces increase ROS formation. In addition, oxygen concentrations, whether hyperoxic or hypoxic may increase superoxide level (Ye *et al.*, 2014). ROS levels in the mitochondria are regulated by the respiratory rate and manganese SOD (Munzel *et al.*, 2010). Hypertensive animals have increased mitochondrial ROS production in the vessels, kidney and central nervous system (Elks *et al.*, 2009).

1.3.2.4. NO synthases (NOS)

It constitute a family of enzyme isoforms (neuronal NOS, nNOS; inducible NOS, iNOS; endothelial NOS, eNOS) that produce $\bullet\text{NO}$ in a reaction that converts L-arginine to L-citrulline (Sugamura and Keaney, 2011). However, in conditions of limited bioavailability of the cofactor tetrahydrobiopterin (BH_4), or the substrate L-arginine, these enzymes become unstable and reduce molecular oxygen to $\text{O}_2\bullet^-$ instead of $\bullet\text{NO}$ production (uncoupled NOS) (Li *et al.*, 2013).

NOS uncoupling is more often described for eNOS and is triggered by oxidative/nitrosative stress (Briones and Touyz, 2010). Numerous experimental studies have shown that arterial hypertension is associated with eNOS dysregulation and endothelial dysfunction (Li *et al.*, 2013).

1.3.2.5. Myeloperoxidase (MPO)

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

1.4. Biological roles of free radicals

The beneficial effects of ROS and RNS occur at low/moderate concentrations and are involved in several normal physiological function against several cellular responses. Most cells can produce superoxide, hydrogen peroxide and nitric oxide constitutively while others have inducible ROS/RNS release system. For example defense against infectious agents by

phagocytosis, killing of cancer cells by macrophages and cytotoxic lymphocytes, detoxification of xenobiotics by cytochrome P450, generation of ATP in mitochondria (energy production), cell growth, and the induction of mitogenic responses at low concentrations are some key beneficial activities of ROS and RNS (Sen and Chakraborty, 2011).

ROS also play important role on different cellular signalling at low concentration like activation of several cytokines and growth factor signalling, non-receptor tyrosine kinases activation, protein tyrosine phosphatases activation, release of calcium from intracellular stores, activation of nuclear transcription factors. Also, ROS exert vital actions such as gene transcription, and regulation of soluble guanylate cyclase activity in cells. NO produced by endothelial cells is essential for regulation of vascular tone and is key for neural plasticity, and mediator of the immune response by activated macrophages (Valko *et al.*, 2007; Rahal *et al.*, 2014). Moreover, recent studies also suggest that the ROS, such as superoxide, hydrogen peroxide may act as second messengers, but may be harmful as the accumulation of these free radicals are increased (Rahal *et al.*, 2014).

1.5. Harmful effect of ROS and RNS on biomolecules

Higher production of ROS in body may change DNA structure, result in modification of proteins, lipids, sugars and activation of several stress-induced transcription factors, and production of proinflammatory and anti-inflammatory cytokines (Birben *et al.*, 2012).

1.5.1. Effects of oxidative stress on lipids

Lipid peroxidation can damage cell membranes by disrupting fluidity and permeability. Peroxidation of lipid is initiated by the attack of a species, which can remove a hydrogen atom from a methylene group, resulting the formation of an unpaired electron on the carbon atom

($\bullet\text{CH}$). Carbon radical thus formed is stabilized by molecular rearrangement to produce a conjugated diene, which then can react with an oxygen molecule to form a lipid peroxyl radical ($\text{LOO}\bullet$) (Birben *et al.*, 2012; Kalyanaraman, 2013).

These radicals can react with other lipid molecules to abstract hydrogen atoms further, so that lipid hydroperoxides (LOOH) form and at the same time propagate other lipid peroxidation further (Figure. 2). Products of lipid peroxidation, such as MDA and unsaturated aldehydes, are capable of inactivating many cellular proteins by forming protein cross-linkages (Kalyanaraman, 2013; Birben *et al.*, 2012; Lavie, 2014).

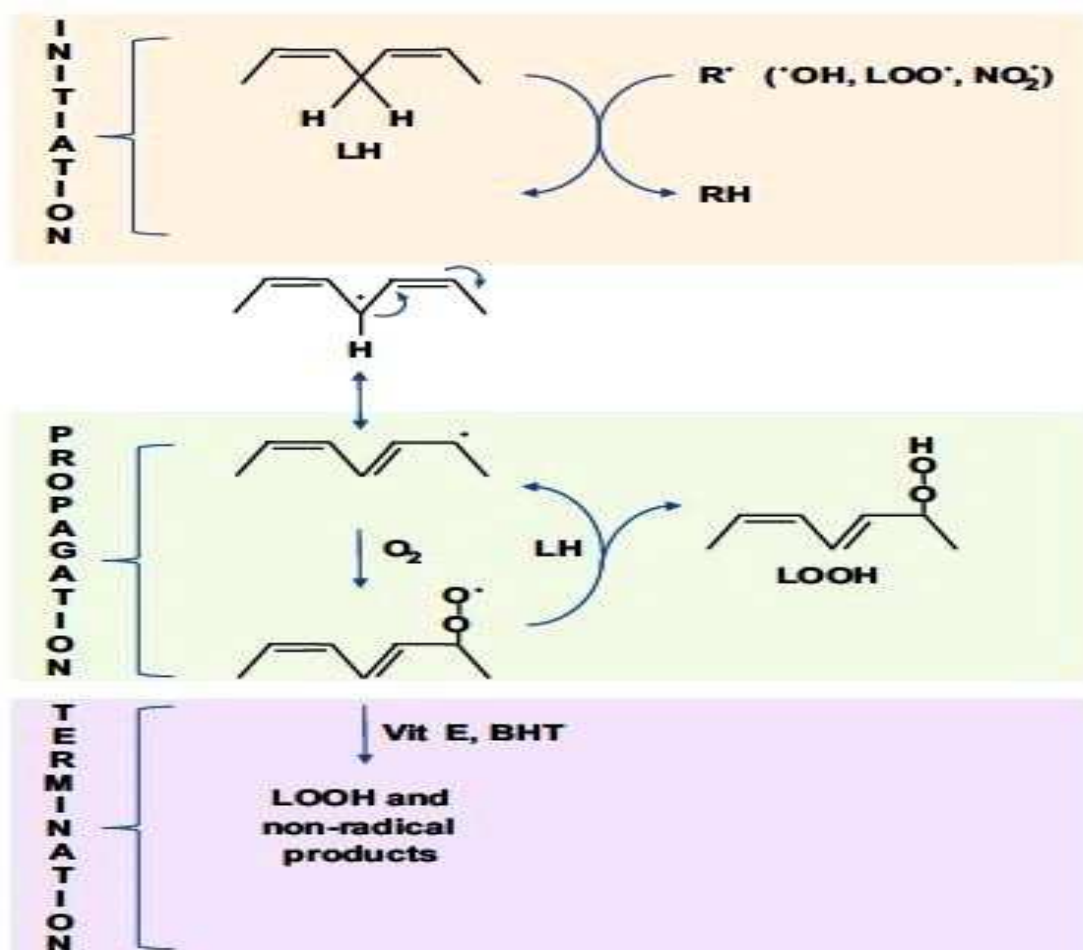


Figure. 2: Lipid peroxidation; oxidation of unsaturated fatty acids to lipid hydroperoxide (Kalyanaraman, 2013).

1.5.2. Effects of oxidative stress on DNA

ROS/RNS interfere with DNA and lead to oxidative damage. DNA is highly susceptible to damage by the free radicals such as $\bullet\text{OH}$ which can react with DNA by addition or loss of hydrogen atoms from the sugar moiety. In particular, the C4-C5 double bond of pyrimidine is very sensitive to attack by $\bullet\text{OH}$, which results generation of a spectrum of oxidative pyrimidine damage products, such as thymine glycol, uracil glycol, urea residue, 5-hydroxydeoxyuridine, 5-hydroxydeoxycytidine, hydantoin and others. Likewise, purines are susceptible to attack by $\bullet\text{OH}$ which leads to the generation of 8-hydroxydeoxyguanosine (8-OHdG), 8-hydroxydeoxyadenosine, formamidopyrimidines and other less characterized purine oxidative products (Sen and Chakraborty, 2011; Birben *et al.*, 2012).

Free radical attack also causes the activation of the poly (ADP-ribose) synthetase enzyme which can lead to fragmentation of DNA and programmed cell death. This process depletes the cellular level of NAD^+ levels thereby disrupting electron transport chain function (Sen and Chakraborty, 2011; Birben *et al.*, 2012; Lavie, 2014).

1.5.3. Effects of oxidative stress on proteins

ROS can cause fragmentation of the peptide chain, alteration of electrical charge of proteins, cross-linking of proteins and oxidation of specific amino acids and therefore lead to increased susceptibility to proteolysis by specific proteases. Also, enzymes that have metals on or close to their active sites are especially more sensitive to metal catalyzed oxidation (Gutiérrez *et al.*, 2014; Lavie, 2014). Cysteine and methionine residues in proteins are particularly more susceptible to oxidation. For example, methionine can be oxidized to methionine sulfoxide and phenylalanine to O-tyrosine; sulfhydryl groups can be oxidized to form disulfide bonds;

and carbonyl groups may be introduced into the side chains of proteins (Gutiérrez *et al.*, 2014; Lavie, 2014).

1.6. Antioxidants

Antioxidants are defined as the substances which at low concentration significantly inhibit or delay the oxidative process, while often being oxidized themselves (Valco *et al.*, 2007). Endogenous and exogenous antioxidants are used to neutralize free radicals and protect the body from free radicals by maintaining redox balance (Pham-Huy *et al.*, 2008). The human body is equipped with a variety of internal antioxidants that serve to counterbalance the effect of oxidants. These can be divided into 2 categories: enzymatic and nonenzymatic antioxidants.

1.6.1. Enzymatic antioxidants

The major enzymatic antioxidants are superoxide dismutase, catalase, GSH peroxidase and GSH reductase (Figure. 3). In addition to these major enzymes, other antioxidants, including glutathione S-transferase (GST), heme oxygenase-1, and redox proteins, such as thioredoxins (TRX), peroxiredoxins (PRXs) and glutaredoxins, have also been found to play crucial roles into antioxidant defenses (Birben *et al.*, 2012; Ye *et al.*, 2014).

1.6.1.1. Superoxide dismutase (SOD)

It is an important endogenous antioxidant enzyme and can exist in three metal containing isoforms which are found in different cellular compartments: SOD1 (copper-zinc SOD) is in the cytoplasm, nucleus and plasma membrane; SOD2 (manganese SOD) is mainly in mitochondria; and SOD3 (copper-zinc SOD) is unique in scavenging superoxide in the extracellular compartment and dismutating superoxides generated during the inflammation

(Athiroh *et al.*, 2014; Ye *et al.*, 2014). SOD acts as the first line defense system against ROS and catalyzes the dismutation of $O_2^{\bullet -}$ to H_2O_2 and O_2 (Sen and Chakraborty, 2011).

1.6.1. 2. Catalase (CAT)

CAT exists as a tetramer composed of 4 identical monomers, each of which contains a heme group at the active site. Degradation of H_2O_2 is accomplished via the conversion between 2 conformations of catalase-ferricatalase (iron coordinated to water) and compound I (iron complexed with an oxygen atom) (Birben *et al.*, 2012). Each CAT molecule can decompose millions of hydrogen peroxide molecules produced by SOD to water and oxygen in every second (Sen and Chakraborty, 2011).

1.6.1. 3. Glutathione peroxidase (GPx)

It is generally present in the cytoplasm of cells and protects the cell against oxidative injury caused by H_2O_2 and prevents the formation of hydroxyl radical from H_2O_2 . GPx remove H_2O_2 by coupling its reduction to H_2O with oxidation of GSH (Navarro-Yepes *et al.*, 2014). GPx1 is present in the cytosol and mitochondria in most mammalian cells and is more effective than catalase in removing intracellular peroxides under many physiological conditions. Besides the SeCys containing GPx, non-selenium GPx are widely represented in mammals as well as most vertebrates and some lower phyla. GPx 5, 7 and 8 are cysteine-containing GPx, while GPx 6 has a selenocysteine active site in humans. cysteine-containing GPxs are much less efficient at detoxifying peroxides than selenodependent GPx (Ye *et al.*, 2014).

1.6.1.4. Glutathione reductase (GR)

It is flavoprotein enzyme and an important cellular antioxidant necessary for the conversion of GSH. The oxidised glutathione is glutathione disulphide (GSSG) which

reduced back to GSH by the presence of enzyme GR which uses NADPH as an electron donor. The ratio of GSH/GSSG is an important general measure of oxidative stress of an organism, very high concentration of GSSG may damage many enzymes oxidatively (Sen and Chakraborty, 2011; Birben *et al.*, 2012; Navarro-Yepes *et al.*, 2014).

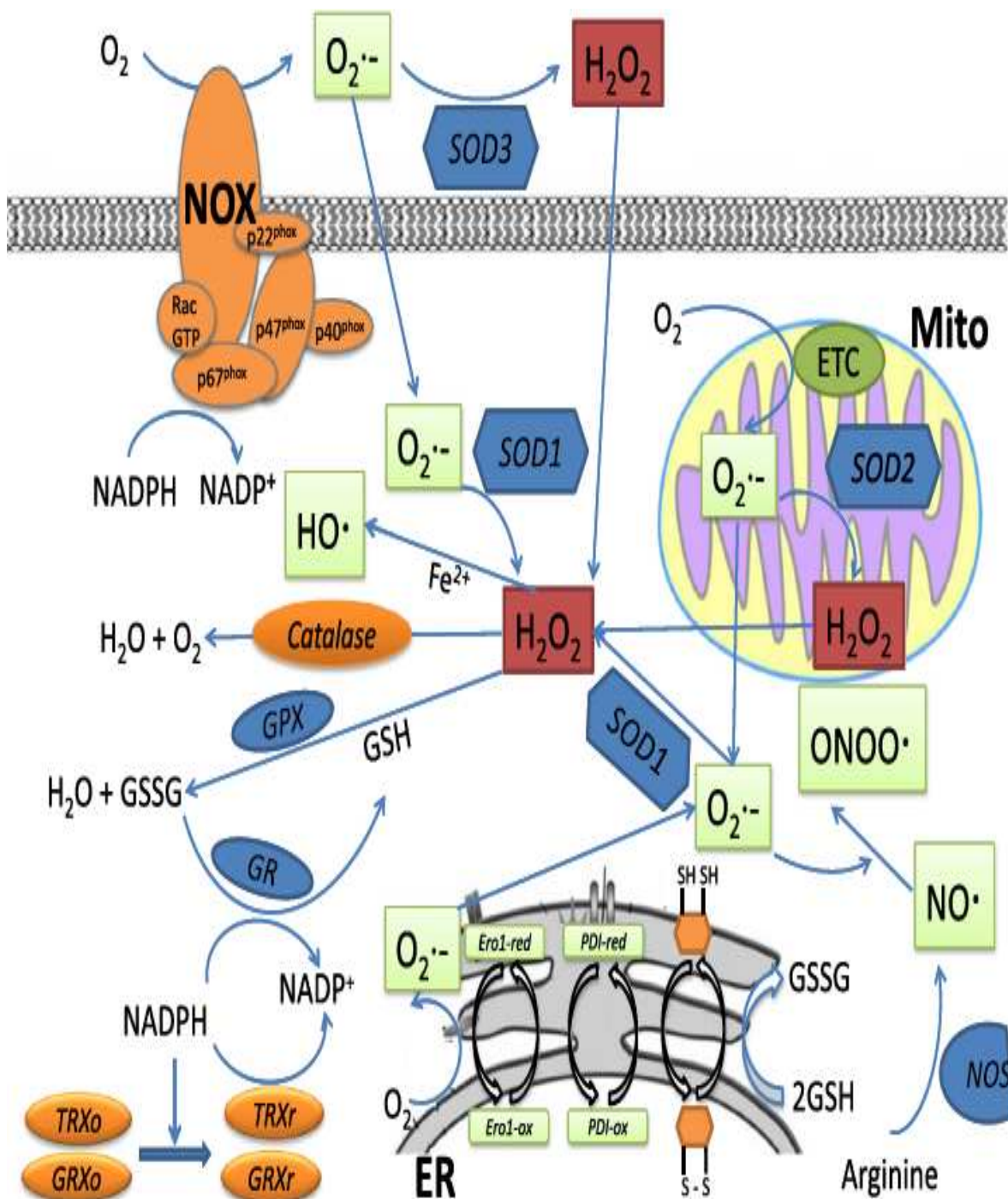


Figure. 3: Cellular enzymatic antioxidants (SOD, Catalase, GPx and GR) (Ye *et al.*, 2014).

1.6.2. Non-enzymatic antioxidants

Non-enzymatic antioxidants are represented by ascorbic acid (Vitamin C), α -tocopherol (Vitamin E), glutathione (GSH), carotenoids, flavonoids, and other antioxidants. Under normal conditions, there is a balance between both the activities and the intracellular levels of these antioxidants. This balance is essential for the survival of organisms and their health.

1.6.2.1. GSH

GSH is a tripeptide (L-g-glutamyl-L-cysteinyl-Lglycine) and the major soluble antioxidant that is highly abundant in all cell compartments. GSH shows its antioxidant effects in several ways. It is also a cofactor for several detoxifying enzymes, such as GSH-Px and GSH transferase, and has a role in converting vitamin C and E back to their active forms. GSH protects cells against apoptosis by interacting with proapoptotic and antiapoptotic signaling pathways. It also regulates and activates several transcription factors, such as AP-1, NF-kB, and Sp-1. Also, reduced glutathione donates protons to membrane lipids and protects them from oxidant attacks (Sousa *et al.*, 2012; Birben *et al.*, 2012).

1.6.2.2. Vitamin E

It is a lipid-soluble vitamin concentrated in the hydrophobic interior site of cell membrane and the principal defense against oxidant-induced membrane injury. Vitamin E donates electron to peroxy radical, which is produced during lipid peroxidation. α -Tocopherol is the most active form of vitamin E and the major membrane-bound antioxidant in cell. Vitamin E triggers apoptosis of cancer cells and inhibits free radical formations by converting $O_2^{\bullet -}$ and $HO^{\bullet}$ to less reactive forms (Birben *et al.*, 2012).

1.6.2.3. Vitamin C

Ascorbic acid is a water-soluble vitamin that provides intracellular and extracellular aqueous-phase antioxidant capacity primarily by scavenging ROS and lipid hydroperoxides. Also, it converts vitamin E free radicals back to vitamin E. Its plasma levels have been shown to decrease with age (Birben *et al.*, 2012).

1.6.2.4. Carotenoids

They are pigments found in plants. Primarily, β -carotene has been found to react with peroxy ($\text{ROO}^\bullet$), hydroxyl ($^\bullet\text{OH}$), and superoxide ($\text{O}_2^{\bullet -}$) radicals. Carotenoids show their antioxidant effects in low oxygen partial pressure but may have pro-oxidant effects at higher oxygen concentrations. Both carotenoids and retinoic acids (RAs) are capable of regulating transcription factors. β -Carotene inhibits the oxidant-induced NF- κ B activation and interleukin (IL)-6 and tumor necrosis factor α production. Carotenoids also affect apoptosis of cells (Birben *et al.*, 2012; Tanumihardjo, 2013).

1.6.2.5. Uric acid

It is a highly abundant aqueous antioxidant, considered to be the main contributor for the antioxidant capacity in the plasma. It has the ability to quench $\text{HO}^\bullet$ and ONOO^- and may prevent lipid peroxidation (Sousa *et al.*, 2012). The scavenging of ONOO^- by uric acid is significantly increased in the presence of vitamin C and cysteine which regenerate the urate radical formed in these reactions. Uric acid also acts as a chelator of iron in extracellular fluids (Sousa *et al.*, 2012).

1.6.2.6. Bilirubin

It is the end-product of heme catabolism, also appears to function as a chain breaking antioxidant. Low circulating bilirubin levels are considered a risk factor for cardiovascular diseases. Plasma albumin, the predominant plasma protein, is also an antioxidant due to its sulfhydryl groups and is able to scavenge MPO-derived chlorinated reactive species and ROO[•] radicals (Sousa *et al.*, 2012).

2.1.2.7. Polyphenols

Polyphenols are naturally available from various dietary sources, and they are increasingly recommended as a daily supplement to prevent oxidative stress. Plants produce more than 8000 different polyphenols as secondary metabolites. Chemically, polyphenols are compounds having one or more hydroxyl groups attached to a benzene ring. They can be categorized as simple phenolics, phenolic acids (derivatives of cinnamic and benzoic acids), coumarins, flavonoids, stilbenes, tannins, lignans and lignins. Chief among these are the flavonoids which have potent antioxidant activities (Fraga *et al.*, 2010; Fadel *et al.*, 2011).

1.6.2.8. Flavonoids

Flavonoids (the term is derived from the Latin word “flavus”, meaning yellow) are ubiquitous plant secondary products that are best known as the characteristic red, blue, and purple anthocyanin pigments of plant tissues. Apart from their physiological roles in the plants, flavonoids are important components of the human diet, but they are not considered as nutrients (Procházková *et al.*, 2011). More than 5000 flavonoids have been identified (Akhlaghi and Bandy, 2009), each with a basic structure containing two benzene rings (A and B) with a pyrane ring in the middle (ring C) (Xiao *et al.*, 2014; Singh *et al.*, 2014). Flavonoids can be divided into several subfamilies according to the degree of oxidation of the oxygenated

heterocycle, being flavonols, flavanones, flavones, flavanes (essentially, flavan-3-ols), isoflavones, and anthocyanidins, the most relevant for human diets (Figure.4) (Xiao *et al.*, 2014; Wu *et al.*, 2015) Starting from a basic chemical structure, plant biosynthetic pathways introduce different hydroxyl group patterns, methyl groups, and sugars (Fraga *et al.*,2010).

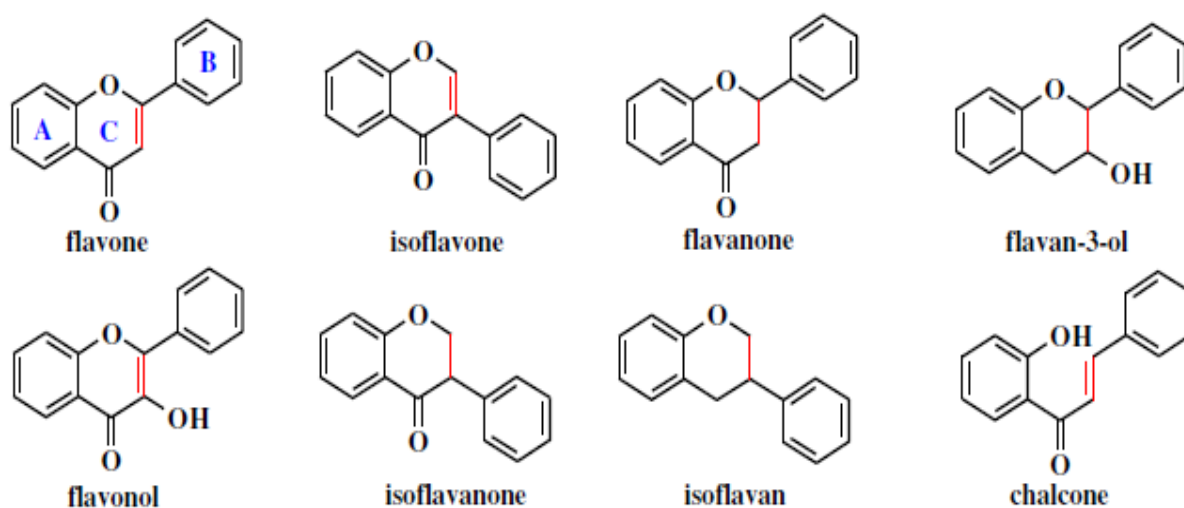


Figure. 4: Basic structure of the main classes of flavonoids (Xiao *et al.*, 2014).

It is already well recognised that flavonoids possess antiviral, antibacterial, anti-inflammatory, vasodilatory, anti-ischemic (Procházková *et al.*, 2011), antilipoperoxidant, antitumoral, antiplatelet and antiallergic activities. In addition, flavonoids exhibit strong antioxidant capacities through scavenging oxygen free radicals, promoting the expression of antioxidant enzymes in the antioxidant system or inhibiting the expression and activity of oxidase enzymes (Figure.5). For these reasons, the biochemistry and medicinal aspects of flavonoids have received increasing attention recently (Wu *et al.*, 2015).

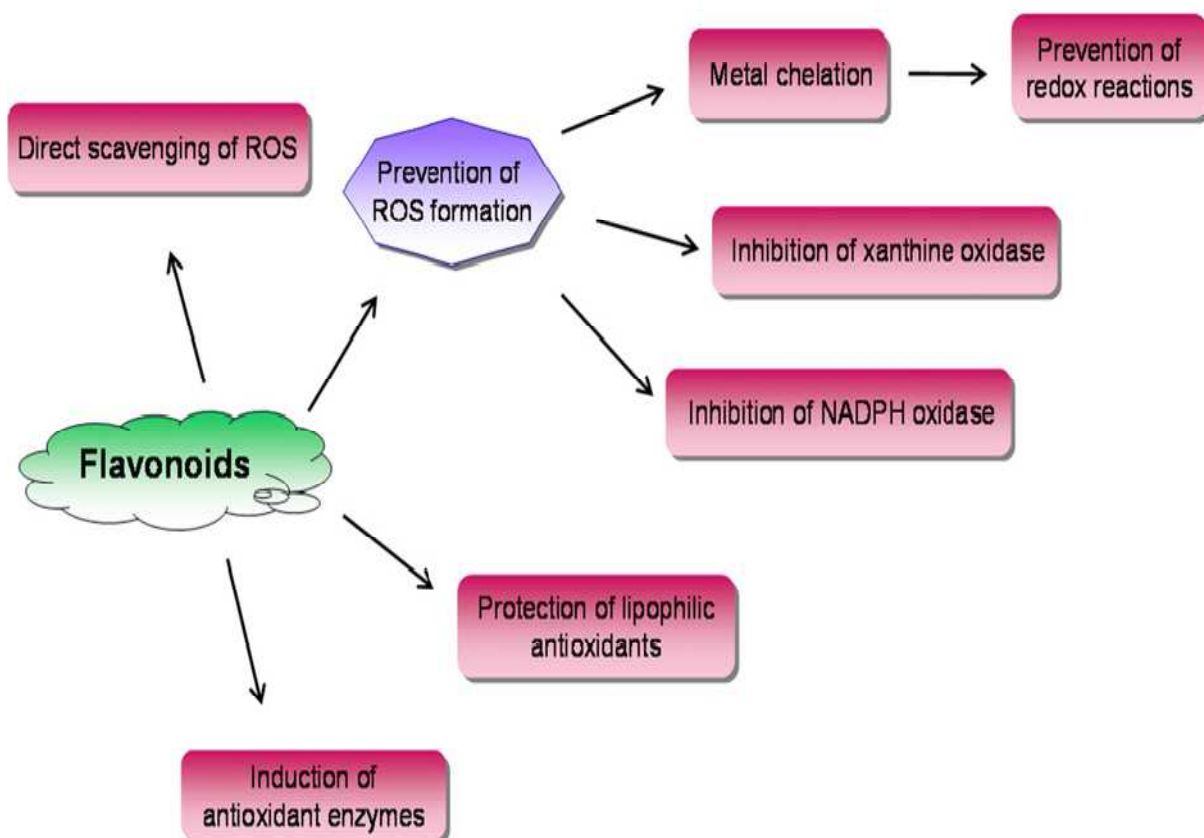


Figure. 5: Mechanisms of antioxidant effects of flavonoids. Flavonoids may exert their antioxidant effects by preventing generation of ROS, direct scavenging of ROS, or indirectly through enhancement of cellular antioxidant enzymes (Akhlaghi and Bandy, 2009).

2. The vascular system

The blood vessels provide the main link between the heart and the tissues and their walls are made up of three layers; the intima (inner layer), the tunica media (middle layer) and the tunica externa (outer layer). The blood vessels are divided depending on function, location and size into arteries, capillaries and veins. Also, arteries can be divided into conducting arteries, conduit arteries and resistance arteries based on their position in the arterial tree and their main function is to supply the organs with blood (Sandoo *et al.*, 2010).

2.1. Endothelium

It is constituted from a monolayer of endothelial cells located on the intima that form a physical barrier between the vessel and the circulating blood. The endothelium releases a variety of messengers that not only regulate blood flow through vasomotion, but are also involved in immune responses, the regulation of coagulation, leukocyte adhesion, vascular smooth muscle cell growth, and also serves as a barrier to the transvascular diffusion of liquids and solutes (Figure.6) (Schildknecht and Ullrich, 2009).

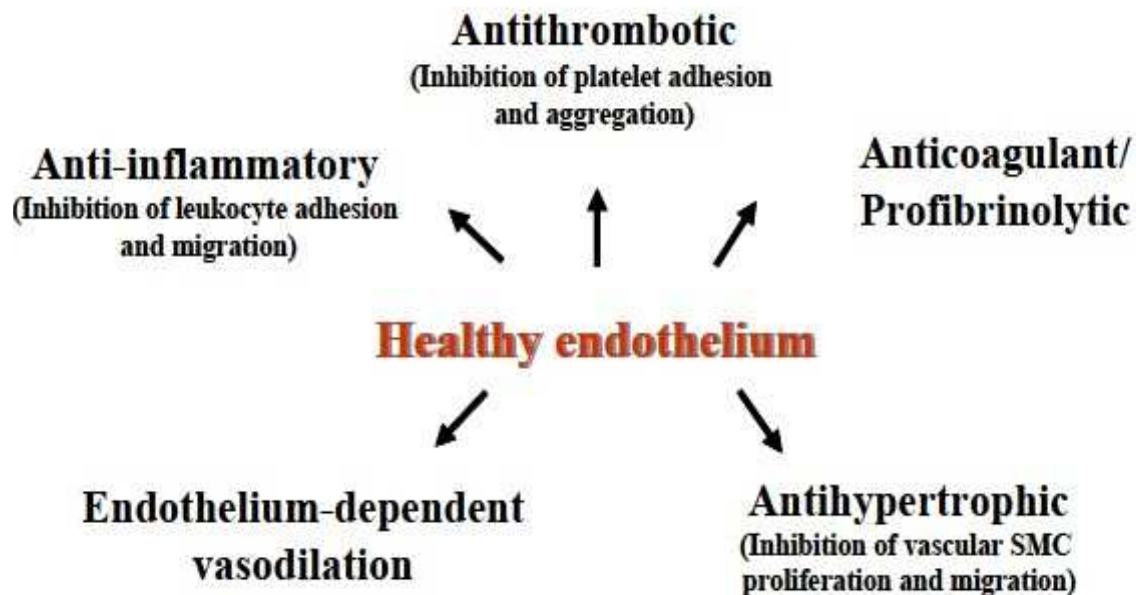


Figure .6: The roles of healthy endothelium (Landmesser *et al.*, 2004).

The endothelium releases various vasoactive factors. These can be vasodilatory factors such as nitric oxide (NO), prostacyclin (PGI₂) and endothelium derived hyperpolarizing factor (EDHF) or vasoconstrictive factors such as thromboxane (TXA₂) endothelin-1 (ET-1), angiotensin II, urotensin II, superoxide, prostaglandins (Figure.7) (Rodrigo *et al.*, 2012; Gutiérrez *et al.*, 2014; Wang *et al.*, 2014). Overall, vascular homeostasis depends on the balance of the bioactive factors released by the endothelium. Thus, a dysfunction of the

endothelial cell disrupts this balance and leads to so called endothelial dysfunction (Perez-Vizcaino *et al.*, 2010).

2.2. Vascular smooth muscle cells

The vascular smooth muscle cells (VSMC) constitute the media layer and are arranged in helical or circular layers around the larger blood vessels and in a single layer around arterioles. VSMC provide active tension in the vessel wall and regulate the diameter of the vessels and are not only involved in the short-term regulation of blood vessel diameter, and therefore in the regulation of blood pressure, but are also involved in long-term adaptation through structural remodeling (Rodrigo *et al.*, 2012; Gutiérrez *et al.*, 2014).

2.3. Contraction mechanisms of VSMC

Contraction in vascular smooth muscle can be initiated by mechanical, electrical, or chemical stimuli. Passive stretching of vascular smooth muscle can cause contraction that originates from the smooth muscle itself and is therefore termed a myogenic response (Shiogai *et al.*, 2010).

A number of chemical stimuli such as norepinephrine, angiotensin II, vasopressin, and endothelin-1 can cause contraction. Whereas acetylcholine, NO, ATP, bradykinin, ADP, substance P, Atrial natriuretic peptide caused dilatation through hyperpolarisation, cAMP or cGMP increase (Figure. 7) (Ferroni *et al.*, 2006; Perez-Vizcaino *et al.*, 2010; Shiogai *et al.*, 2010). Each of these substances binds to specific receptors on the vascular smooth muscle cell (or to receptors on the endothelium), which then leads to vascular smooth muscle contraction or dilatation. Two major biochemical pathways of the endothelium that determine the vascular tone are the NO synthesis pathway and the COX pathway, which generates both vasodilator

and vasoconstrictor prostanoids via the metabolic conversion of arachidonic acid (AA) (Senejoux *et al.*, 2012).

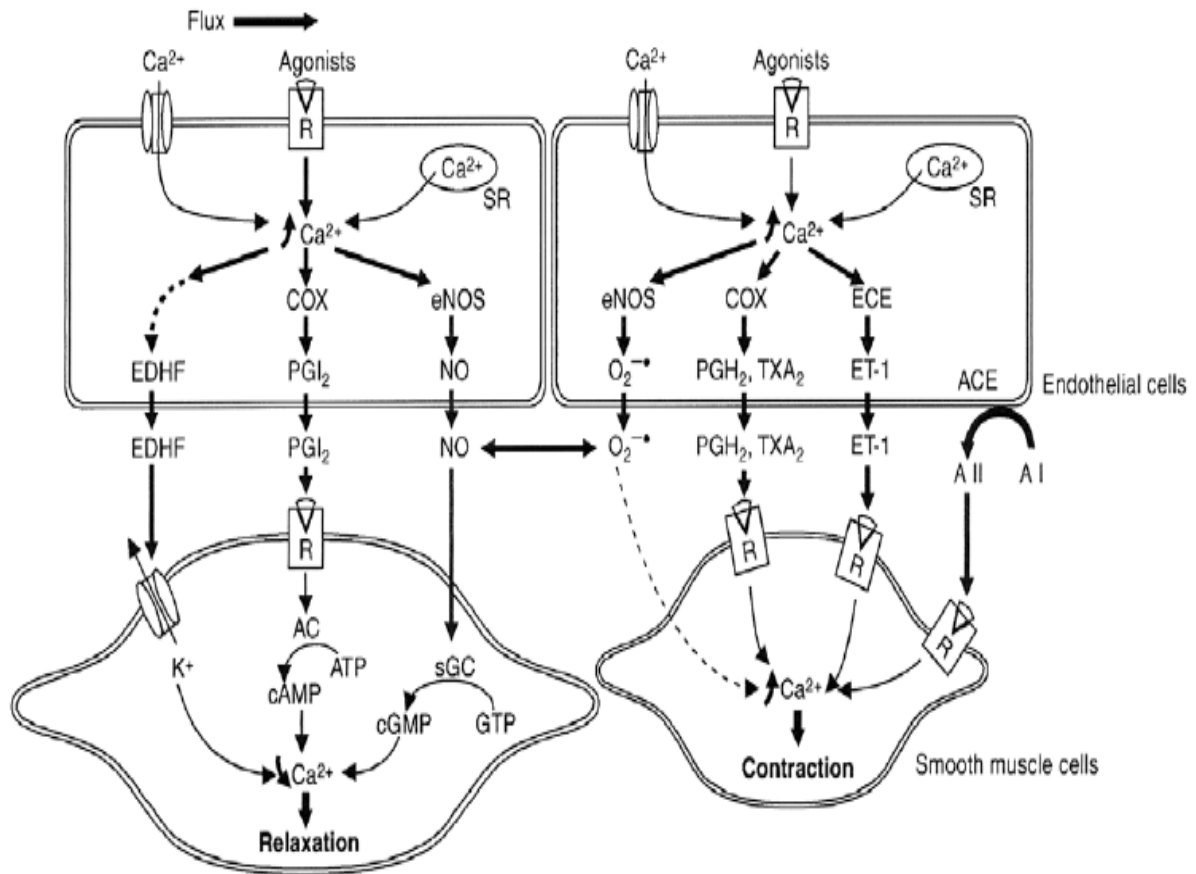


Figure. 7: Release of relaxing and contracting factors from endothelial cells and their effect on vascular smooth muscles. AC: Adenyl cyclase, ACE: angiotensin converting enzyme; ATP: adenosinetriphosphate; AI: angiotensin I; AII: Angiotensin II; cAMP: cyclic adenosine monophosphate; cGMP: cyclic guanosine monophosphate; COX: cyclooxygenase; ECE: endothelin converting enzyme; EDHF: endothelin derived hyperpolarizing factor; eNOS: endothelial nitric oxide synthase; ET-1: endothelin-1; GTP: guanosine triphosphate; NO: nitric oxide; $O_2^{\cdot-}$: superoxide anions; PGH_2 : prostaglandin H_2 ; PGI_2 : prostacyclin; R: receptor; sGC: soluble guanylyl cyclase; SR: sarcoplasmic reticulum; TXA_2 : thromboxane A₂ (Shiogai *et al.*, 2010).

Generally, contraction of VSM in resistance arteries and arterioles is directly influenced by cytoplasmic concentration of free calcium (Ca^{2+}). Activation of protein G leads to the generation of IP_3 that releases Ca^{2+} from the sarcoplasmic reticulum followed by an influx of extracellular Ca^{2+} through voltage-dependent Ca^{2+} channels, resulting in the

formation of the Ca^{2+} –calmodulin complex, which in turn activates the enzyme myosin light chain kinase (MLCK). Activated MLCK phosphorylates the myosin light chain, thereby allowing cycling of cross-bridges between actin and myosin and thus VSM contraction (Wilson *et al.*, 2002; Korthuis *et al.*, 2011). Owing to the circumferential alignment of the long axis of VSM cells around the vessel lumen, contraction results in shortening of VSM and reduces arterial and arteriolar diameter. Smooth muscle contraction is modulated also at constant $[\text{Ca}^{2+}]_i$ by changing the sensitivity of contraction to $[\text{Ca}^{2+}]$. cGKI interferes both with the increase in $[\text{Ca}^{2+}]_i$ and with the Ca^{2+} sensitivity at several levels (Hofmann, 2005).

A decrease in $[\text{Ca}^{2+}]_i$ inactivates MLCK and induces dephosphorylation of regulatory light chains (RLC) by MLCP, leading to relaxation. MLCP is inactivated via Rho-kinase-dependent phosphorylation (Webb *et al.*, 2003).

3. Hypertension

Hypertension is a sustained elevation of systemic arterial pressure to a level greater than or equal to 140 mmHg systolic and 90 mmHg diastolic blood pressure (Wali *et al.*, 2012; Gutiérrez *et al.*, 2014). It is one of the most important risk factors for cardiovascular disease and is associated with coronary heart disease, cerebrovascular disease and renal disease. Therefore, hypertension is a major cause of morbidity and mortality, hence become an increasingly important contributor to the global health burden (Meera and Marcus, 2012; Katoch *et al.*, 2013; Athiroh *et al.*, 2014).

3.1. Types of hypertension

Hypertension may be categorized as either essential or secondary. Essential hypertension is diagnosed in the absence of an identifiable secondary cause (essential

hypertension is also called primary or idiopathic hypertension) and is responsible for nearly 90 % of all hypertension cases. Essential hypertension is the misregulation of a complex interaction between genetic (Katoch *et al.*, 2013) and lifestyle factors affecting the physiological systems regulating blood pressure (Athiroh *et al.*, 2014) as chronic levels of elevated psychological stress, high alcohol intake, smoking, sedentarity, high salt intake as well as obesity (Whitney and Rolfes, 2008).

Secondary hypertension is associated with an underlying disease, which may be renal, neurologic, or endocrine in origin; examples of such diseases, glomerulonephritis, atherosclerosis of blood vessels in the brain, and Cushing's syndrome (Katoch *et al.*, 2013).

3.2. Prevalence

Hypertension is known as the silent killer and is responsible for developing other diseases such as metabolic syndrome, diabetes, renal dysfunction, congestive heart failure, coronary heart and cerebrovascular diseases (Yang *et al.*, 2014).

Actually, it affects approximately 1 billion of the adult population worldwide and is estimated that this figure could increase to 1.5 billion by 2025 (Gutiérrez *et al.*, 2014). Worldwide, around 30% of the population dies from cardiovascular and cerebrovascular events, in which 62% of acute stroke events and 49% of cardiovascular events are directly caused by hypertension (Athiroh *et al.*, 2014). According to the World Health Organization (2010), global status report on non-communicable diseases indicated that the African region has the highest prevalence of hypertension estimated at 46% of adults aged 25 and above (Ikewuchi *et al.*, 2014). In Algeria, it affects 30% of adults based on a survey carried out in two pilot regions, Setif and Mostaganem (Benmohammed *et al.*, 2011).

3. 3. Pathophysiology of hypertension

The exact pathophysiology of hypertension has not been understood completely, but a number of pathophysiological mechanisms have been reported from time to time. The rise in peripheral resistance in established hypertension has been suggested to be attributed to the structural narrowing of small arteries and arterioles (Desir *et al.*, 2012). In addition, decrease in the number and density of capillaries has also been noted to be part of the cause (Montezano and Touyz, 2012).

Further, hypertension has been linked with reduced peripheral venous compliance, enhancing the venous return and cardiac preload, ultimately causing diastolic dysfunction. Moreover, occurrence of high pulse pressure in geriatric patients presented with hypertension or isolated systolic hypertension has been suggested to be caused due to increased arterial stiffness associated with aging. Furthermore, other mechanisms have also been denoted for the development of increased peripheral resistance which includes disturbances in renal salt and water management, particularly abnormalities in the intra-renal renin-angiotensin system, and dysfunctioning of the sympathetic nervous system (Sonu *et al.*, 2013).

Additionally, various evidences have suggested that endothelial dysfunction, aberrant G protein-coupled receptor signaling and vascular inflammation also contribute to enhanced peripheral resistance and vascular damage in essential hypertension. Common to these factors is long-term oxidative stress, which causes overproduction of vascular reactive oxygen species, decrease in nitric oxide bioavailability and a reduction of antioxidant capacity (Viel *et al.*, 2010).

3.4. Oxidative stress and hypertension

Oxidative stress may contribute to the generation and/ or maintenance of hypertension via a number of possible mechanisms. These include quenching of the vasodilator nitric oxide (NO) by ROS such as superoxide; generation of vasoconstrictor lipid peroxidation products, such as F2-isoprostanes; depletion of tetrahydrobiopterin (BH₄), an important NO synthase cofactor, and structural and functional alterations within the vasculature. These vascular changes may be mediated in several ways, including direct damage to endothelial and vascular smooth muscle cells, effects on endothelial cell eicosanoid metabolism, altered redox state, increases in intracellular free calcium concentrations, and stimulation of inflammatory and growth-signaling events (Amanullah *et al.*, 2012 ; Aquil *et al.*, 2013).

Vascular oxidative stress has been demonstrated in many forms of experimentally induced hypertension, such as angiotensin (Ang) II-mediated hypertension, salt-sensitive hypertension, lead-induced hypertension, obesity-associated hypertension, aldosterone-provoked hypertension, and nitric oxide synthase inhibitor-induced hypertension (Tanito *et al.*, 2004; Amanullah *et al.*, 2012). Unlike the findings in animal models, the association between oxidative stress and hypertension in humans is less consistent, and results vary depending on the marker of oxidative damage being investigated (Ward and Croft, 2006; Amanullah *et al.*, 2012; Gutiérrez *et al.*, 2014).

3.4.1. Endothelial dysfunction

Endothelial dysfunction is largely equivalent to the inability of the endothelium to generate adequate amounts of bioactive NO• (Li *et al.*, 2013). In the vasculature, the potential sources of ROS include NADPH oxidase, uncoupled eNOS, xanthine oxidase, cyclooxygenase (COX), the mitochondrial respiratory chain, and neutrophils. Among these,

NADPH oxidase is generally considered the major source of vascular ROS (Rodrigo *et al.*, 2012).

During vascular oxidative stress, superoxide produced by the NADPH oxidase reacts with $\text{NO}^\bullet$ resulting in the formation of peroxynitrite (ONOO^-). This reaction leads to the decrease in amount of $\text{NO}^\bullet$, which as previously mentioned, is one of the main vasodilators of blood vessels. In return, the ONOO^- oxidizes BH_4 , one of the cofactors of eNOS, leading to the uncoupling eNOS, and the production of more $\text{O}_2^{\bullet-}$ which lead to a further decrease in $\text{NO}^\bullet$ levels (Figure.8) (Gutiérrez *et al.*, 2014; Lavie, 2014).

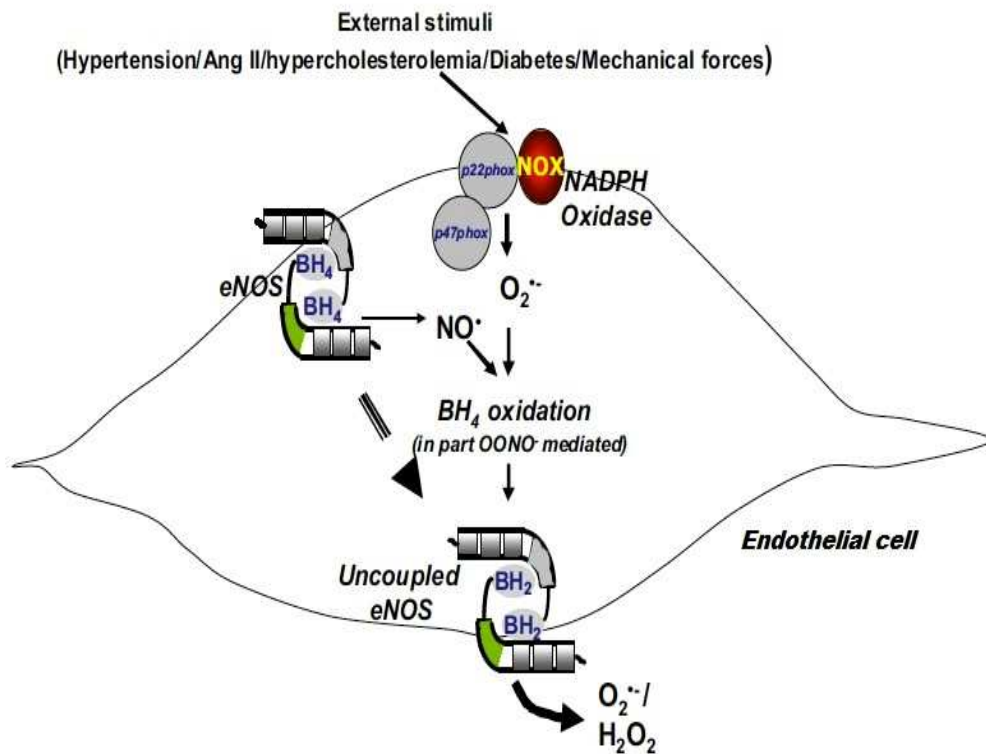


Figure. 8: Uncoupling of endothelial cell nitric oxide synthase (eNOS) by oxidation of tetrahydrobiopterin (BH_4) (Harrison *et al.*, 2007).

As we know that under physiological conditions the eNOS which produces $\text{NO}^\bullet$ in the vasculature acts as an anti-hypertensive and anti-thrombotic factors. It relaxes vascular smooth muscle cells, inhibits leukocyte adhesion, and platelet aggregation and adhesion to endothelial cells in the vascular wall (Lavie, 2014).

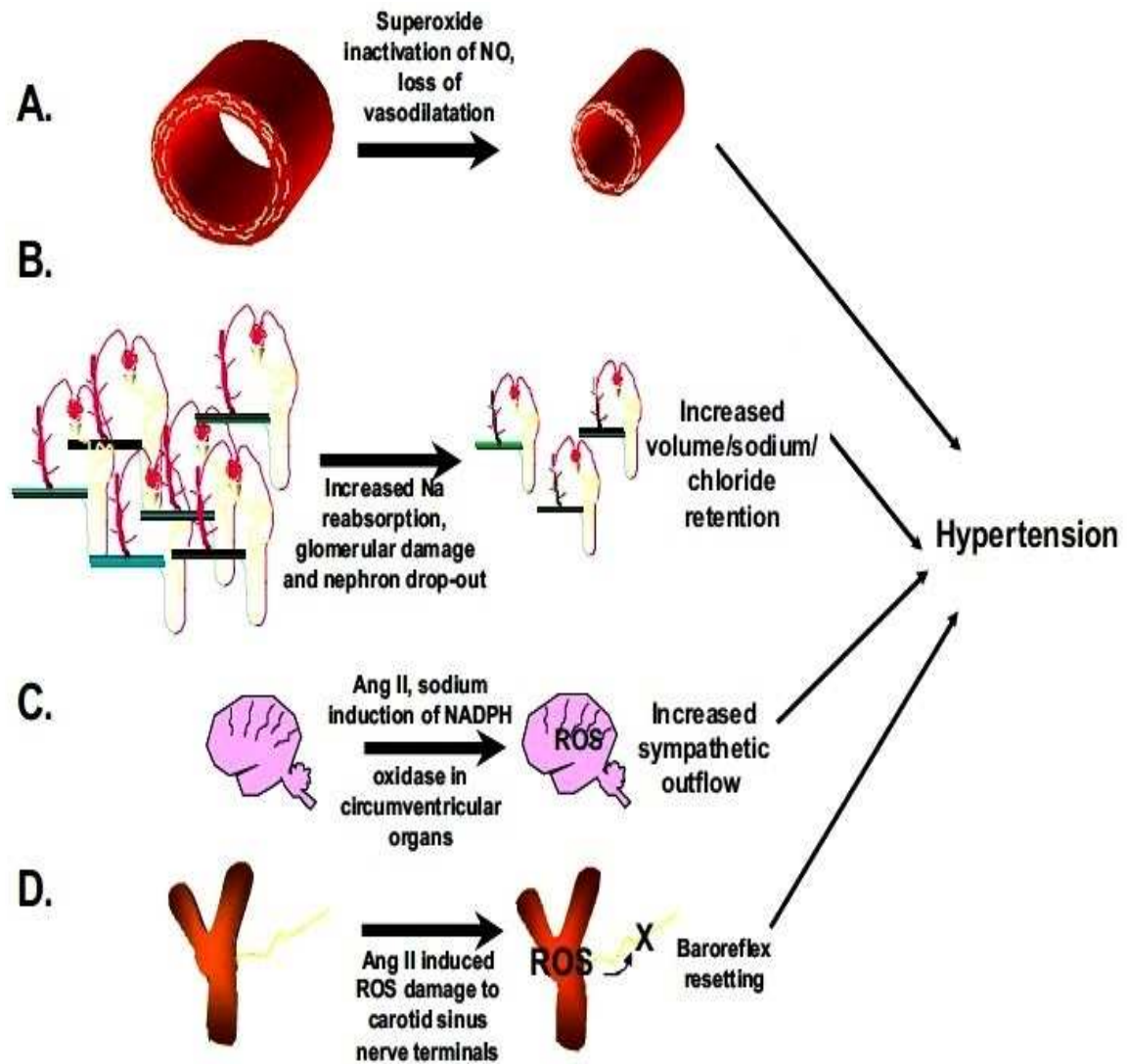


Figure. 9: Effect of reactive oxygen species (ROS) on various organs leading to hypertension. (A): ROS can promote vasoconstriction by inactivation of nitric oxide (NO), leading to an increase in systemic vascular resistance. (B): ROS can cause nephron damage and drop-out, promoting hypertension. (C): In the central nervous system, ROS in the circumventricular organ stimulates sympathetic outflow and hypertension. (D): ROS produced in the vessel wall can damage afferent nerve terminals, leading to alteration of the baroreflex (adapted from Harrison *et al.*, 2007).

Thus, reduction of NO[•] bioavailability promotes vascular smooth muscle cell proliferation and hypertrophy and collagen deposition, leading to thickening of the vascular media and narrowing of the vascular lumen. In addition, increased oxidative stress may damage the endothelium and impair endothelium-dependent vascular relaxation and increases vascular contractile activity (Figure 9. A) (Amanullah *et al.*, 2012; Meera and Marcus, 2012)

and subsequently an increase in systemic vascular resistance (Harrison *et al.*, 2007). Also, decreased levels of NO• can increase the sympathetic activity in the blood vessels leading to vasoconstriction which promotes hypertension (Li *et al.*, 2013).

3.4.2. Renin-Angiotensin system

ROS can act on angiotensin converting enzyme (ACE) to increase its catalytic activity resulting in increase in angiotensin II production, which in turn is a potent vasoconstrictor and is implicated in the pathogenesis of hypertension. Angiotensin II is a major endogenous inducer of NADPH oxidase, increasing NADPH concentration subsequently increases vascular intimal ROS production with resultant increase in oxidative stress and induction of vascular remodeling and endothelial dysfunction, which are associated with high rates of blood pressure (Hitomi *et al.*, 2007; Meera and Marcus, 2012). In rats and mice where hypertension is induced by infusion of angiotensin II, the expression of the subunits of the NADPH oxidase, the oxidizing activity and ROS generation are increased (Landmesser *et al.*, 2003).

This angiotensin II not only increases the activity of NADPH oxidase, but also upregulates the activity of SOD, possibly to compensate the increased levels of ROS. In situations where this compensatory effect is effective, the ROS levels may remain normal, even in pro-oxidant conditions. However, when ROS production becomes uncontrollable, the compensatory mechanisms are insufficient and physiopathologic consequences are triggered (Taniyama *et al.*, 2003). Treatment with liposome-encapsulated SOD or the membrane-permeable SOD diminishes the increase in blood pressure caused by angiotensin II administration, suggesting that stimulation of ($O_2^{\bullet-}$) formation is critically involved in the blood pressure response to angiotensin II (Katoch *et al.*, 2013).

NO• not only antagonizes the effects of angiotensin II on vascular tone, cell growth and renal sodium excretion, but also down-regulates the synthesis of ACE and the expression of angiotensin I receptors. Therefore, ACE inhibition upregulates the expression of the NOS. The ability of angiotensin II to induce endothelial dysfunction is also due to its ability to downregulate the soluble guanylyl cyclase, causing damage in the signaling of NO/ cGMP (Gutiérrez *et al.*, 2014).

3.4.3. Alteration of kidney functions

ROS play a key role in the pathophysiological processes of various kidney diseases, which are considered causes and consequences of hypertension. With respect to the glomerular changes, ROS produce glomerulopathic lipoproteins and other glomerular inflammatory lesions (Rodrigo and Rivera, 2002). A recent study showed that some lipid conglomerates produce NADPH oxidase activation and the production of ROS, which is an important molecular mechanism that stimulates homocysteine, which favors the oxidative damage to podocytes. This damage may represent an early phenomenon that starts glomerulosclerosis during hyper-homocysteinemia (Zhang *et al.*, 2010). One of the underlying mechanisms of tubulointerstitial injury, mediated by ROS, is the exposure of tubular cells to LDL, which may cause alterations in the interstitium tubules due to the production of ROS by NADPH oxidase (Piccoli *et al.*, 2009).

Angiotensin II not only has a pathogenic role in the progression of tubulointerstitial injury, but also in obstructive nephropathy (Grande *et al.*, 2013); it also activates NADPH oxidase and generates $O_2^{\bullet-}$ causing hypertrophy of renal tubular cells (Sachse and Wolf, 2007). There are findings that suggest that a high-fat diet induces renal inflammation and elevation of blood pressure via ROS in rats (Chung *et al.*, 2010).

Also, chronic angiotensin II infusion increases expression of Nox1 and p22^{phox} and decreases expression of extracellular SOD (ec-SOD) in the kidneys in rats. In renal tubular cells, increased levels of $O_2^{\bullet-}$ and diminished NO promote sodium reabsorption and alter tubuloglomerular feedback, leading to sodium and volume-dependent hypertension. There is also evidence that ROS cause glomerular damage and proteinuria. Increased superoxide has been associated with nuclear factor κ B activation and apoptosis in tubulointerstitial cells. Together, these effects of ROS can not only stimulate sodium and H_2O retention but cause nephron loss, leading to hypertension (Figure. 9 B) (Harrison *et al.*, 2007).

3.4.4. Alteration of central nervous system function

Many studies suggest that increased brain oxidative stress is strongly implicated in pathological signaling leading to hypertension in a framework that includes increased sympathetic activity (Zimmerman *et al.*, 2004; Guyenet *et al.*, 2006). In the other hand, it was reported that in male Dahl salt-sensitive rats, increased brain oxidative stress, possibly via $O_2^{\bullet-}$ production by activation of NADPH oxidase, may elevate arterial pressure through central sympathoexcitation (Fujita *et al.*, 2007). Grande *et al.* (2011) have shown also that systemic administration of Tempol, a SOD mimetic, reduced arterial pressure as well as urinary excretion of oxidative stress markers and reduced both angiotensin II and norepinephrine plasma levels in transgenic hypertensive mice. Moreover, administration of apocynin (inhibitors of NADPH oxidase), agrees the above results.

Also, it was reported that overexpression of SOD in the circumventricular organs markedly reduced the hypertension caused by systemic angiotensin II infusion. Moreover, the NADPH oxidase in the hypothalamus has also been implicated in associated with ROS angiotensin-II-induced hypertension (Harrison *et al.*, 2007).

The rostral ventrolateral medulla is the largest vasomotor center, and is essential in the maintenance of basal vascular tone. Some results show that an increase in the ROS at this level increases the vasomotor stimulation of spontaneous hypertension through the sympathetic nervous system activation (Gutiérrez *et al.*, 2014). Taken together, these studies indicate that centrally produced ROS can stimulate sympathetic out flow, leading to hypertension (Figure 9.C).

Finally, there is evidence that carotid baroreflex resetting, an important consequence of hypertension, is mediated by ROS. This can prevent the normal bradycardia and sympathetic withdrawal that accompanies elevations of pressure, and thus plays a permissive role in hypertension (Figure. 9 D) (Harrison *et al.*, 2007).

3.5. Oxidative stress biomarkers of hypertension

Studies in diverse experimental models of hypertension have demonstrated raised pro-oxidant activity and ROS levels, increased lipid peroxidation, altered antioxidant defenses and increased ROS mediated damage (Simic *et al.*, 2003; Grande *et al.*, 2011, Yang *et al.*, 2014; Deoghare *et al.*, 2014). Bilanda *et al.* (2010) showed that treatment with *Allanblackia floribunda* (Clusiaceae) aqueous extract in alcohol- and sucrose-induced hypertensive rats significantly prevented the increase of SOD, malondialdehyde (MDA), catalase and the decrease of GSH concentrations in aorta, heart, kidney and liver of rats.

In human hypertensive patients, there is also evidence of redox dysfunction. It was reported a higher superoxide and hydrogen peroxide production in hypertensive subjects, which returned to levels observed for control subjects after blood pressure reduction (Amanullah *et al.*, 2012; Aquil *et al.*, 2013). Due to reduced activity of catalase and superoxide dismutase, the production of hydroxyl radical is enhanced which may also

contribute to oxidative stress, thereby favoring lipid peroxidation with subsequent rise in MDA level (Meera and Marcus, 2012). Also, hydroxyl radicals may be produced from decomposition of hydroperoxynitrites and may trigger lipid peroxidation (Athiroh *et al.*, 2014). Thus, an elevation of several lipid peroxidation markers, such as MDA, 4-hydroxy-2-nonenal (HNE), thiobarbituric acid reactive substances (TBARs), 8-isoprostanes and oxidized low-density-lipoprotein (oxLDL) has also been observed in plasma or serum, urine or blood cells of hypertensive patients compared to normotensive patients (Bilanda *et al.*, 2010; Amanullah *et al.*, 2012; Wali *et al.*, 2012; Aquil *et al.*, 2013; Kachhawa *et al.*, 2014). Similarly, antioxidant enzymes (SOD, Catalase, GPx) were significantly decreased in prehypertensive and hypertensive group when compared with normotensive group (Meera and Marcus, 2012; Aquil *et al.*, 2013). Furthermore, the negative correlation between SBP, DBP and GSH/ GSSG ratio observed in essential hypertensives subjects indicated the significant oxidation of GSH by increased ROS (Kachhawa *et al.*, 2014; Deoghare *et al.*, 2014).

All, these studies confirmed that essential hypertension is associated with greater than normal of lipoperoxidation and an imbalance in antioxidant status, suggesting that oxidative stress is important in the pathogenesis of essential hypertension or in arterial damage related to essential hypertension.

3.6. Treatment of hypertension

3.6.1. Medication

In severe cases of hypertension, a combination of lifestyle changes as well as medication is needed in order to lower the blood pressure to an acceptable level. Medications that are used for the treatment of hypertension are beta-adrenergic blockers, calcium channel blockers, angiotensin II receptor blockers, Angiotensin-converting enzyme inhibitors

(ACEIs), diuretics, as well as vasodilators (Guyton and Hall, 2006). Some of these classic antihypertensive agents (ACEIs and angiotensin II receptor type 1 blockers) showed ability to decrease the vascular oxidative stress in addition to their blood pressure lowering effect. Thus, these agents may prove beneficial in long term treatment of hypertension (Gutiérrez *et al.*, 2014; Deoghare *et al.*, 2014). In hypertension associated with oxidative stress, pharmacological means to prevent oxidative stress are of major therapeutic interest. Some established drugs and novel therapeutic approaches can prevent oxidative stress and, presumably, vascular disease and hypertension.

Treatments with ROS inhibitors (Apocynin, Gp91ds-tat and Allopurinol) (Heumuller *et al.*, 2008) or antioxidants in experimental models of hypertension are generally more effective in preventing rather than reversing the hypertension (Krug *et al.*, 2008). Once triggered, these pathways may be sufficient to sustain the deleterious effects of oxidative stress even after ROS blockade or elimination (Sousa *et al.*, 2012).

Many natural antioxidants have been suggested for the treatment and prevention of the essential hypertension associated with ROS production which include Vitamin A, C, E, coenzyme Q10 (CoQ-10), L-arginine, α -lipoic acid and flavonoids (Sonu *et al.*, 2013). Also, antioxidant drugs as Tempol, N-acetylcysteine, polyethylene glycol-catalase, ebselen, polyethylene glycol, statins, nebivolol, pentaerithrityl tetranitrate (PETN), resveratrol and BH₄ were effective to scavenge superperoxide and hydroperoxide or to stabilize eNOS and catalase (Sousa *et al.*, 2012, Li *et al.*, 2013).

3.6.2. Phytotherapy of hypertension

The World Health Organization (WHO) defines traditional medicine as "the total combination of knowledge and practices, whether explicable or not, used in diagnosing, preventing or eliminating physical, mental or social diseases and which may rely exclusively

on past experience and observation handed down from generation to generation, verbally or in writing". WHO estimates that 80 % of the populations living in rural areas in developing countries depend on traditional medicine for their health care needs (Hendel *et al.*, 2012).

Ethnopharmacological surveys carried out in many parts of the world, including Algeria, have identified hundreds of plants used in folk medicine for the treatment of hypertension, and studies in experimental animals have confirmed the ethnomedical use of some of these plants (Table .1).

In Morocco, about thirty hypotensive medicinal plants were selected among them were *Allium sativum*, *Olea europea*, *Arbutus unedo*, *Urtica dioica*, *Ajuga iva*, *Allium cepa*, *Artemisia herba-alba*, *Carum caraway* , *Nigella sativa*, *Rosmarinus officinalis*, *Origanum majorana*, *Peganum harmala*, and *Phoenix willdactylifera* (Ziyyat *et al.*, 1997; Tahraoui and Ali, 2007).

In Egypt, the most medicinal plants used for the treatment of hypertension are *Hibiscus sabdariffa*, *Allium sativum*, *Cazrum caraway*, *Hyphaene thebaica*, and *Origanum majorana* (Abou Zid and Mohamed, 2011). Whereas, in the countries of the Middle East, the most hypotensive plants used are *Allium cepa*, *Olea europaea*, *Pistacia palaestina*, *Bryonia dioica* (Aburjai *et al.*, 2007), *Allium satium*, *Viscum album*, *Plantago major*, *Urtica dioica*, and *Scorzonera semicana* (Cakilcioglu *et al.*, 2011).

In Algeria, an ethnopharmacological survey carried out in Setif region revealed the use of 62 medicinal plants belonging to 31 families in the treatment of hypertension and the most plants used were: *Allium sativum*, *Mentha spicata*, *Olea europaea*, *Petroselinum crispum*, *Artemisia herba-alba*, *Laurus nobilis*, *Rosmarinus officinalis*, *Eucalyptus globulus*, *Marrubium vulgare*, *Origanum glandulosum*, *Trigonella foenum-graecum*, *Coriandum sativum*, *Myrtus communis* and *Crataegus azarolus* (Bouaziz *et al.*, 2012).

Table .1: Studies in experimental animals in the world confirming the ethnomedicinal use of some hypotensive plants.

Plants	Family	Extract	Reference
<i>Achillea millefolium</i>	Asteraceae	Hydroethanolic extract (leaves), iv.	Almeida <i>et al.</i> (2011)
<i>Ajuga iva</i>	Lamiaceae	Aq-ext (WP), po.	El-Hilaly <i>et al.</i> (2004)
<i>Allium sativum</i>	Liliaceae	Bulbe (diet)	Malik and Siddiqui (1981)
<i>Artemisia herba-alba</i>	Asteraceae	Aq-ext (AP), p o.	Al-Waili (1988)
<i>Carum copticum</i>	Apiaceae	MeOH ext (seeds)	Gilani(2005)
<i>Coriandrum sativum</i>	Apiaceae	Aq-ext (seed), po.	Medhin <i>et al.</i> (1986)
<i>Crataegus tanacetifolia</i>	Rosaceae	Aq-ext (leaves),p o	Kocyildiz <i>et al.</i> (2006)
<i>Crataegus Oxyacantha</i>	Rosaceae	Aq-ext (leaves), p o.	Abdul-Ghani(1987)
<i>Crataegus azarolus</i>	Rosaceae	MeOH and EA- ext, iv.	Bouaziz <i>et al.</i> (2014)
<i>Cynodon dactylon</i>	Poaceae	Essential oil (rhizome), p o.	Artizzu <i>et al.</i> (1996)
<i>Foeniculum vulgare</i>	Apiaceae	Aq-ext (seed), po.	Beaux <i>et al.</i> (1997)
<i>Herniaria glabra</i>	Caryophyllaceae	Saponin (leaves), po.	Rhiouani <i>et al.</i> (2001)
<i>Hibiscus sabdariffa</i>	Malvaceae	Aq-ext (calyxes),p o.	Herrera-Arellano <i>et al.</i> , (2007)
<i>Juglans regia</i>	Juglandaceae	Aq-ext (leaves), p o.	Perusquia <i>et al.</i> (1995)
<i>Lavandula officinalis</i>	Lamiaceae	Aq-ext (flowers), p o.	Elhajili <i>et al.</i> (2001)
<i>Lepidium sativum</i>	Cruciferae	Aq-ext (WP), p o.	Maghrani <i>et al.</i> (2005)
<i>Marrubium vulgare</i>	Lamiaceae	Aq-ext (AP), p o.	El Bardai <i>et al.</i> (2001)
<i>Myrtus communis</i>	Myrtaceae	MeOH and EA- ext, iv.	Bouaziz <i>et al.</i> (2015)
<i>Nigella sativa</i>	Ranunculaceae	Oil (seed), p o.	El Tahir <i>et al.</i> (1993)
<i>Olea europaea</i>	Oleaceae	Aq-ext (leaf), p o.	Cherif <i>et al.</i> (1996)
<i>Peganum harmala</i>	Zygophyllaceae	Aq-ext (seed), <i>in vitro</i>	Aquel and Hadidi (1991)
<i>Petroselinum crispum</i>	Apiaceae	Aq-ext (AP), p o.	Neuhaus-Carlisle <i>et al.</i> (1993)
<i>Rosmarinus officinalis</i>	Lamiaceae	Aq-ext (AP), p o.	Haloui <i>et al.</i> (2000)
<i>Tribulus terrestris</i>	Zygophyllaceae	MeOH and Aq-ext (WP), iv.	Phillips <i>et al.</i> (2006)
<i>Urtica dioica</i>	Urticaceae	Aq-ext (AP), iv.	Tahri <i>et al.</i> (2000)
<i>Viscum album</i>	Loranthaceae	Aq-ext (leaves) ,p o.	Samochowiec <i>et al.</i> (1955)

AP: aerial part; Aq: aqueous; ext: extract; MeOH: methanol; EA: Ethyl acetate

WP: whole plant. Mode of administration: I v.: intravenous; p o.: oral.

4. Selected Plants

4.1. *Crataegus azarolus* L.

Crataegus species (Rosaceae), known as “Hawthorn” comprise approximately 280 species which are native to Mediterranean region, North Africa, Europe and Central Asia. The genus name *Crataegus* is derived from a Greek word *Kratos* meaning hardness of wood (Arya and Thakur, 2012). Most species of Hawthorn have prominent, long, straight and sharp thorn, ranging from 1-5 inches. In Mediterranean region, the predominant species of the genus *Crataegus* is *Crataegus azarolus* L. (*C. azarolus*) that populates the mountains of these areas (Shatoor, 2011). In Algeria, it is especially localized in the tell of Constantine region, and known under the name of "zaaroura" (Saadoudi *et al.*, 2012).

4. 1.1. Botanical description

Azarole tree (*C. azarolus* L.) is a deciduous tree growing up to 5–8 m high. The young branches are felty, with a brown bark and a thorny tip. The leaves grow spirally arranged on long shoots, and in clusters on spur shoots on the branches or twigs. The leaf is triangular-cunate, and its base narrows gradually towards the petiole. Its end is cleaved into 3-5 lobes. It is 3-5 cm long, and its texture is rather leathery. The flowers are white, typical of the Rosaceae, and are arranged in dense clusters of 5-10 flowers. The calyx is campanulate, and its five lobes are rolled back. The corolla has 5 separate rounded leaves. It has a diameter of 1.5 cm and contains 2 styles and 5-25 stamens. The fruit called the azerole; it is up to 25 mm in diameter and contains 1–3 large seeds. When it matures, its skin color changes from white cream to yellow (Figure. 10) (Koyuncu *et al.*, 2007; Saadoudi *et al.*, 2012).

The botanical systematics of this plant is as the following (Quezel and Santa, 1963):

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Rosales

Family: Rosaceae

Genus: *Crataegus*

Species: *Crataegus azarolus* L.

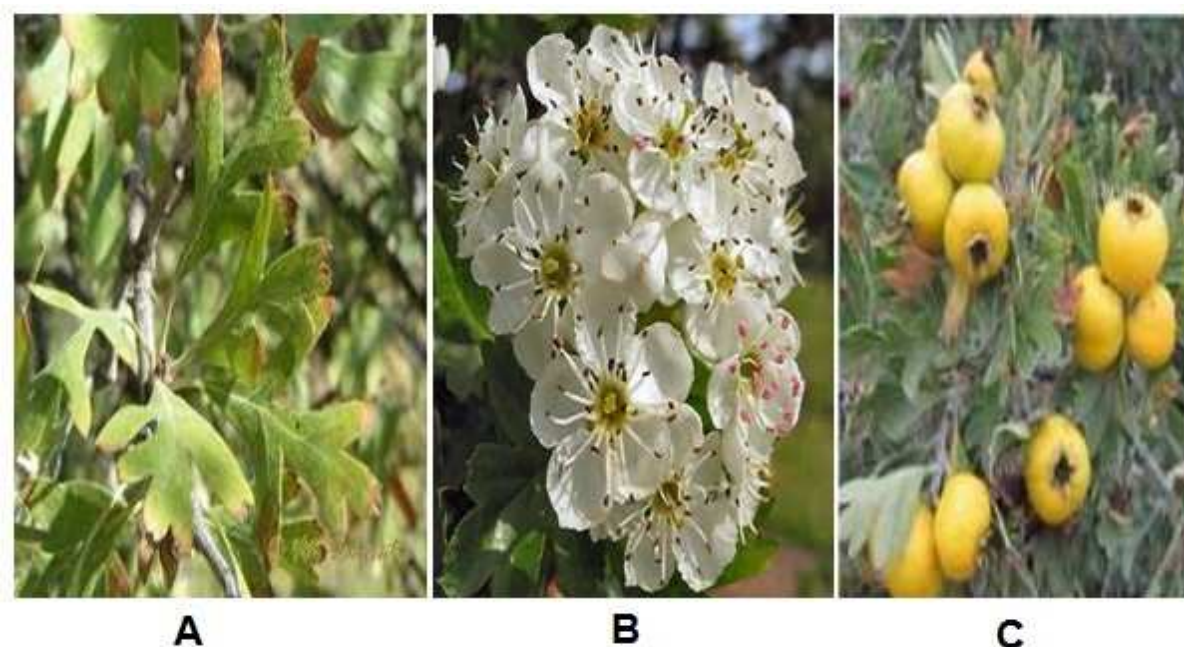


Figure.10. Different parts of *C.azarolus* L.: A: leaves, B: flowers and C: fruits (<http://www.vivaigabbianelli.it/frutti-vari/2122-azzeruolo-bianco-crataegus-azarolus.html>)

4. 1.2.Traditional uses

Crataegus species have been used traditionally for treating cardiovascular diseases as congestive heart failure, angina, hypertension, peripheral vascular disease, hyperlipidaemia and diabetes mellitus (Ling *et al.*, 2007; Abdul Kareem *et al.*, 2009; Shatoor *et al.*, 2012), in addition to their use in Turkey as therapeutic agent for treatment of cough, flu, asthma, stomach ache, rheumatic pain, nephritis, hemorrhoids, cardiac diseases (Kültür, 2007;

Özyürek *et.al.*, 2012). Several ethnopharmacological surveys on the therapeutic use of *C. azarolus*, revealed its use in the Arab traditional medicine to treat cardiovascular diseases, as well as cancer, diabetes and sexual weakness (Ljubuncic *et al.*, 2005). Furthermore, the fruits and flowers of this plant have a hypotensive effect as well as acting as a direct and mild heart tonic. They are especially useful in the treatment of heart conditions associated with high blood pressure (Esmaeili *et al.*, 2013). In Algeria, the leaves of *C. azarolus* are recommended as hypotensive agent (Bouaziz *et al.*, 2012).

4. 1.3. Chemical composition

The phytochemical studies revealed that the major components in hawthorn are polyphenols (Svedström *et al.*, 2006): catechins, mainly (-)-epicatechin, oligomeric proanthocyanidins such as the prominent B2 dimeric procyanidin, and flavonoids such as hyperoside (flowers and fruits) and vitexin-2''-O-rhamnoside (leaves))(Froehlicher *et al.*, 2009). Many of these phenolic compounds have been shown to be cytoprotective by reducing oxidative stress (Rice-Evans, 2004; Ljubuncic *et al.*, 2005).

HPLC analysis of folwers buds and open flowers of *C. azarolus* grown in Tunisia revealed the presence of two flavan-3-ols ((-)-epicatechin and procyanidin B2 dimer), the cholinergic acid and five flavonoids (hyperoside, rutin, spiraeoside, isoquercitrin and quercetin) (Bahri-Sahloul *et al.*, 2009).

4. 1.4. Pharmacological properties

Crataegus species possess immense medicinal applications, but a few species have been screened for their biological activities. The experimental results have shown a wide spectrum of such effects. *Crataegus* may improve coronary artery blood flow and the contractions of the heart muscle, hence used widely in cardiovascular disorders like arrhythmia, myocardial

infarction, congestive heart failure and hypertension (Long *et al.*, 2006; Tadic *et al.*, 2008; Froehlicher *et al.*, 2009; Kumar *et al.*, 2012). *Crataegus* extracts also prevent lipid peroxidation (Ljubuncic *et al.*, 2005). It inhibits ACE and reduces production of the potent blood vessel-constricting substance angiotensin II, hence acts as hypotensive and diuretic (Schroder *et al.*, 2003, Kumar *et al.*, 2012). Also, *C. aronia* demonstrated to have an anti-coagulant effect, and prolongs the bleeding time via the inhibition of thromboxane B2 synthesis (Shatoor *et al.*, 2012).

4.2. *Myrtus communis* L.

The genus *Myrtus* (family Myrtaceae) comprises flowering plants with approximately 16 species native to Southern Europe, North Africa and West Asia (Barboni *et al.*, 2010; Sumbul *et al.*, 2011; Aleksic and Knezevic, 2013). Term "*Myrtus* " comes from Greek word "*myrtos*" (Plant or shrub) and *communis* means common plant growing in groups (Sumbul *et al.*, 2011). *Myrtus Communis* (*M. communis*), known as true myrtle, is typical of Mediteranian flora (Mimica-Dukić *et al.*, 2010) and grows spontaneously in many countries (Spain, France, Tunisia, Algeria and Morocco) (Nassar *et al.*, 2010). In Algeria, *M. communis* L. is widespread especially in the Tell Atlas and in the coastal regions of Algiers and Constantine (Bouzabata *et al.*, 2013 a). It is commonly known under the name of Riehen. Another species of the Myrtle (*Myrtus nivellei* Batt. & Trab) can also be found in the central Sahara (Hoggar) called locally Tefeldest (Moghrani and Maachi, 2008).

4. 2.1. Botanical description

The common myrtle has upright stem, 2.4-3 m high, its branches form a close full head, thickly covered with evergreen leaves. The stem of the plant is branched and dark green leaves are glossy, glabrous, coriaceous, opposite, paired or whorled, ovate to lanceolate with

stiff structure, aromatic, entire margined, acuminate and 2.5-3.8 cm long, glands absent in the lamina. It has axillary white flowers on slender peduncles; medium sized about 2 cm in diam., stiff having yellow anthers. The petals are pure white with glands and somewhat tomentose margin covered with fine hairs (Figure.11). They give off a sweet fragrant smell. The glabrous berry has rounded (vaselike) shape with a swollen central part and remnants of persistent 4-5 partite calyx at the outer part. The developed fruit is initially pale green, then turns deep red and finally becomes dark indigo when fully mature (Figure.11). They are bitter when unripe, sweet when ripe (Sumbul *et al.*, 2011).

Systematic of myrtle is as the following (Quezel and Santa, 1963):

Kingdom: Plantae

Phyllum: Tracheophyta

Class: Magnoliopsida

Order: Myrtales

Family: Myrtaceae

Genus: *Myrtus*

Species: *Myrtus communis* L.



A



B

Figure. 11: Different parts of *M. communis* L.: A: flowers and B: fruits (Aleksic and Knezevic, 2013).

4. 2.2. Traditional uses

Myrtle has been used for medicinal, food and spice purposes since ancient times. Fruits possess antiseptic, astringent, carminative, dessicant, demulcent, analgesic, anti-inflammatory, antiemetic, diuretic, stomachic, haemostatic, nephroprotective, and antidiabetic properties (Sumbul *et al.*, 2011; Aleksic and Knezevic, 2011). Leaves are claimed to be astringent, antiseptic, hypoglycaemic, laxative, analgesic, haemostatic, stimulant and are used also against respiratory diseases (Sumbul *et al.*, 2011; Aleksic and Knezevic, 2011). In Algeria, the decoction of myrtle aerial part is recommended as hypotensive (Boudjelal *et al.*, 2013), hypoglycemic and anti-inflammatory agents (Bouzabata, 2013 b).

4. 2.3. Chemical composition

Many studies have been reported the presence of phenolic acids (ellagic, gallic, caffeic, syringic, vanillic and ferulic acids) and flavonoids (catechin, myricetin, myricetin-3-O-galactoside, myricetin-3-O-rhamnoside, hesperidin, esculin, patuletin, quercetin, quercetin-3-rutinoside, quercetin-3-O-rhamnoside) in leaf and fruit alcoholic extracts. Tannins comprise hydrolysable tannins (gallotannins) and proanthocyanidins (condensed tannins). These latter were represented by delphinidin-3-O-glucoside, petunidin-3-O-glucoside, malvidin-3-O-glucoside, cyanidin-3-O-glucoside, peonidin-3-O-glucoside, delphinidin-3-O-arabinoside, petunidin-3-O-arabinoside and malvidin-3-O-arabinoside (Romani *et al.*, 1999; Montoro *et al.*, 2006; Aidi Wannes *et al.*, 2010; Tuberoso *et al.*, 2010). Furthermore, the main compounds responsible for the flavor of leaves and flowers myrtle oil are monoterpenes: 1,8-cineole, myrtenyl acetate, α -pinene, myrtenol, limonene (Mimica-Dukić *et al.*, 2010, Aidi Wannes *et al.*, 2010).

4. 2.4. Pharmacological properties

Antibacterial properties of myrtle essential oils and extracts against pathogenic bacteria and fungi were reported in many studies (Mansouri *et al.*, 2001 ; Othman *et al.*, 2007; Shan *et al.*, 2007; Rattanachaikunsopon and Phumkhachorn, 2010; Pereira *et al.*, 2012; Taheri *et al.*, 2013). Also, leaves extracts of myrtle showed a negative inotropic (Al-Zohyri *et al.*, 1985), hypoglycaemic as well as hypotriglyceridemic (Elfellah *et al.*, 1984; Dineel *et al.*, 2007) and antimutagenic activities (Hayder *et al.*, 2008; Mimica-Dukić *et al.*, 2010).

Moreover, the plant was assessed for the anti-inflammatory activity by measuring the suppression of carrageenan-induced paw oedema in mice by myrtle decoction. Indomethacin was used as the standard drug. Results showed that it possessed anti-inflammatory activity (Amira *et al.*, 2008). Moreover, Tretiakova *et al.* (2008) reported that EC₅₀ 3–8 μ M of myrtucommulone from *M. communis* induced apoptotic cell death in different cancer cells with low cytotoxicity.

Administration of two doses of aqueous extracts (105 and 175 mg/kg) and methanolic extracts (93 and 154 mg/kg) of dried berries of myrtle in Wistar rats decreased gastric ulcer against ethanol, indomethacin and pyloric ligation induced models (Sumbul *et al.*, 2010). Many reports have described also the antioxidant activities of different extracts of myrtle and certain ingredients thereof (Rosa *et al.*, 2003; Hayder *et al.*, 2004; Romani *et al.*, 2004), implying potential as medicine for the treatment of diseases related to oxidative stress.

4. 3. *Origanum glandulosum* Desf.

The genus *Origanum* (Lamiaceae family) comprises approximately 42 species which are widely distributed in Eurasia and North Africa (Chishti *et al.*, 2013). However, the majority of these species, approximately 80%, are concentrated in the Mediterranean region

(Skoula *et al.*, 2008). Origano is often regarded as the wild shape of marjoram and the origano term comes from two Greek words " oros" and " genos" (glare of the mountain). *Origanum vulgare* ssp *glandulosum* (Desf.) Ietswaart, synonymous with *Origanum glandulosum* Desf. is an endemic spontaneous plant of North Africa (Algeria and Tunisia). In Algeria, this species is called " Zaatar" (Bendahou *et al.*, 2008; Oukil *et al.*, 2011).

4. 3.1. Botanical description

Origanum glandulosum (*O.glandulosum*) is an aromatic, woody-based herb, which grows to up to 50 cm high (Figure. 12). The leaves are ovate, 10-40 mm long and 5-25 mm wide, and borne opposite each other on the stem. The edges of the leaves are smooth or very shallowly toothed, and the leaf tips vary from acute (pointed) to obtuse (rounded). The inflorescence is many-flowered, with flowers grouped into short dense lateral or terminal spikes. The corolla is white to purplish, 4-8 mm long, and has a lower lip which is much longer than the upper lip. The calyx is five-toothed. Each flower has four stamens with purple anthers. The fruits are achenes (Ietswaart, 1980; Baba Aissa, 1999). According to Ietswaart (1980), the *Origanum glandulosum* classification is as follows:

Phylum: Tracheophyta

Class: Magnoliopsida

Family: Lamiaceae

Order: Lamiales

Genus: *Origanum*

Species: *Origanum vulgare* L.

Subspecies : *Origanum vulgare* subsp. *glandulosum* (Desf) Ietswaart.

**A****B**

Figure.12: Morphological aspect of *O. glandulosum*. A: whole plant; B: leaves (http://de.academic.ru/pictures/dewiki/79/Oregano_1.jpg)

4. 3.2. Traditional uses

Origanum species are well known as medicinal and culinary herb (Kaurinovic *et al.*, 2011). they are traditionally used as an antidiabetic, diaphoretic, carminative, antispasmodic, tonic, stimulant, diuretic, sedative, degasifier, antiseptic, and to treat asthma, gastrointestinal diseases and constipation (Nakiboglu *et al.*, 2007; Kordali *et al.*, 2008; Shihab *et al.*, 2013). Baser (2002) and Dundar *et al.* (2008) reported also that *Origanum* has been used as expectorant, antihelminthic and for gastrointestinal complaints in Turkish folk medicine. In Algeria, *O. glandulosum* is used for the treatment of whooping cough, cough, fever, bronchitis, colds (Sari *et al.*, 2006; Bendahou *et al.*, 2008; Khadir *et al.*, 2013), rheumatism (Orhan *et al.*, 2010), hypertension, digestive disorders (Boudjelal *et al.*, 2013) and as antiseptic (Belhattab *et al.*, 2005) as well as stomachic (Bouzabata *et al.*, 2013 b).

4. 3.3. Chemical composition

Secondary metabolites of *O. glandulosum* have been studied in terms of polyphenolic compounds and essential oils (Nakatani and Kikuzaki, 1987; Lin *et al.*, 2003; Koukoulitsa *et al.*, 2006; Liang *et al.*, 2010; Skaltsa *et al.*, 2010).

According to the study carried out by skoula *et al.* (2008) using HPLC coupled to diode-array detection and APCI-MS, the identified phenolic compounds of ethanolic extract of *O. glandulosum* from Blida (Algeria) were apigenin, 5,6,4'-Trihydroxy-7,3'-dimethoxyflavone, ladanein, naringenin, eriodictyol, aromadendrin, taxifolin. However, the study reported by Oukil *et al.* (2011) on methanolic extract of *O.glandulosum* collected from Bejaia (Algeria) had been determined the presence of gallic acid, vanillic acid, coumaric acid, ferrulic acid, rutin and naringenin.

In addition, the study of the chemical composition of *O.glandulosum* of the area of Setif (Algeria) carried out by Belhattab *et al.* in 2005, their allowed to highlight 29 compounds. They found that carvacrol (47%), followed α - terpinene (13.4%), p-cymene (11.2%) and thymol (6.6%) are the major essential oils of this plant. Moreover, many studies reported that the polar phenols; thymol and carvacrol are responsible for many of the properties of the these essential oils (Kulisic *et al.*, 2004; Bozin *et al.*, 2006; Bakkali *et al.*, 2008; Mohamed and Nassier, 2013).

4. 3.4. Pharmacological properties

Several studies have been published on biological properties of *Origanum* spp. as antibacterial (Şahin *et al.*, 2004, Bejaoui *et al.*, 2013; Kotana *et al.*, 2014), antifungal (Khosravi *et al.*, 2011), anti-inflammatory, anticholinesterase (Loizzo *et al.*, 2009), and antimutagenic effects (Mezzouga *et al.*, 2007). In addition, water extract of *O. vulgare* leaves

exerted a significant antihyperglycemic activity (Lemhadri *et al.*, 2004; Mohamed and Nassier, 2013) and showed anti-inflammatory activity using 5-lipoxygenase assay (Albano and Miguel, 2011).

The Algerian *O. glandulosum* oil exhibited a good antioxidant effect (Belhattab *et al.*, 2005; Sari *et al.*, 2006) and showed antimicrobial activities against bacteria, yeast and fungi such as, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus hirae*, *Candida albicans* and *Candida tropicalis* (Sari *et al.*, 2006; Bekhechi *et al.*, 2008; Bendahou *et al.*, 2008 ; Oukil *et al.*, 2011; Khadir *et al.*, 2013). Acetone extract of *O. glandulosum* collected from Setif region possesses cholinesterase inhibitory at 25, 50, and 100µg/mL using an ELISA microplate reader (Orhan *et al.*, 2010).

The essential oil was also screened for α -amylase inhibitory effect and showed a strong capacity to inhibit the degradation of starch by pancreatic and salivary α -amylase. These results show that *O. glandulosum* essential oil could be considered as a natural antidiabetic agent by decreasing glucose release from starch and may have potential for use in the treatment of diabetes mellitus and obesity (Bejaoui *et al.*, 2013).

Furthermore, Khadir *et al.* (2013) proved that the treatment of skin infections especially wounds caused by Panton-Valentine leukocidin (PVL) positive *S. aureus* on Wistar rats is possible with the essential oil (1 and 5%) of *O. glandulosum* collected from Telemcen (Algeria). Also, it has been reported that the Algerian *O. glandulosum* essential oil (Belhattab *et al.*, 2005; Sari *et al.*, 2006) and alcoholic extract (Belhattab *et al.*, 2005; Oukil *et al.*, 2011) exhibited a good antioxidant effectiveness using different methods including DPPH radical-scavenging activity, ferrous ion-chelating ability, and ferric-reducing power.

Materials and methods

1. Materials

1.1. Plant material

The leaves of *C. azarolus* and *O. glandulosum* were collected in June, 2012 from Beni Ourthilane (Setif), and Jebel Megres (Setif), respectively. Whereas, *M. communis* leaves were collected in September, 2012 from Jijel. Plant species were identified by Pr. Hocine Laouar (Laboratory of Botany, Faculty of Natural and Life Sciences, University Ferhat Abbas, Setif 1, Algeria). The leaves were separated, dried under shade and powdered.

1.2. Animals

Healthy male and female adult albino rats weighing 170–300 g were used. For the hypotensive experiments, the rats were obtained from animal house colony of Faculty of science, The university of Jordan, Amman, Jordan. Animals were housed in an air-conditioned animal room, with 12 h/12 h light/dark photoperiod, and maintained with standard dry rodent diet and water *ad libitum*.

However, rats used in the diuretic and *in vivo* antioxidant experiments were purchased from Pasteur institute, Algiers, Algeria. The animals were acclimatized for one week, prior to experiments. They were fed with standard diet and water *ad libitum* and kept in a standard animal facility environment with natural light and dark cycle.

1.3. Chemicals

Linoleic acid, ammonium thiocyanate, β -carotene, butylated hydroxytoluene (BHT), were purchased from Fluka Chemical Co. (Buchs, Switzerland). 2, 2-diphenyl-1-picrylhydrazyl (DPPH), ethylenediamine tetraacetic acid (EDTA), gallic acid, 6-hydroxy-2,5,7,8-

tetramethyl chroman-2-carboxylic acid (trolox), 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-nitrobenzoic acid), H_2O_2 , reduced glutathion (GSH). These chemicals were obtained from Sigma Chemical Co. (St. Louis, MO) or Merck. All other reagents were of analytical grade.

2. Methods

2.1. Phenolic compounds extraction

The extraction procedure for phenolic compounds from dry leaves of plant species was conducted as described by Markham (1982). The powdered plant material (100 g) was extracted with methanol (85% and 50%), at room temperature for 3 days. Then, the suspension was filtered through a Buchner funnel and concentrated under reduced pressure on a rotary evaporator to give crude methanolic extract (ME). The aqueous solution was successively extracted with hexane, chloroform and ethyl acetate. Each fraction was evaporated to dryness under reduced pressure to give hexane (HE), chloroform (CHE), ethyl acetate (EAE), and the remaining aqueous (AqE) extracts. The extracts were stored at $-20\text{ }^{\circ}\text{C}$ until use (Figure. 13).

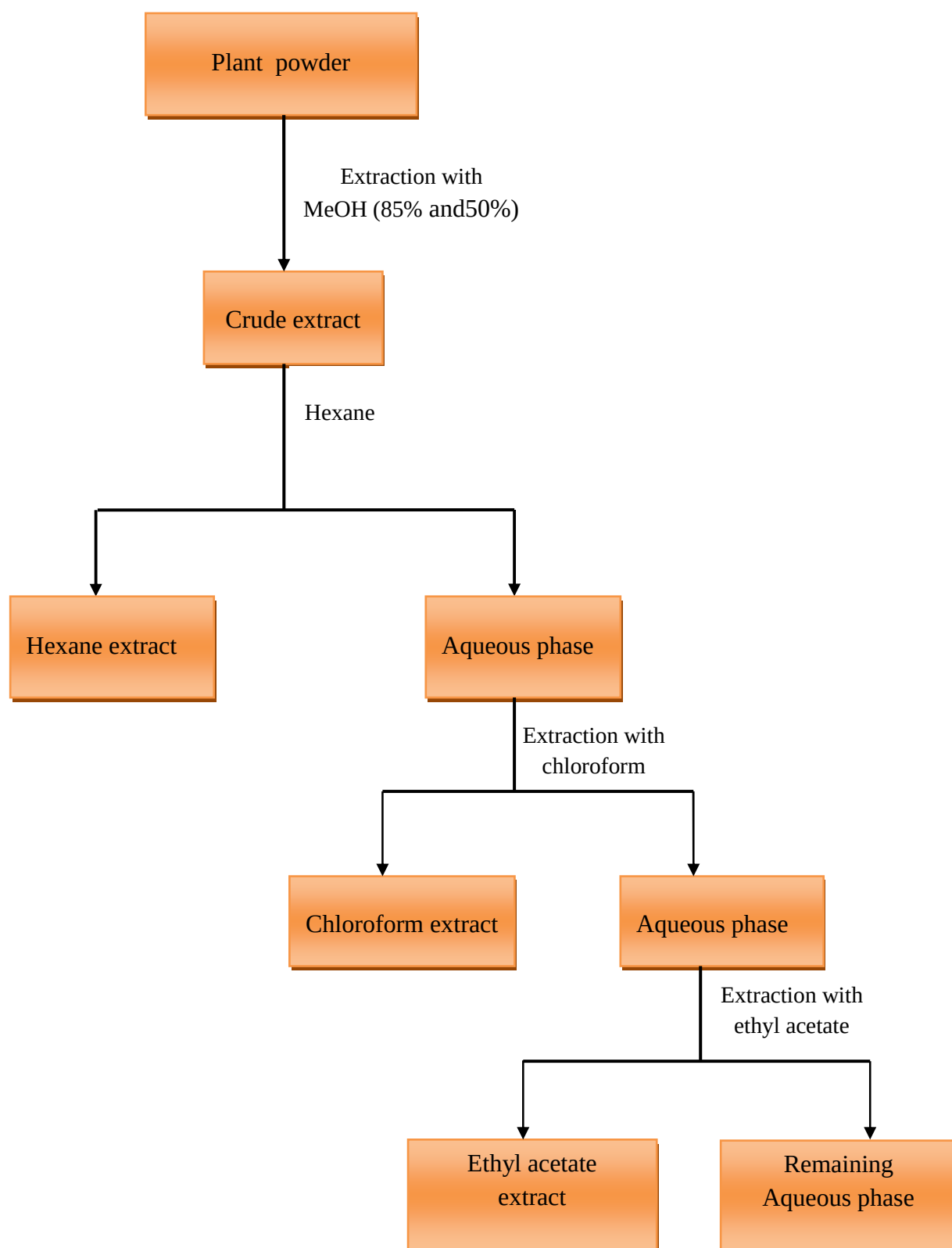


Figure.13: Schematic diagram represents the process of extraction according to Markham (1982) with slight modifications.

2.2. Determination of total polyphenols content in plant extracts

Total polyphenols content was determined by the Folin–Ciocalteu reagent as described by Li *et al.* (1997) with slight modifications. Folin-Ciocalteu phenol reagent consists of a mixture of the hetero-polyphosphomolybdic and phosphotungstic acids in which the molybdenum and tungsten are in the 6+ state. On reduction with certain reducing agents as polyphenols (Figure.14), molybdenum blue and tungsten blue are formed in which the mean oxidation state of the metals is between 5+ and 6+ (Nunes *et al.*, 2012).

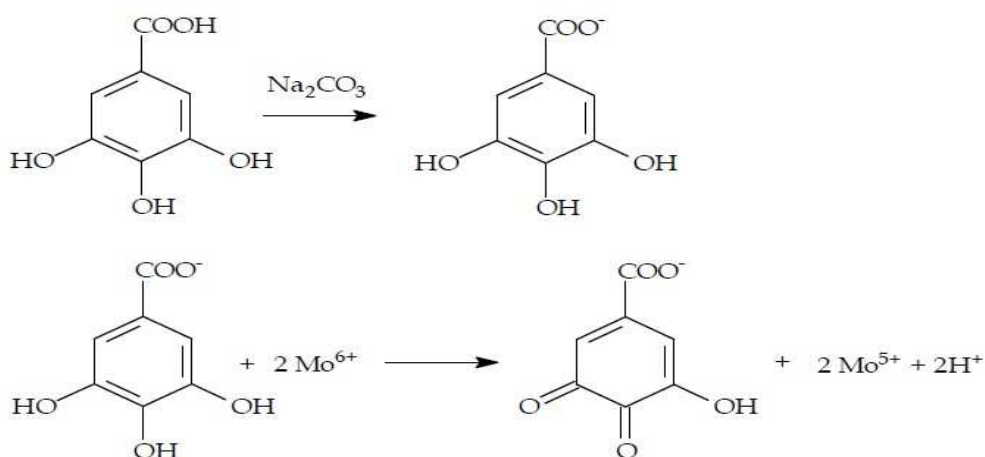


Figure. 14: Reaction of gallic acid with molybdenum, a component of the Folin-Ciocalteu reagent (Nunes *et al.*, 2012).

In brief, 0.1ml of plant extracts were mixed with 0.5 ml of Folin-Ciocalteu reagent (diluted 10 times). After 4 min, 0.4 ml of 7.5% sodium carbonate (Na_2CO_3) solution was added. The final mixture was shaken and then incubated for 90 min in dark at room temperature. The absorbance of all samples was measured at 760 nm. The amount of total polyphenols in different extracts was determined from a standard curve of gallic acid ranging from 0.00 to 160 μg / ml (Figure. 15) and the results were expressed in milligrams of gallic acid equivalents per gram of dry extract (mg GAE/ g of extract).

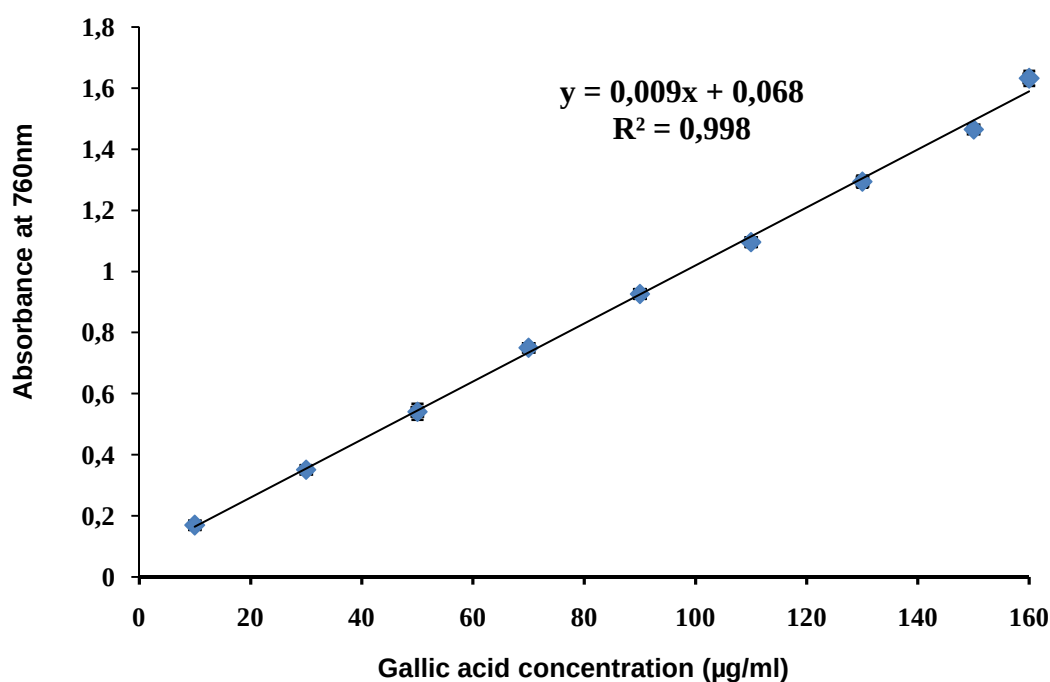


Figure. 15 : Standard curve of gallic acid for the determination of total polyphenols in various plant extracts. Each value represent mean $\pm$ SD (n = 3).

2.3. Determination of total flavonoids content in plant extracts

Total flavonoids content in each extract was determined by a colorimetric method as described by Bahorun *et al.* (1996). Each sample (1 ml) was mixed with 1 ml of 2 % aluminium chloride (AlCl_3) solution and allowed to stand for 10 min. The absorbance of the mixture was then determined at 430 nm versus methanol blank. Results were expressed as quercetin equivalent per gram of dry extract (mg QE / g of extract) using the typical standard curve for the reactivity of quercetin (0-40 µg/ml) with AlCl_3 solution (Figure. 16).

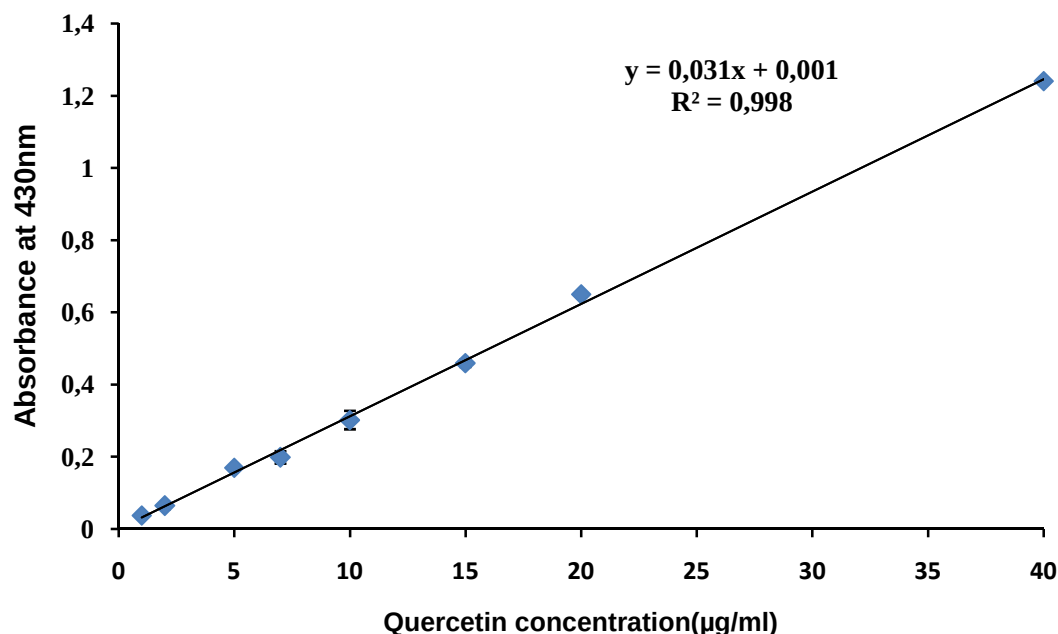


Figure. 16: Standard curve of quercetin for the determination of total flavonoids in various plant extracts. Each value represent mean $\pm$ SD (n=3).

2.4. Phytochemical analysis of phenolic compounds

2.4.1. Extraction

The extraction procedure for phenolic compounds was conducted as described by Markham (1982). The powdered plant material (1kg) of each plant was extracted by maceration with 5 L methanol (85%) at room temperature for 5 days. The filtered solvent was evaporated under vacuum and lyophilized to afford a crude methanolic extract (ME) (144 g, 190.8 g and 102,3 g for *C. azarolus*, *M.communis* and *O. glandulosum*, respectively). Using the same extraction protocol as above, the residue (100 g) of each plant crude extract was dissolved in 1L MeOH– H₂O mixture (9:1) and was then partitioned by successive extractions with hexane, chloroform and ethyl acetate. Each fraction was evaporated to

dryness under reduced pressure to give hexane (HE), chloroform (CHE), ethyl acetate (EAE), and the remaining aqueous (AqE) extracts.

2.4.2. Column Chromatography

2.4.2.1. Chromatography of *C. azarolus* ethyl acetate fraction

The EAE (28 g) was fractionated on column chromatography using silica gel S (0.063-0.2 mm, Riedel-Dehaen, 120g, 3.5 cm i.d.). The column was first eluted with chloroform, and then with chloroform- methanol mixtures of increasing polarity. Totally, 38 fractions (250 ml for each) were obtained and similar fractions were combined according to their thin-layer chromatographic plates (TLC), using different solvent systems. The resulting eight fractions were then purified by preparative thin-layer chromatography and the structures of the isolated compounds were determined by spectral techniques.

2.4.2. 2. Chromatography of *M. communis* ethyl acetate fraction

The EAE (23 g) was chromatographed over silica gel column (0.063-0.2 mm, Riedel-Dehaen, 120g, 3 cm i.d.) eluted with with CHCl_3 ; CHCl_3 : MeOH (95:5–50:50) in a gradient system. On the bases of TLC behavior, 48 fractions (250 ml for each) obtained were combined together in fourteen fractions. Then, the fractions were purified by preparative thin-layer chromatography and the structures of the isolated compound were determined by spectral techniques.

2.4.2.3. Chromatography of *O. glandulosum* ethyl acetate fraction

The EAE (15 g) was dissolved in methanol and adsorbed on 15 g of silica gel (Kieselgel 60 HF_{254}) and then loaded on a silica gel column (0.063-0.2 mm, Riedel-Dehaen, , 2.8 cm i.d.) packed in chloroform. The column was eluted with chloroform, methanol and

water, thirty eight fractions (250 ml each) were collected. Then, the fractions were purified by preparative thin-layer chromatography and the structures of the isolated compounds were determined by spectral techniques.

2.4.3. Silica gel glass plates

Merck silica gel glass (0.25mm) was used to collect the similar fractions together and to test the purity of the isolated compounds. Spots were detected by UV light and anisaldehyde spray reagent.

2.4.4. Structural elucidation

Nuclear Magnetic Resonance (NMR) ;¹H-NMR ¹³C-NMR and DEPT-NMR, 2D-NMR (COSY 45, HMQC, and HMBC) spectra were recorded using a Bruker 500 MHz spectrometer. Chemical shifts were presented as part per million (ppm) or the δ -scale relative to tetramethylsilane (TMS). Electrospray Ionisation Mass spectra were recorded using Bruker Daltonics Apex IV, 7.0 T Ultra shield Plus. These analyses were performed at the Departement of Chemistry, The university of Jordan, Amman, Jordan.

2.5. Antioxidant activity of plant extracts: *in vitro* assays

2.5.1. DPPH radical scavenging assay

Free radical scavenging activity against 2, 2-diphenyl- 1-picrylhydrazyl (DPPH) radical was measured using the method described by Burits and Bucar (2000). In this assay, the purple radical (picrylhydrazyl) is reduced by antioxidant compounds to the corresponding pale yellow hydrazine (picrylhydrazine) (Figure. 17). The discoloration indicates free radical scavenging activity of tested sample (Aktumsek *et al.*, 2013). Briefly, 50 μ l of different

extract dilutions were added to 5 ml of 0.004% solution DPPH dissolved in methanol. After 30 min at room temperature, the absorbance was measured at 517 nm. BHT was used as standard. Inhibition of free radical DPPH in percent (I%) was calculated in following way:

$$I\% = 100 (A \text{ control} - A \text{ sample}) / A \text{ control}$$

where A control is the absorbance of the blank solution (containing all reagents except the test compound), and A sample is the absorbance in the presence of the test compound. Extract concentration providing 50% inhibition (IC₅₀) was calculated from the plot of inhibition percentage against extract concentration.

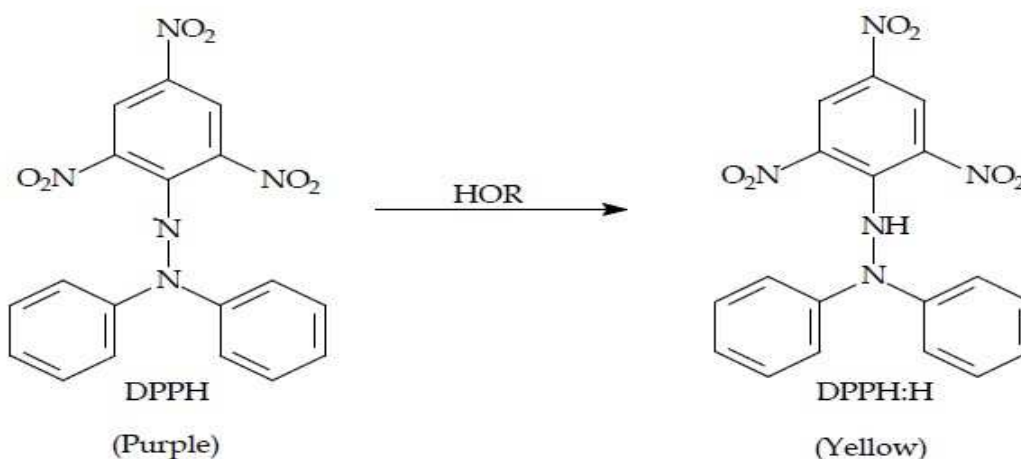


Figure. 17: Radical and non-radical forms of DPPH (Nunes *et al.*, 2012).

2.5.2. ABTS^{•+} radical scavenging activity assay

The spectrophotometric analysis of ABTS^{•+} radical scavenging activity was determined according to the method of Re *et al.*(1999) with some modifications. This method is based on the ability of antioxidants to quench the long-lived ABTS^{•+} radical cation, a blue/green chromophore with characteristic absorption at 734 nm, in comparison to that of Trolox, a water-soluble α -tocopherol analogue.

The ABTS^{•+} solution was produced by the reaction of 7 mM of ABTS solution in 2.45 mM potassium persulfate (final concentration). The mixture was kept in the dark at room temperature for 24 h before use. The solution was diluted with methanol and equilibrated at room temperature to give an absorbance of 0.70 ± 0.02 at 734 nm in a 1 cm cuvette. Then, 50 μ l of sample was mixed with 1ml of ABTS^{•+} solution and kept for 30 min at room temperature, the absorbance of reaction mixture was measured at 734 nm. Trolox was used as positive control. The ABTS^{•+} radical scavenging ability was calculated according to the same equation as that in the DPPH assay.

2.5.3. Hydroxyl radical scavenging assay

The hydroxyl radical scavenging activity was assayed according to the method of Li *et al.* (2008) with slight modifications. In this system, hydroxyl radicals were generated by the Fenton reaction. Hydroxyl radicals could oxidize Fe²⁺ into Fe³⁺, and only Fe²⁺ could combined with 1,10-phenanthroline to form a red compound (1,10-phenanthroline-Fe²⁺) with the maximum absorbance at 536 nm. The concentration of hydroxyl radical was reflected by the degree of decolourization of the reaction solution. Briefly, a mixture of 100 μ l of 1,10-phenanthroline (5.0 mM), 100 μ l of FeSO₄ (5.0 mM) and 100 μ l of EDTA (15 mM) were mixed with 70 μ l of sodium phosphate buffer (0.2 M, pH 7.4). Then, 100 μ l of sample and 140 μ l of H₂O₂ (0.01%) were added. The mixture was incubated at 37 °C for 60 min, and the absorbance was measured at 536 nm. Vitamin C was used as a positive control. To calculate hydroxyl radical scavenging, the following equation was used:

$$\text{Hydroxyl radical scavenging activity (\%)} = (A_s - A_0) \times 100 / (A_c - A_0)$$

A_s is the absorbance of the sample

A_0 is the absorbance of the blank solution using distilled water instead of sample

A_c is the absorbance of a control solution in the absence of H₂O₂.

2.5.4. Ferrous ion chelating activity

Ferrous ion chelating activity was measured by the inhibition of the formation of Fe^{2+} -ferrozine complex after the treatment of various concentration of plant fractions with Fe^{2+} , using the method of Decker and Welch (1990). The reaction mixture (1.50 ml) contained 500 μl test material or EDTA, 100 μl FeCl_2 (0.6 mM in water) and 900 μl MeOH. The control contained all the reaction reagents except the extract and EDTA. The mixture was well shaken and allowed to react at room temperature for 5 min; 100 μL of ferrozine (5 mM in methanol) was then added. The absorbance of the Fe^{2+} -ferrozine complex was measured at 562 nm. The chelating effect was calculated as a percentage, using the same equation as that in the DPPH assay. IC_{50} value defined as the effective concentration of test material that is required to chelate 50% of iron ions.

2.5.5. Reducing power

Fe^{+3} reduction is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action (Ebrahimzadeh *et al.*, 2010). The reducing power of the three plant extracts was determined according to the method of Chung *et al.* (2005). A 0.1 ml aliquot of different concentrations of each fraction, were mixed with an equal volume of 0.2 M phosphate buffer (pH 6.6) and 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min, to reduce ferricyanide into ferrocyanide. After that, 0.25 ml of 10% trichloroacetic acid was added to the mixture to stop the reaction and centrifuged at 3000 rpm for 10 min. The supernatant (0.25 ml) was added to distilled water (0.25 ml) and 0.1% ferric chloride (0.5 ml) and then the absorbance was measured at 700 nm. BHT was used as a positive control and the reducing power was calculated using the same equation as that in the DPPH assay. The IC_{50} value defined as the effective concentration of test material at which the absorbance was 0.5.

2.5.6. β -Carotene bleaching assay

In this assay, antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation (Dapkevicius *et al.*, 1998). A stock solution of β -carotene-linoleic acid mixture was prepared as follows: 0.5 mg β -carotene was dissolved in 1 ml of chloroform and 25 μ l linoleic acid and 200 mg Tween 40. Chloroform was completely evaporated, and then, 100 ml distilled water saturated with oxygen (30 min, 100 ml/min) were added with vigorous shaking. 2500 μ l of this reaction mixture were dispensed into test tubes and 350 μ l of the various extracts, prepared at 2 mg /ml concentrations, were added and the emulsion system was incubated for 48 h at room temperature. The same procedure was repeated with synthetic antioxidant BHT as positive control, and blanks (MeOH and H₂O). The absorbances of the mixtures were measured at 490 nm after; 0, 1, 2, 4, 6, 24 and 48 h. The antioxidant activity (AA) was measured in terms of successful bleaching of β -carotene by using the following equation: $AA\% = A_{\text{sample}} / A_{\text{BHT}} \times 100$.

A sample: absorbance in the presence of the extract

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

2.5.7. Ferric thiocyanate (FTC) method

The antioxidant activity of plant extracts on the inhibition of linoleic acid peroxidation was assayed with a modified thiocyanate method described by Yen *et al.* (2003). FTC method was used to determine the amount of peroxide at the initial stage of lipid peroxidation. The peroxide reacts with ferrous chloride (FeCl₂) to form a reddish ferric chloride (FeCl₃) pigment. In this method, the concentration of peroxide decreases as the antioxidant activity increases. A sample solution (0.5 ml) of plant fractions was mixed with 2.5 ml of 0.02 M

linoleic acid emulsion at pH 7.0 and 2 ml of 0.2 M phosphate buffer at pH 7.0. The linoleic acid emulsion was prepared by mixing 0.2804 g of linoleic acid, 0.2804 g of Tween 20 as emulsifier, and 50 ml of phosphate buffer. The reaction mixture was incubated at 37 °C for 5 days. To 0.1 ml of the reaction mixture at 24 h intervals was added 75% EtOH (4.7 ml), 30% ammonium thiocyanate (0.1 ml), 0.02 M ferrous chloride in 3.5% HCl (0.1 ml). Three minutes after the addition of ferrous chloride to the reaction mixture, the absorbance was measured at 500 nm. The measurement was taken every 24 hours until the absorbance of the control reached its maximum value. BHT was used as a positive control.

2.5.8. Thiobarbituric Acid (TBA) method

The TBA test was conducted on the final day of FTC according to the method described by Kikuzaki and Nakatani (1993) to determine the MDA formation from linoleic acid peroxidation. This method is based on spectrophotometric measurement of the pink color generated by the reaction of two molecules of TBA with one molecule of MDA at a low pH and high temperature (100 °C) (Figure.18). Brefly, the same sample preparation method as described in the FTC method was used. To 1 ml of sample solution, 20% trichloroacetic acid (2 ml) and thiobarbituric acid solution (2 ml) were added. The mixture was placed in a boiling water bath for 10 minutes. After cooling, it was then centrifuged at 3000 rpm for 20 minutes. Absorbance of the supernatant was measured at 532 nm. Antioxidant activity was recorded based on the absorbance of the final day of the FTC assay. Both methods (FTC and TBA) described antioxidant activity by percent inhibition:

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

A control: absorbance of the control (without sample)

A sample: absorbance of the experimental reactions in the presence of extract.

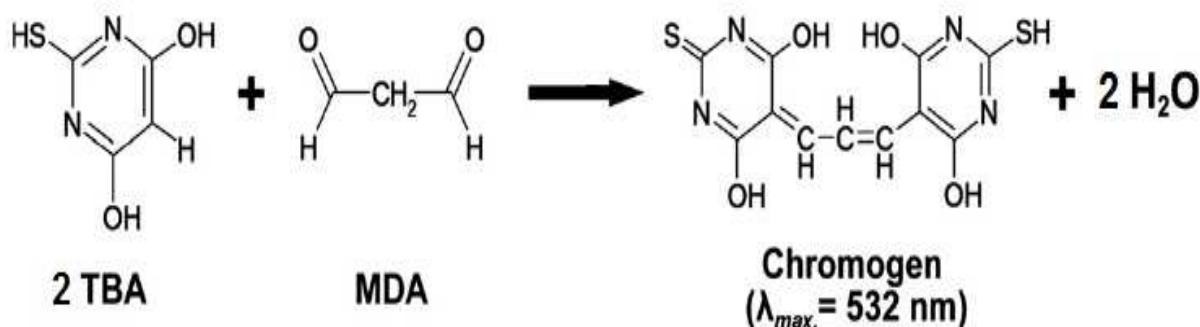


Figure. 18 : Principle of TBA assay (Bastos *et al.*, 2012)

2.6. Antioxidant activity of plant extracts: *in vivo* assays

2.6.1. Treatment protocol

The rats were divided into 8 groups of six animals each, and the treatment was given everyday via orogastric tube for 14 days as the following:

Group 1: received normal saline (0.9%) and served as control group.

Group 2: received ME of *C.azarolus* at the dose of 100 mg/kg.

Group 3: was given ME of *C.azarolus* at the dose of 200 mg/kg.

Group 4: was treated with ME of *M.communis* at the dose of 100 mg/kg.

Group 5: received ME of *M.communis* at the dose of 200 mg/kg.

Group 6: was treated with ME of *O.glandulosum* at the dose of 100 mg/kg.

Group 7: received ME of *O.glandulosum* at the dose of 200 mg/kg.

Group 8: received vitamin C (100 mg/kg) and served as drug control or reference control.

2.6.2. Sacrifice of animals

On day 15, all animals were sacrificed. Blood was collected and centrifuged at 3000 rpm for 15 min for determination of plasma antioxidant capacity. The liver and kidney were

removed and washed with ice-cold saline, blotted with filter paper and kept in plastic vials at -20 °C until use.

2.6.3. Preparation of homogenate

Liver or kidney tissues were cut down into small pieces, placed in 1.15M KCl buffer, and homogenized using dounce homogenizer in ice-cold condition to obtain 10 % homogenate. The homogenate thus obtained was centrifuged at 4000 rpm for 15 min and the supernatant collected was used for the determination of MDA as a lipid peroxidation marker. Catalase, reduced glutathione and total protein were also assayed.

2.6.4. Effect of extracts on plasma antioxydant capacity using DPPH radical

In this test, the capacity of plasma to trap the DPPH radical was evaluated according to the method of Burits and Bucar (2000) with some modifications, 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 at 517 nm is measured and the plasmatic antioxydant capacity was then calculated according to the equation below:

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

A control: is the absorbance of the blank solution

A sample: is the absorbance in the presence of the test compound.

2.6.5. Effect of extracts on plasma reducing power

The reducing power was determined according to the method of Chung *et al.* (2005). In brief, plasma (0.1 ml) was mixed with 0.1 ml of 0.2 M sodium phosphate buffer (pH 6.6)

and 0.1 ml of 1 % potassium ferricyanide. The mixture was then incubated for 20 min at 50°C. After that, 0.250 ml of 1% trichloroacetic were added, the mixture was then centrifuged for 10 min at 3000 rpm. The aliquot (0.250 ml) of the upper layer was mixed with 0.250 ml of distilled water and 0.5 ml of ferric chloride (0.1%), and the absorbance at 700 nm was measured. A higher absorbance indicated a higher reducing power.

2.6.6. Assessment of total protein level

Tissue protein concentration was assayed according to Gornall *et al.* (1949) using the Biuret reagent (potassium iodide, potassium sodium tartrate, copper sulphate and sodium hydroxide) and bovine serum albumin as a standard. In brief, 1 ml biuret reagent was mixed with 25 µl sample or standard (albumin), and the absorbance (540 nm) was measured after 10 min of incubation at 37 °C. Total protein level was calculated through formula:

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

Where n is standard concentration.

2.6.5. Catalase activity assay

Catalase activity was measured by the method of Clairborne (1985). The breakdown of hydrogen peroxide in the presence of catalase was followed by observing the decrease in absorption of peroxide solution in the ultraviolet (UV) region. In brief, an aliquot (50 µl) of each tissue supernatant was added to cuvette containing 2950 µl of 19 mM H₂O₂ prepared in 0.1M phosphate buffer (pH 7.4). The rate of decomposition of H₂O₂ was measured spectrophotometrically at 240 nm in an interval of two minutes time. The decrease in optical density due to decomposition of hydrogen peroxide was measured against a blank at 240 nm. Units of catalase were expressed as the amount of enzyme that decomposes 1 µM H₂O₂ per

minute at 25°C. Thus, the specific activity was expressed in terms of units per gram of tissue according to this formula:

$$U/g \text{ tissue} = (2.3033/ T) \times (\log A1/A2) /g \text{ tissue}$$

A1 : Absorbance at t_0

A2 : Absorbance at t_1 .

T : Interval of time (minute).

2.6.6. Assessment of reduced glutathione concentration

Total reduced glutathione (GSH) content was measured following the method of Ellman's (1959), which measures the reduction of 5,50-dithio-bis (2-nitrobenzoic acid) (DTNB) (Ellman's reagent) by sulfhydryl groups to 2-nitro-5-mercaptobenzoic acid (Figure. 19), which has an intense yellow color and maximum absorption at 412 nm (Huo *et al.*, 2011). Briefly, 50 μ l of supernatant were diluted in 10 ml phosphate buffer (0.1 M, pH 8). Then, to 3 ml of the mixture of dilution, 20 μ l of DTNB (0.01 M) were added. The yellow color developed was read at 412 nm after 5 min. A series of standards GSH (0.4-20 μ mol/ml) were treated in a similar manner along with blank. Reduced glutathione was expressed as μ mol/ g tissue.

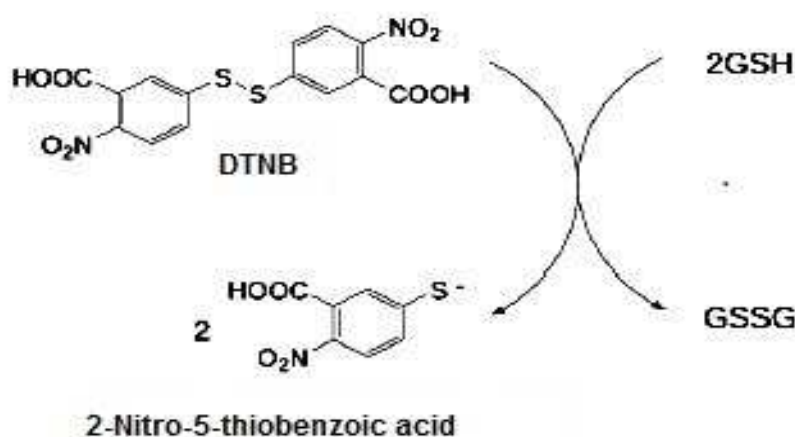


Figure. 19 : Principle of total glutathione assay (Araujo *et al.*, 2008)

2.6.7. Assessment of lipid peroxidation

MDA levels were measured by the method of Ohkawa *et al.* (1979), which is based on spectrophotometric measurement of the pink color generated by the reaction of TBA with MDA. Briefly, 0.5 ml of tissue homogenate was mixed with 0.5 ml of TCA (20 % w/v) and 1ml of TBA (0.67 % w/v). Then, the mixture was boiled in a water bath for 15 min. After cooling to room temperature, 4 ml of n-butanol were added to each sample and centrifuged at 3000 rpm for 15 min. The absorbance was measured at 532 nm with respect to the blank solution. The concentration of MDA was determined from a standard curve of 1, 1, 3,3 tetraethoxypropane in the same conditions and it was expressed as n mol/ g tissue.

2.7. Blood pressure measurements in anesthetized rats

The blood pressure of the anesthetized rats was recorded by method described by Abdalla *et al.*(1994). Male albino rats were anesthetized with sodium thiopental (50 mg/ kg body weight; i.p)(Figure. 20. A). The right common carotid artery was exposed and a catheter was introduced for the recording of blood pressure using P23AA Statham pressure transducer situated at the level of the heart and connected to a Gilson polygraph)(Figure. 20. B). The right femoral vein was also catheterized for the intravenous injection of ME and EAE of each plant. After a steady baseline of blood pressure was obtained (about 15 minutes), ME and EAE were injected in doses of 0.04, 0.12, 0.4, 1.2, 4, and 12 mg/kg body weight. Blood pressure was allowed to return to the resting level before every next dosing. The changes in systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial blood pressure (MABP) were recorded and expressed as percent of their respective control values obtained before drugs administration. Mean arterial blood pressure (MABP) was calculated by adding the values of DBP and one-third of pulse pressure.

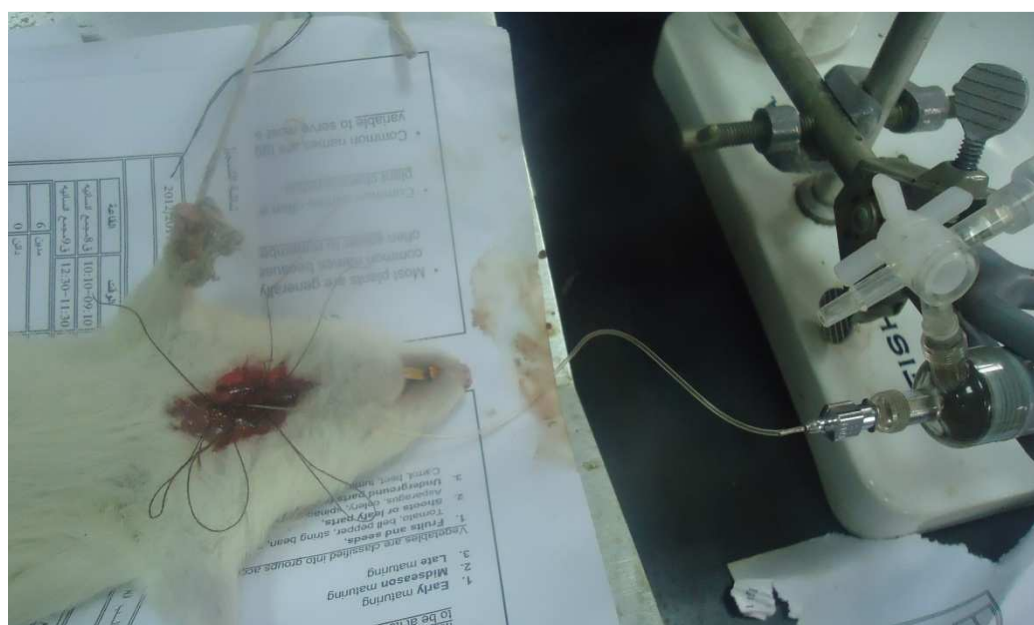
A**B**

Figure. 20: Invasive method of blood pressure recording. (A): rats were anaesthetized with sodium thiopental ; (B) : P23AA Statham pressure transducer was introduced in the carotid artery (Personal photos)

2.8. Evaluation of the diuretic activity

The diuretic activity was determined following reported method of Zhang *et al.*(2010), with some modifications. Animals were divided into 11 groups of five animals each and were fasted 18 hours prior to the test, with free access to water. Before treatment, all animals received physiological saline (0.9% NaCl) at an oral dose of 25 ml /kg of body weight (BW) to impose a uniform water and salt load, and then treated orally in the following manner:

Group 1: received 5 ml/ kg BW of saline and considered as control group.

Groups 2 to 4: received 100, 300 and 600 mg/kg BW of ME of *C. azarolus*, respectively.

Groups 5 to 7: treated with 100, 300 and 600 mg/kg BW of ME of *M. communis*, respectively.

Groups 8 to 10: received 100, 300 and 600 mg/kg BW of ME of *O. glandulosum*, respectively.

Group 11: received 20 mg/kg BW of furosemide and considered as diuretic agent.

Each rat was individually placed in a metabolic cage, and the cumulative urine output and pH were determined at the end of the experimental period (5 h.). The urinary electrolyte (Na^+ , K^+ , Cl^-) concentrations were performed in central laboratory of University Hospital of Setif using Beckman automate.

2.9. Statistical analysis

Statistical analysis was performed using Graph Pad Prism (version 5.01 for Windows). *In vitro* results were expressed as mean $\pm$ standard deviation (SD) and were analyzed by one-way analysis of variance (ANOVA) followed by Dunnet's test. The pharmacological results were presented as mean $\pm$ standard error of mean (S.E.M.). Values were compared by using Student's *t*-test. In all cases, The *P*-values less than 0.05 were considered statistically significant.

Results and discussion

1. Total polyphenols and flavonoids contents in plant extracts

Plant phenolics, tannins and flavonoids represent major groups of plant constituents that work predominantly as powerful antioxidants or scavenger of free radicals. They play beneficial role in human health and cure/prevent ailments such as inflammatory disorders, cancer, cardiovascular diseases and diabetes which occur due to the deregulation of free radicals generation in the cells (Middleton *et al.*, 2000; Chouhan and Singh, 2011). Table 2 presents the total phenolic and flavonoids contents in different extracts of *C. azarolus*, *M. communis* and *O. glandulosum*. The total phenolic content in the extracts was determined by the Folin–Ciocalteu method, which is considered the best method for total phenolic content (including tannins) determination (Wang *et al.*, 2007), whereas the flavonoids content quantified using AlCl_3 method. Total phenolics content were expressed as mg gallic acid equivalents per gram of dry extract (mg GAE/ g of extract) and total flavonoids content as mg quercetin equivalents per gram of dry extract (mg QE/ g of extract). Results obtained in the present study revealed that the level of these phenolic compounds in the various plant extracts was considerable.

As seen in Table. 2, the highest phenolic contents were observed in EAE with values of 514.814, 435.37 and 396.04 mg GAE/ g of extract for *O. glandulosum*, *M. communis* and *C. azarolus*, respectively, whereas the lowest total polyphenols content was signaled for AqE in the three studied plants with the values of 162.74, 157.70, 38.25 mg GAE/g of extract for *O. glandulosum*, *M. communis* and *C. azarolus*, respectively.

Concerning the total flavonoids content, the highest value was recorded in EAE for *M. communis* (130.75 mg QE/g of extract) and *C. azarolus* (32.62 mg QE/g of extract), whereas in *O. glandulosum*, it was found that CHE contained the biggest amount of flavonoids

(22.62 mg QE/g of extract). On the other hand, AqE showed the lowest total flavonoids content in the three studied plants with the values of 2.12, 2.64 and 8.49 mg QE/g of extract for *C.azarolus* , *M. communis* and *O. glandulosum*, respectively.

In general, it was observed that plant extracts contained different amounts of polyphenols and flavonoids and this could be due to different degree of polarity of the solvents used for the extraction of polyphenolic compounds, confirming that ethyl acetate was the best solvent to concentrate phenolic substances of intermediate polarity. Similar results have been previously reported indicated the efficiency of ethyl acetate in the extraction of phenolic compounds in various plants (Chung *et al.*, 1999; Parejo *et al.*, 2002; khennouf *et al.*, 2013).

Table. 2: Total polyphenols and flavonoids contents in various plant extracts of *C.azarolus*, *M. communis* and *O.glandulosum*.

Extracts	<i>C.azarolus</i>		<i>M.communis</i>		<i>O.glandulosum</i>	
	Polyphenols ^(a)	Flavonoids ^(b)	Polyphenols ^(a)	Flavonoids ^(b)	Polyphenols ^(a)	Favonoids ^(b)
ME	188.91±0.63	21.03±0.81	260.44±2. 52	26.77±0.46	179.92±0.83	12.60±0.56
CHE	149.08±0.41	24.46±0 .16	186.96± 1.69	50.81±1.2	239.92± 1.45	22.62±0.79
EAE	396.04±1.20	32.62±0.03	435.37±3.15	130.75±2.86	514.81±4.26	6.23±0.59
AqE	38.25± 0.015	2.12±0.01	157.70± 2.65	2.64±0.22	162.74 ± 1.78	8.49±0.16

ME; methanolic extract, CHE; chloroform extract, EAE; ethyl acetate extract, AqE; aqueous extract.

^(a) mg Gallic acid Equivalents / g of dry extract ; ^(b) mg Quercetin Equivalents / g of dry extract . Results are expressed as means ± SD (n = 3).

In the present study, EAE from *C. azarolus* presented higher contents in total polyphenols and flavonoids in regard to the same extract prepared from flower buds and open flowers of *C. azarolus* grown in Tunisia (16,4 mg gallic acid equivalents/ g of extract; 7,3 mg rutin equivalent/g of extract)(Bahri-Sahloul *et al.*, 2009). To our knowledge, no studies have so far been reported total polyphenols and flavonoids and contents of the leaves of *C. azarolus* leaves. Thus, the present study is the first one.

Also, the phenolic content of methanolic extract from *M. Communis* leaves observed in this study corroborated with the findings of Nassar *et al.* (2010) and Gardeli *et al.* (2008) who studied the total phenol content of the leaf methanolic extract from Egyptian and Greek myrtle, respectively. However, our finding was eight fold higher to that of Amensour *et al.* (2009) and Aidi Wannes *et al.* (2010) who found that the total phenol contents of the leaf methanolic extract were 31.2 and 33 mg GAE /g of extract from Portuguese and Tunisian myrtle, respectively.

The evaluation of total polyphenols and flavonoids contents showed that ME of *O. glandulosum* contains 179.92 ± 0.83 mg GAE / g of extract and 12.6 ± 0.56 mg QE / g of extract, respectively. These results are largely higher than that obtained by oukil *et al.* (2011) from the methanolic extract of Algerian *O. glandulosum* (55.15 mg GAE / g of extract and 6.88 mg QE / g of extract, respectively). It appears that phenolic compounds extracted depend on plant species and the solvent used. Furthermore, environmental factors such as climate, soil, irrigation, temperature range, exposure to diseases and pests, the harvest season, the way of drying and storage or even the part of the plant tissue to be used are parameters that should be taken into consideration. The extraction procedure appears also to affect total phenolic content and antioxidant activity of each plant (Oukil *et al.*, 2011; Skotti *et al.*, 2014).

2. Isolation and identification of phenolic compounds from selected plants

Majority of the drug discovery in pharmaceutical industry relies on the natural product. To identify the phenolic compounds in *C. azarolus*, *M. communis* and *O.glandulosum*, qualitative analysis was performed by a series of different chromatographic methods (column chromatography, silica gel glass plates). Then, the isolated compounds were identified by spectral techniques (^1H -NMR, ^{13}C -NMR, DEPT 90, DEPT 135, 2D-NMR, and Mass spectra).

2.1. Compounds isolated from *C.azarolus*

2.1. 1. (+)- Catechin

The mother liquor of fraction [5] gave one major spot which was purified using preparative TLC eluting with chloroform /methanol(80:20) to give a red –orange powder identified as (+)- catechin or 2S 3R catechin (Figure.21).

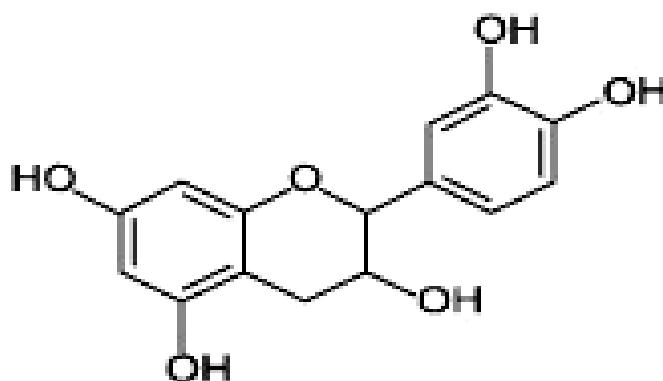


Figure. 21: (+)- Catechin.

The NMR spectroscopic data of (+) - catechin was as the following (Figure.22 and 23; Appendix A):

^1H -NMR (DMSO) δ ppm: 2.49 (1H, m, H-4 α), 2.69 (1H, dd, $J=4.1, 16.2\text{Hz}$, H-4 β), 4.01(1H,br.s, H-3), 6.68 (2H, m, H-5', H-6'), 4.68(1H, br.s,H-2), 6.9 (1H, b.s, H-2') , 5.74(1H, d, $J=1.8\text{Hz}$, H-6), 5.92(1H, d, $J=1.8\text{Hz}$, H-8).

^{13}C -NMR (DMSO) δ ppm: 28.7 (C-4), 65.4 (C-3), 78.5 (C-2), 94.6 (C-6), 95.6 (C-8),99.0(C-10),115.5(C-2' and C-5'),118.4 (C-6'), 131.1 (C-1'), 144.9 (C-3 'and C-4'), 156.1 (C-7), 156.7 (C-5), 157.0(C-9).

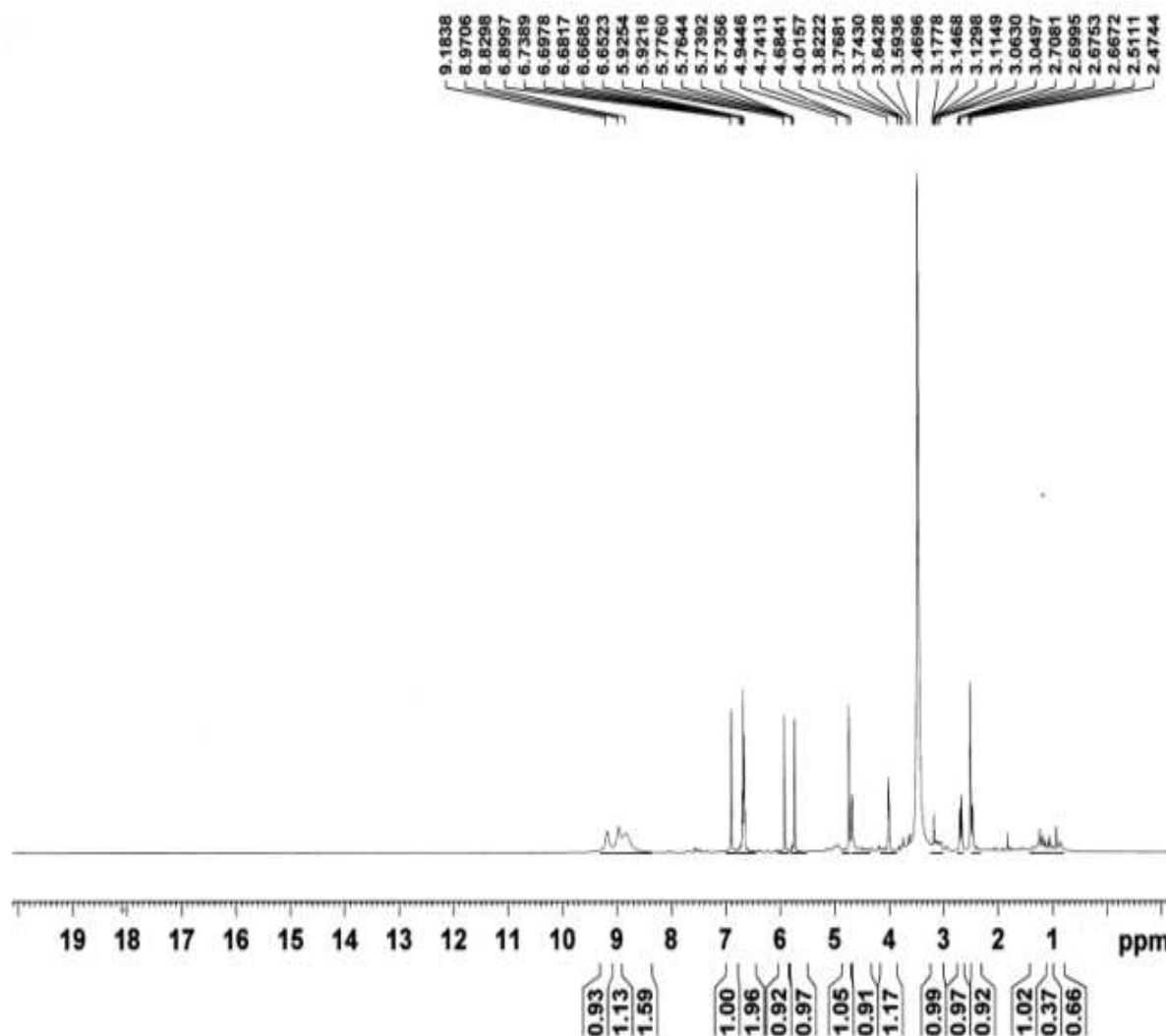


Figure . 22 : ^1H -NMR (500MHz,DMSO) spectrum of (+)- Catechin.

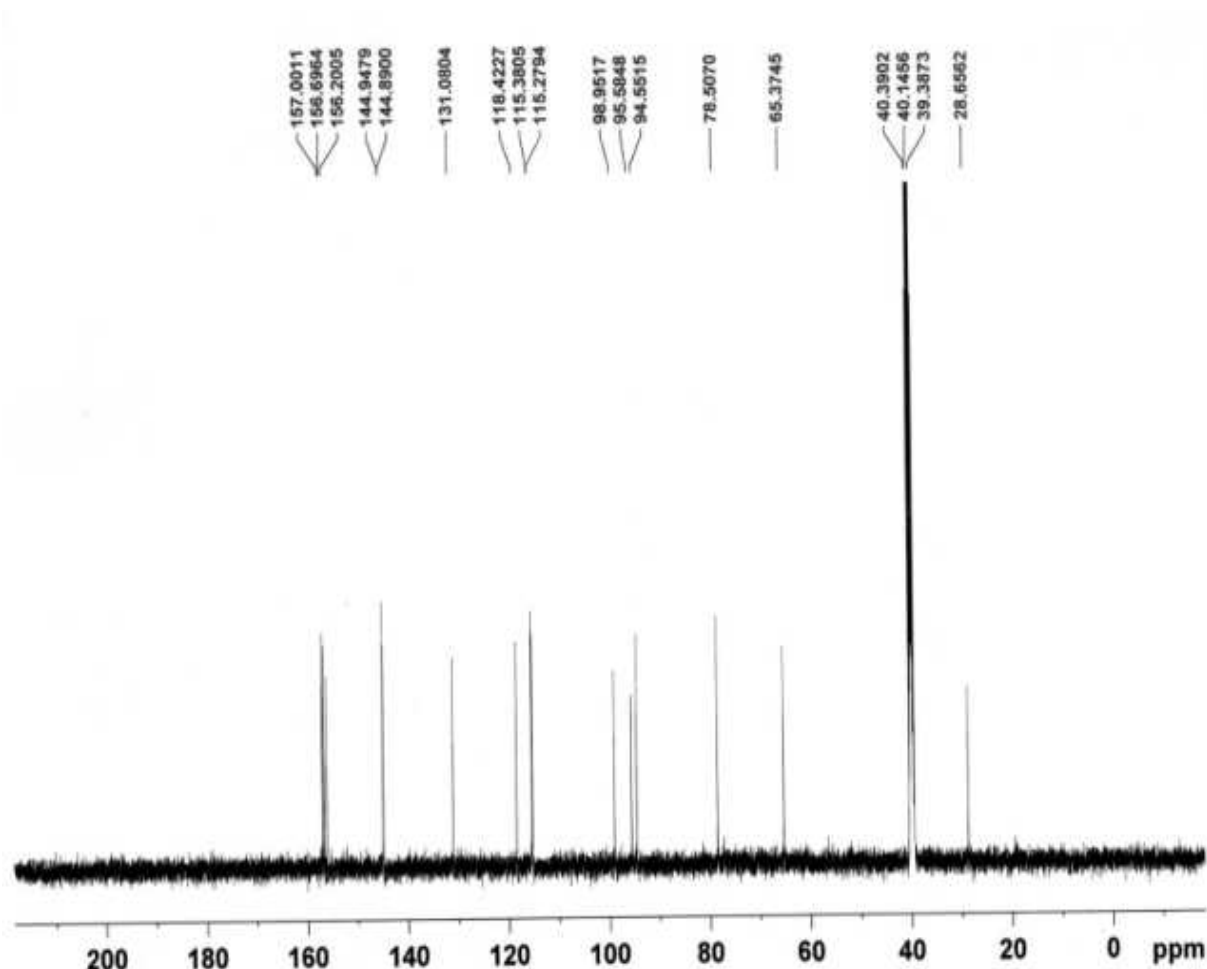


Figure . 23 : ^{13}C -NMR(DMSO) spectrum of (+)- Catechin.

As seen in Figure.22, the ^1H -NMR spectrum showed signals typical of the ring C protons of catechin. The H-2 and H- 3 protons appear as two down field (δ =4.68 and δ = 4.01) broad singlets indicating the cis relationship of these two protons. H-4 α appears as multiplet at δ = 2.49 while H- 4 β appears as a double doublet at δ =2.69. The signals for rings A and B also agree with substitution patterns of the two rings.

The ^{13}C -NMR spectrum was in full agreement with the reported data for catechin which was previously isolated from the same genus (Lee *et al.*, 2003) (Figure.23). Significantly, the DEPT 135 spectrum showed the methylene signal of C-4 at δ =28.7 and the two methane signals of C-2 and C-3 at δ = 78.5 and δ = 65.4, respectively (Figure.24).

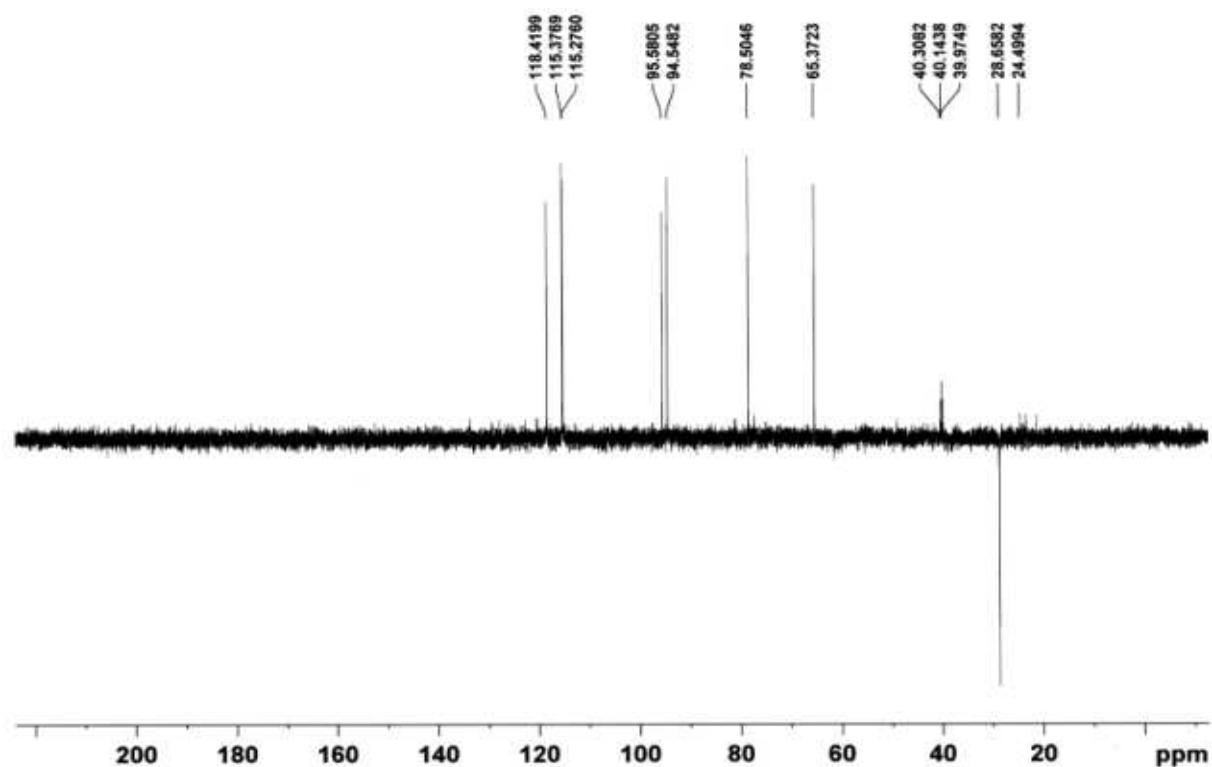


Figure. 24: DEPT 135(500MHz,DMSO) spectrum of (+) - Catechin.

Furthermore, the mass spectrum of catechin gave a molecular ion peak at $m/z=291.08631$, calculated for the molecular formula $[M + H]^+$ (Figure.25).

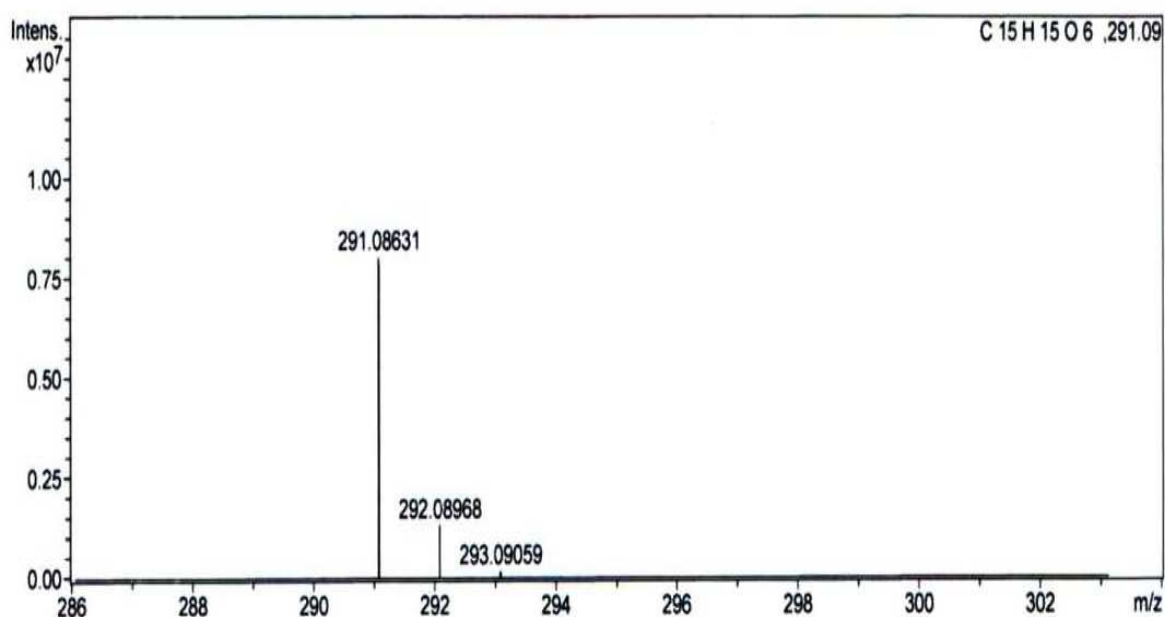


Figure. 25: Mass spectrum of (+)- Catechin.

2.1. 2. Quercetin-3-O-galactoside

Treatment of fraction [6-12] with methanol afforded a pure yellowish solid, which was identified as quercetin-3-O-galactoside or hyperoside (Figure. 26).

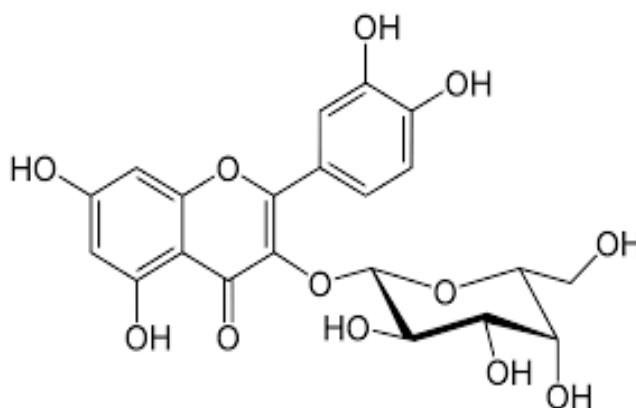


Figure. 26: Quercetin-3-O-galactoside

The NMR spectroscopic data of quercetin-3-O-galactoside was as the following (Figure.27 and 28; Appendix B):

The ^1H -NMR (DMSO) δ ppm: 3.15-5.65 (sugar protons), 5.85(1H, d, J = 7.7 Hz, anomeric), 6.19(1H, d, J = 2Hz, H-8), 6.40 (1H, d, J =2Hz, H-6), 6.80 6.8(1H, d, J = 8.5 Hz, H-5'), 7.48 (1H, d, J = 2.2 Hz, H-2'), 7.62 (1H, dd, J =2.2 and 8.5Hz, H-6').

The ^{13}C -NMR (DMSO) δ ppm: 94.0 (C-8), 99.2 (C-6), 104.4 (C-10), 115.7(C-2'), 116.4 (C-5'), 121.5 (C-6' and C-1'), 133.9 (C-3), 145.3 (C-3'), 149.0 (C-4'), 156.7(C-2 and C-9), 161.7(C-5), 164.6 (C-7), 178 (C-4). Galactose signals: 60.6 (C-6), 68.4 (C-4), 71.7 (C-2), 73.6(C-3), 76.3(C-5), 102.2 (C-1).

The ^1H - NMR (DMSO) spectrum of quercetin-3-O-galactoside showed a doublet at δ = 5.58 ppm (J =7.7 HZ), which is assigned to the anomeric hydrogen of the glycoside. The high

coupling constant reveals that the sugar has β -configuration. The ^{13}C - NMR (DMSO) spectrum showed 21 peaks, 6 of them are assigned to galactose unit. The rest of the peaks are identical to those of quercetin (Lavault and Richomme, 2004; Wong *et al.* , 2012). DEPT 135 showed 1 CH_2 , 10 CH and 10 quaternary carbons (Figure. 29).

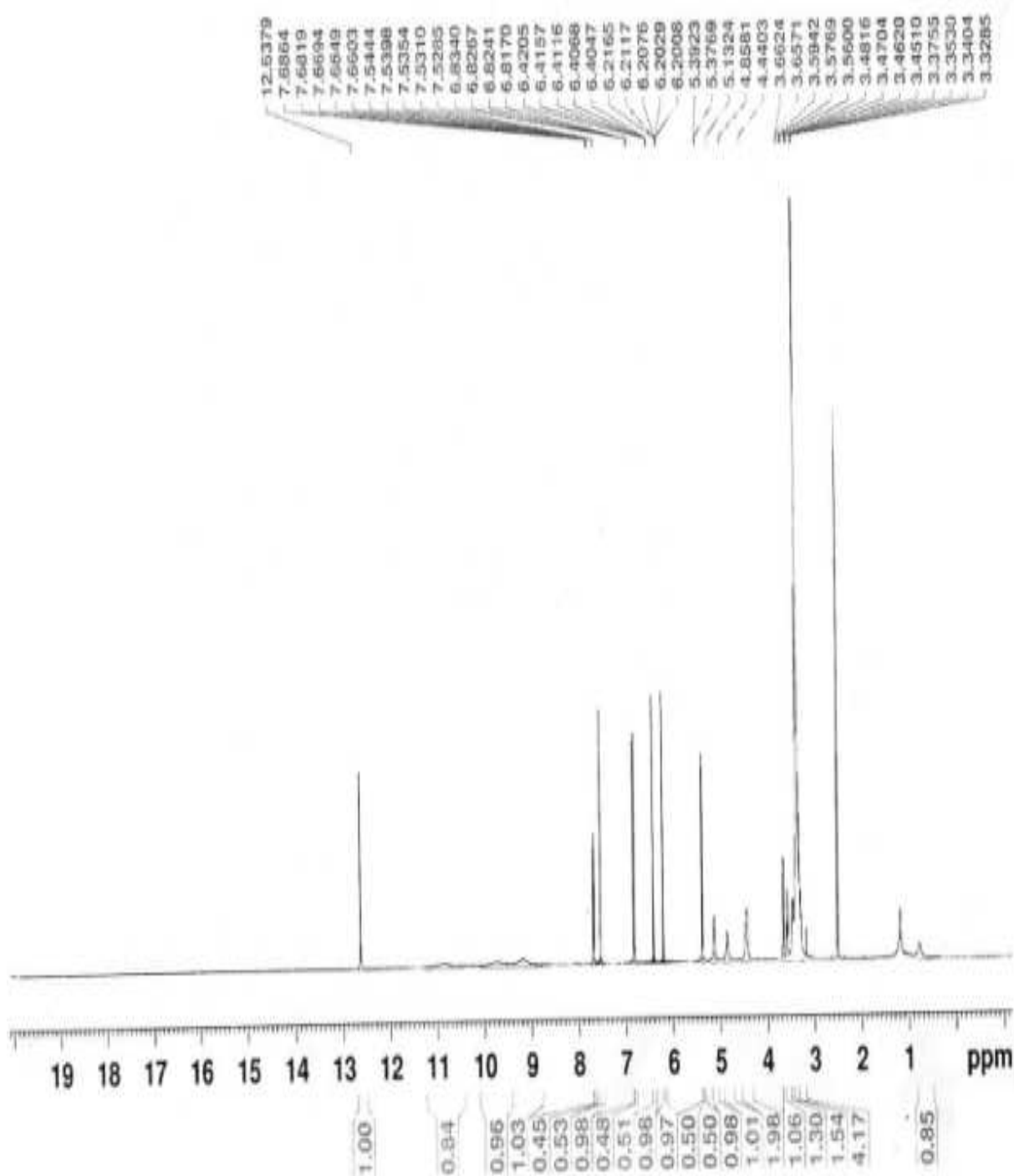


Figure . 27 : ^1H -NMR(500MHz,DMSO) spectrum of quercetin-3-O-galacoside.

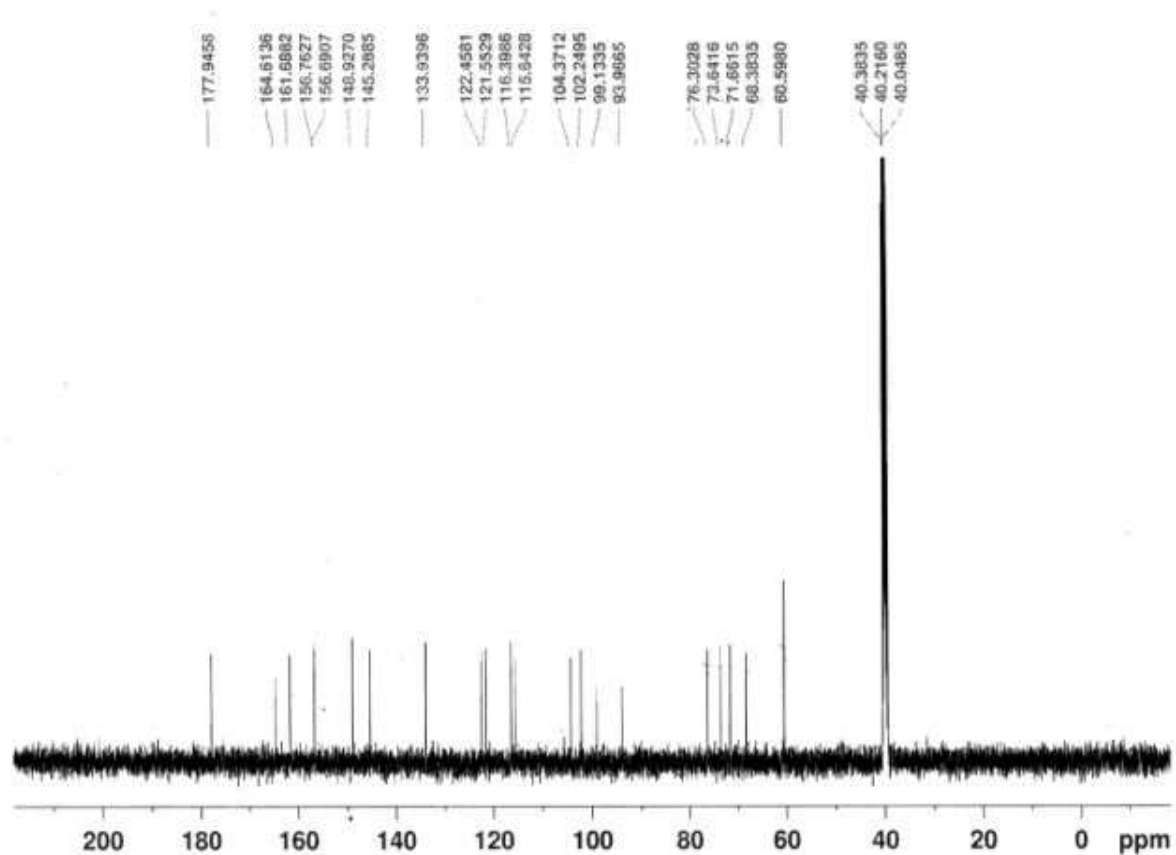


Figure . 28: ^{13}C -NMR (500MHz,DMSO) spectrum of quercetin-3-O-galactoside.

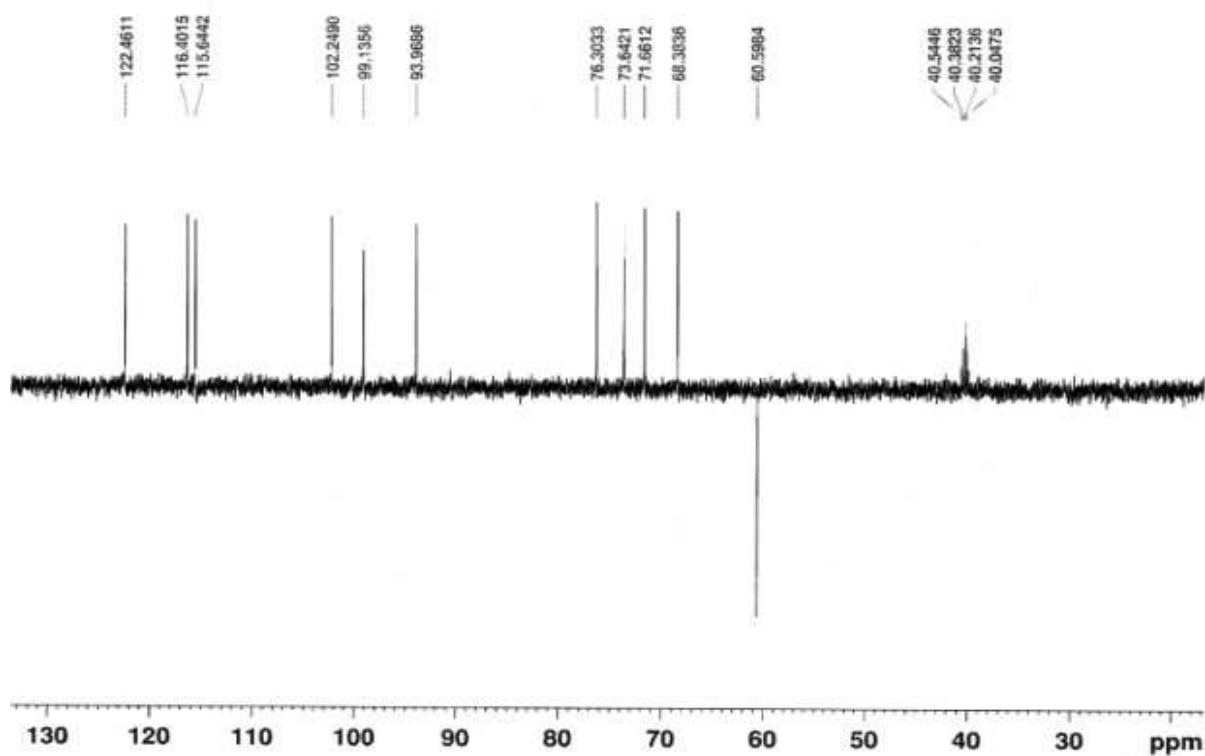


Figure. 29: DEPT 135 (500MHz,DMSO) spectrum of quercetin-3-O-galactoside.

Crataegus species have shown to be a rich source of polyphenols. In particular, dihydroflavonoids (Svedstroma *et al.*, 2006). Well-known naturally occurring flavonoids, i.e., hyperoside and catechin were previously reported from several *crataegus* species. Hyperoside was isolated from the leaves (Belkhir *et al.*, 2013) and flowers of *C. azarolus* (Bahri-Sahloul *et al.*, 2009), *C. aronia* (Al-Hallaq *et al.*, 2012), *C. pinnatifida* fruits (Jurikova *et al.*, 2012), *C. tanacetifolia* (Birman *et al.*, 2011), *C. monogyna* (Prinz *et al.*, 2007), *C. pentagyna*, and *C. laevigata* (Orhan *et al.*, 2007). Catechin was extracted from *C. pinnatifida* (Chang *et al.*, 2006), *C. grayana* (Liu *et al.*, 2011), *C. monogyna* (Bernatoniene *et al.*, 2008), and *C. azarolus* flowers (Bahri-Sahloul *et al.*, 2009). Nevertheless, as far as we know, this is the first report for the isolation of (+)- catechin in *C. azarolus* leaves from Algeria.

2.2. Myrecitin - 3- O - α - rhamnoside isolated from *M. communis*

The mother liquor of fraction [18-28] from EAE gave one major spot which was purified by preparative TLC using CHCl_3 -MeOH- H_2O (65: 35:6) to give four bands, the first band gave the title compound; myricetin - 3- O- α - rhamnoside (Figure. 30) which was isolated previously from myrtle liqueur (Montoro *et al.*, 2008) and myrtle leaves (Hayder *et al.*, 2008).

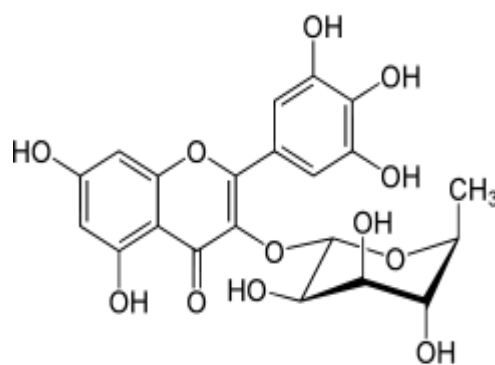


Figure. 30: Myricetin – 3- O- α - rhamnoside.

The NMR spectroscopic data of myricetin – 3- O- α - rhamnoside was as the following (Figure. 31 and 32; Appendix C):

$^1\text{H-NMR}$ (DMSO) δ ppm: 0.85 (3H, d, J = 6.1Hz, H-6''), 3.00-3.70 (4H, m, H-2'', H-3'', H-4'' & H-5''), 3.99 (1H, br s, H-1''), 6.44 (1H, br s, H-8), 6.47 (1H, br s, H-6), 6.91 (2H, s, H-2' & H-6'), 12.66 (1H, br s, 5-OH).

$^{13}\text{C-NMR}$ (DMSO) δ ppm: 18.0 (C-6''), 70.5 (C-2'' & C-5''), 70.9 (C-3''), 71.8 (C-4''), 102.4 (C-1''), 94.0 (C-8), 99.2 (C-6), 104.4 (C-10), 108.4 (C- 2' and C- 6'), 120.1 (C- 1'), 134.8(C-4'), 136.9 (C-3), 146.2 (C-3 'and C- 5'), 156.8 (C-2), 157.9 (C-9), 161.7 (C-5), 164.8 (C-7), 178.2 (C-4).

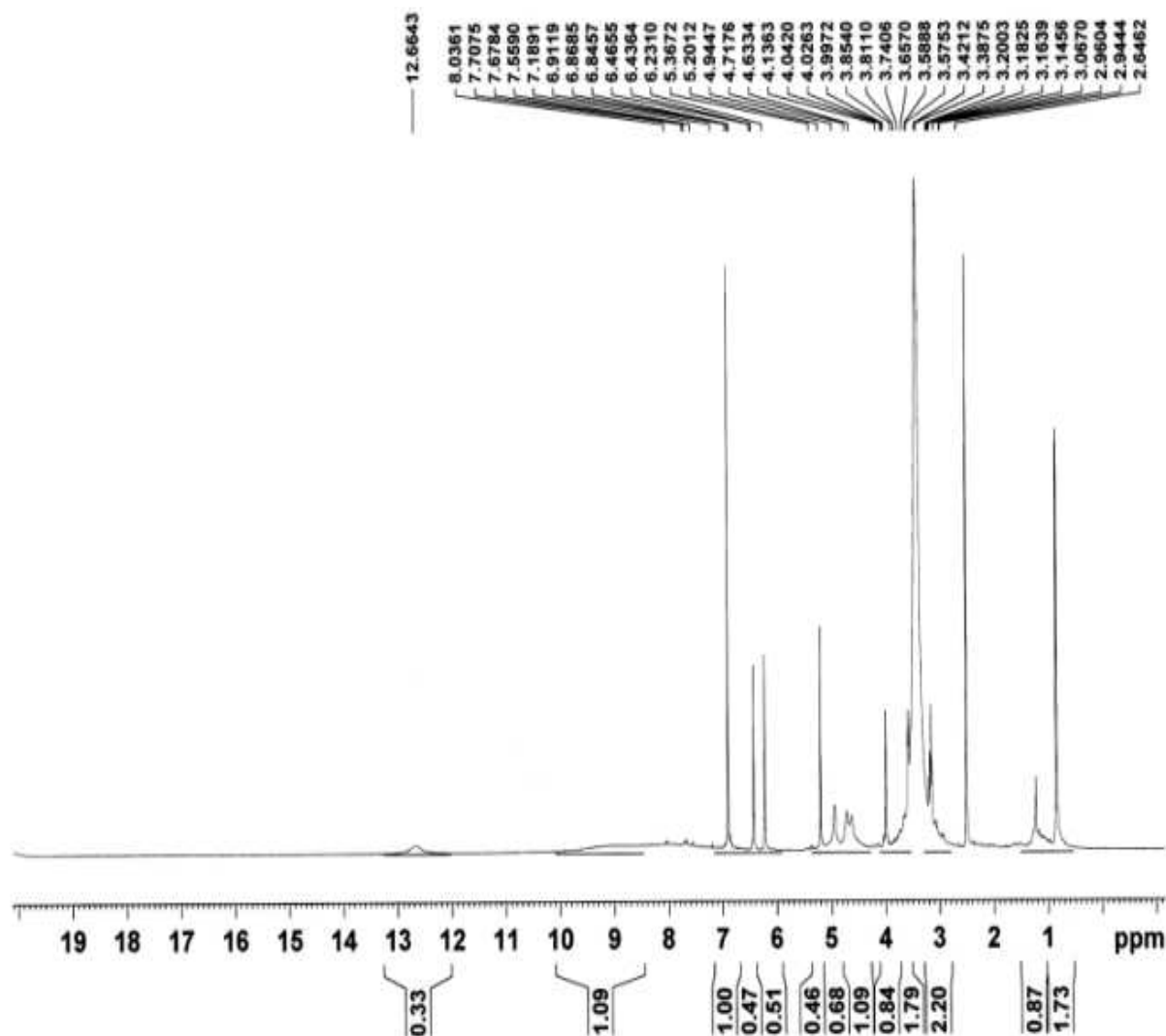


Figure.31: $^1\text{H-NMR}$ (500MHz,DMSO) spectrum of myricetin – 3- O- α - rhamnoside.

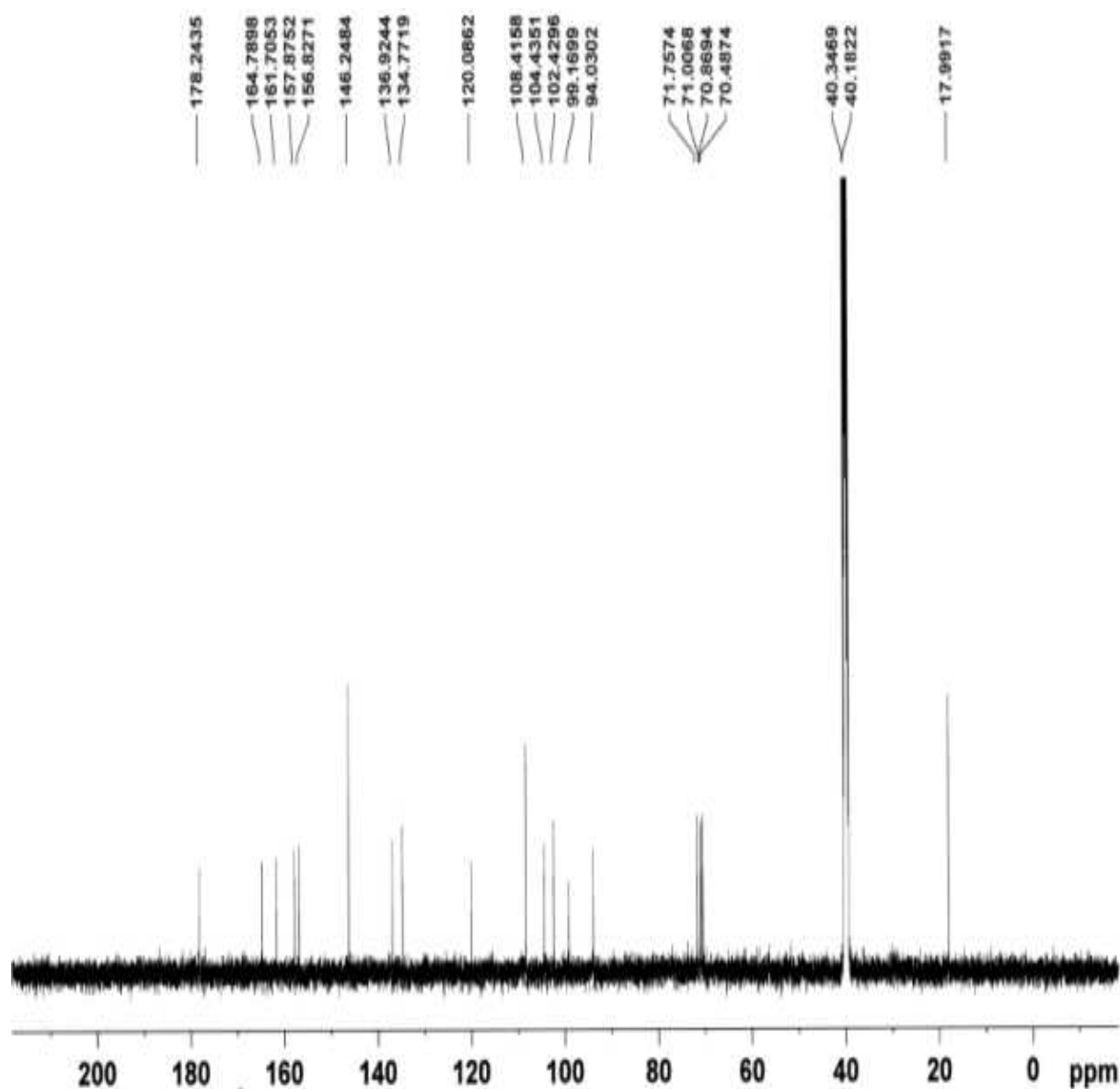


Figure. 32: ^{13}C -NMR (500MHz,DMSO) spectrum of myricetin – 3- O- α - rhamnoside.

The ^1H - NMR spectrum (DMSO) of myricetin - 3- O - α - rhamnoside showed signals for a flavonol glycoside. The upfield three protons double at $\delta = 0.85$ indicated that the compound is a rhamnoside. The anomeric proton appears as broad singlet at $\delta = 3.99$, indicating the glycoside linkage is α . The flavonoid protons appear as two broad singlets for H-8, H-6 and a two protons signal for H-2' and H-6' ($\delta = 6.91$). The 5-OH appears as expected down field at $\delta = 12.66$ due to H- bonding to the carbonyl group.

The ^{13}C - NMR spectrum (DMSO) confirmed the presence of rhamnoside moiety as it showed ^{13}C - NMR signals which are typical of rhamnoside. The rest of ^{13}C - NMR signals are in full agreement with those reported for myricetin (Hinou *et al.*, 1988; David *et al.*, 1996).

As shown in Figure. 33, the structure of myricetin – 3- O- α – rhamnoside was confirmed by mass spectrum which gave a molecular ion peak at $m/z=463.08820$, calculated for the molecular formula $[\text{M} - \text{H}]^+$.

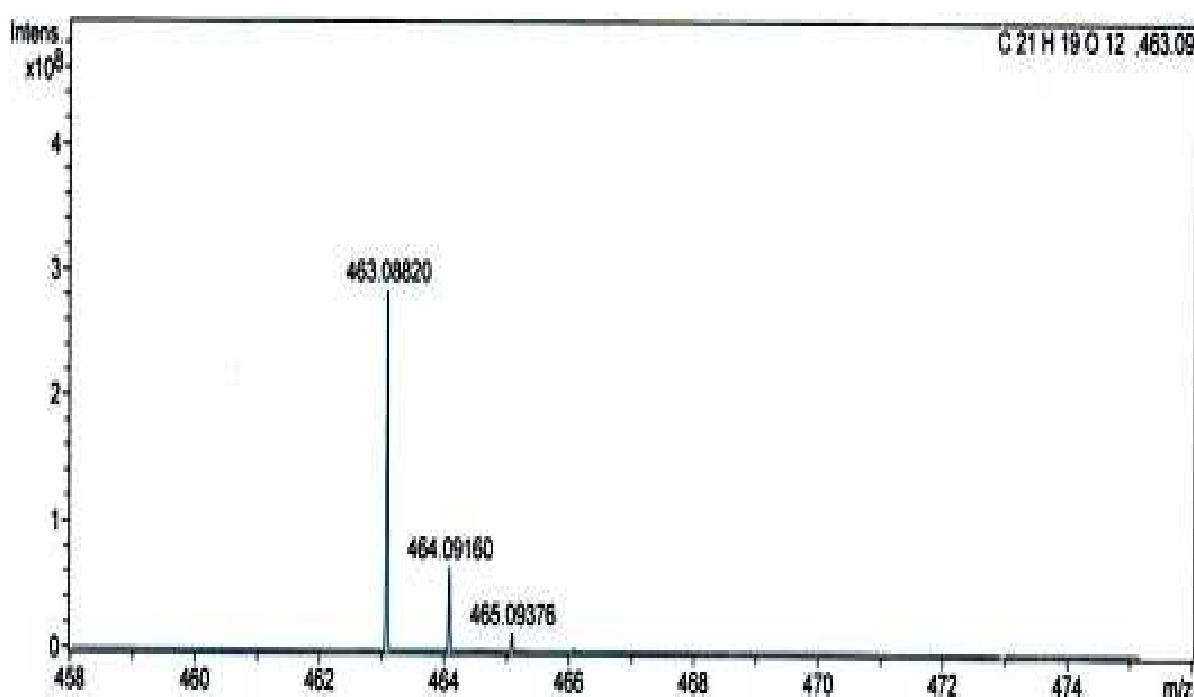


Figure. 33: Mass spectrum of myricetin – 3- O- α - rhamnoside.

2.3. Compounds isolated from *O. glandulosum*

2.3.1. Katuranin

Purification of fraction [5-6] by preparative TLC using 20% MeOH / CHCl_3 gave a pure white powder which was identified as katuranin or aromadendrin (Figure. 34). Katuranin was

isolated previously from greek *O.vulgare* (Vekiari *et al.* , 1993; Exarchou *et al.*, 2003) and *O. glandulosum* by HPLC coupled to diode-array detection and APCI-MS (Skoula *et al.*, 2008).

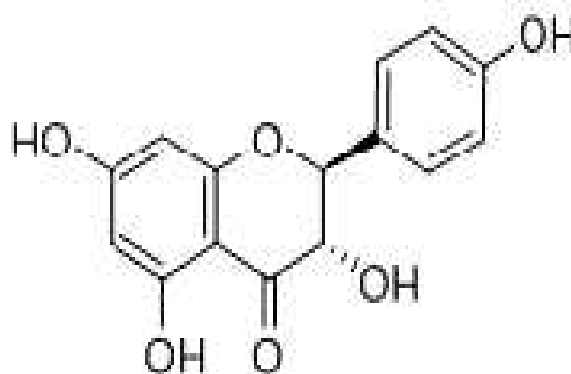


Figure. 34: Katuranin.

The NMR spectroscopic data of katuranin was as the following (Figure.35 and 36; Appendix D):

$^1\text{H-NMR}$ (DMSO) δ ppm: 4.58 (1H, d, $J=11.4$ Hz, H-3), 5.01(H, d, $J=11.4$ Hz H-2), 5.90 (1H, d, $J=1.5$ Hz, H-8), 5.95 (1H, d, $J=1.5$ Hz, H-6), 6.79 (2H, d, $J=8.4$ Hz, H-3' & H-5'), 7.31 (2H,d, $J=8.4$ Hz, H-2' & H-6').

$^{13}\text{C-NMR}$ (DMSO) δ ppm: 71.9(C-3), 83.3 (C-2), 95.5 (C-8), 96.5 (C-6), 100.9 (C-10), 115.4 (C-3' & C-5'), 128.0 (C-1'),129.9(C-2' & C-6'),158.2 (C- 9), 163.0 (C-4'), 163.7 (C-7), 167.4 (C-5), 198.3 (C-4).

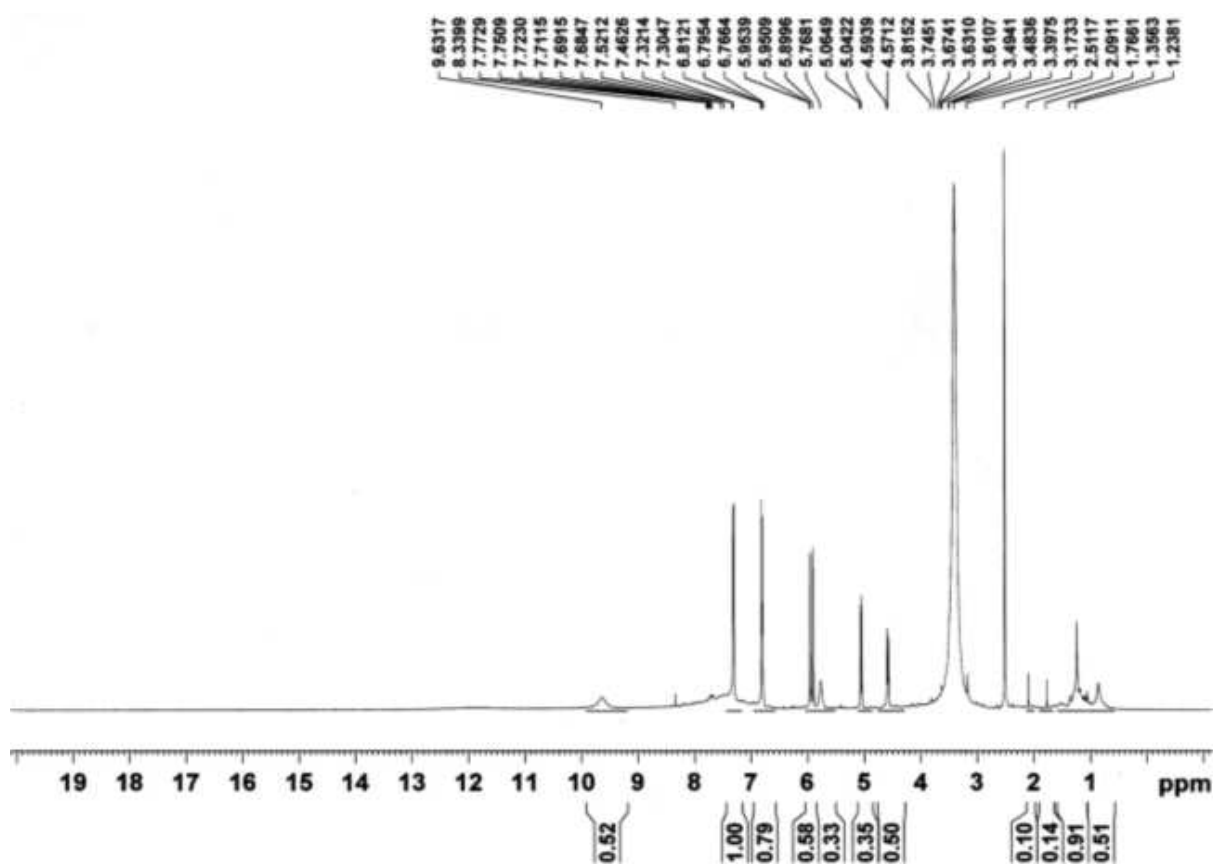


Figure. 35: ^1H -NMR (DMSO) spectrum of katuranin.

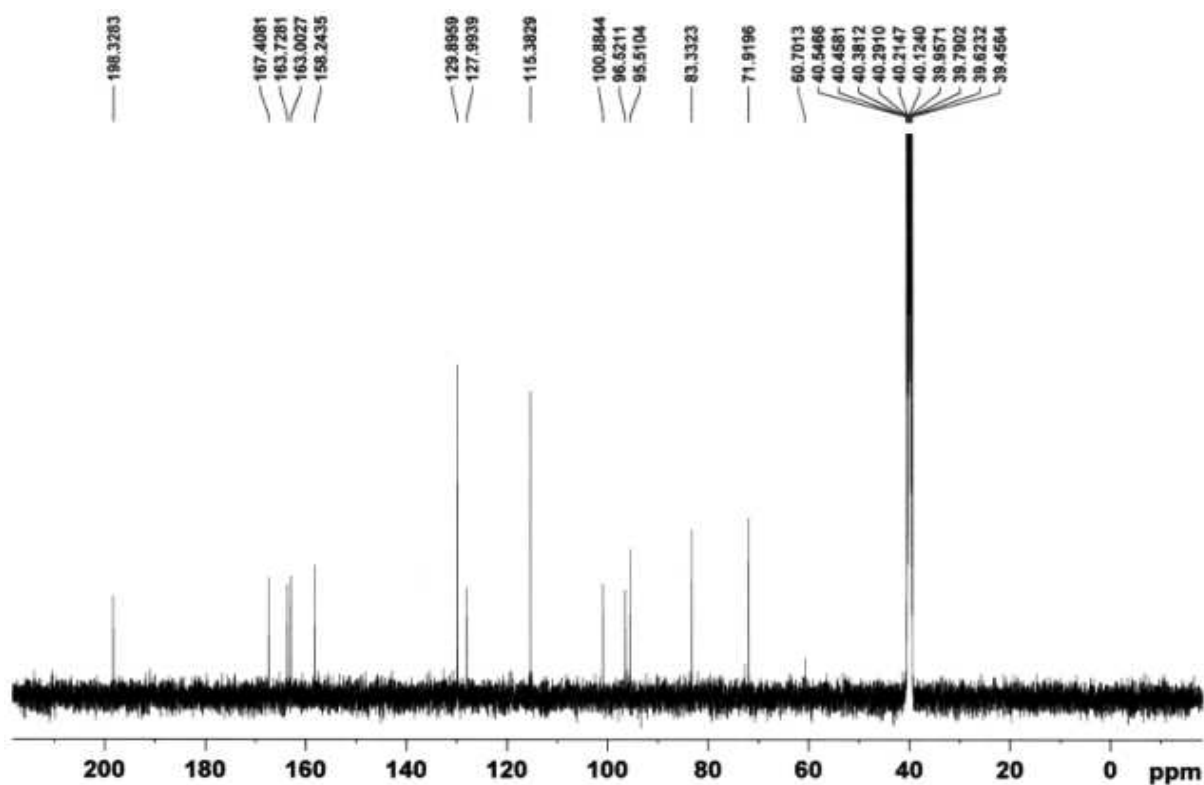


Figure. 36: ^{13}C -NMR (500MHz, DMSO) spectrum of katuranin.

The ^1H - NMR spectrum (DMSO) is typical of tetra-hydroxylated flavanone (Figure.35). It shows H-2 and H-3 as two doublets at $\delta=5.05$ and $\delta=4.58$ with $J=11.4$ Hz. The high value of the coupling constants indicates the trans relationship between the two protons. The spectrum also shows two doublets at $\delta=5.90$ and $\delta=5.95$ Hz ($J=1.5$ Hz) for the meta coupled protons H-8 and H-6, respectively. The protons of para-disubstituted ring B appear as expected as two doublets at $\delta 6.79$ and $\delta 7.31$ ($J=8.4$ Hz) for H-3',H-5' and H-2', H-6', respectively.

The ^{13}C - NMR spectrum (DMSO) of katuranin showed the presence of 15 carbon atoms (Figure.36). The oxygenated methane carbons C-2 and C-3 appear at $\delta=83.3$ and $\delta=71.91$, respectively. The carbonyl signal appears at $\delta=198.3$, which is typical of flavanones.

The DEPT experiment confirmed the presence of four oxygenated aromatic carbons (Figure. 37). The carbon chemical shifts are in full agreement with the structure of katuranin (Vekiari *et al.*, 1993; Exarchou *et al.*, 2003).

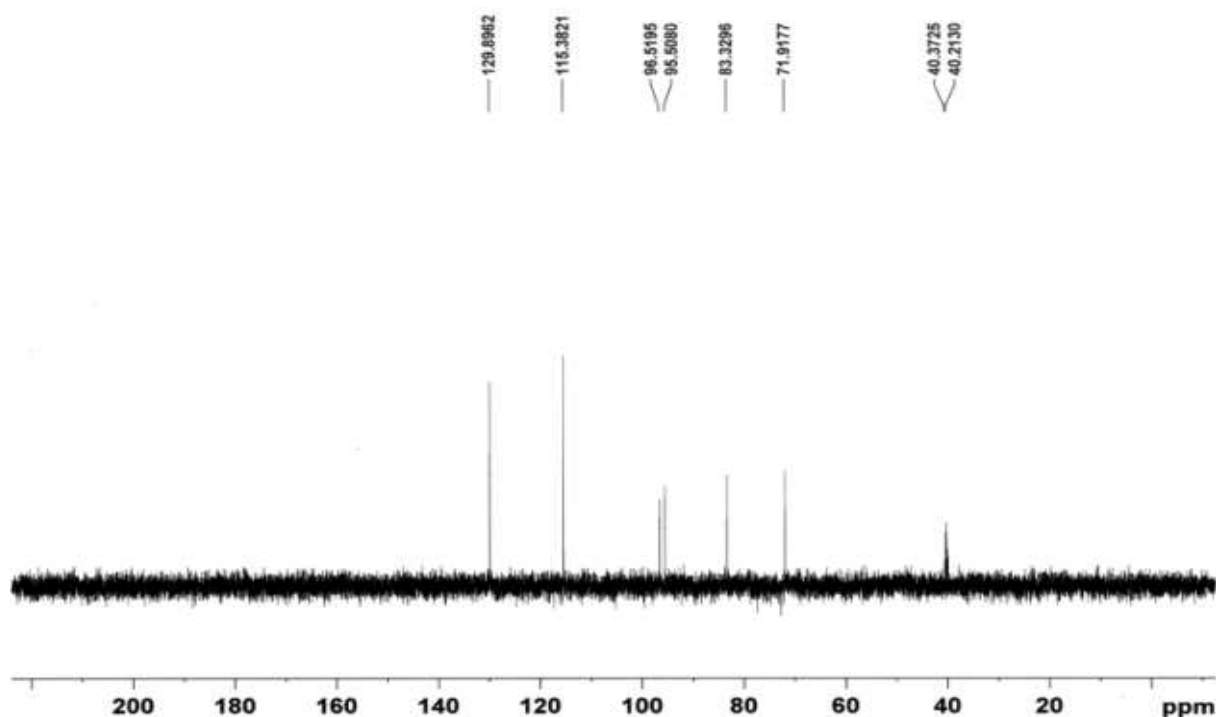


Figure . 37: DEPT 135(500MHz, DMSO) spectrum of katuranin.

As shown in Figure. 38, the mass spectrum of katuranin gave a molecular ion peak at $m/z=287.05611$, which correspond to the formula $[M - H]^+$.

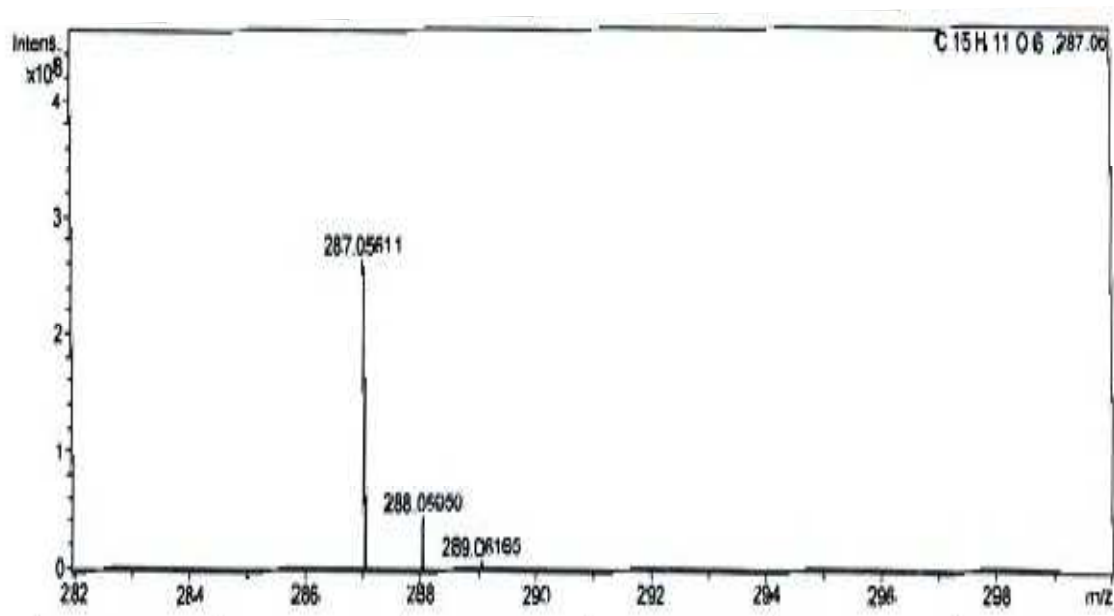


Figure . 38: Mass spectrum of katuranin.

2.3.2. 5-Isopropyl-3-methylphenol

Purification of fraction [1-2] of EAE from *O. glandulosum* using 2 % MeOH/ $CHCl_3$ on TLC plates afforded a pure compound which was identified here as 5-isopropyl-3-methylphenol (Figure.39) for the first time in the species and the genus *Origanum*.

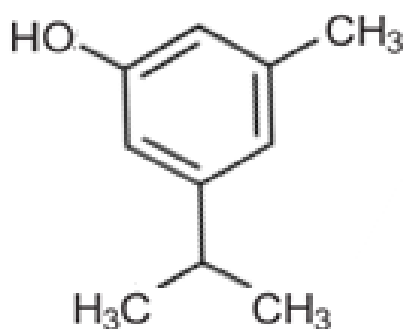


Figure.39: 5-Isopropyl-3-methylphenol.

The NMR spectroscopic data of 5-isopropyl-3-methylphenol was as the following:

^{13}C -NMR (CDCl_3) δ ppm : 16.0 (C-10), 18.0 (C-8 & C-9), 22.0 (C-7), 109.7 (C-6), 116.9 (C-2), 121.5 (C-4), 126.7 (C-3), 131.9 (C-5), 147.8 (C-1) (Figure. 40).

^1H -NMR (CDCl_3) δ ppm: 1.30 (6H, b.s, H-8 and H-9), 2.30 (3H, s, H-10), 3.40 (1H, b.s, H-7), 6.63 (1H, m, H-6), 6.79 (1H, m, H-2), 7.11 (1H, m, H-4) (Figure. 41).

The ^{13}C -NMR spectrum (CDCl_3) of the compound showed 10 signals which indicate a monoterpene skeleton (Figure. 40). Also, the same spectrum showed signals; for 3 methyls and an aliphatic methine carbon ($\alpha\text{-C-H}$).

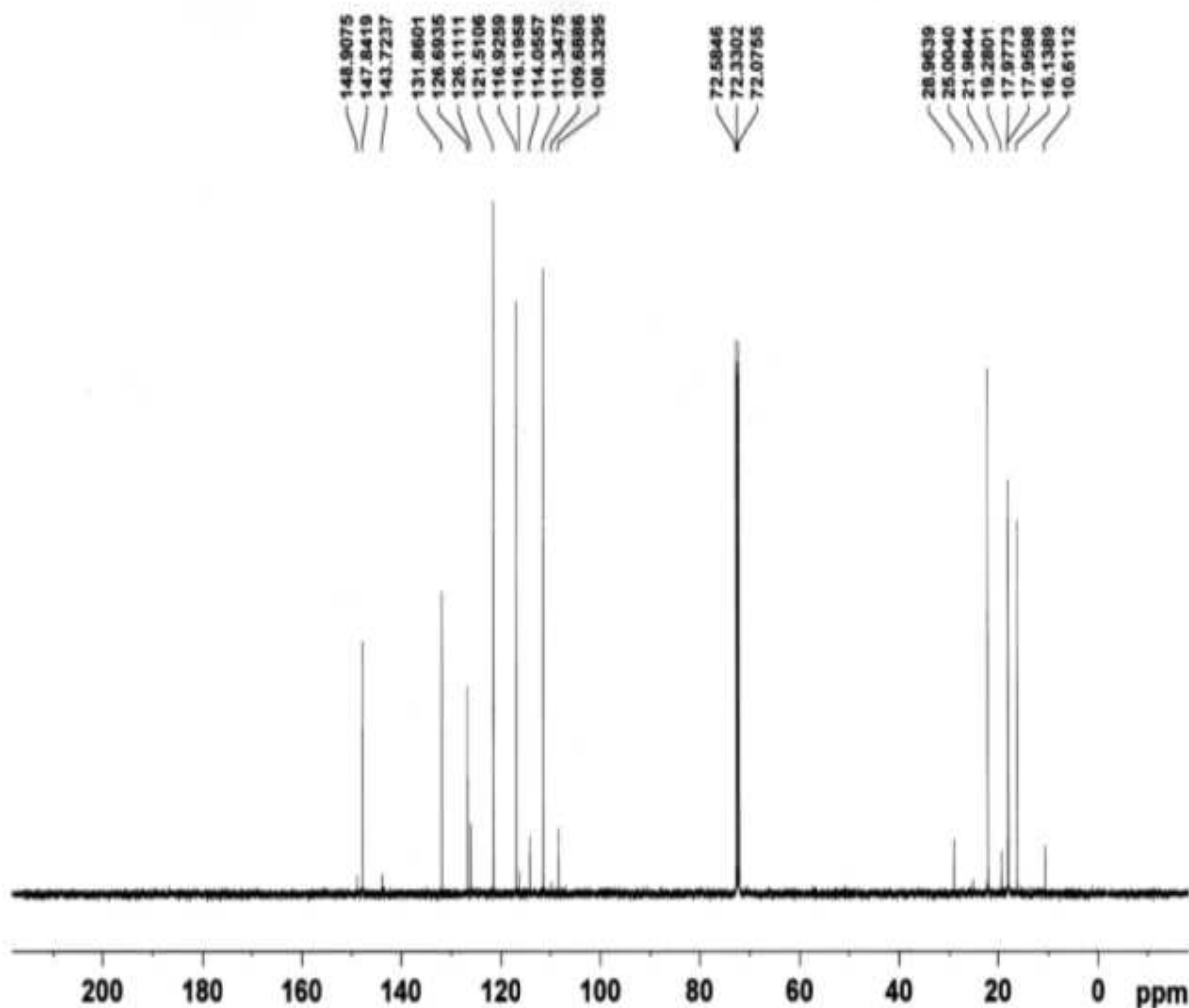


Figure. 40: ^{13}C -NMR (500MHz, CDCl_3) spectrum of 5-isopropyl-3-methylphenol.

^1H - NMR spectrum confirmed the presence of three broad aromatic signals integrating for 1 proton each (Figure. 41). This state confirmed the meta relationship of the protons. Also, the spectrum showed a methyl signal at $\delta=2.30$ (on a benzene ring) and a broad signal for the 2 methyl groups at $\delta=1.30$. The substitution pattern was confirmed by 2D-NMR (COSY, HMQC, and HMBC) (Appendix E). The COSY spectrum showed correlation between the C-H ($\delta=3.4$) and the CH_3 signal at $\delta=1.3$ (Figure. 42). Also, the location of the aromatic methyl and the quaternary carbon at $\delta=126.7$ was confirmed by the correlation with C-H at $\delta=3.40$. The correlation between CH_3 at $\delta=2.30$ and the carbons at $\delta=131.9$, 121.5 and 109.7 confirmed the location of these three carbons. The DEPT 135 experiment showed 3 methine signals in the aromatic region in addition to 3 quaternary carbons one of them is oxygenated ($\delta=147.8$) which indicated the presence of phenolic group (Figure. 43).

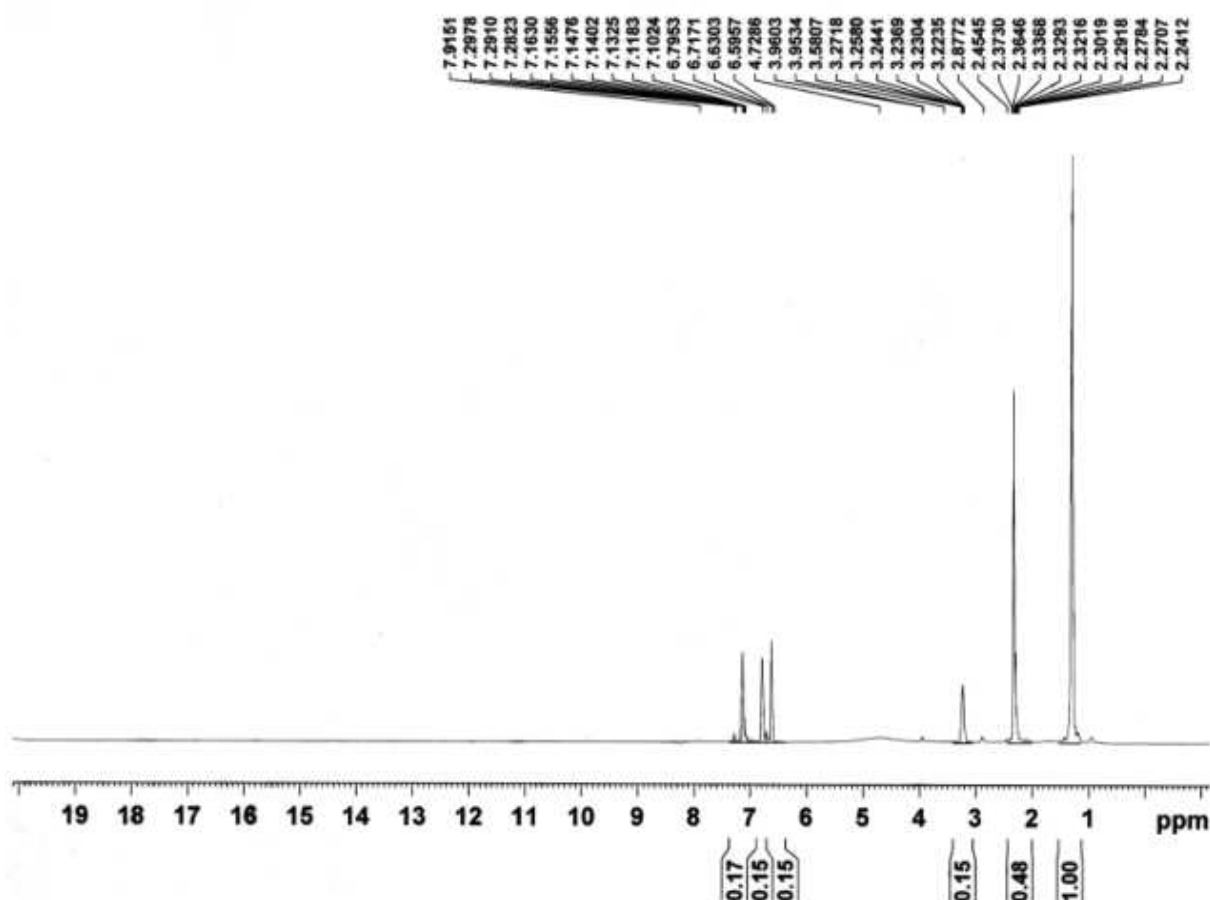


Figure. 41: ^1H -NMR (500MHz, CDCl_3) spectrum of 5-isopropyl-3-methylphenol.

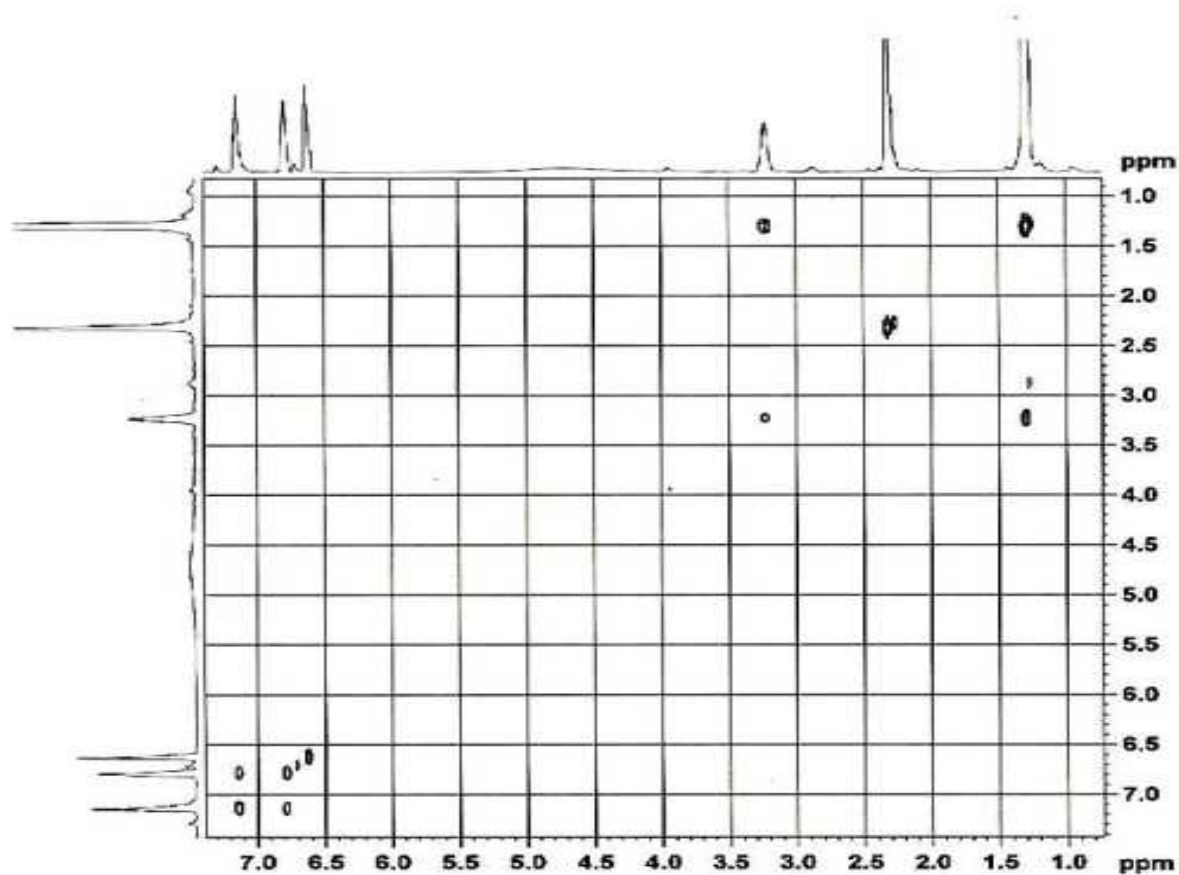


Figure. 42: COSY (500MHz, CDCl_3) spectrum of 5-isopropyl-3-methylphenol.

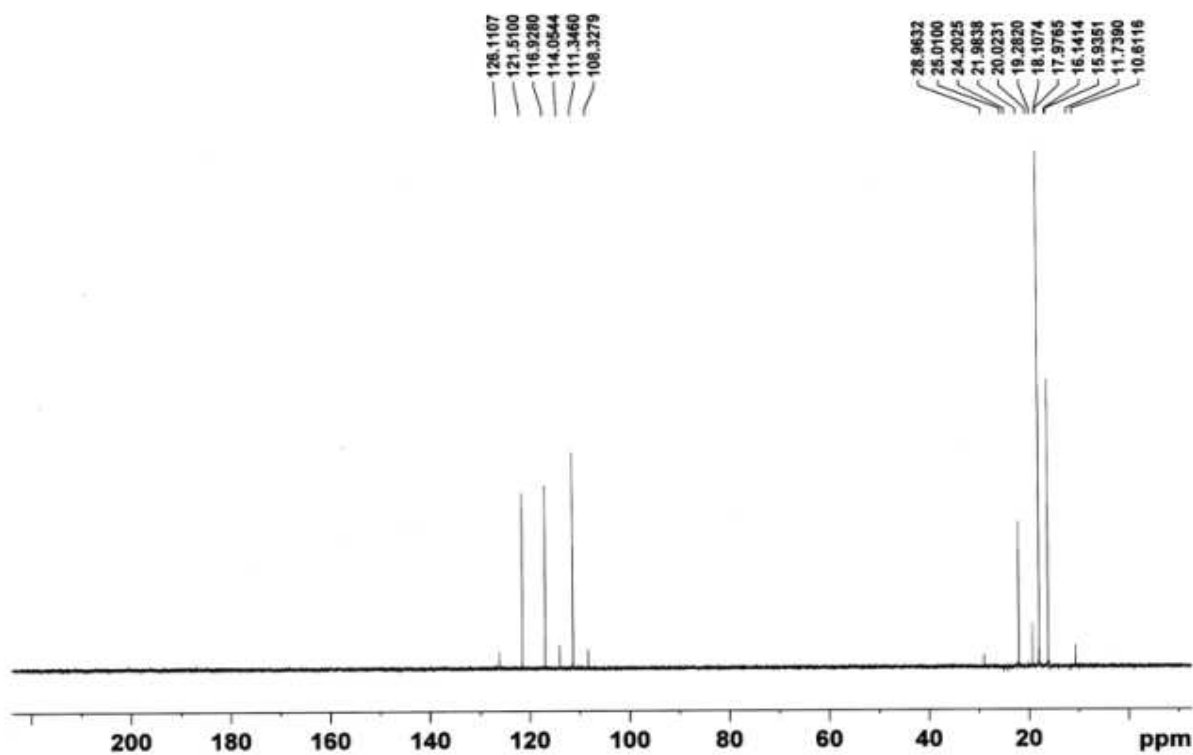


Figure. 43: DEPT 135 (500MHz, CDCl_3) spectrum of 5-isopropyl-3-methylphenol.

3. Antioxidant activity of extracts: *in vitro* assays

The diversity of nature and the complexity of phytochemical compounds of plant extracts impose the development of many methods to evaluate the antioxidant activity and to estimate the effectiveness of these substances. The majority of these methods are based on the colouring or the discolouration of a reagent in the reactional medium. They can be classified into two groups: those assays used in food and biological system to evaluate lipid peroxidation while measuring the degree of oxidation inhibition and those assays used to measure free radical scavenging ability. Some can be based on metal reducing power (ferric reducing antioxidant power, FRAP), peroxy radical scavenging (oxygen radical absorbance capacity, ORAC; total radical trapping antioxidant parameter, TRAP), hydroxyl radical scavenging, organic radical scavenging like ABTS and DPPH (trolox equivalent antioxidant capacity, TEAC), quantification of products formed during the lipid peroxidation (thiobarbituric acid reactive substance, TBARS; LDL oxidation) (Benhammou *et al.*, 2009).

3.1. DPPH radical scavenging activity of extracts

The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples (Ebrahimzadeh *et al.*, 2009). DPPH is a stable nitrogen-centered free radical whose color changes from violet to yellow upon reduction by either the process of hydrogen or electron donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers (Brand-Williams *et al.*, 1995; Ebrahimzadeh *et al.*, 2010; Moyo *et al.*, 2012).

The results show that all fractions from the three studied plants can reduce the signal intensity of DPPH and their radical-scavenging activities increased with increasing concentration (Figure. 44).

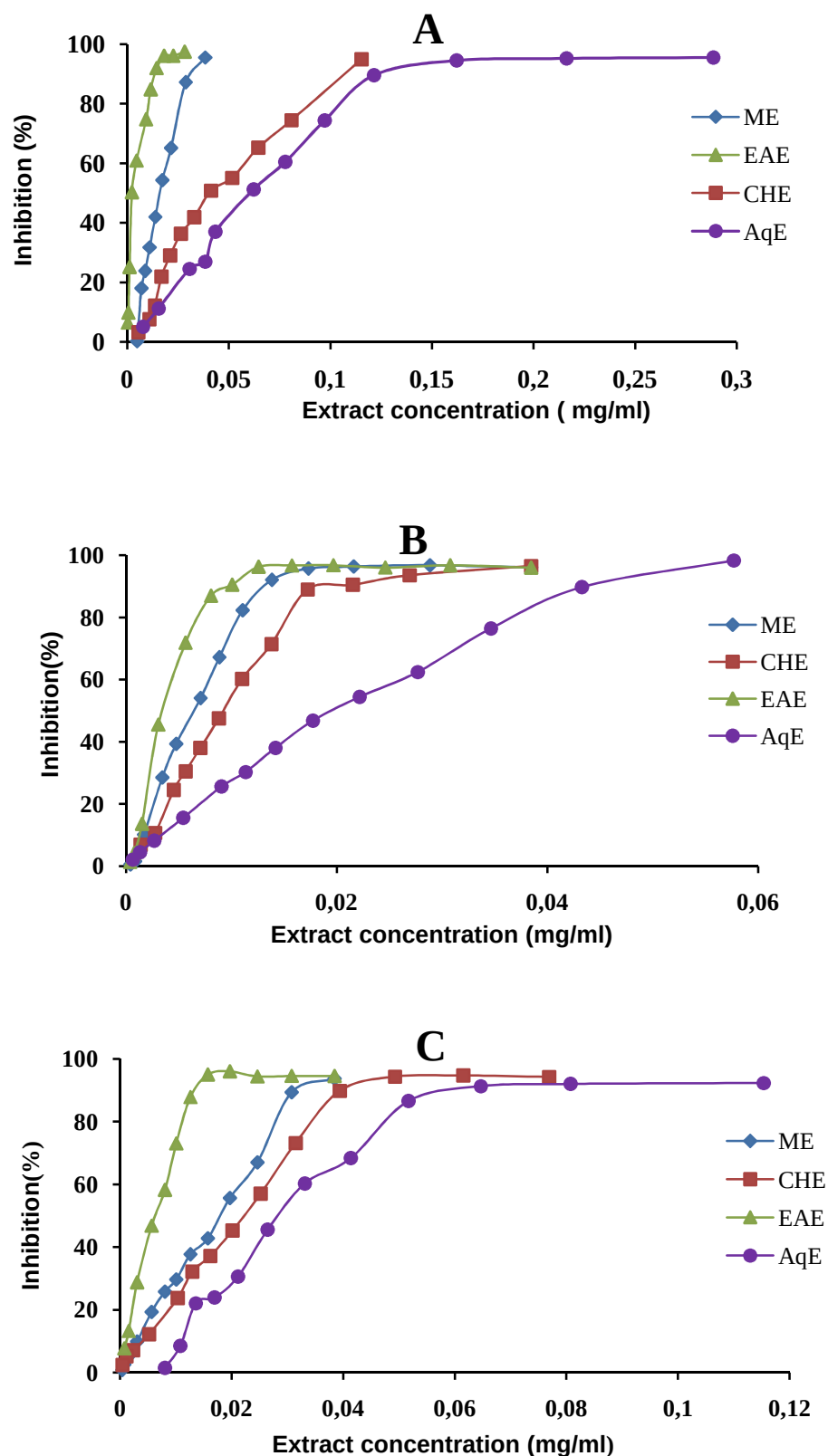


Figure. 44: DPPH free radical scavenging activity of different plant extracts. (A): *C.azarolus*; (B); *M.communis* ; (C): *O.glandulosum*. ME: methanolic extract, CHE: chloroform extract, EAE: ethyl acetate extract, AqE: aqueous extract.

EAE, ME and CHE fractions from *C. azarolus* were significantly more active than our positive control, BHT ($IC_{50} = 0.031 \pm 0.00005 \text{ mg/ml}$) ($p < 0.001$) and the IC_{50} of *C. azarolus* fractions were in this order: EAE ($0.006 \pm 0.0001 \text{ mg/ml}$) < ME ($0.016 \pm 0.0004 \text{ mg/ml}$) < CHE ($0.029 \pm 0.0019 \text{ mg/ml}$) < AqE ($0.066 \pm 0.0004 \text{ mg/ml}$) (Figure. 45). In previous studies, it was found that ethyl acetate extract prepared from the floral buds and open flowers of *C. azarolus* (Bahri-Sahloul *et al.*, 2009) and fruit extracts of *C. monogyna* prepared with ethanol and water (Bernatoniene *et al.*, 2008) were effective in scavenging DPPH and ABTS radicals.

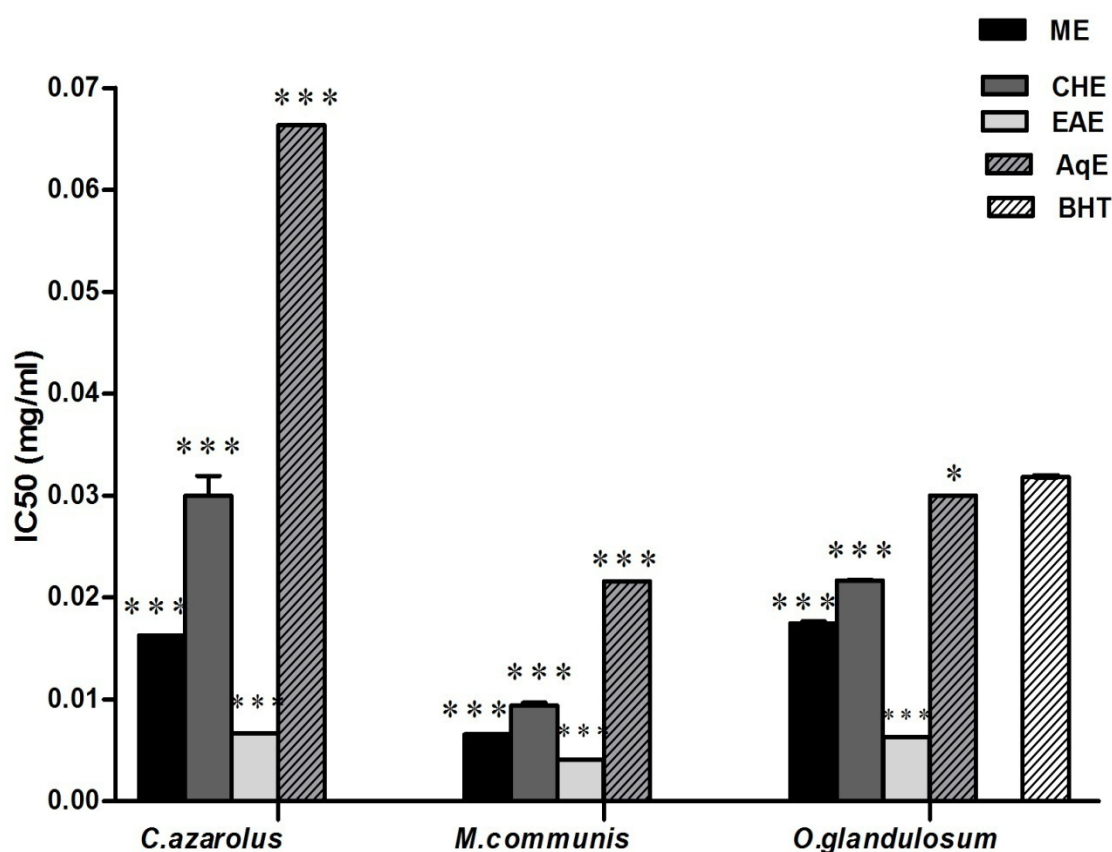


Figure. 45: A comparison between different plant extracts in DPPH free radical scavenging activity. ME: methanolic extract, CHE: chloroform extract, EAE: ethyl acetate extract, AqE: aqueous extract. Data were presented as IC_{50} means $\pm$ SD ($n = 3$). (***) $p < 0.001$; * $p < 0.05$) compared to BHT as standard.

Also, it was found that the four myrtle fractions scavenge significantly DPPH radical ($p < 0.001$) with IC_{50} in increasing order of EAE (0.004 ± 0.0003 mg/ml), ME (0.006 ± 0.00005 mg/ml), CHE (0.009 ± 0.0002 mg/ml) and AqE (0.021 ± 0.001 mg/ml), respectively (Figure. 45). These values from myrtle DPPH scavenging activity are in agreement with those obtained by Hayder *et al.* (2004), who studied the effect of extraction solvent on antiradical activity of myrtle leaf extracts from Tunisia.

In the case of *O. glandulosum*, the EAE, ME and CHE showed a stronger ability to neutralize DPPH radicals than reference standard ; BHT ($p < 0.001$) with followed IC_{50} values of 0.006 ± 0.00001 mg/ml, 0.017 ± 0.0001 mg/ml, 0.021 ± 0.00007 mg/ml, respectively. However, AqE showed the lowest scavenging activity with value of IC_{50} equal to 0.029 ± 0.0019 mg/ml (Figure. 45).

Oukil *et al.* (2011) found that IC_{50} to scavenge DPPH free radical for methanolic extract of the same *Origanum* collected from Bejaia (Algeria) was equal to 6730 μ g/ml which is four hundred fold lower than our finding. The content of polyphenols in their study was very low (55.15 mg GAE / g of extract). This partly explains the low scavenging effect of *O.glandulodum* extract in their study.

According to recent reports, there is a highly positive relationship between polyphenols, flavonoids, and antioxidant activities in many plant species (Kumaran and Karunakaran, 2007; Ozsoy *et al.*, 2008; Kim *et al.*, 2012).

Polyphenols and flavonoids are able to scavenge free radicals directly by hydrogen atom donation. Radicals are made inactive according to the following equation, where $R\bullet$ is a free radical and $Fl-O\bullet$ is a flavonoid phenoxyl radical (Figure. 46).

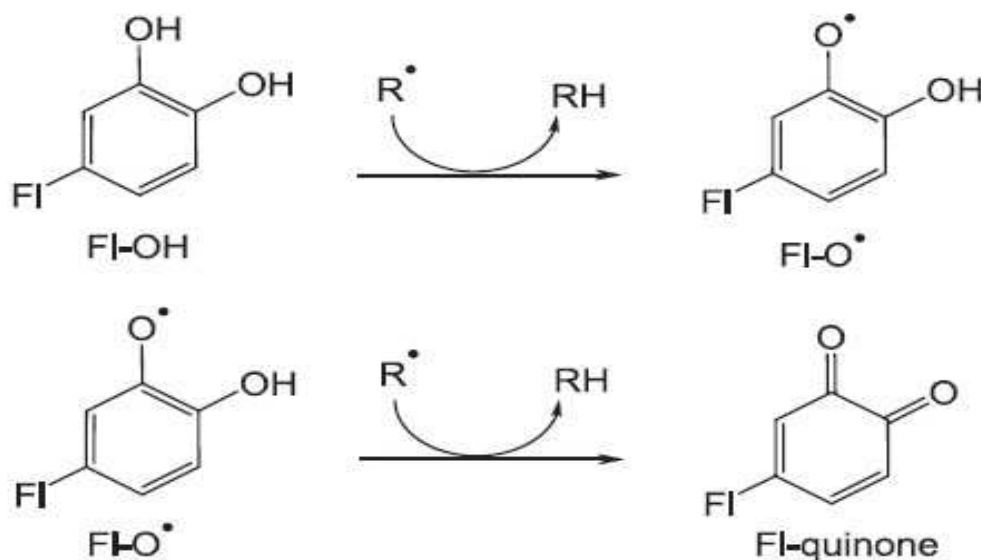


Figure. 46: Scavenging of reactive oxygen species (R•) by flavonoid. The free radical FI-O• may react with a second radical, acquiring a stable quinone structure (Procházková *et al.*, 2011).

Based on the obtained results, it is highly possible that the polyphenols and flavonoids contents may contribute to the observed antioxidant activity, as indicated by the good significant correlation coefficients (*C. azarolus*: $R^2 = 0.80$, *M. communis*: $R^2 = 0.55$, *O. glandulosum*: $R^2 = 0.50$).

Furthermore, it is obvious that, although the content of polyphenol of *O. glandulosum* in CHE was significantly higher than in the ME, their DPPH activity were close. This confirmed once again that the antioxidant capacity depends not only on the quantity, but also on the type of flavonoids present in the extracts.

The *in vitro* flavonoid antioxidant activity depends on the arrangement of functional groups on its core structure. Both the configuration and total number of hydroxyl groups substantially influence the mechanism of the antioxidant activity (Tuberoso *et al.*, 2010; Fraga *et al.*, 2010). The B ring hydroxyl configuration is the most significant determinant of ROS scavenging, whereas substitution of the rings A and C has little impact on superoxide anion

radical scavenging rate constants. Also, the *in vitro* antioxidant activity could be increased by polymerization of flavonoid monomers, e.g. proanthocyanidins (also known as condensed tannins), the polymers of catechins, are excellent *in vitro* antioxidants due to the high number of hydroxyl groups in their molecules (Procházková *et al.*, 2011).

It is known in the literature that the glycosylation of flavonoids also reduces their *in vitro* antioxidant activity when compared to the corresponding aglycons (Benhammou *et al.*, 2009; Tuberoso *et al.*, 2010). Comparison of TEAC values of quercetin (4.42 mM) and rutin (2.02 mM), quercetin-3-O-rutinoside, shows that glycosylation of the 3-OH group has strongly suppressive effect on the antioxidant activity. Similar results were observed also for other pairs of flavonoid aglycon and glycoside (e.g. hesperetin–hesperidin, luteolin–luteolin 4'-glucoside; luteolin–luteolin 7-glucoside; baicalein–baicalin; and quercetin–quercitrin) (Procházková *et al.*, 2011).

Consequently, the main structural features of flavonoids required for efficient radical scavenging could be summarized as follows (Figure.47); a) an ortho-dihydroxy structure (catechol) in the B ring, for electron delocalization; b) 2,3-double bond in conjugation with a 4-oxo function in the C ring provides electron delocalization from the B ring; c) hydroxyl groups at positions 3 and 5 provide hydrogen bonding to the oxo group (Benhammou *et al.*, 2009; Fraga *et al.*, 2010; Xiao *et al.*, 2014; Singh *et al.*, 2014).

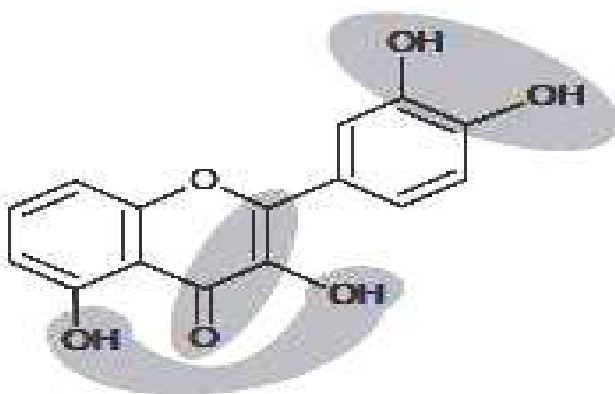


Figure. 47: Structural features defining antioxidant actions of flavonoids. Structural requirements associated with antioxidant possibilities of flavonols as chain breaking antioxidant (Fraga *et al.*, 2010).

3.2. ABTS radical scavenging activity of extracts

ABTS assay is a decolorizing assay, which involves the direct generation of ABTS radical into monocation, which has a long wavelength absorption spectrum without involvement of any intermediary radical. The scavenging activity against cationic ABTS radical indicates the ability of fractions to act as electron donors or hydrogen donors in free radical reactions (Prior *et al.*, 2005, Liu *et al.*, 2009; Tanzadehpanah *et al.*, 2012). To assay the ABTS radical scavenging of *C.azarolus*, *M. communis* and *O. glandulosum* fractions, cationic ABTS radical decolorization was carried out.

Similar to DPPH assay, the results shown in Figure. 48 revealed that all plant fractions are effective ABTS radical scavengers in a concentration dependent manner. EAE from the three plants exhibited a strongest antioxidant activity which is comparable to trolox as standard (no significant difference, $p > 0.05$). The other fractions also showed a good ABTS radical-scavenging activities.

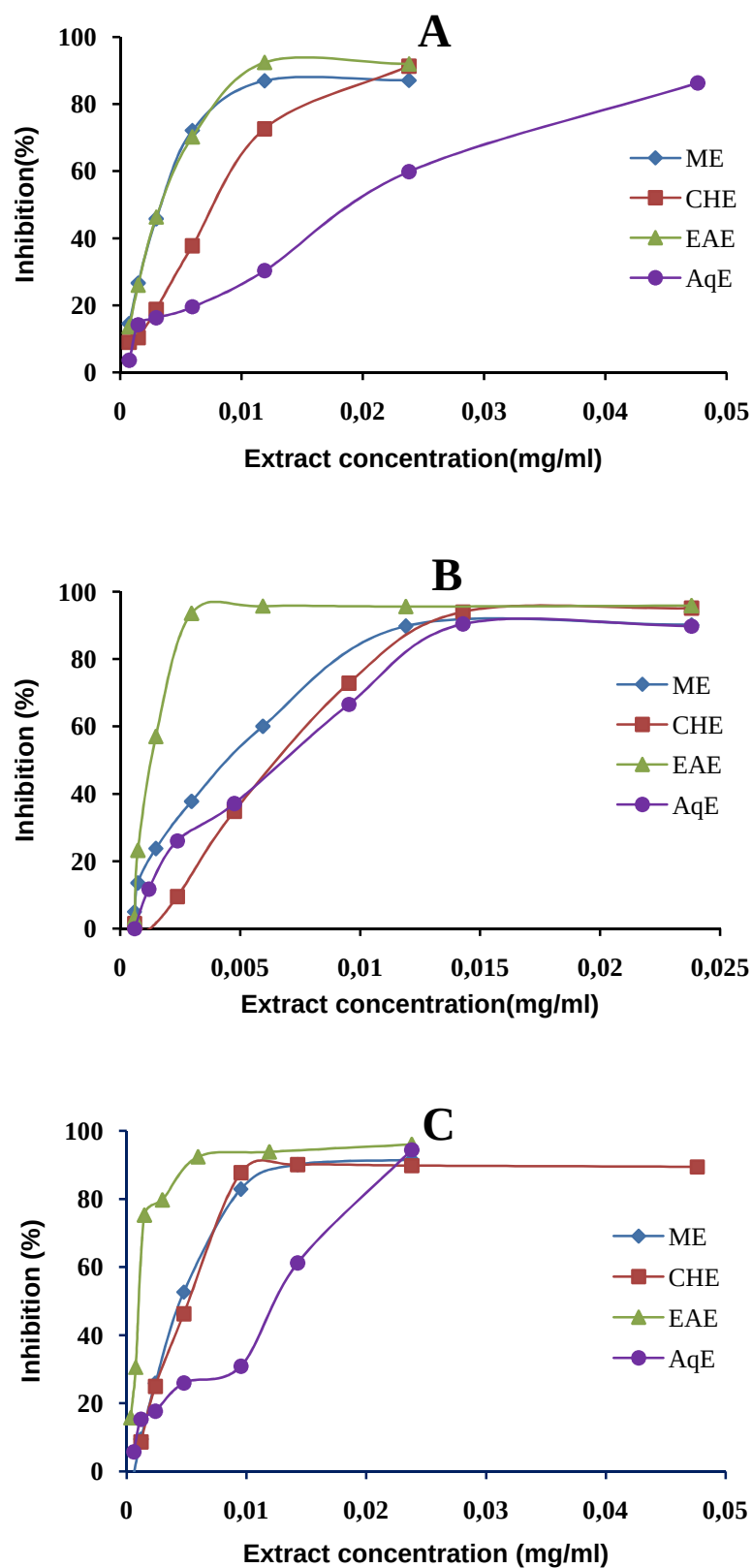


Figure. 48: ABTS radical scavenging activity of different plant extracts. (A): *C. azarolus*; (B): *M. communis*; (C): *O. glandulosum*. ME: methanolic extract, CHE: chloroform extract, EAE: ethyl acetate extract, AqE: aqueous extract.

Concerning *C. azarolus*, it was found that no significant difference ($p > 0.05$) in antioxidant activities between EAE (0.0035 ± 0.0007 mg/ml), ME (0.0037 ± 0.0001 mg/ml) and trolox (0.0025 ± 0.00005 mg/ml). While, the CHE (0.008 ± 0.00005 mg/ml) and AqE (0.024 ± 0.0008 mg/ml) showed a significant difference ($p < 0.001$) when compared to trolox as positive control (Figure.49).

On the other hand, all myrtle fractions effectively are able to donate electrons to ABTS cations with IC_{50} values ranging from 0.001 to 0.008 mg/ml (Figure.49). Such values are significantly lower ($p < 0.05$) than our standard trolox. The EAE and ME had strong activity to quench ABTS radicals with IC_{50} values of 0.0015 ± 0.00009 mg/ml and 0.0047 ± 0.001 mg/ml, respectively.

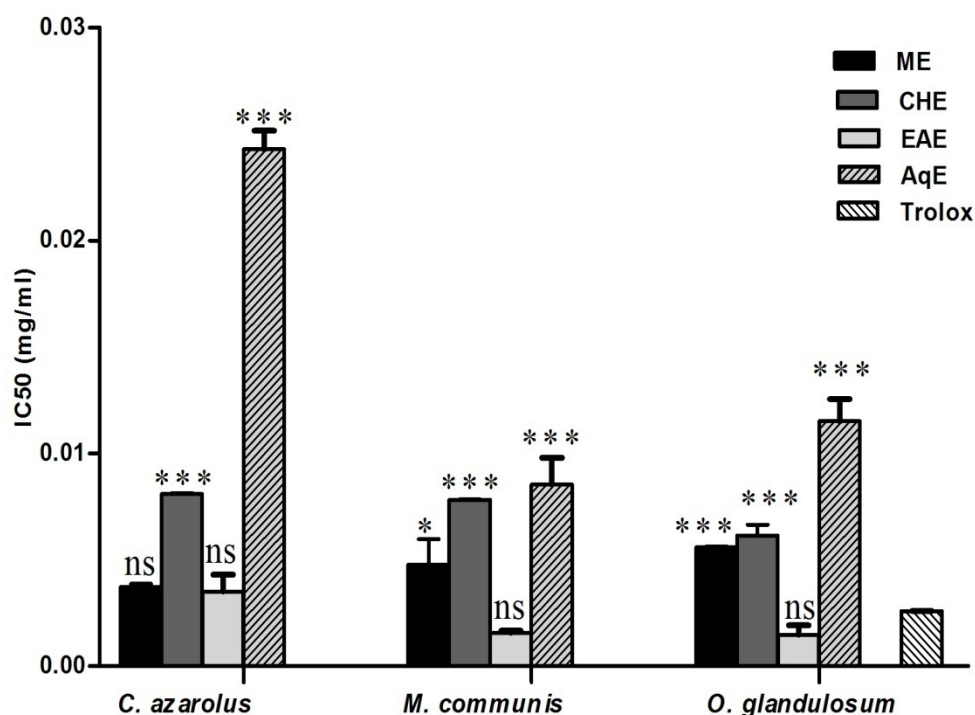


Figure. 49: A comparison between different plant extract in ABTS free radical scavenging activity. ME: methanolic extract, CHE: chloroform extract, EAE: ethyl acetate extract, AqE: aqueous extract. Data were presented as IC_{50} means $\pm$ SD ($n = 3$). (ns: no significant difference; * $p < 0.05$; *** $p < 0.001$) compared to Trolox as standard.

Evaluation of *O. glandulosum* fractions indicated that only EAE showed stronger ABTS scavenging activity as the standard, trolox with IC_{50} of 0.001 ± 0.001 mg/ml (no significant difference, $p > 0.05$), but neither of other extracts showed better antioxidant properties than trolox, their IC_{50} were in the following order: 0.011 ± 0.001 mg/ml, 0.0006 ± 0.0005 mg/ml and 0.005 ± 0.00004 mg/ml for AqE, CHE and ME, respectively (Figure.49).

Comparing the ABTS test results and total polyphenols content in the extracts, it could be concluded that there is a correlation between them (*C. azarolus*: $R^2 = 0.60$, *M. communis*: $R^2 = 0.96$, *O. glandulosum*: $R^2 = 0.68$). Thus, the strong scavenging capacity of plant extracts was possibly due to the hydrogen/ electron donating ability of the polyphenolic compounds present in these extracts which made them good antioxidants acting as free radical inhibitors or scavengers.

In agreement with our findings, the close correlation between antioxidant activity and phenolic content of extracts obtained from various natural sources has been previously demonstrated (Liu *et al.*, 2009; Ben Farhat *et al.*, 2013). Therefore, these polyphenols should be considered to contain conjugated ring structures and hydroxyl groups that have the potential to function as antioxidant *in vitro* by scavenging free radicals, lipid peroxy radicals and stabilizing free radicals involved in these oxidative processes (Priyadharshini *et al.*, 2012).

3.3. Hydroxyl radical scavenging activity of extracts

Reactive oxygen species as hydroxyl and superoxide free radicals have been implicated in the etiology of many diseases and conditions, and eventually resulted in the cell injury/death. In the biological systems, cells' injury causes leakage of transition metal ions

viz. Fe^{2+} ion from the cellular protein which further generates hydroxyl radicals by the process 'Fenton chemistry' ($\text{Fe}^{2+} + \text{H}_2\text{O}_2 \longrightarrow \text{Fe}^{3+} + \text{OH}^- + \text{OH}^\bullet$).

Above reaction is a cardinal mechanism for the generation of hydroxyl radical in cells. However, presence of antioxidant prevents the cellular damage either by quenching of $\text{OH}^\bullet$ radicals or by chelating transition metal ions (Chouhan and Singh, 2011).

Phenanthroline- Fe^{2+} is one of oxidation–reduction indicator in common use, where the change of color can be used to indicate the alteration of the state of oxidation–reduction reaction. In this assay, hydroxyl radicals produced in $\text{H}_2\text{O}_2/\text{Fe}^{2+}$ system can oxidise phenanthroline- Fe^{2+} into phenanthroline- Fe^{3+} , and the absorption peak at 532 nm was reduced significantly.

When the drugs were added into the system, the change of absorbance can be alleviated, so the inhibitory effect on hydroxyl radical could be the criterion for estimating the relative strength of anti-oxidation of extract (Zhou and Li, 2009). As shown in Figure.50, all plant fractions possess good *in vitro* scavenging activity and exhibited concentration dependent scavenging activities against hydroxyl radicals generated in a Fenton reaction.

The results indicated that hydroxyl radical scavenging activity of *C. azarolus* extracts were significantly ($p < 0.001$) lower than vitamin C (IC_{50} was 0.084 mg/ml) and the higher activity was found in EAE (Figure.51). The IC_{50} values of EAE, CHE, ME and AqE were 0.283 mg /ml, 0.294 mg/ml, 0.330 mg/ml and 1.869 mg/ml, respectively.

This result was consistent with those of other researchers who found that *C. oxycantha* extract (Swaminathan *et al.*, 2010) and *C. pinnatifida* var. *major* fruit extract (Liu *et al.*, 2010) inhibited hydroxyl radical generation.

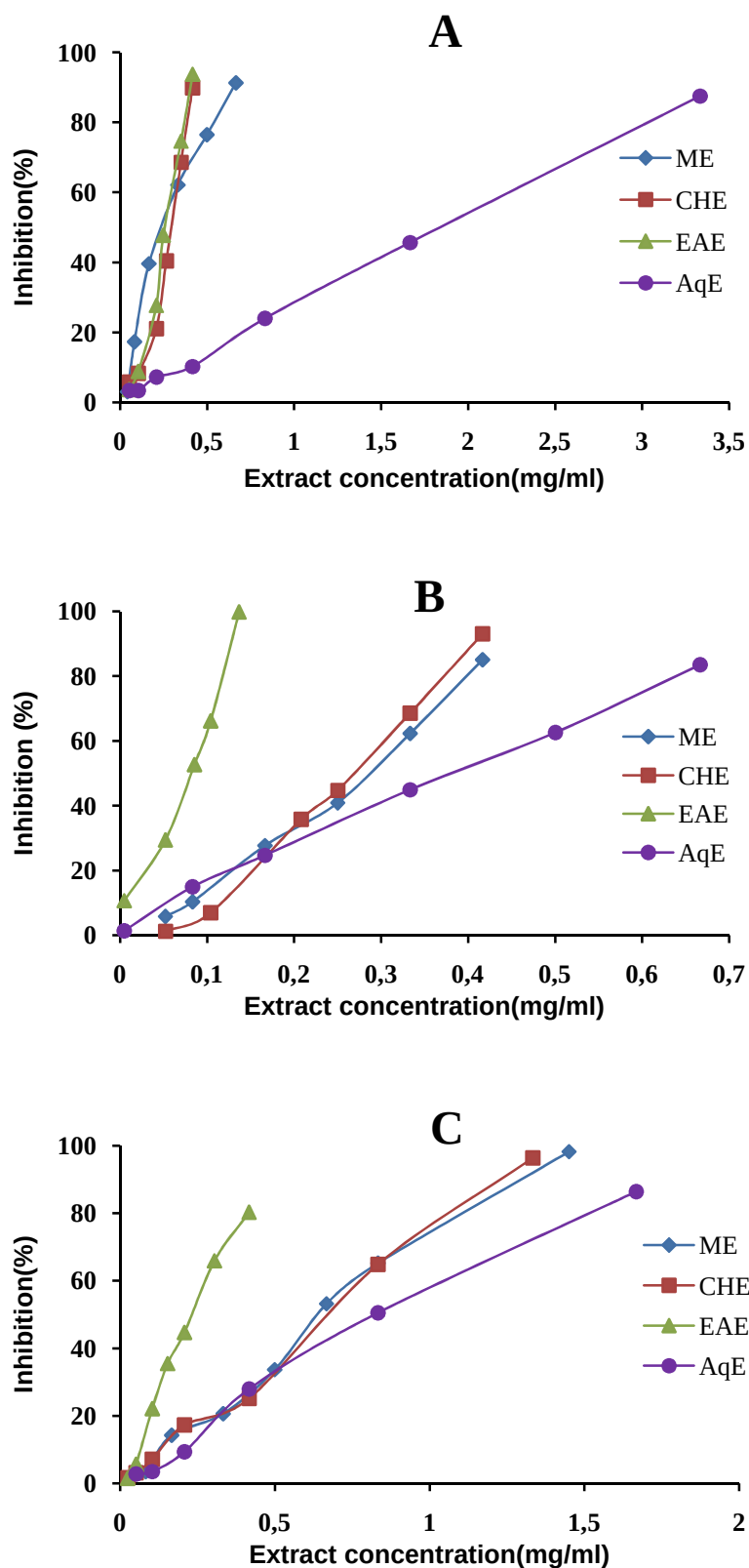


Figure. 50: Hydroxyl radical scavenging activity of different plant extracts. (A): *C. azarolus*; (B): *M. communis*; (C): *O. glandulosum*. ME: methanolic extract, CHE: chloroform extract, EAE: ethyl acetate extract, AqE: aqueous extract.

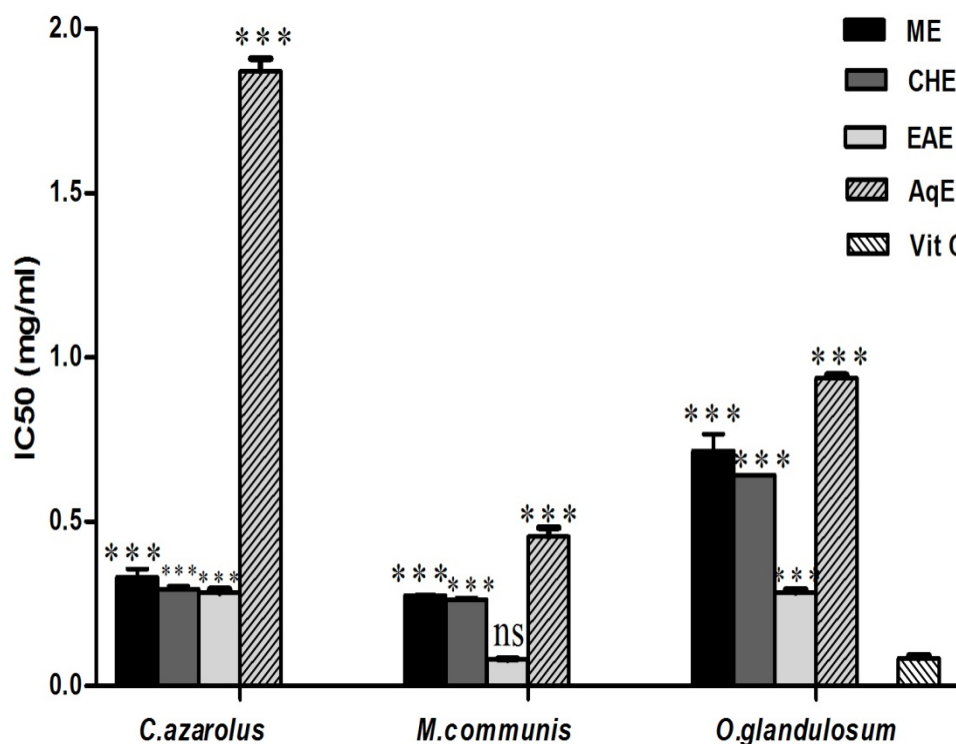


Figure. 51: A comparison between different plant extracts in hydroxyl radical scavenging activity. ME: methanolic extract, CHE: chloroform extract, EAE: ethyl acetate extract, AqE: aqueous extract. Data were presented as IC₅₀ means $\pm$ SD (n = 3). (ns: no significant difference ;*** p<0.001) compared to vitamin C as standard.

On the other hand, the degradation of H_2O_2 by Fe^{+2} 1, 10-phenanthroline/(EDTA) system was significantly decreased by all *M. communis* leaves fractions. The antioxidant activity values of these extracts increased in the order of EAE > CHE > ME > AqE and their IC₅₀ values were found to be 0.08 ± 0.007 mg/ml, 0.26 ± 0.005 mg/ml, 0.27 ± 0.0008 mg/ml and 0.45 ± 0.02 mg/ml, respectively (Figure.51).

Similar to the above results, all of the assessed fractions of *O. glandulosum* were able to reduce the generation of hydroxyl radicals (Figure. 51) with IC₅₀ values as follows: 0.285 ± 0.008 mg/ml, 0.642 ± 0.0003 mg/ml, 0.715 ± 0.05 mg/ml and 0.936 ± 0.01 mg/ml for EAE, CHE, ME, and AqE, respectively .

Hydroxyl radical is most reactive oxygen species, causing lipid peroxidation and enormous biological damage. It is a potent cytotoxic agent, able to attack and damage almost every molecule found in living tissues (Hazra *et al.*, 2008; Sen *et al.*, 2013). Therefore, the scavenging of hydroxyl radical by extracts seems to be directly related to the prevention of propagation of the process of lipid peroxidation and may provide a significant protection to biomolecules against free radicals (Govindan *et al.*, 2013).

In this study, plant extracts displayed different potential in scavenging hydroxyl radical which is positively related to their amount of total phenolic and flavonoids contents (*C. azarolus* : $R^2 = 0.50$, *M. communis*: $R^2= 0.82$, *O. glandulosum*: $R^2=0.90$) and this might be due to their active hydrogen donor ability of hydroxyl substitution (Bose *et al.*, 2011). Phenols 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).

3.4. Metal chelating activity of extracts

The metal chelating capacity is important, since it reduces concentration of the catalyzing transition metal in lipid peroxidation. It has been reported that the chelating agents form bonds with a metal are effective as secondary antioxidants because they reduce the redox potential, thereby stimulating the oxidized form of the metal ion. Among the transition metals, iron is known as the effective factor in lipid oxidation due to its high reactivity. The ferrous iron via the Fenton reaction generates hydroxyl radical. In addition, Fe^{3+} ion produces radicals from peroxides, although the rate is tenfold less than that of Fe^{2+} ion (Gülçin, 2006).

In the presence of chelating agents, the ferrozine-Fe²⁺ complexes are disrupted, resulting in a decrease in the red color of the complex (Rajapakse *et al.*, 2005).

In this study, the fractions from *C. azarolus*, *M. communis* and *O. glandulosum* showed chelating activity as demonstrated by their effectiveness in inhibiting the formation of Fe²⁺-ferrozine complex, and the absorbance of this complex was dose dependently decreased (Figure. 52).

As seen in Figure. 53, *C. azarolus* fractions showed chelating activity in the order of AqE (IC₅₀= 0.35 ± 0.05 mg/ml) > ME (IC₅₀= 0.57 ± 0.04 mg/ml) > EAE (IC₅₀= 2.09 ± 0.15 mg/ml) > CHE (IC₅₀= 3.19 ± 0.11 mg/ml).

By comparing myrtle extracts chelating activity (Figure.53), ME (IC₅₀ of 0.39 ± 0.06 mg/ml) and AqE (IC₅₀ of 0.40 ± 0.05 mg/ml) were found to be strong as positive control, EDTA (IC₅₀ of 0.0186 ± 0.003 mg/ml) (no significant difference, *p* > 0.05) while CHE and EAE presented a very low ability to chelate ferrous irons with IC₅₀ values of 3.05 ± 0.21 mg/ml and 16.05 ± 0.88 mg/ml, respectively comparing to that of EDTA (*p* < 0.001).

Furthermore, CHE from *O. glandulosum* showed the lowest chelating effects on ferrous ions (IC₅₀ of 4.97 ± 0.02 mg/ml). However, the other extracts showed an excellent chelating activity, especially AqE and ME which gave IC₅₀ of 0.330 ± 0.004 mg/ml and 0.38 ± 0.07 mg/ml, respectively, and were too close to each other and statistically similar to EDTA (*p* > 0.05), followed by EAE (IC₅₀ of 2.68 ± 0.371 mg/ml) (Figure.53).

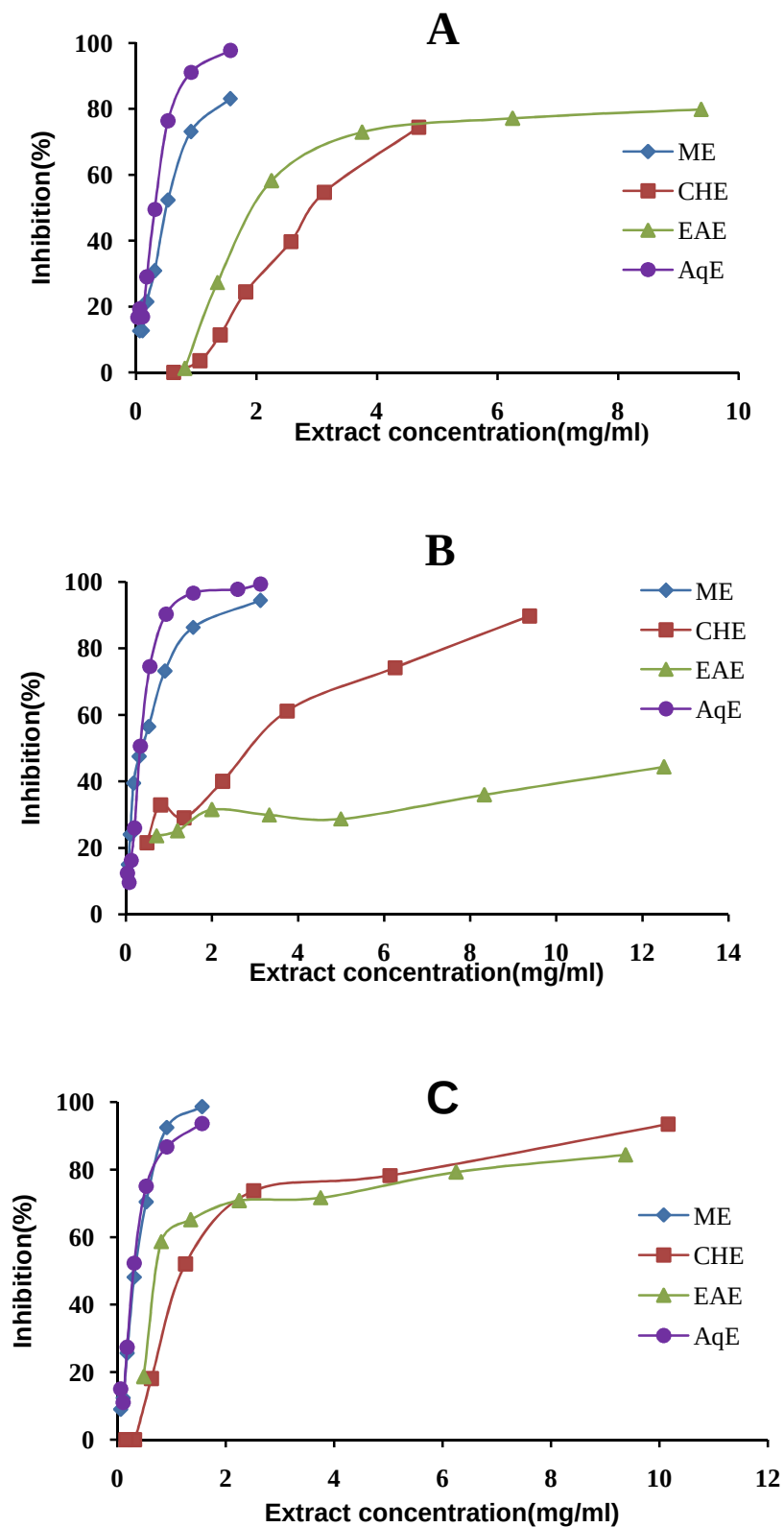


Figure. 52: Chelating activity of different plant extracts. (A): *C. azarolus*; (B): *M. communis*; (C): *O. glandulosum*. ME: methanolic extract, CHE: chloroform extract, EAE: ethyl acetate extract, AqE: aqueous extract.

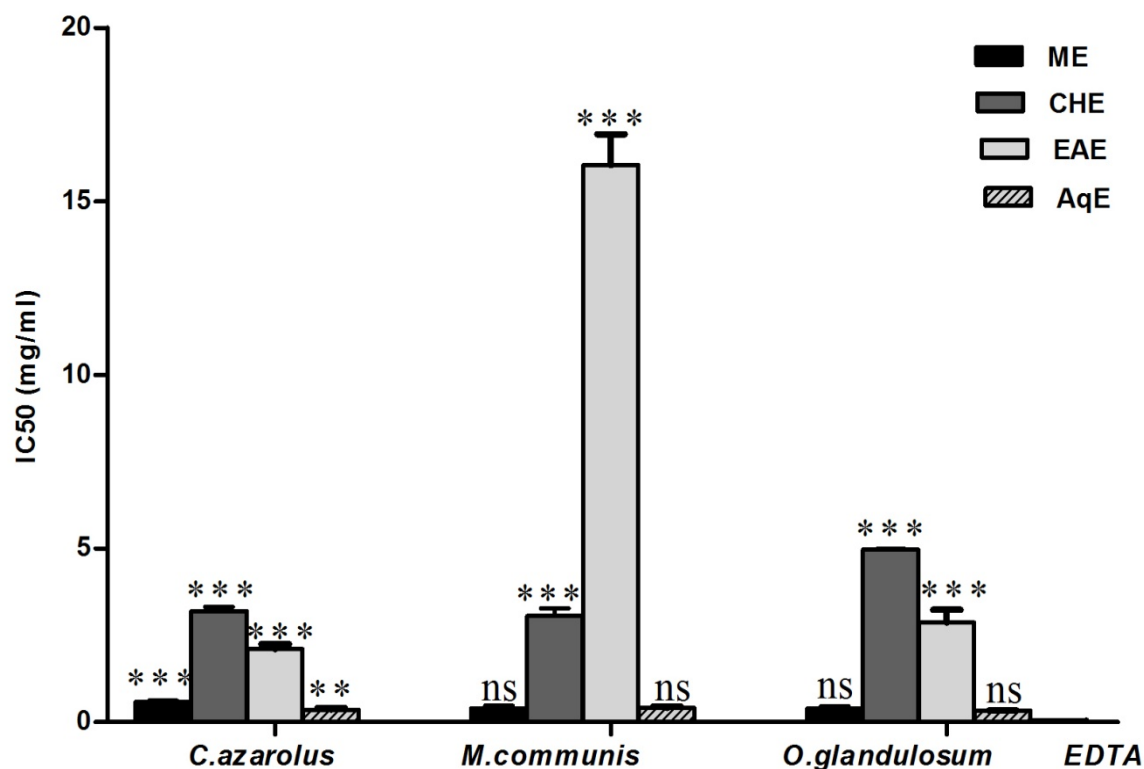


Figure. 53: A comparison between different plant extracts in metal chelating activity. ME: methanolic extract, CHE: chloroform extract, EAE: ethyl acetate extract, AqE: aqueous extract. Data were presented as IC₅₀ means $\pm$ SD (n = 3). (ns: no significant difference; ** p<0.01;*** p<0.001) compared to EDTA as standard.

The chelating capacity is very important because it reduces the concentration of transition metal catalysts of the lipid peroxidation. Indeed, iron can stimulate lipid peroxidation by the Fenton reaction, and accelerates peroxidation by decomposing lipid hydroperoxides into peroxy and alkoxy radicals that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation (Elmastas *et al.*, 2006, Hazra *et al.*, 2008, Sen *et al.*, 2013). The observed results demonstrate that all extracts interfered with the formation of ferrous and ferrozine complex suggesting that they have chelating activity through capturing the ferrous ion before it reacts to form a complex with ferrozine.

Although phenolic contents in ME and AqE are lower than in EAE, they showed the better chelating activity than that of EAE and this finding was supported with the correlation

analysis showing that there is a weak correlation between phenolic content and chelating activity for *C. azarolus* ($R^2 = 0.15$) and *O. glandulosum* ($R^2=0.16$). However, a negative correlation was shown for *M. communis* ($R^2= 0.82$). In fact, although phenolic compounds are the most abundant and potent antioxidants in plants, there is also a pool of non-phenolic compounds that contribute to the overall antioxidant activity of extracts plant (Gonçalves *et al.*, 2013).

Catechol moieties and combinations of hydroxyl and carbonyl groups present in polyphenols are centers of high affinity for metal ions; however, large differences have been observed in the metal-chelating capacity of different polyphenols (Perron and Brumaghim, 2009). Iron and flavonoids are the most studied transition metal and polyphenol family, respectively. From those studies, there is a consensus on the presence of “iron chelation sites” or “iron binding motifs” in an important number of flavonoids (Figure. 54): flavonoid-metal binding occurs preferentially at the 3-hydroxyl-4-carbonyl group, followed by 4-carbonyl-5-hydroxyl group, and the 3'-4' hydroxyl (Fraga *et al.*, 2010; Procházková *et al.*, 2011). Recently, the 6-7 hydroxyl groups have been indicated as the iron chelation site for baicalein (Fraga *et al.*, 2010).

Furthermore, it was reported that the compounds with structures containing two or more of the following functional groups: –OH, –SH, –COOH, –PO₃H₂, –C=O, –NR₂, –S– and –O– in a favourable structure–function configuration can show metal chelating activity (Gülçin *et al.*, 2010). Kazazica *et al.* (2006) demonstrated that flavonoids like kaempferol chelated Cu²⁺ and Fe²⁺ on the functional carbonyl groups.

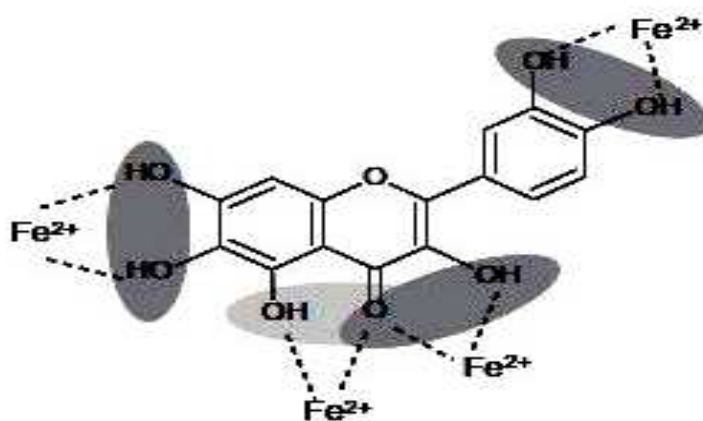


Figure. 54: Structural features defining antioxidant actions of flavonoids acting as metal chelators (Fraga *et al.*, 2010).

3.5. Reducing power capacity of extracts

In this assay, the yellow color of the test solution changes to various shades of green and blue, depending on the reducing power of each compound. The presence of reducers (i.e. antioxidants) causes the reduction of the Fe^{3+} /ferricyanide complex to the ferrous form. Therefore, measuring the formation of Perl's Prussian blue at 700 nm can monitor the Fe^{2+} concentration (Ferreira *et al.*, 2007; Sen *et al.*, 2013). Figure. 55 shows the dose-response curves for the reducing powers of various extracts from *C. azarolus*, *M. communis* and *O. glandulosum*. It was found that all these extracts exhibited ferric reducing power greatly in concentration dependent manner. The reducing power capacity of the extracts may provide a significant indication about the potential antioxidant capacity of the plant.

As shown in Figure. 56, there was significant difference ($p < 0.001$) among the different extracts of *C. azarolus* in reducing power compared to BHT as positive control ($\text{IC}_{50} = 0.05 \pm 0.006$ mg/ml). EAE (0.07 ± 0.0006 mg/ml) showed the better reducing power than other extracts followed by ME (0.13 ± 0.007 mg/ml), CHE (0.24 ± 0.01 mg/ml) and AqE (0.44 ± 0.01 mg/ml).

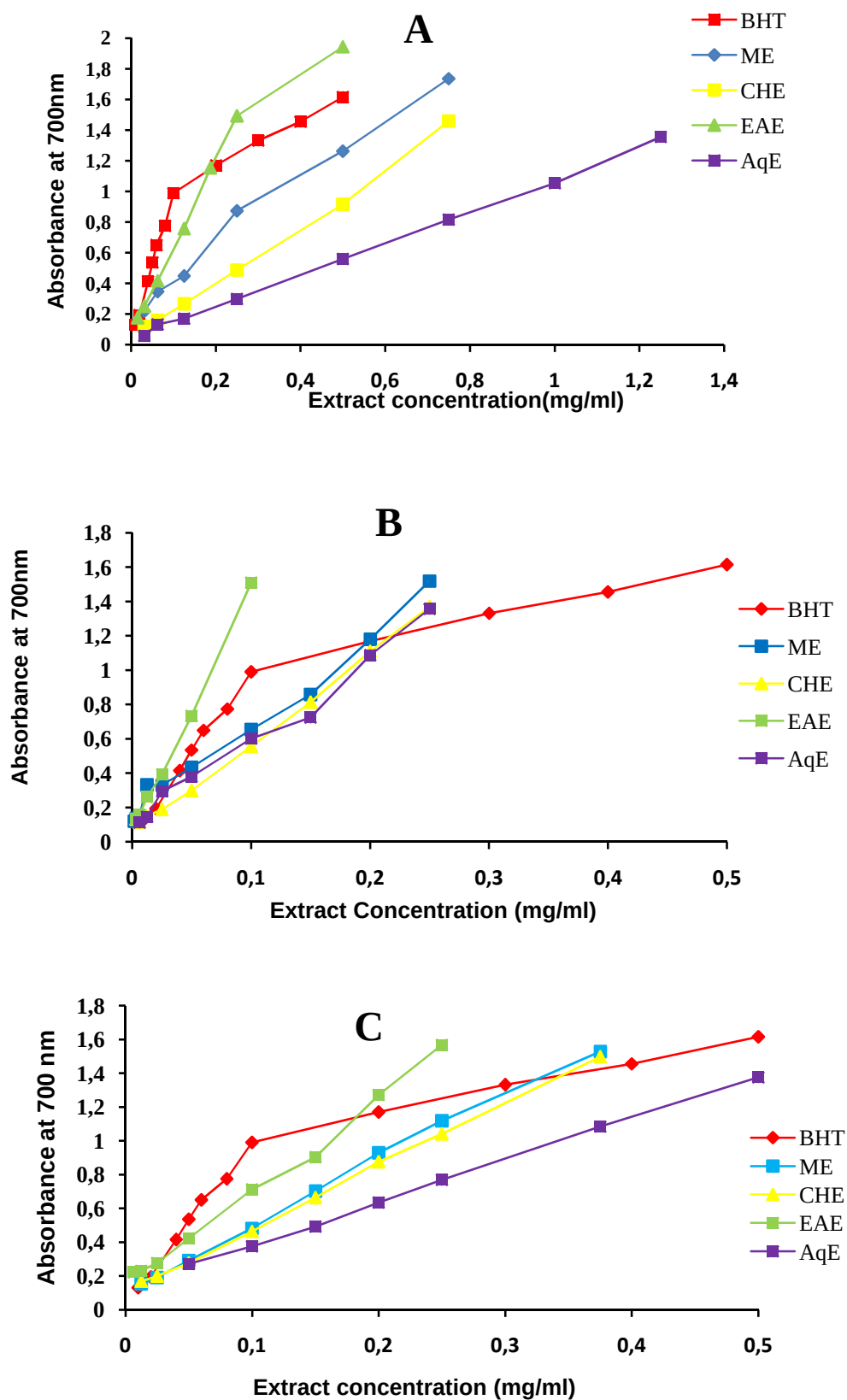


Figure. 55: Reducing power of different plant extracts. (A): *C. azarolus*; (B): *M. communis*; (C): *O. glandulosum*. ME: methanolic extract, CHE: chloroform extract, EAE: ethyl acetate extract, AqE: aqueous extract.

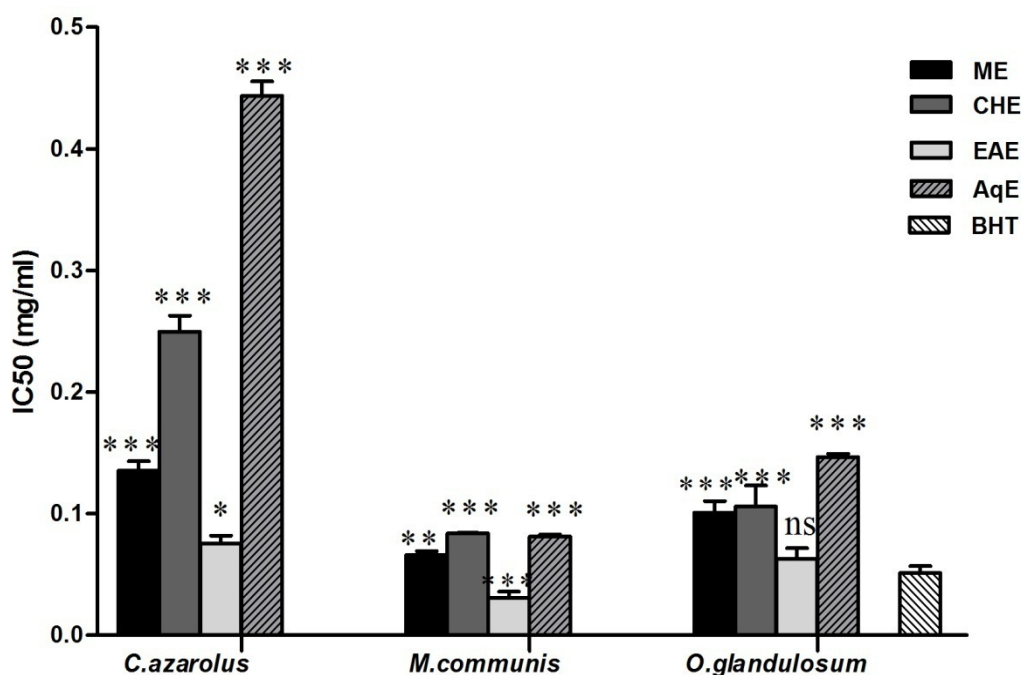


Figure. 56: A comparison between different plant extracts in reducing power assay. ME: methanolic extract, CHE: chloroform extract, EAE: ethyl acetate extract, AqE: aqueous extract. Data were presented as IC_{50} means $\pm$ SD ($n = 3$). (ns: no significant difference ; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) compared to vitamin C as standard.

Moreover, the extracts of the leaves of *M. communis* showed a promising result in this assay (Figure.56), and IC_{50} of extracts exhibited the following order: EAE (0.03 ± 0.005 mg/ml) < ME(0.06 ± 0.003 mg/ml) < AqE(0.081 ± 0.001 mg/ml) < CHE(0.083 ± 0.0005 mg/ml). The EAE exhibited significantly potent reducing power when compared to BHT ($p < 0.001$).

For *O. glandulosum*, we can notice that the best reducing power was signaled for EAE with IC_{50} value of 0.06 ± 0.008 mg/ml, which was statistically similar ($p > 0.05$) to that of the standard, BHT. The reducing power of other extracts was generally lower than that of BHT ($p < 0.05$) and their IC_{50} values were in the following order: 0.10 ± 0.009 mg/ml, 0.11 ± 0.01 mg/ml and 0.14 ± 0.002 mg/ml for ME, CHE and AqE, respectively (Figure.56).

The statistical analysis indicated that there was a significant correlation between the ferric reducing antioxidant power and phenolic contents of the extracts (*C. azarolus*: $R^2 = 0.80$, *M. communis*: $R^2=0.98$, *O. glandulosum*: $R^2=0.78$). This emphasizes the importance of phenolic contents in reducing power observed in this study, and this may be due to their potent electron donating abilities (Bilto *et al.*, 2012). The reducing properties are generally associated with the presence of reductones (Mathew and Abraham , 2006) , and therefore should be able to terminate radical chain reaction by converting free radicals and reactive oxygen species to more stable products (Vinson *et al.*, 1998). Nevertheless, as far as we know, this is the first report of reducing power of *C. azarolus*, *M. communis* and *O. glandulosum* leaves extracts.

3.6. Antioxidant activity of extracts using β -Carotene bleaching assay

The basis of β -carotene-linoleate assay is discolouration of β -carotene in reaction with linoleic acid free radical formed upon removal of hydrogen atom located between two double bonds of linoleic acid. The consequence is the loss of conjugation and accordingly, a decrease in absorbance at 490 nm. Antioxidants can reduce the extent of β -carotene destruction by reacting with the linoleate free radical or any other free radical formed within the system. Thus, by simulation of the oxidation of the membrane lipid components in the presence of antioxidants, this test gives an insight of the inhibitory effect of extracts on the lipid peroxidation (Sarikurku *et al.*, 2009; Petlevski *et al.*, 2013).

Figure. 57 shows the changes in the percentage of the inhibition ratios of linoleic acid oxidation under the influence of *C.azarolus*, *M.communis* and *O. glandulosum* extracts (2 mg/ml) compared to that of synthetic antioxidant BHT as a positive control during 24 h under the same condition. In this assay, most of the extracts and BHT were able to prevent degradation of β -carotene during the assay (24 hours). This effect is due to either; the

inhibition of linoleic acid peroxidation or the radical scavenging hydroperoxides formed during the peroxidation of linoleic acid (scavenger effect) (Aslan *et al.*, 2006).

As shown in Figure.58, all *C. azarolus* extracts inhibit the oxidation of β -carotene in a very important level compared to BHT (95.4% inhibition). EAE is the best inhibitor of the oxidation of β -carotene in 24 h (89.21 ± 2.08 %), which was as strong as the positive reference, BHT (no significant difference, $p > 0.05$), followed by CHE (88.52 ± 2.48 %), AqE (87.96 ± 1.76 %) and ME (73.51 ± 4.72 %), respectively. The same results were reported by Ljubuncic *et al.* (2005) who demonstrated that aqueous extract from a decoction of leaves and unripe fruits of *C. aronia*, a hawthorn indigenous to Jordan and Palestine, inhibited the oxidation of β -carotene in the coupled oxidation of β -carotene and linoleic acid.

Similarly, there was a significant difference ($p < 0.001$) between the means of total antioxidant activity among the myrtle extracts and BHT as standard (Figure.58). The order of decreasing of relative antioxidant activity was CHE (91.19 ± 0.51 %) followed by EAE (90.73 ± 1.9 %), AqE (89.54 ± 1.2 %) and ME (79.13 ± 1.2 %), respectively.

Among oregano fractions, EAE showed the highest antioxidant activity as BHT, while other extracts showed significantly low antioxidant activity compared to BHT ($p < 0.01$), and the calculated % inhibition capacity of oregano extracts; CHE, EAE, AqE and ME were found to be 89.81 ± 2.8 %, 89.14 ± 1.7 %, 86.75 ± 5.5 % and 85.2 ± 0.92 %, respectively (Figure.58).

Among oregano fractions, EAE showed the highest antioxidant activity as BHT, while other extracts showed significantly low antioxidant activity compared to BHT ($p < 0.01$),

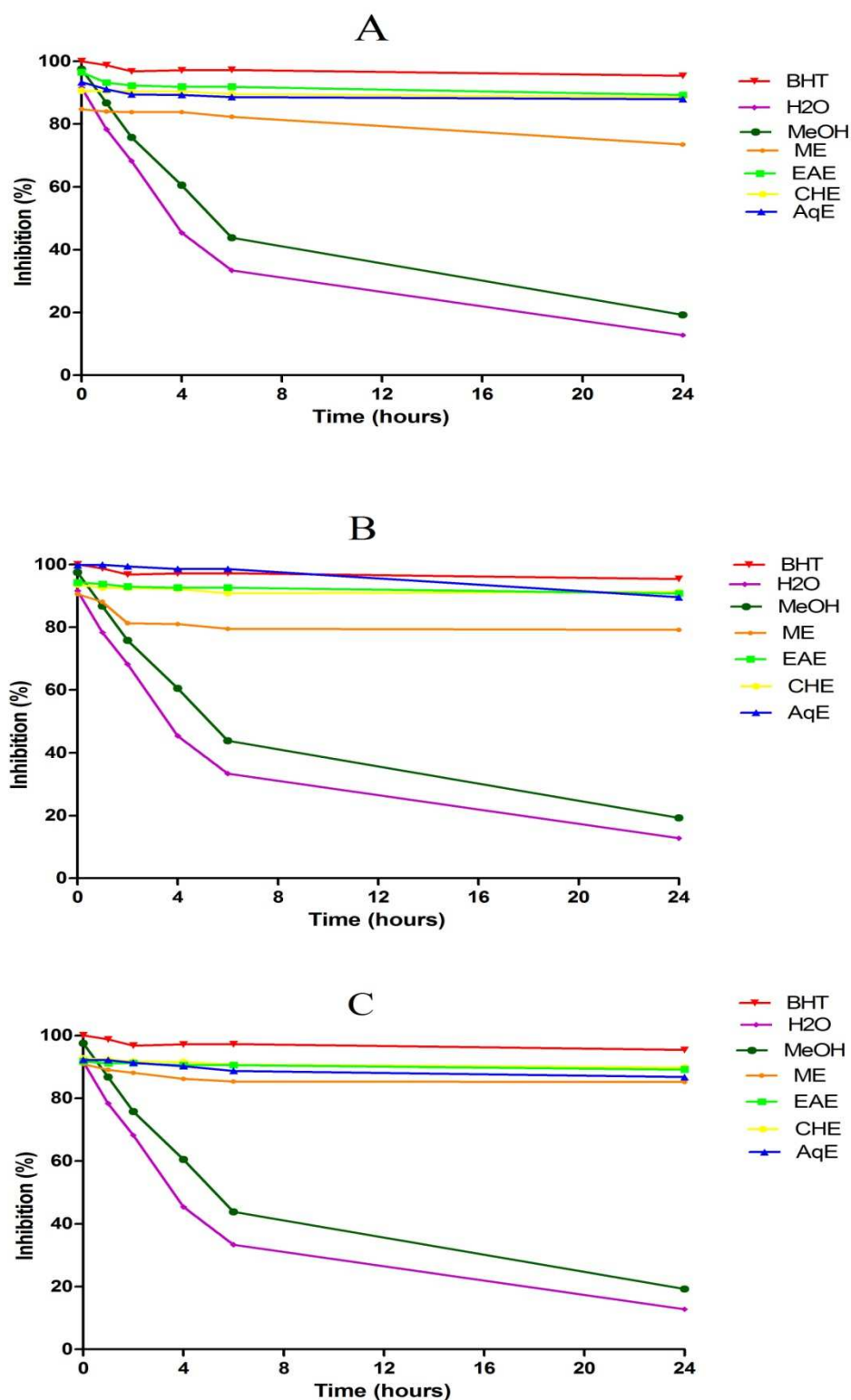


Figure. 57: The changes in the percentage of the inhibition ratios of linoleic acid oxidation of different plant extracts (2mg/ml) using β -carotene bleaching method, compared to BHT as a positive control during 24 h. (A): *C. azarolus*; (B): *M. communis*; (C): *O. glandulosum*. ME: methanolic extract, CHE: chloroform extract, EAE: ethyl acetate extract, AqE: aqueous extract.

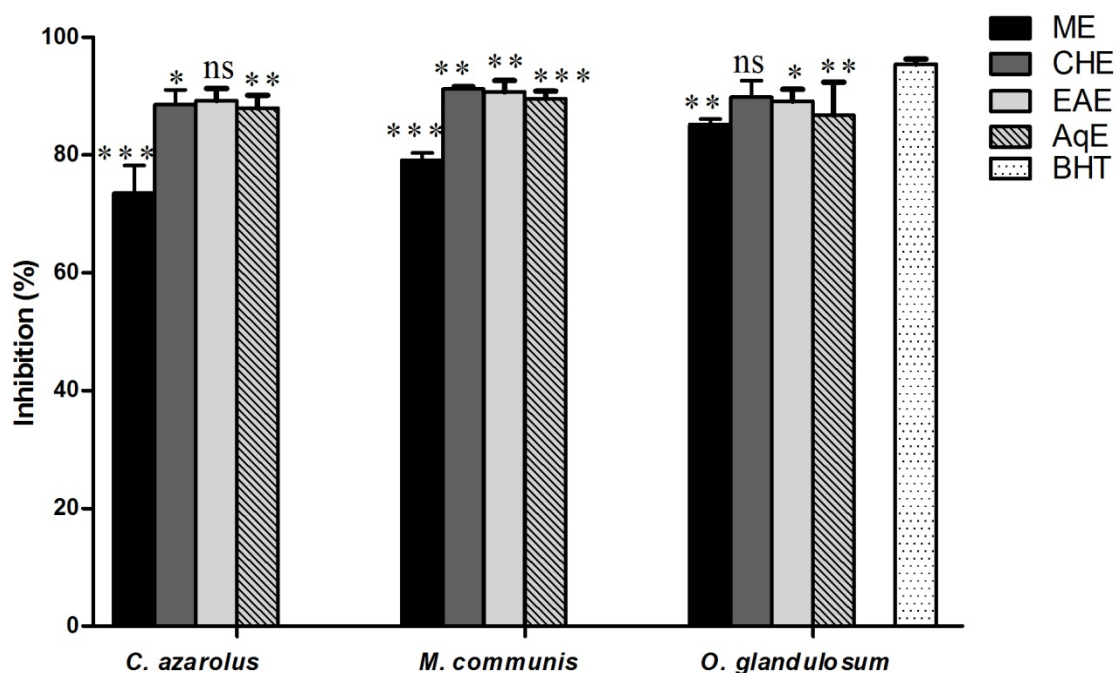


Figure.58: Antioxidant activities of different plant extracts (2 mg/ml at 24 h of incubation) measured by β -carotene bleaching method. ME: methanolic extract, CHE: chloroform extract, EAE: ethyl acetate extract, AqE: aqueous extract. BHT was used as reference antioxidant. Values are means $\pm$ SD (n = 3). (ns: no significant difference, *p < 0.05, ** p < 0.01, *** p < 0.001) compared to BHT as standard.

Several studies have shown some kind of relationship between the antioxidant activity and total phenolic compounds (Djeridane *et al.*, 2006; Hayouni *et al.*, 2007; Özyürek *et al.*, 2012; Medini *et al.*, 2013). However, in the present study, a fair correlation was observed for *O. glandulosum* ($R^2=0.32$), but no correlation was found for *C.azarolus* ($R^2 = 0.007$) and *M.communis* ($R^2=0.001$).

This weak correlation may be attributed to the followings: (i) antioxidant activity on this test is due to specific polyphenols present in extracts, (ii) apart from polyphenols, antioxidant activity is due also to other phytochemical compounds, and/or (iii) there is a synergism between polyphenols and other phytochemical compounds. For example a number of studies have shown that plant polyphenols when combined between them or with other antioxidants

exhibit stronger antioxidant activity compared to their individual one (Dai *et al.*, 2008; Apostolou *et al.*, 2013).

Although, flavonoids can inhibit lipid peroxidation, there are some reports that terpenoids have more potent activity in lipid peroxidation inhibition (Olatunde *et al.*, 2003; Asgarpanah *et al.*, 2011) and this could explain partly the good lipid peroxidation of origano extracts because of the presence of the terpenoids as 5-isopropyl-3-methylphenol isolated in the present study. Furthermore, synergistic effects of different compounds, existing in the extracts of *C. azarolus*; *M. communis* and *O. glandulosum* might be responsible for their significant activities against lipid peroxidation. It is very well to note that, besides the structural effects, other factors, such as the hydrophobicity of the compounds should be taken into account (Silva *et al.*., 2005).

Hernandez-Hernandez *et al.* (2009) indicated that ethanol extract of oregano contain high amounts of phenols and that this high phenol concentration is not correlated with its high antioxidant activity. Its effect on lipid and myoglobin oxidation is also related to the extraction and solvent used, the structure of the antioxidant compound, storage temperature, and initial oxidation state of the sample.

Moreover, these results indicated that all the extracts can significantly inhibit oxidation of linoleic acid, but the apolar extracts are more effective (CHE and EAE). In conclusion, the activity of tested extracts in terms of polarity activity, the apolar extract; CHE of *M. communis* and *O. glandulosum* are more active than EAE, the richest one by phenolic contents. These results are in agreement with those reported by Frankel and Meyer (2000) who have suggested that the polarity of an extract is important in water: lipid emulsions, viz., apolar extract exhibit most important antioxidant properties as they are concentrated within lipid-water interface, thus helping to prevent radical formation and lipid oxidation of β -

carotene. While polar extract are diluted in the aqueous phase and are thus less effective in protecting lipids peroxidation.

3.7. Antioxidant activity of extracts using FTC method

Membrane lipids are rich in unsaturated fatty acids that are most susceptible to oxidative processes. Specially, linoleic acid and arachidonic acid are targets of lipid peroxidation (Yu, 2001). The inhibitory capacity of extracts was tested against oxidation of linoleic acid by FTC method. This method was used to measure the ability of antioxidants to scavenge peroxy radicals through hydrogen donation during polyunsaturated fatty acid (PUFA) oxidation. Peroxides are generated during linoleic acid oxidation, and react with Fe^{2+} to form Fe^{3+} . Ferric ions then react with thiocyanate to produce a red-colored complex, and this complex has a maximum absorbance at 500 nm (Liu and Yao, 2007; Nabavi *et al.*, 2008; Norhaizan *et al.*, 2011).

Figure. 59 shows the changes in the absorbance for each fraction during 5 days of incubation at 40°C, in comparison with BHT. The absorbance of the control increased in proportion to the incubation time, and the absorbance of other samples also increased with increasing incubation time.

As seen in Figure. 60, *C. azarolus* extracts exhibited good antioxidant potential with percent inhibition in the range of 12.5% – 90.13% as compared with BHT. The results indicated that both EAE and CHE exerted marked effects on inhibition of linoleic acid oxidation, which were as strong as the positive reference, BHT (no significant difference, $p > 0.05$), while AqE showed significantly ($p < 0.001$) lower percent inhibition (12.5%) compared with BHT ($89.19 \pm 1.88 \%$).

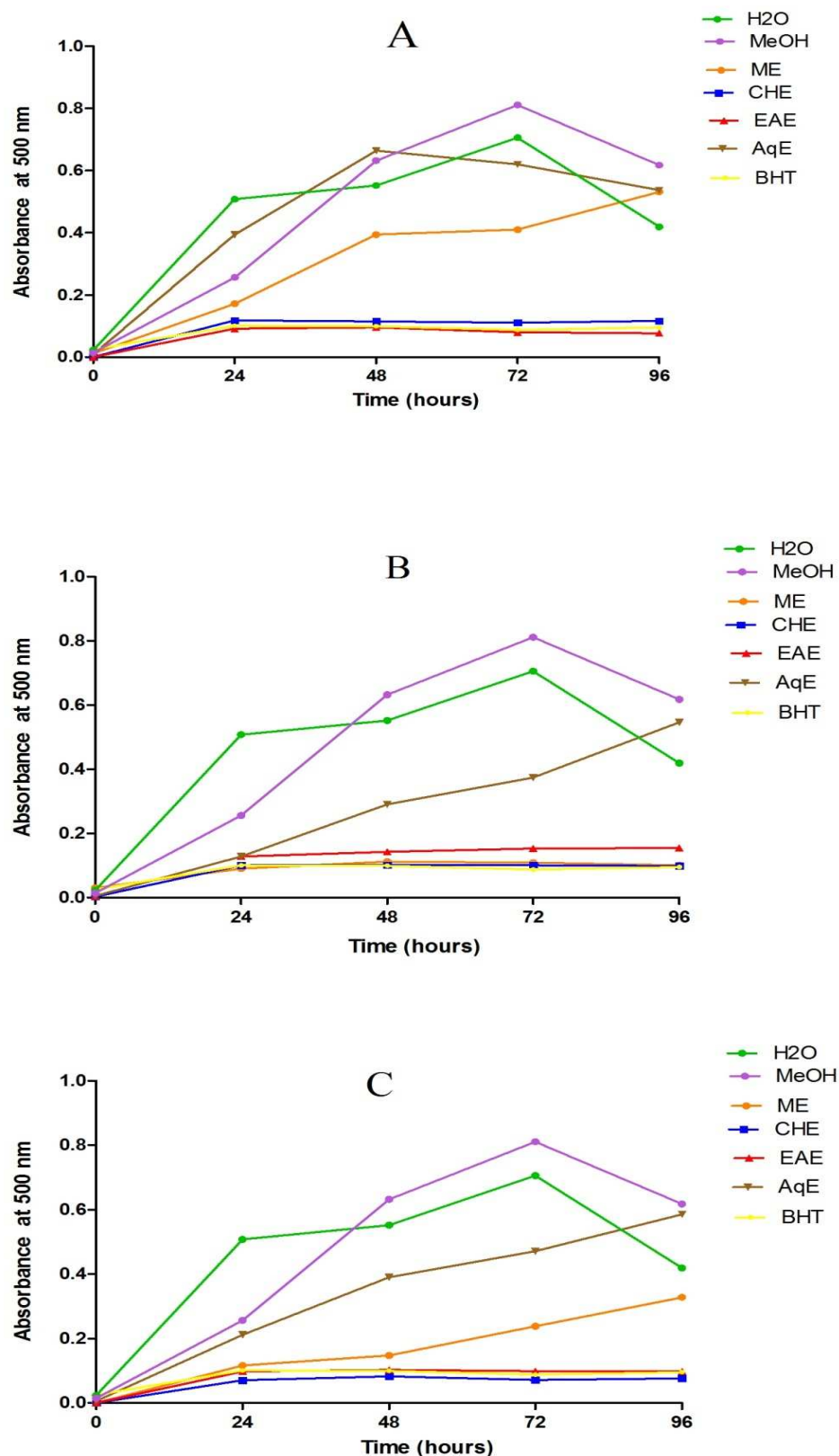


Figure. 59: Absorbance of different plant extracts (2 mg/ml) using FTC method, compared to BHT as a positive control during 96 h. (A): *C. azarolus*; (B): *M. communis*; (C): *O. glandulosum*. ME: methanolic extract, CHE: chloroform extract, EAE: ethyl acetate extract, AqE: aqueous extract.

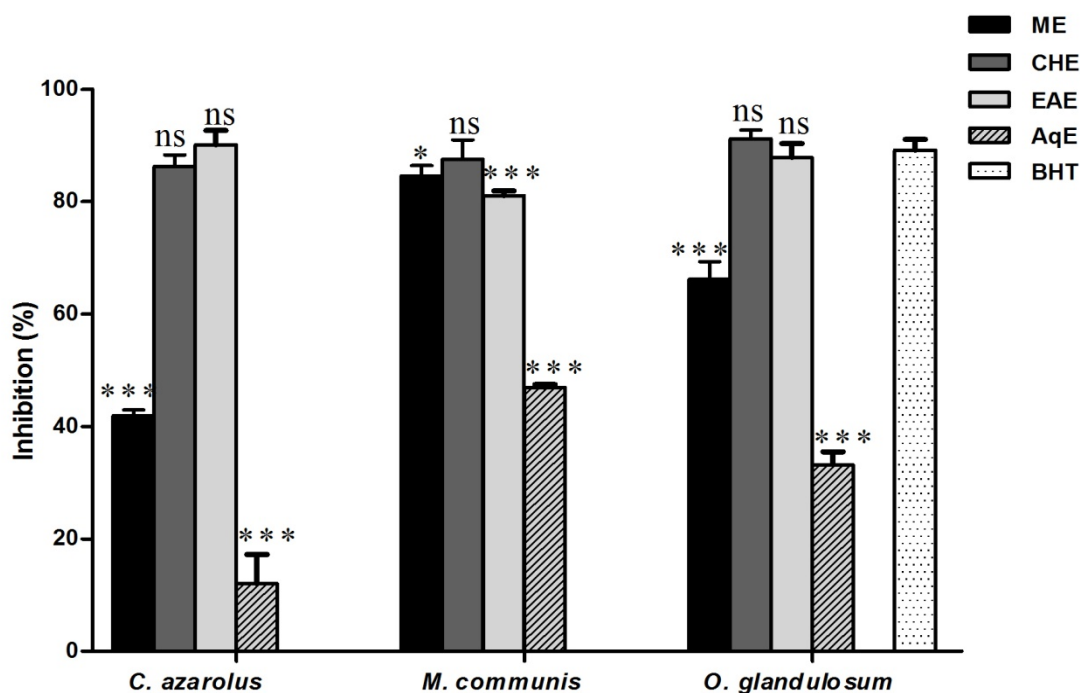


Figure.60: Antioxidant activities of different extracts (2 mg/ml at 96 h of incubation) measured by FTC method. ME: methanolic extract, CHE: chloroform extract, EAE: ethyl acetate extract, AqE: aqueous extract. BHT was used as reference antioxidant. Values are % means $\pm$ SD (n = 3). (ns: no significant difference, * $p < 0.05$, *** $p < 0.001$) compared to BHT as standard.

Furthermore, the best activity of myrtle extracts on lipid peroxidation inhibition was observed with CHE ($87.55 \pm 1.4\%$) which was as strong as the positive control, BHT (no significant difference, $p > 0.05$) followed by ME ($84.59 \pm 1.8\%$), EAE ($81.05 \pm 0.87\%$) and AqE ($46.95 \pm 0.49\%$) (Figure.60). Our study is in agreement with the work of Gonçalves *et al.* (2013) and Kumar *et al.* (2011) who demonstrated that extracts of myrtle prepared using different organic solvents were effective inhibitors of lipid peroxidation.

However, the effect of different fractions of *O. glandulosum* (2 mg/ml) on lipid peroxidation of linoleic acid emulsion were found to be $91.16 \pm 1.58\%$, $87.83 \pm 2.49\%$, $66.19 \pm 3.12\%$ and $33.14 \pm 2.33\%$ for CHE, EAE, ME, AqE respectively (Figure. 60). The activity of both extracts, CHE and EAE was similar to that of BHT (no significant difference, $p > 0.05$).

Consequently, these results clearly indicated that the different plant fractions had effective and powerful antioxidant activity which could be attributed to their phenolic contents at least for *C.azarolus* ($R^2 = 0.55$) and *O.glandulosum* ($R^2=0.37$) but a low correlation was observed for *M.communis* ($R^2=0.18$). Phenolic compounds in plant are powerful free radical-scavengers which can inhibit lipid peroxidation by neutralizing peroxy radicals or decomposing peroxides generated during the oxidation of lipids (Adedapo *et al.*, 2008). Therefore, inhibition of lipid peroxidation by the *C. azarolus* and *O.glandulosum* leaves extracts can be a fundamental property through which they can mitigate the initiation and/or propagation of oxidative stress related diseases. Moreover, the antioxidant activity of an extract could not be explained on the basis of its phenolic content, which also needs its characterization. A high antioxidant activity could also be due to other compounds besides phenolics which are soluble in different solvents (Baghiani *et al.*, 2012) and this could partly explain the higher antioxidant activity of myrtle and oregano extracts.

3.8. Antioxidant activity of extracts using TBA test

One of the major targets of reactive oxygen species is the cellular biomembranes, where they induce lipid peroxidation resulting in the production of MDA which can react with biomolecules and exert cytotoxic and genotoxic effects (Ardestani and Yazdanparast 2007; Wang *et al.*., 2009; Patel *et al.*, 2011).

Different from the FTC test, which is related to the peroxide formation in the initial stage of lipid oxidation, the TBA test measures the amount of MDA produced after the decomposition of the lipid peroxide during the oxidation process (Zin *et al.*, 2002).

As seen in Figure. 61, all fractions of *C. azarolus*, except for AqE, showed a significantly ($p < 0.05$) high antioxidant activity within the range of 8.27 – 74.23 % when

compared with BHT ($79.75 \pm 0.61\%$). The inhibition of lipid peroxidation decreased in the following order of EAE ($74.23 \pm 0.61\%$) > CHE($71.91 \pm 5.94\%$)>ME ($32.33 \pm 2.03\%$)>AqE ($8.27 \pm 0.24 \%$).

Moreover, The percentage inhibition of MDA formation of myrtle fractions exhibited the following order: CHE ($82.59 \pm 1.9\%$) > ME ($74.64 \pm 2.47 \%$) > EAE($71.78 \pm 0.61 \%$) > AqE ($28.96 \pm 0.49 \%$)(Figure. 61).

For *O. glandulosum*, the CHE and EAE presented the highest antioxidant activity in inhibiting MDA formation with values of $80.6 \pm 4.18 \%$ and $74.54 \pm 2.24 \%$, respectively which are similar to that of BHT (Figure.61), whereas ME and AqE had significantly low antioxidant activity ($p < 0.001$) with values of $48.24 \pm 1.75\%$ and $24.29 \pm 1.87\%$, respectively.

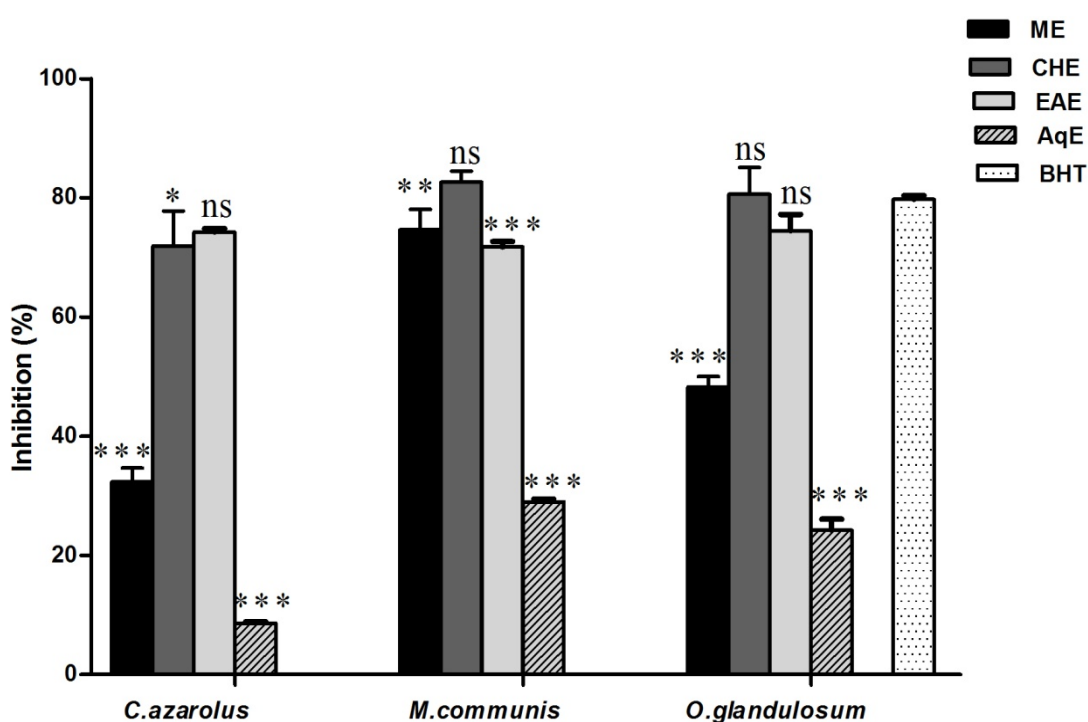


Figure. 61: Antioxidant activities of different plant extracts (2 mg/ml) measured by TBA method. ME: methanolic extract, CHE: chloroform extract, EAE: ethyl acetate extract, AqE: aqueous extract. BHT was used as reference antioxidant. Values are % means $\pm$ SD ($n = 3$). (ns: no significant difference, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) compared to BHT as standard.

The analysis of the correlation between the total phenolic compounds and antioxidant activities using TBA test showed a moderate correlation for *C.azarolus* ($R^2 = 0.53$) and *O. glandulodum* ($R^2=0.38$) and a low correlation was also observed for *M.communis* ($R^2=0.16$). The antioxidative effectiveness in natural sources has been reported to be mostly due to phenolic compounds. Some authors have found a correlation between the phenolic content and the antioxidant activity (Duh and Yen, 1997), while others have found no such relationship (Sun and Ho, 2005). Thus, lipid peroxidation inhibition of *C.azarolus* and *O.glandulosum* could mainly be due to their phenolic components such as flavonoids, phenolic acids, phenolic diterpenes, that have ability to act as antioxidant due to the presence of conjugated ring structures and carboxylic groups which have been reported to inhibit lipid peroxidation (Priyadharshini *et al.*, 2012; Moyo *et al.*, 2012). Different phenolic compounds also vary in their antioxidant activities, and may work cooperatively, antagonistically or synergistically with other compounds present in crude extracts (Ni'ciforovi'c *et al.*, 2010).

Our results are in accordance with previously published reports which indicated that the antioxidant activity of the FTC method is higher than the antioxidant activity of the TBA assay and the two assays were strongly correlated (Ismail *et al.*, 2010; Norhaizan *et al.*, 2011; Rezaeizadeh *et al.*, 2011), and this is mainly due to the amount of peroxide in the initial stage of lipid peroxidation was greater than the amount of peroxide in the secondary stage. Furthermore, the secondary product such as malonaldehyde is not stable for a period of time. It will turn into alcohol and acid which can not be detected by spectrophotometer (Rahmat *et al.*, 2003; Saha *et al.*, 2004; Rezaeizadeh *et al.*, 2011).

Also, we can conclude from the results of both tests, that CHE and EAE were the most effective in inhibiting the lipid peroxidation than the other separated fractions (ME and

AqE), which were lower than the activity of BHT. Hence, these antioxidant activities were in agreement with those found in the β -carotene linoleic acid system. An indication that the antioxidant activities measured in the three model systems complemented each other.

4. Antioxidant activity of extracts: *in vivo* assays

4.1. Plasma antioxidant capacity

Several methods have been used to evaluate the antioxidant capacity of flavonoids and extracts of flavonoid-rich foods and that of human plasma before and after consumption of flavonoid-rich foods. These methods include the ferric-reducing antioxidant potential (FRAP); oxygen radical absorbance capacity (ORAC), without or with prior precipitation of plasma proteins with perchloric acid (ORAC-PCA); trolox equivalent antioxidant capacity (TEAC); and total radical-trapping antioxidant parameter (TRAP). All these assays assess either the radical-scavenging (hydrogen atom transfer) or reducing (electron transfer) capacity of the compound or (biological) fluid under investigation (Lotito and Frei, 2006).

4.1.1. Effect of extracts on plasma antioxidant capacity using DPPH radical

To evaluate the effectiveness of an antioxidant treatment, the scavenger effect against DPPH radical was assessed; this last 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.

In this study, the obtained results (Figure. 62) showed that the administration *per os* of ME from *C. azarolus* at doses of 100 and 200 mg/Kg in rats increased significantly the plasma antioxidant capacity with values of $13.28 \pm 1.36 \%$ and $16.65 \pm 1.40 \%$, respectively compared to control group ($7.37 \pm 1.36 \%$).

On the other hand, the administration of the same doses of ME from *M. communis* showed low scavenger activity compared to control group but this decrease is still statistically no significant. However *O.glandulosum* treatment resulted in the elevation of plasma antioxidant activity with percent inhibition of (9.50 ± 1.56 % and 13.96 ± 1.54 %) for the the doses 100 mg/kg and 200 mg/kg , respectively compared to control group.

In general, it was found that 100 and 200 mg/kg doses of ME from *C. azarolus* and 200 mg/kg dose of ME from *O.glandulosum* were as stronger (no significant difference ; $p > 0.05$) as vitamin C (14.90 ± 1.24 %) to increasing the total plasma antioxidant capacity.

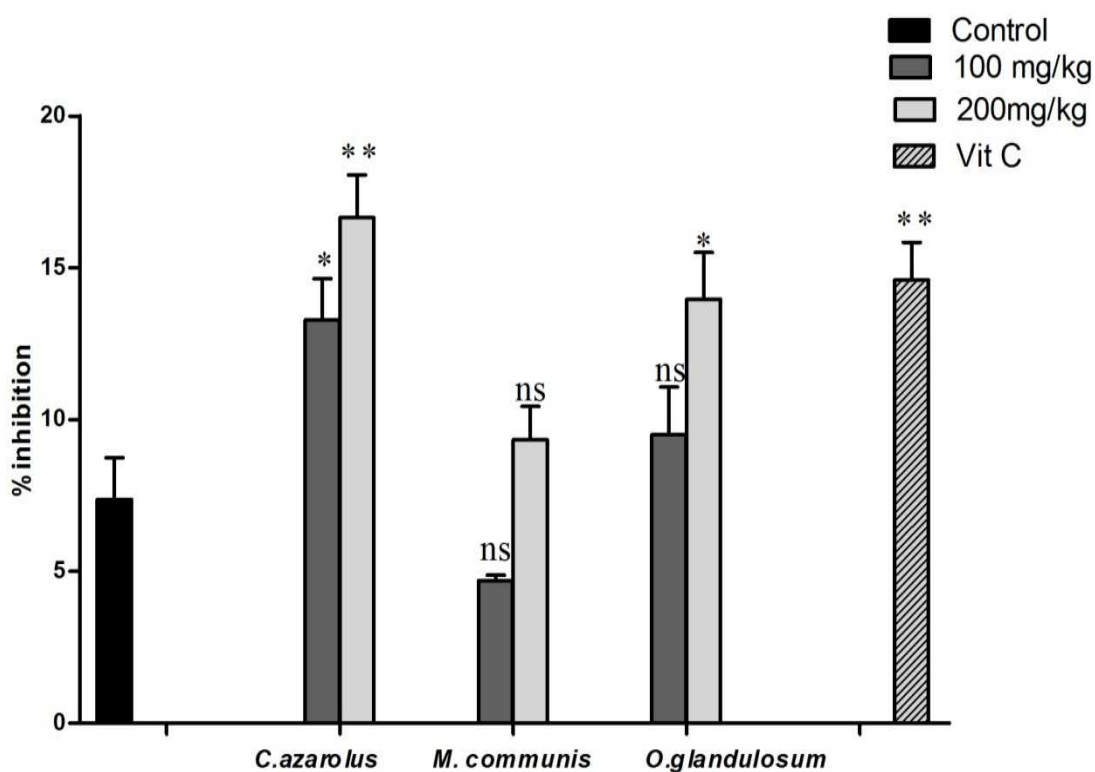


Figure. 62: A comparison between different plant extracts in plasma antioxidant capacity using DPPH radical. Data were presented as % means $\pm$ SEM ($n = 6$). (ns: no significant difference ; ** $p < 0.01$; * $p < 0.05$) compared to control group.

4.1. 2. Effects of extracts on plasma reducing power

Plasma antioxidant capacity of the selected plants were reinforced by the reducing power assay. Data demonstrated that administration of ME of *C. azarolus*, *M. communis* and *O. glandulosum* possess similar DPPH-free radical scavenging pattern. As seen in Figure. 62, all extracts doses showed electron donation capacity dose dependently, but only the reducing power of the dose 200 mg/kg from *C.azarolus* and *O. glandulosum* (0.64 ± 0.08 and 0.46 ± 0.05 , respectively) were significantly higher than that of control group.

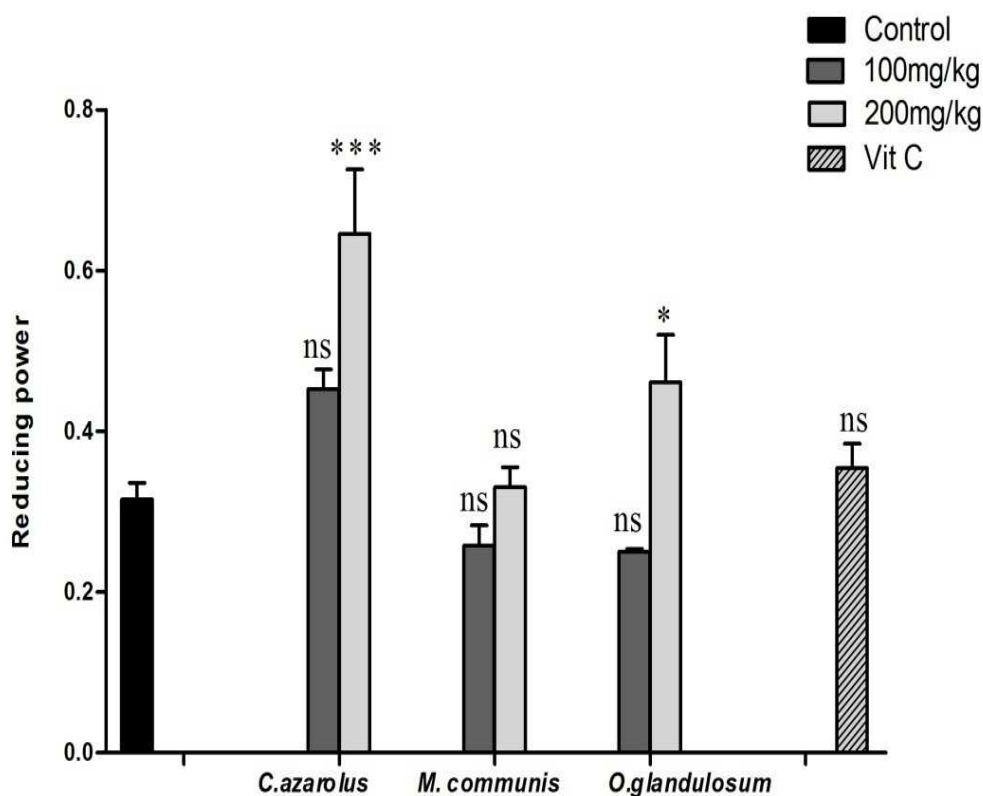


Figure. 63: A comparison between different plant extracts in plasma antioxidant capacity using DPPH radical. Data were presented as means $\pm$ SEM (n = 6). (ns: no significant difference; * p < 0.05; *** p < 0.001) compared to control group.

However, in rats that were orally treated with *C. azarolus* (100 mg/kg), *M. communis* (100 and 200 mg/kg) and *O.glandulosum* (100 mg/kg), their plasma reducing capacity values were 0.45 ± 0.02 , 0.25 ± 0.02 , 0.33 ± 0.02 , 0.25 ± 0.003 , respectively. These values did not show any important alteration in plasma reducing power (no significant difference, $p > 0.05$) when compared to control and positive groups which had values of 0.31 ± 0.02 and 0.35 ± 0.03 , respectively.

These obtained results showed a positive association between plant extracts administration and plasma antioxidant capacity using DPPH scavenging assay and reducing power and these effects may be primarily attributed to the polyphenolic contents of these plant extracts. Many studies have found highly significant correlation between the measured plasma antioxidant capacity and the total phenol content of plant-derived extracts and beverages (Gladine *et al.*, 2007; Oliveira *et al.*, 2010; Vicente *et al.*, 2014; Avila-Nava *et al.*, 2014).

It was reported that the consumption of extra virgin olive oil rich in phenolic compounds (30 days, 50 ml/day) increases the antioxidant activity by 40% (Oliveras-López *et al.*, 2014) and the consumption of 482 ml/day coffee rich in phenolic acids such as caffeic, ferulic, chlorogenic, and p-coumaric acids for 4 weeks increases also the plasma total antioxidant status subjects by 26 % (Corrêa *et al.*, 2012). Addition of catechins (Rivero *et al.*, 2005) or tea extract to human plasma and tea consumption by humans (Bors *et al.*, 2001) were also reported to increase the total antioxidant capacity of plasma.

Furthermore, the administration of 80 g of procyanidin-rich semisweet chocolate caused elevation of plasma (-)-epicatechin from 22 to 257 nM, and significant increase of 31% in plasma total antioxidant capacity, corresponding to about 90 µM trolox equivalents (Rein *et al.*, 2000).

One of the most important properties of an antioxidant substance is that, besides being a scavenger *in vitro*, the antioxidant compound should be able to reach the site of action in its active form and consequently act on the target molecules. The results for plasma antioxidant activity *in vivo* in rats using DPPH radical scavenging activity and reducing power assay showed remarkable increase in antioxidant activity due to administration of methanolic extracts of selected plant and their *in vivo* efficiency could be classified as: ME - *C.azarolus* > ME - *O. glandulosum* > ME - *M.communis*.

These data indicate that treatment with ME of *M. communis* (200 mg/kg) did not make a significant contribution to the change in plasma antioxidant capacity in our study. However ME of *M. communis* at dose of 100 mg/kg decreased plasma DPPH and reducing power values, suggesting an effect of food intake *per os*, in agreement with previous reports (Cao and Prior, 2000; Mazza *et al.*, 2002).

Another interesting finding of the present study was that a weak correlation occurred between the *in vitro* and the *in vivo* antioxidant capacity of plant extracts, especially for myrtle methanolic extract which was found to exhibit a strong antioxidant activity *in vitro*. Despite the strong antioxidant capacity of polyphenols *in vitro*, their antioxidant efficacy *in vivo* is limited by several factors including low bioavailability and metabolic conversion of the absorbed polyphenols.

Firstly, the maximum plasma concentration of polyphenols rarely exceeds 1µM following ingestion of 10-100 mg of a single phenolic compound (Veskoukis *et al.*, 2012). Thus, the maximal plasma concentrations of flavonoids in humans, reached usually between 1 and 3 h after consumption of flavonoid-rich foods, are between 0.06 and 7.6 µM for flavonols, flavanols, and flavanones, and less than 0.15 µM for anthocyanidins (Manach *et al.*, 2005). In addition, the half-lives of flavonoids in human plasma are short, usually in the range of a few

hours. These factors severely limit the capability of dietary flavonoids to act as antioxidants in plasma *in vivo*, especially compared to other antioxidants present in plasma at high steady-state concentrations, e.g., 30–150 μM ascorbate (vitamin C), 160–450 μM urate, or 15–40 μM α -tocopherol (vitamin E) (Lotito and Frei, 2006).

Secondly, the low effect of flavonoids in the *in vivo* study may be attributed to their biotransformation in liver to non active compounds. Following ingestion, polyphenols are mainly absorbed as their aglycones (Avila-Nava *et al.*, 2014) and appear in plasma largely as conjugates with glucuronic acid or as sulfated and methylated compounds. Other modifications also occur, such as the metabolism of chlorogenic acid to caffeic acid by esterases. These metabolic modifications can substantially alter their antioxidant properties (Procházková *et al.*, 2011; Xiao *et al.*, 2014).

4.2. Effect of extracts on hepatic and renal antioxidant status

It is well known that free radicals can cause cell damage through covalent binding and lipid peroxidation with subsequent tissue injury. Liver and kidney are most vulnerable to injury by free radicals resulting in serious health problems (Sreenivasamurthy *et al.*, 2012).

Antioxidant agents of natural source have attracted special attention because they can protect the human body from these free radicals (Patrick-Iwuanyanwu *et al.*, 2011; Sreenivasamurthy *et al.*, 2012). Thus, in this study, the selected plant extracts were screened for their possible *in vivo* antioxidant activity, which served as a significant indicator of their potential antioxidant activity to maintain redox status.

4.2.1. Effect of extracts on total proteins level

Liver is one of the largest glands in the human body and the chief site for intense metabolism and excretion. So, it has the surprising role in the maintenance, performance and regulating the homeostasis of body. It is involved also in almost all biochemical pathways to growth, to fight against infections, diseases, nutrient supply, energy provision etc. The major functions of liver are carbohydrate, protein and fat metabolism, detoxification, storage of vitamin and formation of bile (Huo *et al.*, 2011; Rayese *et al.*, 2013). Thus, we tried in this study to evaluate the effect of extracts on total proteins level in liver and kidney.

As seen in Figure 64, the treatment with ME at the doses of 100 and 200 mg/kg and vitamin C (100 mg/kg) resulted in significant ($p < 0.05$) increase of total proteins level in liver except for the dose of 100 mg/kg of ME of *M.communis* when compared to control group (26.87 ± 0.74 mg/ml). The total protein levels for *C.azarolus*, *M.communis* and *O.glandulosum* at doses of 100 mg/kg and 200 mg/kg were found to be (30.50 ± 0.77 mg/ml, 30.51 ± 0.32 mg/ml), (27.99 ± 1.05 mg/ml, 33.53 ± 1.23 mg/ml), and (29.82 ± 0.77 mg/ml, 31.84 ± 0.68 mg/ml), respectively.

However, the administration of the same doses of ME of the three studied plants and vitamin C had no effect (no significant difference, $p > 0.05$) on proteins level in kidneys compared to control (26.43 ± 2.91 mg/ml) and vitamin C groups (28.99 ± 0.82 mg/ml). ME of *C. azarolus* (200 mg/ml) showed the lowest total proteins content (24.24 ± 0.82 mg/ml), whereas the highest content of 28.87 ± 0.65 mg/ml was signaled for *O.glandulosum* (200 mg/kg).

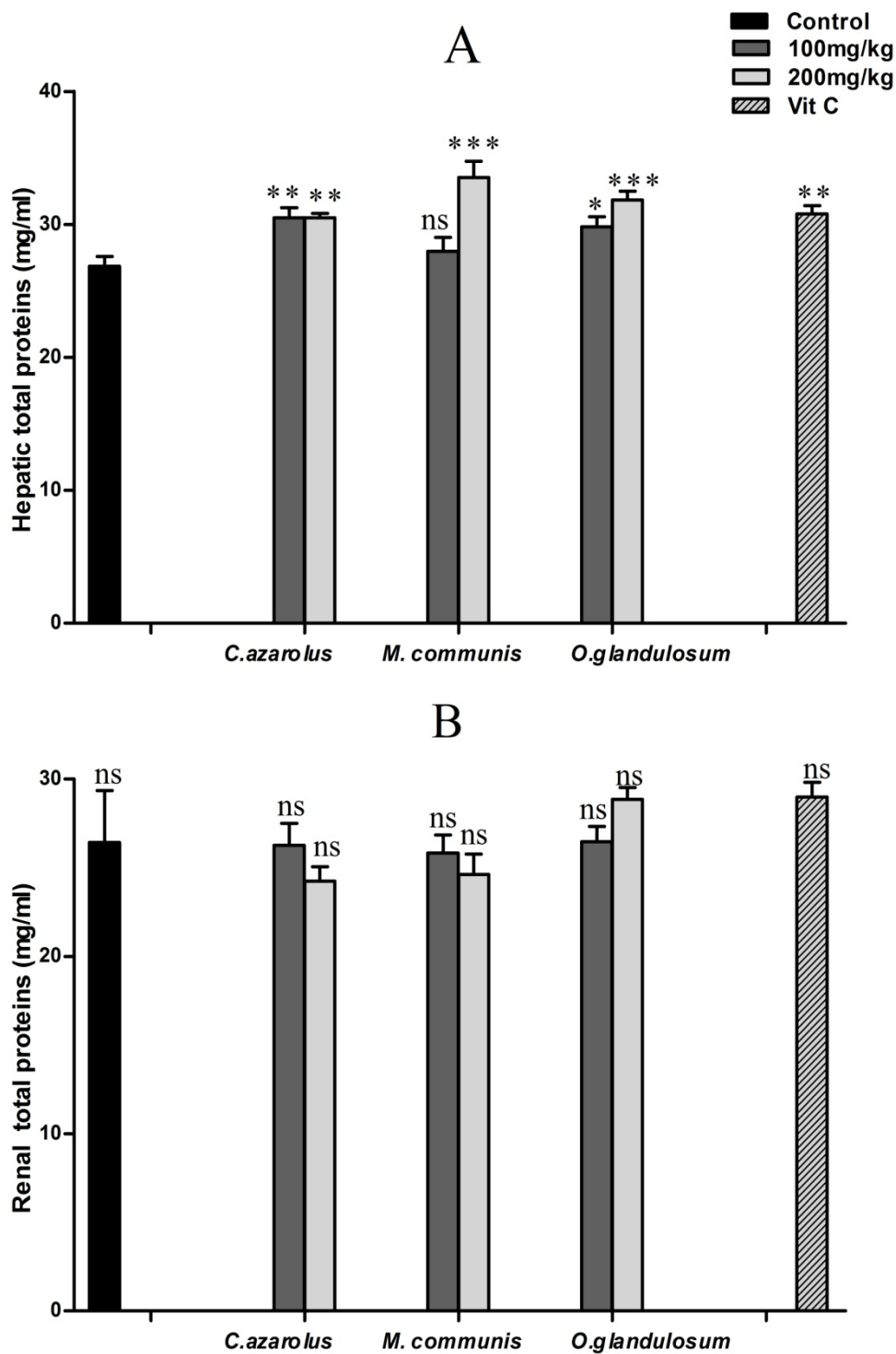


Figure. 64: Effect of methanolic extract of *C.azarolus*, *M.communis*, *O. glandulosum* and vitamin C on total proteins level in liver (A) and kidney (B) of rats . Values are given as mean $\pm$ SEM (n=6). (ns: no significant difference; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) compared to control group.

As mentioned above, liver is the site of synthesis, storage, and metabolism for proteins and a healthy functioning liver is required for the synthesis of the serum proteins except for the γ globulins. Hypoproteinemia is a feature of liver damage due to significant fall in protein synthesis (Akilavalli *et al.*, 2011). It has been reported that stimulation of protein synthesis has been advanced as a contributing hepatoprotective mechanism which accelerates regeneration of cells (Patrick-Iwuanyanwu *et al.*, 2011). Thus, an increase in total proteins level evaluated in this study suggests the stabilisation of endoplasmic reticulum by plant extracts treatment, leading to protein synthesis (Nasir *et al.*, 2013).

Kidney plays an essential role in health, disease and overall development and growth. The main function of kidney is to maintain total body fluid volume, its composition and acid base balance. A number of environmental variables including certain xenobiotics (e.g. pesticides) influence these functions (Refaie *et al.*, 2014). As mentioned above, there was no significant alteration of renal proteins level and this may be due to the protective and safety effects of the administered plant extracts on renal tissues.

4.2.2. Effect of extracts on catalase activity

Endogenous antioxidant, SOD detoxifies the super anion radicals into H_2O_2 and catalase converts this by-product into water and molecular oxygen, which contribute to the protective function by scavenging for any excess lipid peroxidation products generated (Pandanaboina *et al.*, 2012; Ye *et al.*, 2014). Thus, *in vivo* effects of methanolic extract of *C. azarolus*, *M. communis*, *O. glandulosum* and vitamin C on catalase activity in liver and kidney of rats were evaluated and shown in Figure. 65.

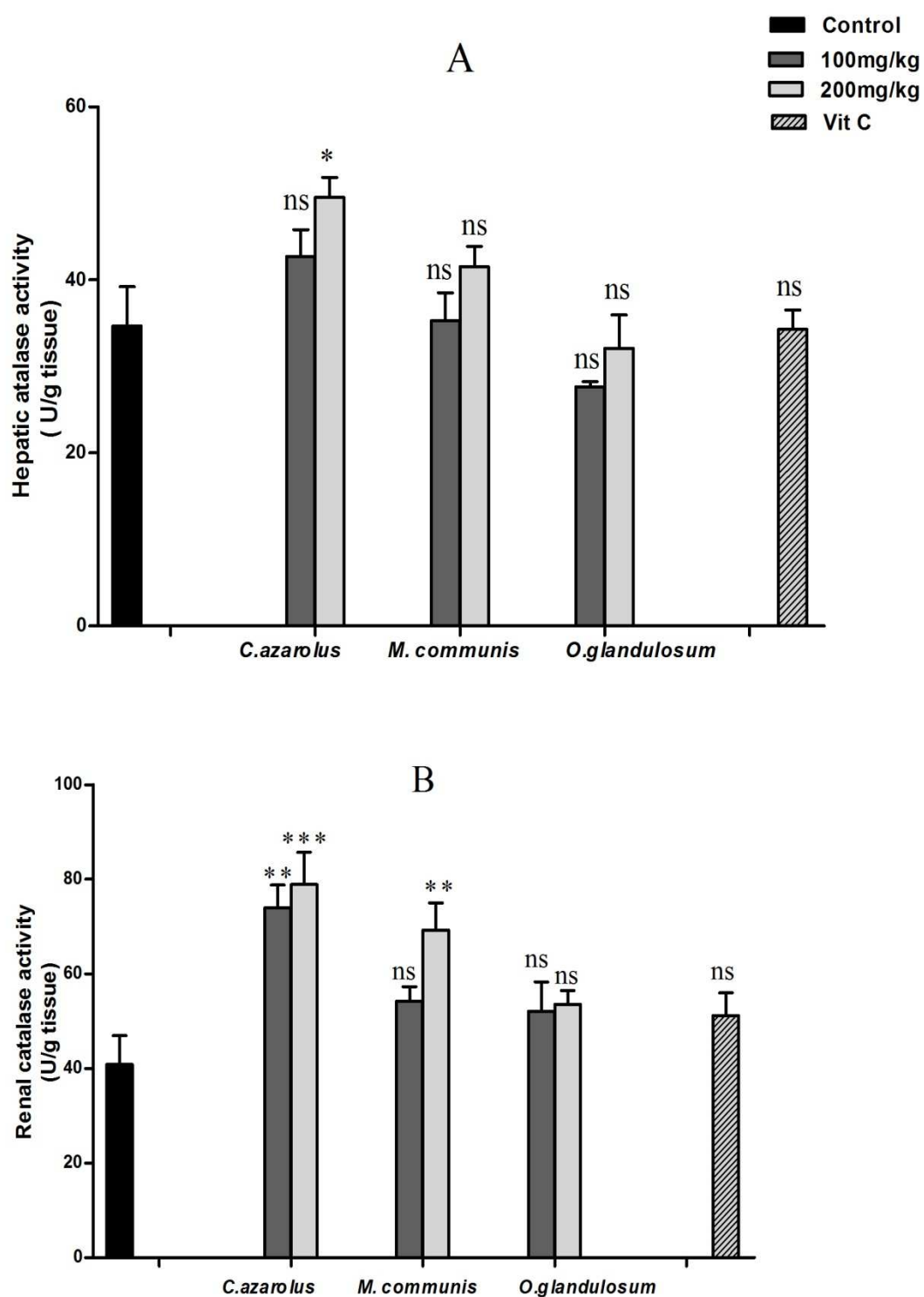


Figure. 65: Effect of methanolic extract of *C.azarolus*, *M.communis*, *O. glandulosum* and vitamin C on catalase activity in liver (A) and kidney (B) of rats . Values are given as means $\pm$ SEM (n=6). (ns: no significant difference; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) compared to control group.

In our study, it was observed that the treatment of rats with the ME of *C. azarolus*, *M. communis*, *O. glandulosum* at doses of 100 and 200 mg/kg exhibited increased activity of catalase in liver, but this increase didn't reach statistically significant difference except for the dose 200 mg/kg of *C. azarolus* when compared to control group (34.67 ± 4.53 U/g tissue). The values of catalase activity of the two administered doses of 100 and 200 mg/kg were (42.68 ± 3.101 U/ g tissue; 49.53 ± 2.31 U/ g tissue), (35.26 ± 3.228 U/ g tissue; 41.45 ± 2.31 U/ g tissue) and (27.66 ± 2.18 U/ tissue; 32.09 ± 3.84 U/ g tissue) for *C. azarolus*, *M. communis* and *O. glandulosum*, respectively.

Also, it was observed a significant increase ($p < 0.01$) in the activity of renal catalase in the groups treated with *C. azarolus* at doses of 100 and 200 mg/kg (73.93 ± 4.89 U/ g tissue and 78.88 ± 6.84 U/ g tissue, respectively) when compared with control group (40.84 ± 6.11 U/g tissue). Similarly, administration of *M. communis* at dose of 200 mg/kg significantly ($p < 0.01$) increased the activity of this enzyme (69.26 ± 5.7 U/g tissue). However, no significant difference was observed between rat groups treated with ME of *O. glandulosum* (100 and 200 mg/kg) and vitamin C (100 mg/kg) when compared to control group.

In the present study, the increase in catalase activity observed after the administration of ME of selected plants may be due to the high levels of polyphenols in these extracts. Similar studies have shown a positive effect of different classes of polyphenols on antioxidant enzyme activities (SOD, CAT and GPx) in animal model and human studies (Feng *et al.*, 2010; Srivastava and Shivanandappa, 2010; Kim *et al.*, 2013; Avila-Nava *et al.*, 2014; Khan *et al.*, 2014; Kardum *et al.*, 2014; Wu *et al.*, 2015).

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 (Mitjavila and Moreno, 2012; Oliveras-López *et al.*, 2014; Nakayama *et al.*, 2015).

Nakayama *et al.* (2015) demonstrated that pine bark extract treatment resulted in increases in the expressions of antioxidant enzymes, glutathione peroxidase-1, catalase, and heme oxygenase-1 in THP-1 monocytic cells. In addition, pine bark extract induced activation of Nrf2, which was accompanied by the activation of extracellular signal-regulated kinase and Akt despite a down-regulation of reactive oxygen species.

Oral extra virgin oil intake also was found to increase CAT and GPx activities and their mRNA expressions in healthy adults (Oliveras-López *et al.*, 2014). These findings that may support our hypothesis in that the methanolic extract of these plants might exert antioxidant activities based on its ability to stimulate activity of antioxidant enzymes and an induction of gene expression.

4.6.3. Effect of extracts on GSH level

ME of *C. azarolus*, *M. communis* and *O. glandulosum* *per os* treatment to rats at doses of 100 and 200 mg/kg did not alter the level of GSH in liver compared to control (18.34 ± 2.47 $\mu\text{mol/g}$ tissue) and vitamin C groups (17.61 ± 0.80 $\mu\text{mol/g}$ tissue) (Figure.66). It was found that at dose of 200 mg/kg, *C.azarolus* extract had a slightly higher effect on GSH level (22.11 ± 1.72 $\mu\text{mol/g}$ tissue) followed by *M. communis* (21.18 ± 0.96 $\mu\text{mol/g}$ tissue) and *O.glandulosum* (17.42 ± 0.66 $\mu\text{mol/g}$ tissue).

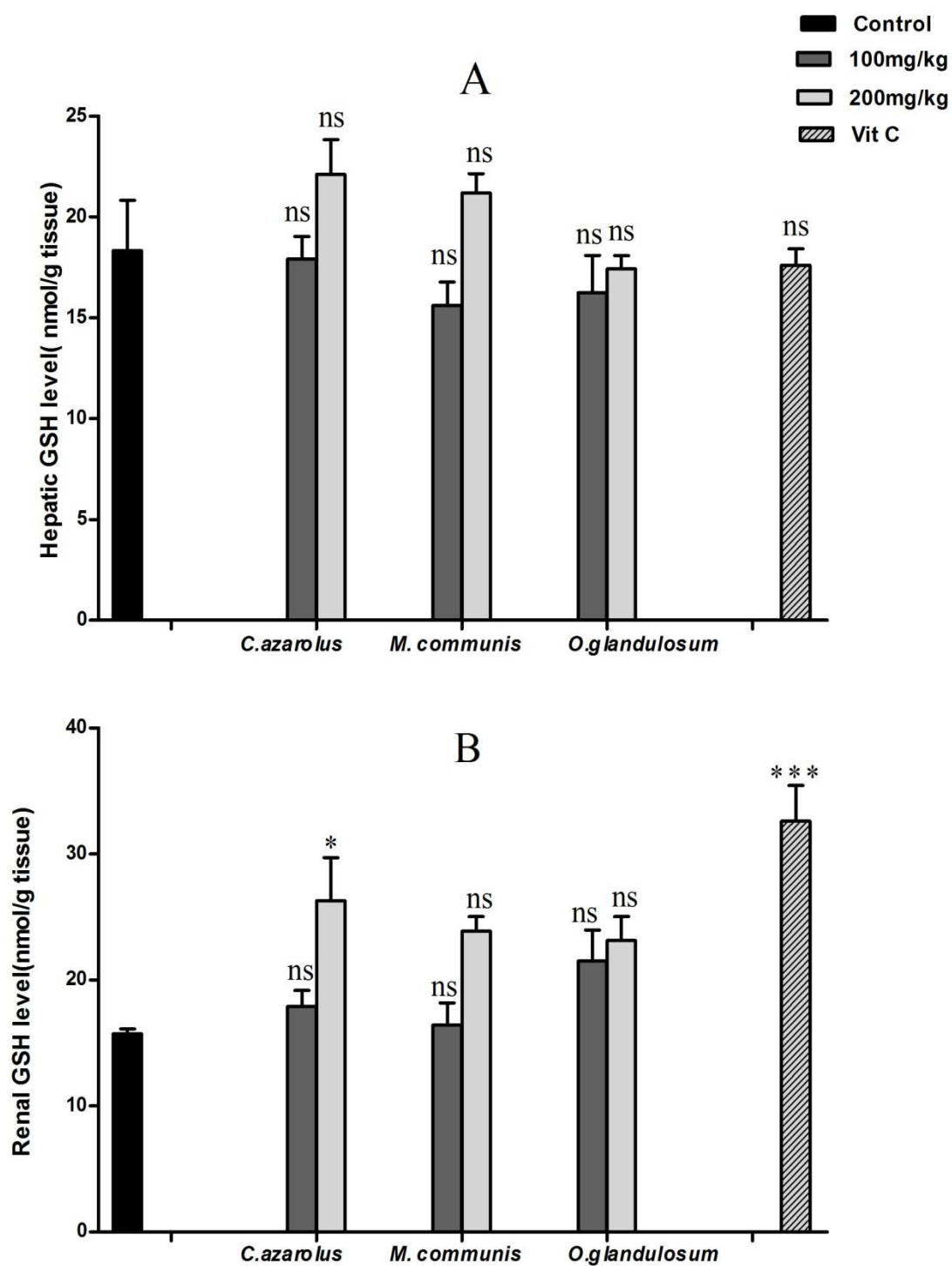


Figure. 66: Effect of methanolic extract of *C.azarolus*, *M.communis*, *O. glandulosum* and vitamin C on reduced glutathione level in liver (A) and kidney(B) of rats. Values are given as means $\pm$ SEM (n=6). (ns: no significance difference; * $p < 0.05$; *** $p < 0.001$) compared to control group.

Also, administration of *C. azarolus*, *M. communis* and *O. glandulosum* at two different doses (100 and 200 mg/kg) in rats didn't produce significant increase in renal GSH levels except in the case of ME of *C. azarolus* (200 mg/kg) which had the value of 26.29 ± 3.41 $\mu\text{mol/g}$ tissue. The treatment with the vitamin C showed a considerable effect in increasing GSH level (32.60 ± 2.822 $\mu\text{mol/g}$ tissue) compared to control group and other extracts treated groups (Figure.66).

GSH antioxidant system plays a fundamental role in cellular defense against reactive free radicals and other oxidant species. This system consists of GSH and an array of functionally related enzymes, of which GR is responsible for the regeneration of GSH, whereas GPx and GST work together with GSH in the decomposition of hydrogen peroxide, superoxide anions and alkoxy radicals (Srivastava and Shivanandappa, 2010; Huo *et al.*, 2011; Lavie, 2014). Thus, GSH plays a primary role in the maintenance of a balanced redox status. Hence, it can serve as a potential marker of susceptibility, early and reversible tissue damage, and of decrease in antioxidant defense (Kaushik *et al.*, 2011; Jain and Singhai, 2012). Also, depletion of GSH level has been shown to be associated with enhanced lipid peroxidation (Akilavalli *et al.*, 2011; Nasir *et al.*, 2013).

In this study, all plant extracts administered at dose of 100 and 200 mg/kg increased slightly the hepatic and renal reduced glutathione except for the *C. azarolus* (200 mg/kg), where the increase in GSH was found significant.

Akila and Devaraj (2008) demonstrated that oral administration of tincture of *Crataegus* (TCR) made from alcoholic extract berries of *Crataegus oxyacantha* (0.5 ml/100 g body weight) to rats fed with an atherogenic (4% cholesterol, 1% cholic acid, 0.5% thiouracil) diet caused a significant decrease in lipid accumulation in the liver and aorta and significantly restored the activity of antioxidant enzymes such as superoxide dismutase, catalase,

glutathione peroxidase, and glutathione. This effect could be attributed to the synergistic activity of flavonoids in TCR

Taking into account the fact that ME of the three studied plants contained high concentration of phenolics and flavonoids, such constituents have demonstrated previously significant increase of GSH level (Wang and Chen, 2010; Srivastava *et al.*, 2011; Jain and Singhai, 2012), thus, their presence could explain the elevated content of GSH by ME of *C.azarolus* treatment in rats or maintaining the normal ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG) and a normal redox state in liver and kidney of *M. communis* and *O. glandulosum* treated groups.

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.

4.2.4. Effect of extracts on lipid peroxidation

The concentration of MDA was measured as an index of the extent of lipid peroxidation in different extracts treated groups. It was observed that treatment (Figure.67) with methanolic extract of *C. azarolus* at dose of 200 mg/kg significantly reduced ($p < 0.001$) the level of MDA in liver (52.1 ± 2.16 nmol/ g tissue) compared to control group (81.69 ± 3.49 nmol/ g tissue). Similarly, the doses (100 and 200 mg/kg) of ME of *O.glandulosum* significantly ($p < 0.01$) decreased the levels of MDA with values of 58.96 ± 5.88 nmol/ g tissue and 54.83 ± 5.08 nmol/ g tissue, respectively. ME of *M.communis* decreased MDA formation with level of 73.64 ± 4.68 nmol/ g tissue and 65.93 ± 3.76 nmol/ g tissue for the dose of 100 and 200 mg/kg, respectively, but this decrease was statistically not significant when compared to control group.

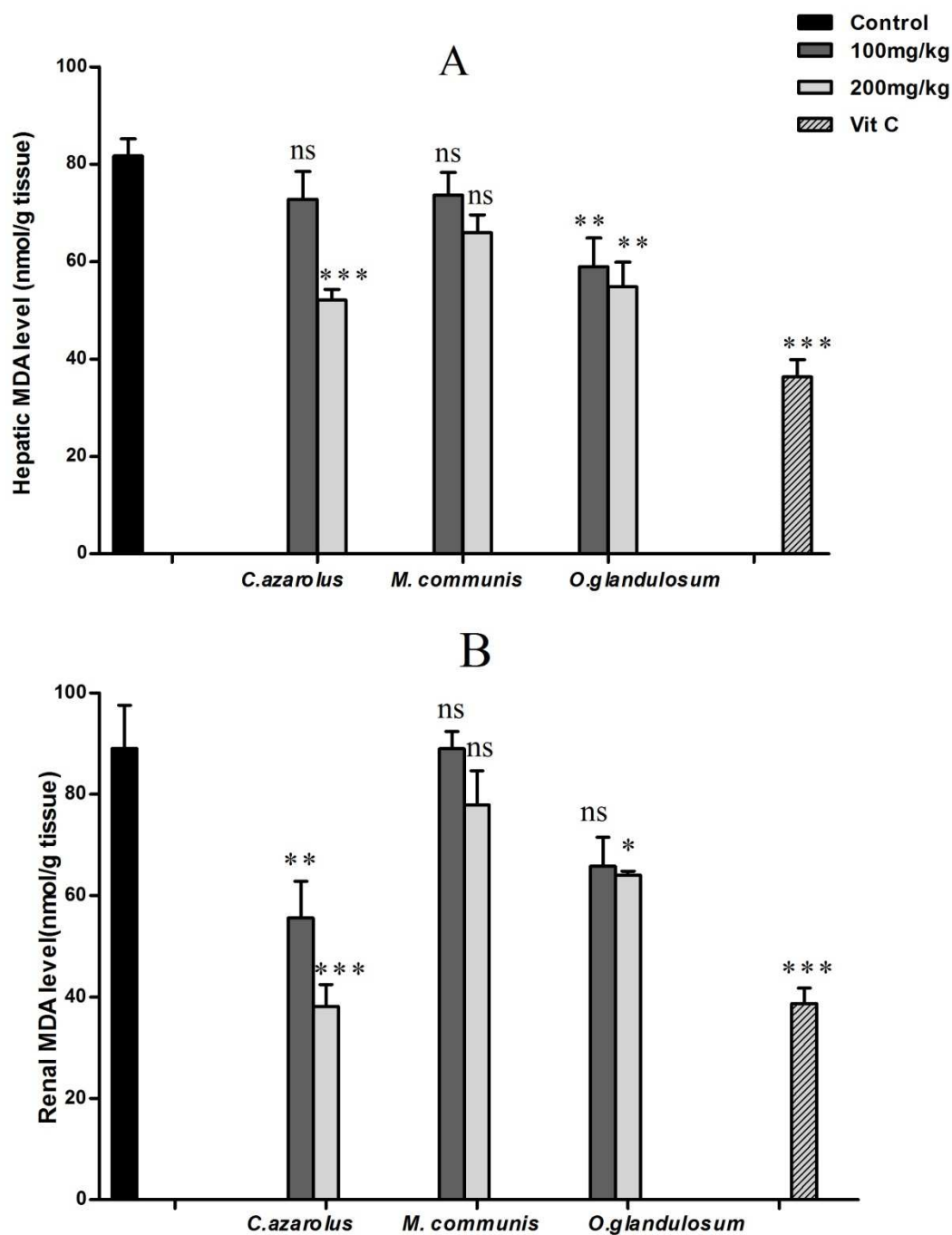


Figure . 67 : Effect of methanolic extract of *C. azarolus*, *M. communis*, *O. glandulosum* and vitamin C on MDA level in liver (A) and kidney (B) of rats . Values are given as means $\pm$ SEM (n=6). (ns: no significant difference; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) compared to control group.

As seen in Figure 67, the same results were obtained in kidney homogenates of rats treated with methanolic extract of selected plants. There was significant decrease in the levels of MDA in rat groups treated with 100 and 200 mg/kg of ME of *C.azarolus* with values of 55.56 ± 7.23 and 38.13 ± 4.29 nmol/ g tissue, respectively when compared with control group (89.04 ± 8.52 nmol/ g tissue). The same doses exhibited similar effect to that of rats receiving the standard drug; vitamin C (38.74 ± 2.98 nmol/ g tissue). Also, the dose of 200 mg/kg of ME of *O. glandulosum* decreased lipid peroxidation (64.05 ± 0.77 nmol/ g tissue). However, the groups treated with *M. communis* (100 and 200 mg/kg) and *O.glandulosum* (100 mg/kg) decreased the MDA level without reaching the statistical significance when compared with control group.

Propagative lipid peroxidation is a degenerative process that affects cell membranes and other lipid containing structures under conditions of oxidative stress (Najeeb *et al.*, 2012). Oxidation LDL resulting from increased oxidative stress is an essential early event in the pathogenesis of atherosclerosis which is a major complication of hypertension. Hence, lipid profile has been reported to be the important predictor for metabolic disturbances including hypertension, dyslipidaemia, diabetes, hyperinsulinaemia and cardiovascular diseases (Wali *et al.*, 2012).

In our study, we observed that the level of hepatic MDA formation in rats treated groups followed the decreasing order : *M.communis* treated group > *C.azarolus* treated group > *O.glandulosum* treated group. Whereas the renal MDA level were in this order: *M.communis* treated group > *O.glandulosum* treated group > *C.azarolus* treated group.

These observed results may be due to the high polphenolic contents of the plant extracts. Many *in vivo* studies suggested that polyphenols and flavonoids rich extracts could

exhibit antioxidant ability through affecting the production of free radicals in the progress of aerobic respiration and lipid peroxidation in the organism. The antioxidant mechanism used by these reductones breaks free radical chain reactions by donating a hydrogen atom, or by reacting with certain precursors to prevent peroxide formation (Jiang *et al.*, 2011). Therefore, antioxidants can delay or prevent the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. Different antioxidants have a wide range of capacities to scavenge various reactive oxygen species (Wang and Chen, 2010).

Accordingly, a lower level of oxidative stress and improvement in antioxidant status was found in many researches after polyphenol-rich food administration mainly based on the reduction of TBARS levels (Bernabé *et al.*, 2013; Boaventura *et al.*, 2013; Athiroh *et al.*, 2014; Kardum *et al.*, 2014).

Flavonoids such as catechin or quercetin may directly capture $O_2^{\bullet -}$, H_2O_2 or $HOCl$ and may also chelate and remove transition metal ions such as iron or copper, thus avoiding the formation of ROS produced by the Fenton reaction and inhibiting lipid peroxidation (Quiñones *et al.*, 2013).

The differences in the inhibition of lipid peroxidation between plant extracts may be attributed to their different phenolic contents and compositions (Bonarska-Kujawa *et al.*, 2011). Furthermore, changes in structure of isolated phenolic compounds, such as glycosylation, resulted in a decrease of antioxidant activity, probably due to its weaker binding to biological membranes (Kardum *et al.*, 2014).

Moreover, the reduction of the lipid peroxidation level in groups treated with methanolic extract of *C.azarolus* and *O.glandulosum* could be due to the increase in the antioxidant status, resulting in an increase in the activity of catalase and concentration of the

GSH compared to the control group (Figure. 65 and 66). It was reported that if the catalase and GSH activities are not sufficiently enhanced to metabolize hydrogen peroxide, this can lead to increased hydrogen peroxide and TBARS levels. It is noteworthy that, in mice supplemented with ferulic acid and clofibrate, the increase of antioxidant enzyme activities resulted in significant reduction of hepatic TBARS levels compared with the control group (Khalifa and Elmazny, 2013).

5. Hypotensive effects of extracts in anesthetized rats

The present study demonstrated for the first time that the ME and EAE of *C. azarolus*, *M. communis* and *O. glandulosum* possessed hypotensive effect, thus underscoring their potential usefulness for the management of hypertension, especially in the reduction of cardiovascular complications, morbidities or mortalities that may accompany adverse changes in any or a combination of systolic, diastolic, pulse and mean arterial pressures.

Our results revealed that the intravenous administration of all plant extracts experimented at the range doses from 0.04 to 12 mg/kg body weight induced dose-dependent and transitory decrease in systolic, diastolic blood pressure and mean arterial blood pressure of the anesthetized rats (Appendix F).

At the maximal dose of the ME (12 mg/kg) injected, average SBP and DBP were decreased significantly by ($28.12 \pm 2.34\%$; $26.50 \pm 2.03\%$), ($18.67 \pm 2\%$; $21.57 \pm 3.17\%$) and ($14.24 \pm 2.69\%$; $15.67 \pm 4.31\%$), respectively for *C. azarolus*, *M. communis* and *O. glandulosum* (Figure.68), whereas a percent decrease in SBP and DBP for EAE was about (38.29 ± 5.69 ; $41.54 \pm 5.13\%$), ($30.46 \pm 2.88\%$; $33.51 \pm 1.07\%$) and ($21.44 \pm 2.58\%$; $22.33 \pm 4.26\%$), respectively *C. azarolus*, *M. communis* and *O. glandulosum* (Figure.68).

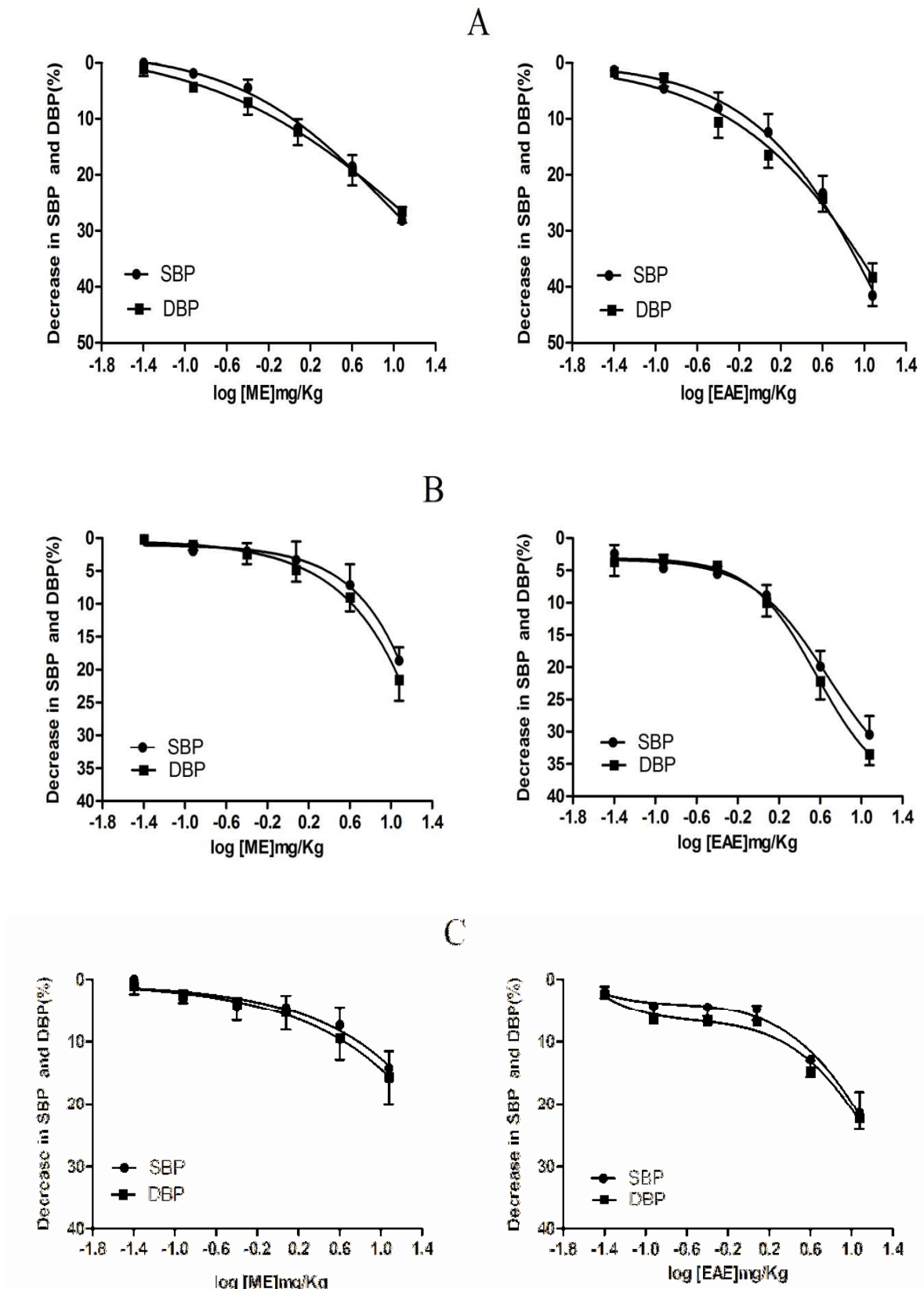


Figure. 68: Concentration-response curves of ME and EAE of different plants on systolic (SBP) and diastolic (DBP) blood pressure of anesthetized rats. (A): *C. azarolus*; (B); *M. communis*; (C): *O. glandulosum*. Values are means $\pm$ SEM (n=6).

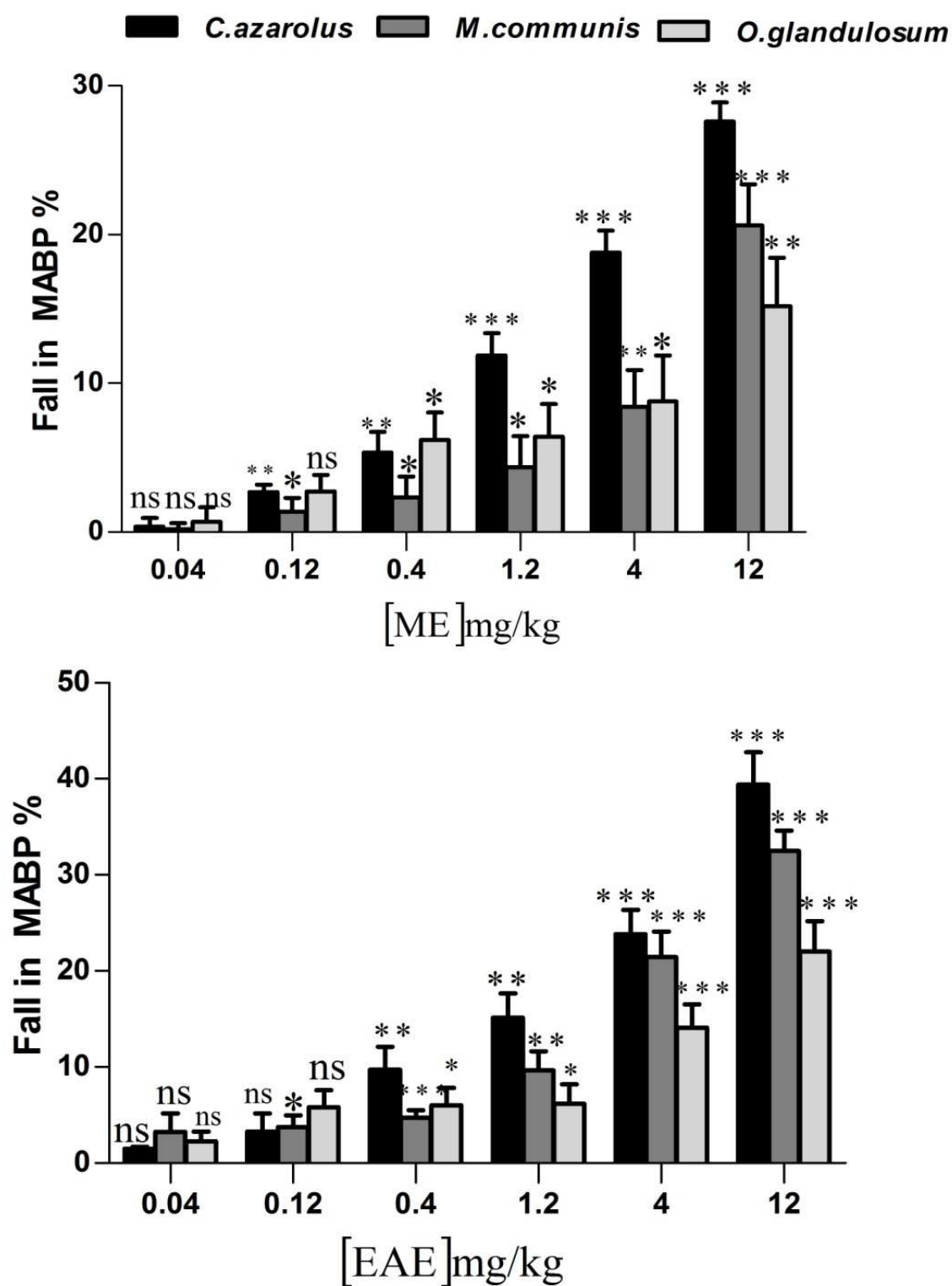


Figure. 69: Dose-dependent hypotensive effect of ME and EAE of *C. azarolus*, *M. communis* and *O. glandulosum* on mean arterial blood pressure (MABP) in anaesthetized rats. Values shown are means $\pm$ SEM (n=6). (ns: no significant difference; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

The hypotensive response at dose of 12 mg/kg was found to be more potent with the EAE for which the maximal fall in MABP was about ($39.37 \pm 3.41\%$), ($32.49 \pm 2.88\%$) and, ($22.04 \pm 3.14\%$) for *C. azarolus*, *M. communis* and *O. glandulosum*, respectively in comparison with the maximal fall observed with the ME; ($27.58 \pm 1.3\%$), ($20.6 \pm 2.78\%$), and ($15.19 \pm 3.23\%$) for *C.azarolus*, *M.communis* and *O. glandulosum*, respectively (Figure.69).

From the above results, it was found that ME from *C.azarolus* and *M.communis* decreased significantly ($p < 0.05$) the MABP at all doses except for 0.04 mg/kg dose, whereas ME from *O. glandulosum* decreased significantly the MABP at the dose of 0.4 mg/Kg to 12 mg/kg.

Similarly, it was found that EAE from *C.azarolus* and *O.glandulosum* decreased significantly the MABP ($p < 0.05$) at the dose of 0.4 mg/kg to 12 mg/kg, whereas EAE from *M.communis* decreased the MABP at the dose of 0.12 mg/kg to 12 mg/kg.

The results obtained from the hypotensive effect of *C. azarolus* are consistent with the effect of related *Crataegus* species in various hypertensive rat models and in clinical studies. It has been shown that the injection of an aqueous methanol extract (70 %) of the flowering tops of *C. meyeri* (Garjani *et al.*, 2000) and aqueous extract of *C. aronia* (Shatoor, 2013) caused a dose-dependent reduction in mean arterial blood pressure in Wistar rats. Similar results were also obtained for the aqueous extract of *C. tanacetifolia* leaves and its hyperoside-rich fraction which significantly attenuated the increase in blood pressure in (L-NAME)-induced hypertensive rats (Koçyildiz *et al.*, 2006). Moreover, Chen *et al.* (1998) demonstrated that ethanol extract from the fruits of *Crataegus* spp. counteracted vasoconstriction in U46619- precontracted rat mesenteric arteries, suggesting an increased production of NO by the endothelial cells as the key mechanism of this vasodilating effect.

Reportedly, in a double-blind, randomised, placebo-controlled clinical trial, supplementation with an extract of the flowering tops of *C. laevigata*, 1200 mg daily for 16 weeks, reduced diastolic blood pressure compared with placebo in hypertensive type 2 diabetes patients (Walker *et al.*, 2006). This finding was also noted by Asgary *et al.* (2004) in subjects with primary mild hypertension treated with an aqueous ethanol extract of leaves and flowers of *C. curvisepala* for three months.

The leaves of *M. communis* and *O. glandulosum* are commonly used in traditional Algerian medicine as antihypertensive therapy, but the pharmacological evidences of their activity are lacking. Therefore, our major finding of this study is that the hypotensive effect of ME and EAE from *M.communis* and *O. glandulosum* leaves was reported for the first time.

Our data reveal that EAE elicited a significant hypotensive response than ME. These significant effects may be solidly related to their phenolic and flavonoids contents and the effect of the major compounds identified in these extracts.

Previous studies reported that these secondary metabolites are capable of exerting their antihypertensive effects by different pathways, such as reduction of intracellular Ca^{2+} , endothelium dependent vasodilation involving NO production (Akhlaghi and Bandy, 2009; Torres-Piedra, 2011; Nugroho *et al.*, 2013). NO exerts its vasodilator effects by activating soluble guanylyl cyclase in vascular smooth muscle cells and the subsequent increase in cGMP. Several flavonoids have also been reported to inhibit several PDE isoforms. Thus, inhibition of PDEs may represent another potential mechanism for flavonoid induced prevention of endothelial dysfunction. In addition, quercetin and its sulfated metabolite selectively enhanced cyclic-GMP-dependent relaxations by a mechanism not involving PDE 5 inhibition (Suri *et al.*, 2010; Ojeda *et al.*, 2010).

Furthermore, some studies have shown that these compounds may even inhibit the angiotensin converting enzyme and reduced oxidative status caused by the antioxidant capacity of these compounds which could also explain their vasodilator and cardioprotective effects (Schmitt and Dirsch, 2009; Alarcón-Alonso *et al.*, 2012).

It was reported that chronic administration of antioxidant flavonoids such as quercetin reduce blood pressure and enhance endothelium-dependent relaxation in various animal models of hypertension. This vascular beneficial effects of quercetin and related bioflavonoids were suggested to derive from their protective antioxidant effects which lead to improved bioavailability of endothelium-derived NO (Belmokhtar *et al.*, 2009).

In this study, the phytochemical screening confirmed the presence of two flavonoids from *C. azarolus* ((+) - catechin and hyperoside), one from *M. communis* (myricetin -3-O-rhamnoside) and one from *O. glandulosum* (katuranin), thus, these bioactive principles, at least in-part, may be responsible for the observed hypotensive effect of the studied plants.

Hawthorn extracts have been reported to induce endothelium-dependent NO-mediated vasodilatation (Brixius *et al.*, 2006; Shatoor *et al.*, 2012) and inhibition of Ca^{2+} influx to the smooth muscle (Chen *et al.*, 1998). Indeed, hyperoside has been shown to inhibit calcium influx induced by the activation of G-protein-coupled receptors leading to vasorelaxant effect (Li *et al.*, 2013). The findings of Schroeter *et al.* (2006) suggest that oral administration of pure (-)-epicatechin showed similar acute vascular effects as catechin-rich cocoa. These effects were likely due to augmentation of NO synthesis by flavanols.

The observed hypotensive is more important with the EAE from *C. azarolus* and *M. communis* in comparison with the maximal hypotensive observed with EAE from *O. glandulosum* which have the higher phenolic contents. However, the effect of ME

from *M. Communis* is less important than that of ME from *C. azarolus* which have lower phenolic contents, and that of ME from *O.glandulosum* is even less. In light of these results, it appears that our study confirmed the finding of heim *et al.* (2002) who reported that the chemical structure of flavonoids affects their metabolism and biological activity.

Structure-activity relationships have been proposed for vasorelaxing activity (Calderone *et al.*, 2004). Thus, series of flavones like 3-hydroxyflavone, 6- hydroxyflavone, 7-hydroxyflavone, chrysin have been evaluated for *ex-vivo* and *in-vitro* vasorelaxant effect, therefore, can be the drugs of interest as novel antihypertensive agents. As example of this, it was found that intravenous administration of isoquercitrin (0.5–4 mg/kg), but not kaempferol, caused a dose-dependent reduction in MAP (Gasparotto Junior *et al.*, 2012). It seems that all the flavone derivatives possess endothelium-dependent vasorelaxant effect, with an increased production of NO and prostacyclin PGI₂ in a concentration-dependent manner (Akhlaghi and Bandy, 2009; Torres-Piedra *et al.*, 2011).

Hydroxyl group at C-5 and C-7 positions in the ring-A, C-4 carbonyl group, C2=C3 double bond and hydroxyl groups at different positions in ring-B are important features for the vasorelaxant activity, while the presence of C-glycosyl group at C-8, hydroxyl group at the C-3 position, greatly reduce relaxation effect (Singh *et al.*, 2014).

On the other hand, bulky substituent's at ortho- and meta- positions on ring-B of flavone derivatives decrease the potency of vasorelaxant activities. 5, 7-dihydroxy-3'-bromo-flavone exhibited the highest vasodilator activity (Singh *et al.*, 2014). It was reported also that prenyl groups could increase the lipophilicity and confer to the molecule a strong affinity to biological membranes, and result in the significant enhancement of bioactivities. Hydrophobic substituent's (as Br and trimethoxyl) in ring-B of flavone exhibit medium activities, while the 3-hydroxyl substituent attenuated the relaxation activity (Dong *et al.*, 2008).

The most effective vasorelaxing agents, luteolin and apigenin exhibit lower energy, and small molecular volume size so have diverse interactions like steric and electronic between the compounds and receptors (Xu *et al.*, 2007; Singh *et al.*, 2014).

6. Diuretic activity of plant extracts

Diuretics, either alone or in combination with other drugs, are valuable in the treatment of hypertension, congestive heart failure, pulmonary edema, nephritic syndrome, renal failure, and pregnancy toxemia. Two widely used diuretics, thiazides and the high ceiling loop diuretic as furosemide, have been associated with a number of adverse effects, such as, electrolyte imbalance, metabolic alkalosis, hyperuricemia, hypomagnesaemia, metabolic alterations, development of new-onset diabetes, and activation of the rennin–angiotensin–neuroendocrine systems. (Lahlou *et al.*, 2007; Kateel *et al.*, 2014). Hence, there is a need for new diuretics with lower potential for adverse effects, such as the plant-based substances which are considered to be relatively safe.

In the present study, the diuretic effect of orally administered methanolic extract of *C. azarolus*, *M. communis* and *O. glandulosum* was evaluated in normal rats after one oral dose for 5 hours. The pharmacological response was compared with that produced by control group. The effect on electrolyte balance was also determined. The mechanism of action by which diuresis was induced by these plant extracts was also assessed by comparing their effect with that of furosemide, a high-ceiling loop diuretic, which is widely used in clinical practice

6.1. Effect of plant extracts on urinary output in rats

The results of diuretic assay are summarized in Table. 3. The volume of urine excreted by rats in 5 h for control group treated with normal saline (5 ml/Kg) was compared with other groups. Furosemide (20 mg/Kg) was used as standard diuretic, which increased urine volume significantly by 297.02% ($p < 0.001$), when compared to the saline treated control group.

The results demonstrated that both *C.azarolus* and *O.glandsulosum* extracts showed an interesting dose-dependent diuretic activity. After administration doses of 100 , 300 and 600 mg/kg of ME from *C. azarolus*, the urinary output of the experimental groups was remarkably higher than that of the control group (78.73%; $p < 0.05$, 201.11%; $p < 0.01$ and 262,45 %; $p < 0.001$, respectively).

Also, the three doses of ME of *M.communis* (100, 300 and 600 mg/kg) produced a slight increase in urine excretion (3.11%, 42% and 43.38 %, respectively), but did not arrive at statistical significance in 5 h. Therefore, we suggest that this extract does not exhibit diuretic activity.

ME of *O. glandulosum* at doses of 100 and 300 mg/kg did not significantly increase the urine output (15.98% and 37.54%, respectively). However, the dose of 600 mg/kg contributed to remarkable urine excretion (76.95%) with statistical significance ($p < 0.01$) when compared to control group.

Table. 3: Effects of ME of *C. azarolus* , *M. communis* and *O. glandulosum* , normal saline and furosemide on urine output, pH and electrolytes excretion in rats.

Experimental group/dose	Volume(ml /rat)	pH	Urine electrolytes level(mmol/l)			Na ⁺ /K ⁺
			Na ⁺	K ⁺	Cl ⁻	
Control (5ml/kg)	2.69±0.25	6.41±0.36	64.8±5.47	51.50± 2.17	66.20±4.64	1.27±0.13
<i>C.azarolus</i> (100mg/ml)	5.05±0.71*	5.58±0.08	89.6±2.22**	48.28±1.86	79.60±3.93	1.86±0.09**
<i>C.azarolus</i> (300mg/kg)	8.1±1.51**	5.92±0.12	113.2±18.8**	44.53± 0.51	86±6.09*	2.54±0.39**
<i>C.azarolus</i> (600 mg/kg)	9.57±0.60***	7.60±0.30	1405± 106.5***	456.7± 65.91***	4011± 466.1***	3.47±0.43**
<i>M.communis</i> (100mg/kg)	2.77±0.80	6.09±0.20	63.20±5.61	54.54±4.68	85.00±2.00	1.15±0.08
<i>M.communis</i> (300mg/kg)	3.82±0.69	5.97 ± 0.14	73.40 ± 2.97	64.68 ±1.43*	76.75±1.31	1.12 ±0.05
<i>M.communis</i> (600mg/kg)	3.85 ±0.55	7.47±0.32	76.80±6.58	66.83±2.25*	72.89±14.95	1.14±0.13
<i>O.glandulosum</i> (100mg/kg)	3.12±0.18	6.17±0.25	77.25±0.62	58.22±4.31	58.80±5.40	1.35±0.07
<i>O.glandulosum</i> (300mg/kg)	3.70±0.37	6.02±0.08	84.40±1.50**	52.88±2.29	80.80±7.46	1.61±0.08
<i>O.glandulosum</i> (600mg/kg)	4.76±0.64*	6.386±0.21	92.00±3.82**	35,54± 2.25***	155.6±22.80**	2.64± 0.24**
Furosemide (20mg/kg)	10.67±0.89***	5.67±0.05	116.6±12.1**	46.63±2.08	645.4±94.43***	2.72±0.34**

The values are mean ± SEM of five determinations. P-values are compared with control group as * p< 0.05, **p< 0.01, ***p< 0.001 .

6.2. Effect of plant extracts on urinary electrolyte excretion and urinary pH

The results demonstrated that each plant extract dose had a different effect on electrolytes excretion (Table. 3). Furosemide increased Na⁺ and Cl⁻ excretion (79.93%; p <0.01 and 847.92%; p <0.001, respectively) but not that of K⁺: actually there was a small decrease in urinary concentration of K⁺ (-9.45%) when compared to control group.

ME of *C.azarolus* doses (100, 300 and 600 mg/kg) significantly increased Na^+ excretion (38.27 %; , $p < 0.01$, 74.69% ; $p < 0.01$ and 2068.51% ; $p < 0.001$) and only the doses; 300 and 600 mg/kg were found to increase significantly Cl^- excretion (29%; $p < 0.05$ and 5342.29 %; $p < 0.001$). Also, ME of *C.azarolus* at doses of 100 and 300 mg/kg decreased K^+ excretion (6.26 % and -13.53%), but this decrease did not reach statistical significance; however, 600 mg/kg dose increased significantly the kaliuresis when compared to control group (786.79 %; $p < 0.001$). Similarly, this dose caused more urinary Na^+ , K^+ and Cl^- excretion than the standard diuretic; furosemide ($p < 0.001$).

Administration of ME of *M. communis* at doses of 300 and 600 mg/kg increased slightly natriuresis (13.27% and 18.51%), whereas 100 mg/kg dose decreased urinary Na^+ excretion by -2.46% compared to control group. Moreover, the three doses (100, 300 and 600 mg/kg) caused an increase in Cl^- excretion (32.93%, 22.05% and 28.39%), but in all cases, they still had statistically no significant difference when compared to control group. The doses (300 and 600 mg/kg) increased K^+ excretion (25.59% and 29.76%, respectively; $p < 0.05$) without alteration of the Na^+/K^+ ratio.

The Na^+ excretion was increased remarkably ($p < 0.01$) by the 300 and 600 mg/kg doses of ME of *O.glandulosum* (30.24 % and 41.97 %, respectively), which was in agreement with the urine output. A 600 mg/kg dose produced a pronounced decrease in K^+ excretion (30.99%; $p < 0.001$) and strong increase in Cl^- (135.04 %; $P < 0.01$).

The ratio Na^+/K^+ determines the natriuretic activity and value equal or greater than 2 indicates favourable natriuretic effect (Jabeen and Aslam, 2013). The Na^+/K^+ ratio of all treated groups was calculated in the range of 1.12 and 3.47.

Among all administered doses, the doses of 100 , 300 and 600 mg/kg of *C. azarolus* and the dose of 600 mg/kg of *O. glandulosum* increased significantly ($p < 0.01$) the Na^+/K^+ ratios, which were similar to that of reference diuretic; furosemide which had Na^+/K^+ ratio of 2.72 ± 0.34 .

Concerning urinary pH, a weak acidification in urinary pH profile was observed after 5 h duration following treatment with furosemide and different doses of ME of selected plants except for 600 mg/kg dose of *C. azarolus* and *M. communis* which had pH values of 7.60 ± 0.30 and 7.47 ± 0.32 , respectively , but they were too weak to reach statistical significance compared to control group which had pH value of 6.41.

In the present study, ME of *C. azarolus* and *O. glandulosum* , each, showed significant diuresis in rats after a single dose for 5 h when compared with the reference diuretic drug, furosemide (20 mg/kg). While, the different results obtained on electrolytic excretion by both extracts suggest a difference in their comparative diuretic profile. As result, all evidences in this work corroborate the ethnopharmacological application of *C.azarolus* and *O.glandulosum* and may lay the foundation for the use of these two plant species in folk medicine as diuretic agents. In our study, the diuretic activity of ME of *C. azarolus*, *M. communis* and *O. glandulosum* was reported for the first time .

Concerning *C. azarolus*, our result is consistent with the finding of Dizaye *et al.*, (2011) who reported that intravenous infusion of a single dose of procyanidine isolated from related species, *C. monogyna* (50 mg/kg i.v), an Iraqi endogenous plant, produced marked diuretic and natriuretic effects without any significant actions on the potassium excretion in the rats.

Also, diuretic effect of *O.glandulosum* obtained in this study, is in agreement with other previous studies reported by Khan *et al.* (2011) who stated that crude extract of *O.vulgare* from Pakistan increased significantly ($p < 0.05$) the urine output in Wistar rats at the dose of 10 mg/kg, while there was no effect seen at lower (3 mg/kg) or higher dose (30 mg/kg).

Diuresis has two components: increase in urine volume (water excretion) and a net loss of solutes in the urine. These processes result from suppression of renal tubular reabsorption of water and electrolytes into the blood stream. The reference drug, furosemide, increases urine output and urinary excretion of sodium by inhibiting $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ symporter (co-transporter system) in the thick ascending limb of the loop of Henley, while the thiazide diuretics inhibit the Na^+/Cl^- symporter (co-transporter system) in the distal convoluted tubule, by competing for the Cl^- binding site, and increasing the excretion of Na^+ and Cl^- (Mekonnen *et al.*, 2010; Bhavin *et al.*, 2011; Gasparotto Junior *et al.*, 2012). In this study, the mechanism of the diuretic action of ME *C.azarolus* and *O.glandulosum* was investigated by its comparison with the effects of furosemide, the reference drug.

In accordance to the finding of Lahlou *et al.* (2007) and Gasparotto Junior *et al.* (2009), the current results have shown that furosemide (20 mg/kg, p.o.) increases the volume of urine and excretion of sodium and chloride but not that of K^+ .

Although the thiazide diuretics, like hydrochlorothiazide, increase only the urinary K^+ and Na^+ levels (Jalalpure and Gadge, 2011), the administration ME of *C.azarolus* increased urinary K^+ levels along with urinary Na^+ and Cl^- levels. Further, the urine was slightly acidified and Na^+/K^+ ration was altered. These features strongly suggest that ME appears to have several active components, with at least one having a furosemide-like activity and the other a thiazide-like activity. Hydrochlorothiazide has been reported to increase the

urinary excretion of both Na^+ and K^+ by 50–60% over controls after a single oral dose in normal rats (Benjumea *et al.*, 2005; Lahlou *et al.*, 2007).

Also, the current results have shown that ME from *O.glandulosum* had furosemide-like effect, and the higher dose of 600 mg/kg produced electrolyte and water excretion profile qualitatively similar to that of furosemide, albeit at a lower potency, due to the crude nature of the extract. Furthermore, at the same dose, ME decreased significantly K^+ excretion with alteration of Na^+/K^+ ratio. Based on this pattern of excretion of water and electrolytes, it appears that there are at least two types of active principals present in ME, one having a frusemide-like activity and the other as potassium sparing diuretic. However, further systematic phytochemical studies are necessary to elucidate the probable structure activity relationship of biomolecules.

Previous studies demonstrated that several secondary metabolites of plants could be responsible for the diuretic effects, such as alkaloids, tannins, flavonoids, saponins, terpenoids or organic acids (Jabeen and Aslam, 2013). Thus, phenolic and flavonoids compounds detected in *C. azarolus* and *O. glandulosum* may be considered as responsible for this effect because of their high phenolic contents.

The contribution and responsibility of flavonoids and caffeic acid derivatives to diuretic properties have been reported previously (Arafat *et al.*, 2008). Therefore, it is worth noting that both flavonoids and caffeic acid derivatives presented in this two extracts may account for the prominent effect of the ME over the diuresis parameters and these components may act synergistically.

Several isoflavonoids, including genistein and daidzein, have been reported to cause inhibition of the $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransporter, as well as an increase in natriuresis and kaluresis.

Moreover, the flavonoid crisine has been shown to induce a significant increase in urine flow, glomerular filtration and Na^+ and K^+ excretion.

Recently, it was reported that seven methoxyflavonoids actively bound to adenosine receptor A1, provoking antagonism and therefore diuresis and sodium excretion (Montejano-Rodríguez *et al.*, 2013). In the same way, isoquercitrin (0.5 – 4 mg/kg, i.v.) exhibited an antihypertensive effect involving the inhibition of the angiotensin-converting enzyme and a diuretic effect comparable with that of standard diuretics (Gasparotto Junior *et al.*, 2012). In addition, chronic administration of quercetin has been reported to inhibit ATPase activity in rats, which impacts the Na^+/K^+ concentration gradient in epithelial cells of the nephron tubular segment (Alarcón-Alonso *et al.*, 2012).

In fact, there is an association between urine volume and Na^+ concentration in the urine. This is logical, considering that the action mechanism of a great number of diuretics on the market is by stimulation of regional blood flow or initial vasodilation through NO and l-arginine stimulation (Alarcón-Alonso *et al.*, 2012), or by producing inhibition of tubular reabsorption of water and anions, which induces osmosis of water and electrolytes out of the organism, resulting in diuresis (Boffil *et al.*, 2006; Patel *et al.*, 2009, Montejano-Rodríguez *et al.*, 2013). Taken together, these findings support the potential of ME of *C.azarolus* and *O.glandulosum* as a candidate to be a phytomedicine used in those cardiovascular diseases mainly hypertension where increased diuresis is desired.

Although, ME of *M. communis* is rich in phenolic and flavonoid contents (260.44 mg GAE/ g of Extract and 26.77 QE/g of Extract, respectively) but did not significantly increase the urine and urinary electrolytes outputs, in spite of moderate increase of K^+ at dose of 600 mg/kg.

It was reported by Nilveses *et al.* (1989) that the increase of urinary output in rats might result from high potassium content in the plant extract. The diuretic effect may be due to this potassium excess which acts individually or synergistically with natural products such as flavonoids, saponins and organic acids. Thus, it is probably that the observed effect of *M. communis* might be due to K^+ , present in considerable quantities in the plant extract, which exert their action on renal epithelium. The theory that the majority of the medicinal plants have a diuretic effect only due to the presence of the potassium seems somewhat doubtful. In fact, pharmacodynamic studies performed on medicinal plants emphasized that frequently no correlation exists between the diuretic effect observed and the K^+ content of the extract (Sadki *et al.*, 2010).

Also, we hypothesize that the obtained result might be due to the weak bioavailability of active constituents of *M. communis*. It was reported that the oral dosing could affect the bioavailability and metabolism of compounds due to intestinal absorption and hepatic transformation of the active components, which may influence their pharmacological effects (Aissaoui *et al.*, 2008; Valentova *et al.*, 2014). It is also well known that the *in vivo* bioavailability and biological activity of a compound depends not only on its mere solubility, but also on the interphase partitioning. Lipophilicity and hydrophilicity controls protein and membrane interactions, transport and binding activity, influencing absorption and excretion of the compound (Amira *et al.*, 2008; Valentova *et al.*, 2014). Flavonoid glycosides with high solubility and low permeability are commonly hydrolyzed to their aglycones to produce effects *in vivo*. Thus, deglycosylation by small intestinal epithelial cell β -glucosidases is a critical step in the absorption and metabolism of flavonoid glycosides (Xiao *et al.*, 2014).

Conclusion and perspectives

Medicinal plants typically contain mixtures of different secondary metabolites that may act individually, additively or in synergy to improve health and protect the body against various diseases, including cancer, cardiovascular, and neurodegenerative diseases. Such a therapy prevents or minimizes the appearance of the side effects of drugs intake. For this reason, natural products have received huge attention in recent years, and there has been a worldwide surge towards the identification and use of phytotherapeutic principles from these medicinal plants, which will be of a great contribution for pharmaceutical industry. The present study was undertaken to valorize *C.azarolus*, *M. communis* and *O. glandulosum* extracts as antioxidant, hypotensive and diuretic agents.

Phytochemical analysis of ethyl acetate extract from these selected plants revealed the isolation of five compounds; two flavonoids were isolated from *C. azarolus* ((+) - catechin and hyperoside), one flavonol glycoside was isolated from *M.communis* (myricetin -3-O-rhamnoside), one flavanone (katuranin), and one monoterpene (5-isopropyl-3-methylphenol) were isolated from *O. glandulosum*. The compound, (+) - catechin was isolated for the first time from *C. azarolus* , and the compound; 5-isopropyl-3-methylphenol was isolated for the first time from the genus *Origanum*.

Phenolic compounds are widely distributed in plants and have gained much attention due to their antioxidant activities and free radical scavenging abilities. Results obtained in the present study revealed that the level of these phenolic compounds in various extracts of plants were considerably higher in EAE than that in other extracts, whereas CHE of *M.communis* and *O.glandulosum*, and EAE of *C.azarolus* contained the higher amount of flavonoids. These

findings could be due to different degree of polarity of the solvents used for the extraction of polyphenolic compounds, and to the nature of compounds present in these plant extracts.

A number of methods have been developed and applied for the assessment of the antioxidant capacity. However, evaluation of antioxidant potentials of plant products or antioxidant compounds cannot be carried out accurately by any single universal method. Thus, we applied several antioxidant assays that would provide a better insight into the true antioxidant potential of the extracts. In our study, the extracts of selected plants seem to be of real and potential interest due to their huge antioxydant activities which were established *in vitro* as well as *in vivo*.

It can be noted that different extracts from *C. azarolus*, *M. communis* and *O. glandulosum* exhibited different, but effective degrees of *in vitro* antioxidant activity using different methods. Among all extracts, ethyl acetate extract was shown to possess significant radical scavenging activity against DPPH, ABTS, hydroxyl radicals and reducing power, evidencing its ability of having extracted a considerable amount of polyphenols and flavonoids with specific structure with many hydroxyl groups. Remaining extracts (ME, CHE and AqE) can't be considered to be inferiors because satisfactory phenolic contents were obtained in this study and enough to show high antioxidant activity.

Concerning the metal chelating activity, AqE and ME from the three studied plants showed better activity, while the lowest chelating effects on ferrous ions were showed for CHE and EAE which had the highest phenolic content. These obtained data were confirmed by low correlation between phenolics content and chelating activity, suggesting that polyphenols might not be the main ion chelators or the chelating activity could be due to different qualitative and quantitative compositions of phenolic constituents. It is very

important to evaluate the molecular mechanisms of the radical-scavenging activities of polar extracts for better understanding of the mode of action.

Lipid peroxidation is a marker of oxidative damage to membranes, producing derivatives such malonaldehyde that can be used as biomarker to quantify the amount of damage in the cell. We therefore tested the ability of different plant extracts to inhibit lipid peroxidation by three methods (β -carotene bleaching test, FTC method and TBA method). The results showed that all plant extracts inhibited lipid peroxidation, but apolar extracts (CHE and EAE) were found to be more effective than the polar extracts (ME and AqE) in the three tested methods.

To confirm the antioxidant potential of the methanolic extract of *C. azarolus*, *M. communis* and *O. glandulosum*, *in vivo* antioxidant properties were investigated. The results showed that the oral administration of methanolic extract of the three selected plants for 14 days at a daily dose of 100 and 200 mg/kg body weight resulted in an increase in the total plasma antioxidant capacity. It was found that 100 and 200 mg/kg doses of ME from *C. azarolus* and 200 mg/kg dose of ME from *O. glandulosum* increased significantly total plasma antioxidant capacity using DPPH radical scavenging test. Also, all extracts doses showed a good reducing power, but the higher electron donation capacity was noticed for *C. azarolus* and *O. glandulosum* at the dose of 200 mg/kg.

Furthermore, the administration of different extracts showed an improvement of antioxidant status in tissues (liver and kidneys) which might help to better cope with oxidative stress related diseases such hypertension. But, it was found that *C. azarolus* extract was more effective in increasing the catalase activity and GSH level, and decreasing lipid peroxidation by lowering MDA level. These differences between the *in vivo* antioxidant

activity of plants could be due to their intrinsic antioxidant potential but the bioavailability of the polyphenols present in each extract could be also involved.

In this study, the hypotensive effect of methanol and ethyl acetate extracts from *C. azarolus*, *M. communis* and *O. glandulosum* leaves was reported for the first time, providing a rational basis to understand the use of these plants in hypotensive treatment in folk medicine. It was found that the intravenous administration of ME and EAE at the range doses of 0.04 to 12 mg/kg body weight induced dose-dependent and transitory decrease in mean arterial blood pressure in anesthetized rats. EAE showed a significant hypotensive effect than ME and this is most possibly due to its high total contents of phenolic compounds and flavonoids.

Several plants used for the treatment of hypertension have shown diuretic activity when tested on animal models. The present study evaluated the diuretic effect of *C.azarolus* and *O.glandulosum* for the first time. Both plant extracts enhanced the urine output and the electrolytes excretion of sodium and chloride. This finding may explain the blood pressure lowering effect of these two plants which, at least in part, may be mediated through their diuretic activities. Further studies are recommended to find out the involvement of other possible mechanisms in their blood pressure lowering activity.

M.communis did not alter the urine output and this may be due to the low administered doses which may be higher than 600 mg/kg or may be due to the biological properties of polyphenols which are greatly dependent on their bioavailability. Although flavonoids display a potent antioxidant activity *in vitro*, the bioactive forms of flavonoids *in vivo* are not those forms found in plants due to their extensive biotransformation in the small intestine and hepatic metabolism on absorption.

In conclusion, the key research areas to be considered in future in relation to the present study include:

- Investigation of the cellular and molecular mechanisms through which plant extracts act in arterial hypertension and diuresis.
- Isolation and identification of more active constituent (s) responsible for the high observed antioxidant and pharmacological activities.
- The use of these extracts as food additives represents one of our future aims.
- Studying the toxicity of the selected plants.
- Investigating *in vivo* effect of plant extracts on oxidative stress induced hypertension, such as angiotensin II-mediated hypertension, and nitric oxide synthase inhibitor-induced hypertension to further confirm the advantageous quality of these plant extracts to treat hypertension.
- Other *in vivo* kinds of antioxidant assays are needed to evaluate lipid peroxidation and the antioxidant enzymes levels in plasma and aorta.

References

- Abdalla S, Abu Zarga M, Sabri S.** 1994. Effects of flavone luteolin, isolated from *Colchicum ritchii*, on guinea pig smooth muscle and heart and on blood pressure and blood flow. *Phytother Res*, 8(5): 265-270.
- Abdul Ghani A, Amin R, Suleiman M.**1987. Hypotensive effect of *Crataegus oxyacantha*. *Int J Crude Drug Res*, 25: 216-220.
- Abdul Kareem M, Krushna G S, Hussain S A, Devi K L.** 2009. Effect of aqueous extract of nutmeg on hyperglycaemia, hyperlipidaemia and cardiac histology associated with isoproterenol-induced myocardial infarction in rats. *Trop J Pharm Res*, 8(4):337–44.
- Abou Zid S and Mohamed L.** 2011. Survey on medicinal plants and spices used in Beni-Sueif. *J Ethnobiol Ethnomed*, 7: 18-23.
- Aburjai T, Hudaib M, Tayyema T, Yousef M, Qishawi M.** 2007. Ethnopharmacological survey of medicinal herbs in Jordan, the Ajloun heights region. *J Ethnopharmacol*, 110:294-304.
- Adedapo A A, Jimoh F O, Afolayan A J, Masika PJ.** 2008. Antibacterial and antioxidant properties of the methanol extracts of the leaves and stems of *Calpurnia aurea*. *BMC Complement Alter Med*, 8: 53-58.
- Aidi Wannes W, Mhamdi B, Sriti J, Ben Jemia M, Ouchikh O, Hamdaoui G, Kchouk M E.** 2010. Antioxidant activities of the essential oils and methanol extracts from myrtle (*Myrtus communis* var. *italica* L.) leaf, stem and flower. *Food Chem Toxicol*, 48(5):1362–70.
- Aissaoui A, Jaouad E, Zafar H I, Badiâ L.** 2008. Acute diuretic effect of continuous intravenous infusion of an aqueous extract of *Coriandrum sativum* L. in anesthetized rats. *J Ethnopharmacol*, 115: 89–95.
- Akhlaghi M and Bandy B.** 2009. Mechanisms of flavonoid protection against myocardial ischemia–reperfusion injury. *J Mol Cell Cardiol*, 46: 309–317.
- Akila M and Devaraj H.** 2008. Synergistic effect of tincture of *Crataegus* and *Mangifera indica* L. extract on hyperlipidemic and antioxidant status in atherogenic rats. *Vasc Pharmacol*, 49: 173–177.
- Akilavalli N, Radhika J, Brindha P.**2011. Hepatoprotective activity of *Ocimum sanctum* linn. against lead induced toxicity in albino rats. *Asian J Pharm Clin Res*, 4 (2): 84-87.
- Aktumsek A, Zengin G, Guler J O, Cakmak Y C, Duran A.** 2013. Antioxidant potentials and anticholinesterase activities of methanolic and aqueous extracts of three endemic *Centaurea* L. species. *Food Chem Toxicol*, 55: 290–296.
- Alarcón-Alonso J, Zamilpa A, Alarcón Aguilar F, Herrera-Ruiz M, Tortoriello J, Jimenez-Ferrer E.** 2012. Pharmacological characterization of the diuretic effect of *Hibiscus sabdariffa* Linn (Malvaceae) extract. *J Ethnopharmacol*, 139: 751– 756.
- Albano S A and Miguel M G.**2011. Biological activities of extracts of plants grown in Portugal. *Ind Crops Prod*, 33: 338–343.

- Aleksic V and Knezevic P.** 2013. Antimicrobial and antioxidative activity of extracts and essential oils of *Myrtus communis* L. *Microbiol Res*, 168(6): 311-332.
- Al-Hallaq E K, Afifi F U, Abdalla S S.** 2012. Evaluation of the hypocholesterolemic effect and phytochemical screening of the hydroethanolic extract of *Crataegus aronia* from Jordan. *Nat prod commun*, 7(1):35-38.
- Almeida E L S , Filho V C , Niero R V , Clasen B K, Balogun S O, Domingos, Martins D T.** 2011. Pharmacological mechanisms underlying the anti-ulcer activity of methanol extract and canthin-6-one of *Simaba ferruginea* A. St-Hil. in animal models. *J Ethnopharmacol*, 134: 630–636.
- Al-Waili N S D.** 1988. *Artemisia herba-alba* Asso for elevated blood pressure and heart rate. *Med Sci Res*, 16: 889.
- Al-Zohyri A M, Al-Jeboory AA, Jawad ALM.** 1985. Cardiovascular and antimicrobial effect of *Myrtus communis*. *Indian J Pharmacol*, 17(4):233–235.
- Amanullah M, Zaman G S, Rahman G L, Rahman S S.** 2012. Lipid peroxidation and the levels of antioxidant enzymes in hypertension. *Free Radic Antiox*, 2(2):12-18.
- Amensour M, Sendra E, Abrini J, Bouhdid S, Pérez-Alvarez JA, Fernández- López J.** 2009. Total phenolic content and antioxidant activity of myrtle (*Myrtus communis*) extracts. *Nat Prod Commun*, 4 (6), 819–824.
- Amira S, Rotondo A, Mulè F.** 2008. Relaxant effects of flavonoids on the mouse isolated stomach: Structure-activity relationships. *Eur J Pharmacol*, 599: 126–130.
- Apostolou A, Stagos D, Galitsiou E, Spyrou A, Haroutounian S, Portesis N, Trizoglou I, Hayes A W, Tsatsakis A M, Kouretas D.** 2013. Assessment of polyphenolic content, antioxidant activity, protection against ROS-induced DNA damage and anticancer activity of *Vitis vinifera* stem extracts. *Food Chem Toxicol*, 61:106-111.
- Aquel M and Hadidi M.** 1991. Direct relaxant effect of *Peganum harmala* seed extract on smooth muscles of rabbit and guinea pig. *Int J Pharmacogn*, 29: 176–182.
- Aquil A, Mohd M H , Usha S , Najmul I.** 2013. Comparative study of marker of oxidative stress among normotensive, pre-hypertensive and hypertensive subjects. *Biomed Res*, 24 (4): 493-497.
- Arafat O M, Tham S Y, Sadikun A, Zhari I, Haughton PG, Asmawi MZ.** 2008. Studies on diuretic and hypouricemic effects of *Orthosiphon stamineus* methanol extracts in rats. *J Ethnopharmacol*, 118: 354–360.
- Araujo A, M. Saraiva ´ L, Lima J.** 2008. Determination of total and oxidized glutathione in human whole blood with a sequential injection analysis system. *Talanta*, 74: 1511–1519.
- Ardestani A and Yazdanparast R.** 2007. Antioxidant and free radical scavenging potential of *Achillea santolina* extracts. *Food Chem*, 104: 21–29.
- Artizzu N, Bonsignore L, Cottiglia F, Loy G.** 1996. Studies on the diuretic and antimicrobial activity of *Cynodon dactylon* essential oil. *Fitoterapia*, 67: 174–176.
- Arya V and Thakur N.** 2012. Standardisation of Hawthorn fruits. *Am Eurasian J Sci*, 7 (1): 16-22.

- Asgarpanah J, Motamed SM, Farzaneh A, Ghanizadeh B, Tomraee S.** Antioxidant activity and total phenolic and flavonoid content of *Astragalus squarrosus* Bunge. *African J Biotechnol*,10(82): 19176-19180.
- Asgary S, Naderi G H, Sadeghi M, Kelishadi R, Amiri M.** 2004. Antihypertensive effect of Iranian *Crataegus curvisepala* L.: a randomized, double-blind study. *Drugs Exp Clin Res*, 30(5–6): 221–225.
- Aslan A, Güllüce M, Sökmen M, Adigüzel A, Sahin F, and Özkan H.** 2006. Antioxidant and antimicrobial properties of lichens *Cladonia foliacea*, *Dermatocarpon miniatum*, *Everinia divaricata*, *Everinia prunastri* and *Neofuscella pulla*. *Pharm Biol*, 44: 247- 252.
- Athiroh N, Permatasari N, Sargowo D, Widodo M A.** 2014. Antioxidative and blood pressure lowering effects of *Scurrula atropurpurea* on deoxycorticosterone acetate-salt hypertensive rats. *Biomarkers and Genomic Medicine*, 6: 32-36.
- Avila-Nava A, Calderón-Oliver M, Medina-Campos O N, Zou T, Gu L, Torres N, Tovar A R, Pedraza-Chaverri J.** 2014. Extract of cactus (*Opuntia ficus indica*) cladodes scavenges reactive oxygen species *in vitro* and enhances plasma antioxidant capacity in humans. *J funct foods*, 10: 13–24.
- Baba Aissa F.** 1999. Encyclopédie des plantes utiles, flore d'Algérie et du Maghreb. Librairie moderne-Rouiba, pp. 194-231.
- Baghiani A, Ameni D, Boumerfeg S, Adjadj M, Djarmouni M, Charef N, Khennouf S, Arrar L.** 2012. Studies of antioxidants and xanthine oxidase inhibitory potentials of root and aerial parts of medicinal plant *Capparis Spinosa* L. *Am J Med Medical Sci*, 2(1): 25-32.
- Bahorun T, Gressier B, Trotin F, Brunete C, Dine T, Vasseur J, Luyckx M, Cazin M, Pinkas M.** 1996 . Oxygen species scavenging activity of phenolic extract from Hawthorn fresh plant organs and pharmaceutical preparations. *Arzneim Forsch/Drug Res*. 46: 1086-1089.
- Bahri-Sahloul R, Ammar S, Fredj R B, Saghem S, Grec S, Trotin F, Shhiri F H.** 2009. Polyphenol contents and antioxidant activities of extracts from flowers of two *Crataegus azarolus* L. varieties. *Pakistan J biol sci*, 12 (9): 660-666.
- Bakkali F, Averbeck S, Averbeck D, Idaomar M.** 2008. Biological effects of essential oils: a review. *Food Chem Toxicol*, 46: 446–475.
- Barboni T, Venturini N, Paolini J, Desjobert J M, Chiaramonti N, Costa J.** 2010. Characterisation of volatiles and polyphenols for quality assessment of alcoholic beverages prepared from Corsican *Myrtus communis* berries. *Food*, 122:1304–1312.
- Baser K H C.** 2002. The Turkish *Origanum* species. *Oregano*, the Genera *Origanum* and *Lippia*, Ed., Kintzios SE, London and New York, Taylor and Francis, pp.109-126.
- Bastos A S, Loureiro A P, Franco de Oliveira T, Corbi S C T, Caminaga R M C, Rossa Júnior C, Orrico S.** 2012. Quantitation of malondialdehyde in gingival crevicular fluid by a high-performance liquid chromatography-based method. *Anal Biochem*, (423): 141–146.
- Beaux D, Fleurentin J, Mortier F.** 1997. Diuretic action of hydroalcohol extracts of *Foeniculum vulgare* var. *dulce* (D.C.) roots in rats. *Phytother Res*, 11: 320–322.
- Bedard K and Krause K H.** 2007. The NOX family of ROS-generating NADPH oxidases: Physiology and pathophysiology. *Physiol Rev*, 87: 245-313.

- Bejaoui A, Boulila A, Boussaid M.** 2013. Chemical composition and biological activities of essential oils and solvent extracts of *Origanum vulgare* subsp. *glandulosum* Desf. from Tunisia. *J Med Plants Res*, 7(32): 2429-2435.
- Bekhechi C, Atik-Bekkara F and Abdelouahid DE.**2008. Composition et activité antibactérienne des huiles essentielles d'*Origanum glandulosum* d'Algérie. *Phytothérapie*,6:153–159.
- Belhattab R, Larous L, Figueiredo A C, Santos P A G, Barroso J G, Pedro L G.** 2005. *Origanum glandulosum* Desf. grown wild in Algeria: Essential oil composition and glycosidic bound volatiles. *Flav Fragr J*, 20: 209-212.
- Belkhir M, Rebai O, Dhaouadi K, Congiu F, Tuberoso C I G, Amri M, Fattouch S.** 2013. Comparative analysis of Tunisian wild *Crataegus azarolus* (yellow azarole) and *Crataegus monogyna* (red azarole) leaf, and traditionally derived syrup: phenolic profiles and antioxidant and antimicrobial activities of the aqueous –acetone extracts . *J Agr Food chem*,61(41):9594-601.
- Belmokhtar M, Bouanani N, Ziyat A, Mekhfi H, Bnouham M, Aziz M, Mateo P, Fischmeister R, Legssyer A.**2014. Antihypertensive and endothelium-dependent vasodilator effects of aqueous extract of *Cistus ladaniferus*.*Eur J Med Chem*, 84: 206-239.
- Ben Farhat M, Landoulsia A, Chaouch H, Sotomayorc J A, Jordánc M J.** 2013. Phytochemical composition and *in vitro* antioxidant activity of by-products of *Salvia verbenaca* L. growing wild in different habitats. *Ind Crops Prod*, 49: 373– 379.
- Bendahou M, Muselli A, Grignon-Dubois M, Benyoucef M, Desjobert J M, Bernardini A F, Costa J.**2008. Antimicrobial activity and chemical composition of *Origanum glandulosum* Desf. essential oil and extract obtained by microwave extraction: Comparison with hydrodistillation. *Food Chem*, 106: 132–139.
- Benhammou N, Atik Bekkara F, PanovskaT K.** 2009. Antioxidant activity of methanolic extracts and some bioactive compounds of *Atriplex halimus* .*C. R. Chimie*, 12: 1259–1266.
- Benjumea D, Abdala S, Hernandez-Luis F, P´erez-Paz P, Martin-Herrera D.** 2005. Diuretic activity of *Artemisia thuscula*, an endemic canary species. *J Ethnopharmacol*, 100: 205–209.
- Benmohammed K, Nguyenb M T, Khensal S, Valensi P, Lezzar A.**2011. Arterial hypertension in overweight and obese Algerian adolescents: Role of abdominal adiposity. *Diabetes Metab*, 37: 291–297.
- Bernabé J, Mulero J, Cerdá B, García-Viguera C, Moreno D A, Parra S, Avilés F, Gil-Izquierdo A, Abellán J, Zafrilla P.** 2013. Effects of *Citrus* based juice on biomarkers of oxidative stress in metabolic syndrome patients. *J FuncFoods*, 5: 1031–1038.
- Bernatoniene J, Masteikova R, Majiene D, Savickas A, Kevelaitis E, Bernatoniene R, Dvorackova K, Civinskiene G, Lekas R, Vitkevicius K, Peciura R.** 2008. Free radical-scavenging activities of *Crataegus monogyna* extracts. *Medicina- Lithuania*, 44 (9):706-712.
- Bhavin V A, Ruchi V B, Shrikant J V, Payal S D , Santani Dev D.**2011.Effect of aqueous extract of *Pergularia daemia* on urine production. *Der Pharmacia Lettre*, 3 (4)207-214.
- Bilanda D C, Dimo T, Djomeni P D D, Bella N M T, Aboubakar O B F, Nguelefack T B, Tan P V, Kamtchouing P V.** 2010.Antihypertensive and antioxidant effects of *Allanblackia floribunda* Oliv.(Clusiaceae) aqueous extract in alcohol- and sucrose-induced hypertensive rats. *J Ethnopharmacol*,128: 634–640.

- Bilto Y Y, Suboh S, Aburjai T, Abdalla S.** 2012. Structure-activity relationships regarding the antioxidant effects of the flavonoids on human erythrocytes. *Nat Sci*, 9: 740-747.
- Birben E, Sahiner U M, Sackesen C, Erzurum S, Kalayci O.** 2012. Oxidative stress and antioxidant defense. *World Allergy Organization Journal*, 9-12.
- Birman H, Dar K H, Aysegul K, Olgaç V, Gürel E.** 2011. Effects of *Crataegus tanacetifolia* and hyperoside on salivary gland and kidney tissue and relation to nitric oxide (NO) in experimental hypertensive rats. *Nobel Medicus*, 7 (1): 17-22.
- Boaventura B C B, Di Pietro P F, Klein G A, Stefanuto A, de Moraes E C, de Andrade F, Wazlawik E, Da Silva E L.** 2013. Antioxidant potential of mate tea (*Ilex paraguariensis*) in type 2 diabetic mellitus and prediabetic individuals. *J Func Foods*, 5: 1057–1064.
- Boffil M, Lorenzo G, Monteagudo E, Sueiro M and Matos J.** 2006. Diuretic activity of five medicinal plants used popularly in Cuba. *Parmacologyonline*, 3:435-441.
- Bonarska-Kujawa D, Pruchnik H, Oszmian´ski J, Sarapuk J, Kleszczyn´ska H.** 2011. Changes caused by fruit extracts in the lipid phase of biological and model membranes. *Food Biophys*, 6: 58–67.
- Bors W, Michel C, Stettmaier K.** 2001. Structure–activity relationships governing antioxidant capacities of plant polyphenols. *Methods Enzymol*, 335:166–180.
- Bose S, Maji S, Chakraborty P.** 2011. Comparative study of *in vitro* and *in vivo* antioxidant property of different *Ixora* species. *J Adv Pharm Educ Res*, 2: 90-103.
- Bouaziz A, Khennouf S, Bentahar A, Djidel S, Amira S.** 2012. Phytotherapy of hypertension in Setif region (Eastern algeria). Proceedings book. The second African congress on biology and health, Setif. Algeria.pp. 39-42.
- 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*, 5(2): 286-305.
- Bouaziz A, khennouf S, Abu zarga M, Abdalla S, Baghiani A, Charef N.** 2015. Phytochemical analysis, hypotensive effect and antioxidant properties of *Myrtus communis* L. growing in Algeria. *Asian Pac J Trop Biomed*, 5(1):19-28.
- Boudjelal A, HENCHIRI C, Sari M, Sarri D, Hendel N, Benkhalel A, Ruberto G.** 2013. Herbalists and wild medicinal plants in M'Sila (North Algeria): An ethnopharmacology survey. *J Ethnopharmacol*, 148 : 395–4022.
- Bouzabata A, Castola V, Bighelli A, Lahouari A, Casanova J, Tomi F.** 2013 a. Chemical Variability of Algerian *Myrtus communis* L. *Chem Biodiv*, 10 :129-137
- Bouzabata A.** 2013 b. Traditional treatment of high blood pressure and diabetes in Souk Ahras District. *J Pharmacogn Phytother*, 5: 12–20.
- Bozin B, Mimica-Dukic N, Simin N, Anackov G.** 2006. Characterization of the volatile composition of essential oils of some lamiaceae spices and the antimicrobial and antioxidant activities of the entire oils. *J Agr Food Chem*, 54:1822-1828.
- Brand-Williams W, Cuvelier M, Berset C.** 1995. Use of a free radical method to evaluate antioxidant activity. *LWT-Food Sci Technol*, 28: 25-30.

- Briones A M and Touyz R M.** 2010. Oxidative stress and hypertension: current concepts. *Curr Hypertens Rep*, 12: 135-142.
- Brixius K, Willms S, Napp A, Tossios P, Ladage D, Bloch W, Mehlhorn U, Schwinger RH.** 2006. *Crataegus* special extract WS 1442 induces an endothelium-dependent, NO-mediated vasorelaxation via eNOS-phosphorylation at serine 1177. *Cardiovasc Drugs Ther*, 20:177–84.
- Burits M, Bucar F.** 2000. Antioxidant activity of *Nigella sativa* essential oil. *Phytother Res*, 14:323–328.
- Cakilcioglu U, Khatun S, Turkoglu I, Hayta S.** 2011. Ethnopharmacological survey of medicinal plants in Maden (Elazig-Turkey). *J Ethnopharmacol*, 137: 469– 486.
- Calderone V, Chericoni S, Martinelli C, Testai L, Nardi A, Morelli I, Breschi MC, Martinotti E.** 2004. Vasorelaxing effects of flavonoids: investigation on the possible involvement of potassium channels. *Arch Pharmacol*, 370: 290–298.
- Cao G and Prior R L.** 2000. Postprandial increases in serum antioxidant capacity in older women. *J Appl Physiol*, 89:877–883.
- Chang Q, Zuo Z, Chow M S S, Ho W K.** 2006. Effect of storage temperature on phenolics stability in hawthorn (*Crataegus pinnatifida* var. *major*) fruits and a hawthorn drink. *Food Chem*, 98 (3): 426-430.
- Chen Z Y , Zhang Z S , Kwan KY, Zhu M, Ho WK, Huang Y.** 1998. Endothelium-dependent relaxation induced by hawthorn extract in rat mesenteric artery. *Life Sci*, 63(22): 1983–1991.
- Cherif S, Rahal N, Haouala M, Hizaoui B, Dargouth F, Gueddiche M, Kallel Z, Balansard G, Boukef K. A.** 1996. A clinical trial of a titrated *Olea* extract in the treatment of essential arterial hypertension. *J Pharm Belg*, 51:69-71.
- Chishti S, Kaloo Z A, Sultan P.** 2013. Medicinal importance of genus *Origanum*: A review. *J pharmacogn phytother*, 5(10):170-177.
- Chouhan H S and Singh S K.** 2011. Phytochemical analysis, antioxidant and anti-inflammatory activities of *Phyllanthus simplex*. *J Ethnopharmacol*, 137: 1337– 1344.
- Chung H S, Chang L C, Lee SK, Shamon L A, Van Breemen R B, Mehta RG, Farnsworth N R, Pezzuto J M, Kinghorn AD.** 1999. Flavonoid constituents of *Chorizante diffusa* with potential cancer chemopreventive activity. *J Agr Food Chem*, 47(1) : 36–41.
- Chung S, Park CW, Shin S J, Lim J H, Chung HW, Youn DY.** 2010. Tempol or candesartan prevents high-fat diet-induced hypertension and renal damage in spontaneously hypertensive rats. *Nephrol Dial Transplant*, 25(2):389-399.
- Chung YC, Chen S J, Hsu CK, Chang CT, Chou ST.** 2005. Studies on the antioxidative activity of *Graptopetalum paraguayense*. *Food Chem*, 91:419–424.
- Clairborne A.** 1985. Catalase activity. In handbook of method for oxygen radical research. Green world, R.A.ed: Boca Raton .Fla CRC press, pp.283-284.
- Corrêa T A, Monteiro M P, Mendes T M, Oliveira D M, Rogero M M, Benites C I, Vinagre C G, Mioto B M, Tarasoutchi D, Tuda V L, César L A, Torres E A.** 2012. Medium light and

medium roast paper-filtered coffee increased antioxidant capacity in healthy volunteers: Results of a randomized trial. *Plant Foods Hum Nutr*, 67: 277–282.

Dai F, Chen WF, Zhou B. 2008. Antioxidant synergism of green tea polyphenols with alpha-tocopherol and L-ascorbic acid in SDS micelles. *Biochimie*, 90 (10): 1499–1505.

Dapkevicius A, Venskutonis R, Van Beek TA, Linssen PH.1998. Antioxidant activity of extracts obtained by different isolation procedures from some aromatic herbs grown in Lithuania. *J Sci Food Agr*, 77:140–146.

Datla S R and Griendling K K. 2010. Reactive oxygen species, NADPH oxidases, and hypertension. *Hypertens*, 56: 325-330.

David J M, Cruz f G, Guedes M L S, Chavez J P. 1996. Flavonol glycosides from *Davilla flexuosa*. *J Braz Chem Soc*, 7(2): 115–118.

Decker E A and Welch B.1990. Role of ferritin as lipid oxidation catalyst in muscle food. *J Agr Food Chem*, 36: 674-677.

Deoghare S, Tandel K, Zala A, Malam P, Sharma A, Solanki P. 2014. Study of oxidative stress parameters in hypertension and their correlation with blood pressure. *Int J Pharm Sci Rev Res*, 25(1): 114-117.

Desir GV .2012. Human renalase: a review of its biology, function, and implications for hypertension”. *J Am Soc Hypertens*, 37(8):829–33.

Dineel A S, Acikgoz S, Cevik C, Sengelen M, Yesilada E. 2007. Effects of *in vivo* antioxidant enzyme activities of myrtle oil in normoglycemic and alloxan diabetic rabbits. *J Ethnopharmacol*, 110(3):498–503.

Dizaye K. 2011. Antihyperuricemic and diuretic effects of procyanidins extracted from *Crataegus monogyna*. *Zanco J of Med Sci*, 37(8):829–833.

Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, Vidal N. 2006. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chem*, 97: 654-660.

Dong X, Liu Y, Yan J, Jiang C, Chen J, Liu T, Hu Y.2008. Identification of SVM based classification model, synthesis and evaluation of prenylated flavonoids as vasorelaxant agents. *Bioorg Med Chem*, 16: 8151-8160.

Duh P D and Yen G C. 1997. Antioxidative activity of three herbal water extracts. *Food Chem*, 60: 639–645.

Dundar E, Olgun EG, Isiksoya S, Kukcuoglu M, Baser K H C, Bal C. 2008. The effects of intra-rectal and intra-peritoneal application of *Origanum onites* L. essential oil on 2, 4, 6 -Trinitrobenzene sulfonic acid induced colitis in the rat. *Exp Toxicol Pathol*, 59:399–408.

Hernández-Hernández E , Ponce-Alquicira E, Jaramillo-Flores M E, Guerrero Legarreta I. 2009. Antioxidant effect rosemary (*Rosmarinus officinalis* L.) and oregano (*Origanum vulgare* L.) extracts on TBARS and colour of model raw pork batters. *Meat Sci*, 81: 410–417.

Ebrahimzadeh M A, Nabavi S F, Nabavi S M, Eslami B, Asgarirad H. 2010. *In vitro* antioxidant and free radical scavenging activity of *Leonurus cardiaca* subsp. *Persicus*, *Grammosciadium platycarpum* and *Onosma demawendicum*. *African J Biotechnol*, 9(51) :8865-8871.

- El Bardai S, Lyoussi B, Wibo M, Morel N.** 2001. Pharmacological evidence of hypotensive activity of *Marrubium vulgare* and *Foeniculum vulgare* in spontaneously hypertensive rat. *Clin Exp Hypertens*, 23: 329–343.
- El Tahir K E H, Ashour M M S, Al-Harbi M M.** 1993. The cardiovascular actions of the volatile oil of the black seed (*Nigella sativa*) in rats: elucidation of the mechanism of action. *Gen Pharmacol*, 24: 1123–1131.
- Elfellah MS, Akhter MH, Khan MT.** 1984. Antihyperglycaemic effect of an extract of *Myrtus communis* in streptozotocin-induced diabetes in mice. *J Ethnopharmacol*, 11(3):275–281.
- Elhajili M, Baddouri K, Elkabbaj S, Meiouat F, Settaf A.** 2001. Diuretic activity of the flowers infusion of *Lavandula officinalis*. *Reprod Nutr Dev*, 41: 393–399.
- El-Hilaly J, Lyoussi B, Wibo M, Morel N.** 2004. Vasorelaxant effect of the aqueous extract of *Ajuga iva* in rat aorta. *J Ethnopharmacol*, 93:69–74.
- Elks C M, Mariappan N, Haque M, Guggilam A, Majid D S, Francis J.** 2009. Chronic NF- κ B blockade reduces cytosolic and mitochondrial oxidative stress and attenuates renal injury and hypertension in SHR. *Am J Physiol Renal Physiol*, 296: 298–305.
- Ellman G L.** 1959. Tissue sulfhydryl groups. *Arch biochem biophys*, 82: 70–77.
- Elmastas M, Gülçin İ, Isildak Ö, Küfrevioğlu Öİ, İbaoglu K, Aboul-Enein HY.** 2006. Radical scavenging activity and antioxidant capacity of bay leaf extracts. *J Iran Chem Soc*, 3:258–266.
- Esmaeili A, Rahnamoun S, Fariba Sharifnia.** 2013. Effect of O/W process parameters on *Crataegus azarolus* L nanocapsule properties. *J Nanobiotech*, 11:16–24.
- Exarchou V, Godejohann M, Van Beek T, Gerothanassis P, Vervoort J.** 2003. LC–UV–Solid-phase extraction–NMR–MS combined with a cryogenic flow probe and its application to the identification of compounds present in Greek oregano. *Anal Chem*, 75: 6288–6294.
- Fadel O, El Kirat K, Morandat S.** 2011. The natural antioxidant rosmarinic acid spontaneously penetrates membranes to inhibit lipid peroxidation in situ. *Biochim Biophys Acta*, 1808: 2973–2980.
- Feng L, Jia X, Zhu M, Chen Y and Shi F.** 2010. Antioxidant activities of total phenols of *Prunella vulgaris* L. *in vitro* and in tumor-bearing Mice. *Molecules*, 15: 9145–9156.
- Ferreira I C F R, Baptista P, Vilas-Boas M, Barros L.** 2007. Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: Individual cap and stipe activity. *Food Chem*, 100:1511–1516.
- Ferroni P, Basili S, Paoletti V, Davi G.** 2006. Endothelial dysfunction and oxidative stress in arterial hypertension. *Nutr Metab Cardiovasc Dis*, 16: 222–233.
- Fraga CG, Galleano M, Verstraeten S V, Oteiza P I.** 2010. Basic biochemical mechanisms behind the health benefits of polyphenols. *Mol Aspects Med*, 31: 435–445.
- Frankel E N and Meyer A S.** 2000. The Problems of using one-dimensional Methods to evaluate multifunctional food and biological antioxidants. *J Sci Food Agr*, 80(13): 1925–1940.

- Froehlicher T, Hennebelle T, Martin-Nizard F, Cleenewerck P, Hilbert J, Trotin F, Grec S. 2009.** Phenolic profiles and antioxidative effects of Hawthorn cell suspensions, fresh fruits, and medicinal dried parts. *Food Chem*, 115: 897–903.
- Frombaum M, Le Clanche S, Bonnefont-Rousselot D, Borderie D. 2012.** Antioxidant effects of resveratrol and other stilbene derivatives on oxidative stress and NO bioavailability: potential benefits to cardiovascular diseases. *Biochimie*, 94: 269–276.
- Fujita M, Ando K, Nagae A, Fujita T. 2007.** Sympathoexcitation by oxidative stress in the brain mediates arterial pressure elevation in salt-sensitive hypertension. *Hypertens*, 50:360–367.
- Gardeli C, Papageorgiou V, Mallouchos A, Theodosios K, Komaitis M. 2008.** Essential oil composition of *Pistacia lentiscus* L. and *Myrtus communis* L.: evaluation of antioxidant capacity of methanolic extracts. *Food Chem*, 107: 1120–1130.
- Garjani A, Nazemiyeh H, Maleki N, Valizadeh H. 2000.** Effects of extracts from flowering tops of *Crataegus meyeri* A. Pojark. on ischaemic arrhythmias in anaesthetized rats. *Phytother Res*, 14: 428–431.
- Gasparotto Junior A, Boffo MA, Lourenco E L, Stefanello M E, Kassuya, C A, Marques M C. 2009.** Natriuretic and diuretic effects of *Tropaeolum majus* (Tropaeolaceae) in rats. *J Ethnopharmacol*, 122: 517–522.
- Gasparotto Junior A, Prando TBL, Leme T, Gasparotto DS V, Lourenc F M, Rattmann E L B, Da Silva-Santos Y D, Kassuya J E, Marques C A L. 2012.** Mechanisms underlying the diuretic effects of *Tropaeolum majus* L. extracts and its main component isoquercitrin. *J Ethnopharmacol*, 141: 501–509.
- Gilani A H, Khan A U, Shah A J, Connor J, Jabeen Q. 2005.** Blood pressure lowering effect of olive is mediated through calcium channel blockade. *Int J Food Sci Nutr*, 56: 613–620.
- Gladine C, Morand C, Rock E, Gruffat D, Bauchart D, Durand D. 2007.** The antioxidative effect of plant extracts rich in polyphenols differs between liver and muscle tissues in rats fed n-3 PUFA rich diets. *Anim Feed Sci Technol*, 139:257–272.
- Gonçalves S, Gomes S, Costa P, Romano A. 2013.** The phenolic content and antioxidant activity of infusions from Mediterranean medicinal plants. *Ind Crops Prod*, 43: 465–471.
- Gönenç A, Hacısevki A, Taviş Y, Çengel A, Torun M. 2013.** Oxidative stress in patients with essential hypertension: A comparison of dippers and non-dippers. *Eur J Int Med*, 24: 139–144.
- Gornall A, Charles G, Bardawill J, Maxima M D. 1949.** Determination of serum proteins by means of the biuret reaction. *J Biol Chem*, 177: 751–766.
- Govindan P and Muthukrishnan S. 2013.** Evaluation of total phenolic content and free radical scavenging activity of *Boerhavia erecta*. *J Acute Med*, 3 (3): 103–109.
- Grande M T, Pascual G, Riobobos A S, Clemente-Lorenzo M, Bardaji B, Barreiro L, Tornavaca O, Meseguer A, López-Novoa J M. 2011.** Increased oxidative stress, the renin-angiotensin system, and sympathetic overactivation induce hypertension in kidney androgen-regulated protein transgenic mice. *Free Radic Biol Med*, 51:1831–1841.
- Grande M T, Pérez-Barriocanal F, López-Novoa J M. 2013.** Role of inflammation in tubulointerstitial damage associated to obstructive nephropathy. *J Inflamm*, 22(7): 19.

- Gülçin, İ.** 2006. Antioxidant and antiradical activities of L-carnitine. *Life Sci*, 78: 803–811.
- Gu'lıçin I, Huyut Z, Elmastas M, Aboul-Enein H Y.** 2010. Radical scavenging and antioxidant activity of tannic acid. *Arabian J Chem*, 3: 43–53.
- Gutiérrez Y P, Gutiérrez A P, Leónb A R, García K C.** 2014. Role of oxidative stress in the pathogenesis of hypertension. *Cor Salud*, 6(2):181-192.
- Guyenet PG.** The sympathetic control of blood pressure. 2006. *Nat Rev Neurosci*, 7(5):335-346.
- Guyton A C and Hall J E.** 2006. Textbook of Medical Physiology. (11th ed). Elsevier Saunders. pp. 220.
- Haloui M, Louedec L, Michel J B, Lyoussi B.** 2000. Experimental diuretic effects of *Rosmarinus officinalis* and *Centaurium erythraea*. *J Ethnopharmacol*, 71: 465–472.
- Harrison D J, Gongora M C, Guzik T G, Widder J.** 2007. Oxidative stress and hypertension. *J Am Soc Hypertens*, 1(1): 30–44.
- Hayder N, Abdelwahed A, Kilani S, Ben Ammar R, Mahmoud A, Ghedira K, Chekir-Ghedira L.** 2004. Anti-genotoxic and free radical scavenging activities of extracts from (Tunisian) *Myrtus communis*. *Mutat Res*, 564 : 89–95.
- Hayder N, Bouhlel I, Skandrani I, Kadri M, Steiman R, Guiraud P, Mariotte AM, Ghedira K, Dijoux-Franca MG, Chekir-Ghedira L.** 2008. *In vitro* antioxidant and antigenotoxic potentials of myricetin-3-O-galactoside and myricetin-3-O-rhamnoside from *Myrtus communis*: Modulation of expression of genes involved in cell defense system using cDNA microarray. *Toxicol in vitro*, 22: 567–581.
- Hayouni EA, Abedrabba M, Bouix M, Hamdi M.** 2007. The effects of solvents and extraction method on the phenolic contents and biological activities *in vitro* of Tunisian *Quercus coccifera* L. and *Juniperus phoenicea* L. fruit extracts. *Food Chem*, 105: 1126-1134.
- Hazra B, Biswas S, Mandal N.** 2008. Antioxidant and free radical scavenging activity of *Spondias pinnata*. *BMC Complement Altern Med*, 8: 63-69.
- Heim K E, Tagliaferro A R, Bobilya D J.** 2002. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *J Nutr Biochem*, 13(10): 572-584.
- Hendel N, Larous L, Sari M, Boudjelal A, Sarri D.** 2012. Place of labiates in folk medicine of the area of M'sila (Algeria). *Glob J Res Med Plants Indig Med*, 1(8): 315–322.
- Herrera-Arellano A, Aguilar-Santamar'ia L, Garc'ia-Hern'andez B, Nicasio-Torres P, Tortoriello J.** 2004. Clinical trial of *Cecropia obtusifolia* and *Marrubium vulgare* leaf extracts on blood glucose and serum lipids in type 2 diabetics. *Phytomed*, 11: 561–566.
- Heumuller S, Wind S, Barbosa-Sicard E, Schmidt H H, Busse R, Schroder K, Brandes R P.** 2008. Apocynin is not an inhibitor of vascular NADPH oxidases but an antioxidant. *Hypertens*, 51: 211-217.
- Hinou J, Lakkas N, Philianos S.** 1988. Les constituants polyphénoliques de *Myrtus communis* L. *Plantes médicinales et phytothérapie*, 22 : 98–103.
- Hitomi H, Kiyomoto H, Nishiyama A.** 2007. Angiotensin II and oxidative stress. *Curr Opin Cardiol*, 22(4): 311-315.

- Hofmann F.**2005. The Biology of Cyclic GMP-dependent Protein Kinases. *J Biol Chem*, 280:1-4.
- Huo H Z, Wang B, Liang Y K, Bao Y Y and Gu Y.**2011. Hepatoprotective and antioxidant effects of licorice extract against CCl₄-induced oxidative damage in rats. *Int J Mol Sci*, 12: 6529-6543.
- Ietswaart J H.** 1980. A taxonomic revision of the genus *Origanum* (Labiatae), chemical composition and antioxidant activity of essential oils from Algerian *Origanum glandulosum* Desf. In *Leiden Botanical series 4*, Leiden University Press: Le Hague.
- Ikewuchi G C, Ikewuchia C C, Ifeancha M O, Igbohb N M.** 2014. Blood pressure lowering activity of a flavonoid and phytosterol rich extract of the sclerotia of *Pleurotus tuberregium* (Fr) Sing in salt-loaded rats. *Biomed Prev Nutr*, 4: 257–263.
- Ismail H I, Chan K W, Mariod A A, Ismail M.** 2010. Phenolic content and antioxidant activity of cantaloupe (*Cucumis melo*) methanolic extracts. *Food Chem*, 119: 643–647.
- Jabeen Q and Aslam N.** 2013. Hypotensive, angiotensin converting enzyme (ACE) inhibitor and diuretic activities of the aqueous-methanol extract of *Ipomoea reniformis*. *Iran J Pharm Res*, 12 (4): 769-776.
- Jain N K and Singhai A K.** 2012. Hepatoprotective activity of *Chenopodium album* Linn: *in vitro* and *in vivo* studies. *J Exp Integr Med*, 2(4):331-336.
- Jalalpure S S and Gadge N B.**2011. Diuretic effect of young fruit extracts of *Bombax ceiba* L. in rats. *Indian J Pharm Sci*, 73(3): 306–311.
- Jiang M Z, Yan H, Wen Y, Li X M.** 2011. *In vitro* and *in vivo* studies of antioxidant activities of flavonoids from *Adiantum capillus-veneris* L. *African J Pharm Pharmacol*, 5(18): 2079-2085.
- Jurikova T, Sochor J, Rop O, Mlcek J, Balla S, Szekeres L, Adam V, Kizek R.** 2012. Polyphenolic profile and biological activity of Chinese hawthorn (*Crataegus pinnatifida* Bunge) fruits. *Molecules*, 17 : 14490–14509.
- Kachhawa K, Varma M, Sahu A, Kachhawa P, Jha R K.** 2014. Oxidative stress and antioxidant enzyme levels in hypertensive chronic kidney disease patients. *Int J Biomed Adv Res*, 5 (10):488-490.
- Kalyanaraman B.** 2013. Teaching the basics of redox biology to medical and graduate students: Oxidants, antioxidants and disease mechanisms. *Redox Biol*, 1: 244–257.
- Kardum N, Takic´ M, Šavikin K, Zec M, Zdunic´ G, Spasic´ S, Konic´-Ristic´ A.** 2014. Effects of polyphenol-rich chokeberry juice on cellular antioxidant enzymes and membrane lipid status in healthy women. *J funct foods*, 9: 89–97.
- Kateel R, Rai M S, Ashok Kumar J.**2014. Evaluation of diuretic activity of gallic acid in normal rats. *J Sci Innov Res*, 3 (2): 217-220.
- Katoch N, Kaur P, Kashyap P, Gupta S, Dahiya R S.**2013. Role of oxidative stress in cardiovascular diseases. *Res J Pharm Biol Chem Sci*, 4 (3): 870-881.
- Kaurinovic B, Popovic M, Vlasisavljevic S and Trivic S.**2011. Antioxidant capacity of *Ocimum basilicum* L. and *Origanum vulgare* L. extracts. *Molecules*, 16: 7401-7414.

- Kaushik P, Kaushik D, Khokra S L.** 2011. *In vivo* antioxidant activity of plant *Abutilon indicum*. *J Pharm Educ Res*,2 (1): 50-53.
- Kazazica S P , Butkovica V, Srazica D, Klasinc D.**2006. Gas-phase ligation of Fe^+ and Cu^+ ions with some flavonoids.*J Agr Food Chem*, 54: 8391–8396.
- Khadir A, Bendahou M, Benbelaïd F, Ghellai L, Abdoune M A, Bellahsene C, Zenati F, Abdelouahid D , Muselli A , Paollini J, Desjobert J M, Costa J.** 2013.Efficacy of the essential oil of *Origanum glandulosum* Desf against skin infections With PVL-producing *Staphylococcus aureus* strains in mice. *African J Microbiol Res*,7(21): 2656-2661.
- Khalifa F K and Elmazny W N.**2013. Antioxidant mediated defense role of the dietary phytochemical ferulic acid, against carbon tetrachloride (CCl_4) induced toxic hepatitis. *Adv Res*, 1(1): 11-22.
- Khan A, Bashir S, Khan S R, Gilani A H.** 2011. Antirolithic activity of *Origanum vulgare* is mediated through multiple pathway.*BMC Complement Altern Med*, 11: 96-111.
- Khan I, Yousif A M, Johnson S K, Gamlath S.** 2014. Acute effect of sorghum flour-containing pasta on plasma total polyphenols, antioxidant capacity and oxidative stress markers in healthy subjects: A randomised controlled trial. *Clin Nutr*.(In press)doi: 10.1016/j.clnu.2014.08.005.
- Khenouf S, Benchiekh D, Djidel S, Dahamna S, Amira S, Charef N, Baghiani A, Harzallah D, Arrar L.** 2013. Polyphenols and antioxidant properties of extracts from *Mentha pulegium* L. and *Matricaria chamomilla* L. *Pharmacogn Commun*,3 (2):35-40.
- Khosravi A R, Shokri H, Kermani S, Dakhili M, Madani M, Parsa S.** 2011. Antifungal properties of *Artemisia sieberi* and *Origanum vulgare* essential oils against *Candida glabrata* isolates obtained from patients with vulvovaginal candidiasis. *J Mycol Med*, 21: 93-99.
- Kikuzaki H and Nakatani N.** 1993.Antioxidant effects of some ginger constituents. *J Food Sci*, 58(8):1407–1410.
- Kim Y S, Lee S J, Hwang J W, Kim U K, Kim S E, Kim E H, Moon S H, Jeon B T, Park J P.**2012.*In vitro* protective effects of *Thymus quinquecostatus* celak extracts on t-BHP-induced cell damage through antioxidant activity. *Food Chem Toxicol*,50:4191–4198.
- Kim B, Ku C K, Pham T X, Park Y, Martin D A, Xie L, Taheri R, Lee J, Bolling B W.**2013. *Aronia melanocarpa* (chokeberry) polyphenol-rich extract improves antioxidant function and reduces total plasma cholesterol in apolipoprotein E knockout mice. *Nutr Res*, 33: 406 – 413.
- Koçyildiz Z C, Birman H, Olgaç V, Akgün-Dar K, Melikoğlu G, Meriçli A H.** 2006. *Crataegus tanacetifolia* leaf extract prevents L-NAME-induced hypertension in rats: a morphological study. *Phytother Res*, 20(1): 66–70.
- Kordali S , Cakir A, Ozer H, Cakmakci R, Kesdek M, Mete E.** 2008. Antifungal, phytotoxic and insecticidal properties of essential oil isolated from Turkish *Origanum acutidens* and its three components, carvacrol, thymol and p-cymene.*Bioresour Technol*,99: 8788–8795.
- Korthuis R J.** 2011. Skeletal muscle circulation. Chapter 3: Regulation of vascular tone in skeletal muscle. San Rafael (CA): Morgan & Claypool Life Sciences. Available from: <http://www.ncbi.nlm.nih.gov/books/NBK57140/>
- Kotana R, Cakirb A, Ozerc H, Kordalia S, Cakmakcic R, Dadasoglud F, Dikbase N, Aydin T, Kazazg C.** 2014. Antibacterial effects of *Origanum onites* against phytopathogenic bacteria:

Possible use of the extracts from protection of disease caused by some phytopathogenic bacteria. *Scientia Horticulturae*, 172: 210–220.

Koukoulitsa C, Karioti A, Bergonzi MC, Pescitelli G, di Bari L, Skaltsa H. 2006. Polar constituents from aerial parts of *Origanum vulgare* L. ssp. *Hirtum* growing wild in Greece. *J Agr Food Chem*, 54 : 5388–5392.

Koyuncu T, Pinar Y, Lule F. 2007. Convective drying characteristics of azarole red (*Crataegus monogyna* Jacq.) and yellow (*Crataegus aronia* Bosc.) fruits. *J Food Eng*, 78: 1471–1475.

Krug S, Zhang Y, Mori T A, Croft K D, Vickers J J, Langton L K, Whitworth J A. 2008. N-Acetylcysteine prevents but does not reverse dexamethasone-induced hypertension. *Clin Exp Pharmacol Physiol*, 35: 979-981.

Kulisic T, Radoni A, Katalinic V, Milos M. 2004. Use of different methods for testing antioxidative activity of oregano essential oil. *Food Chem*, 85: 633–640.

Kültür S. 2007. Medicinal plants used in Kırklareli Province (Turkey). *J Ethnopharmacol*, 111: 341-364.

Kumar D, Arya V, Ali Bhat Z K, Khan N A, Prasad DN. 2012. The genus *Crataegus*: chemical and pharmacological perspectives. *Brazilian J Pharmacogn*, 22(5): 1187-1200.

Kumar R, Phaneendra P, Bodhanapu S, Rahiman F, Oiyas M K, Tamizmani T. 2011. Antioxidant and hepatoprotective activity of the aqueous extract of *Myrtus Communis* (Myrtle) Linn. leaves. *Pharmacol Online*, 1: 1083-1090.

Kumaran A, Karunakaran R J. 2007. *In vitro* antioxidant activities of acetone extracts of *Phyllanthus* species from India. *Lebens-Wiss Techonologie*, 40: 344–352.

Lahlou S, Tahraoui A, Israili Z, Lyoussi B. 2007. Diuretic activity of the aqueous extracts of *Carum carvi* and *Tanacetum vulgare* in normal rats. *J Ethnopharmacol*, 110: 458–463.

Landmesser U, Dikalov S, Price SR, McCann L, Fukai T, Holland SM, Mitch WE, Harrison DG. 2003. Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. *J Clin Invest*, 111(8):1201-1209.

Landmesser U, Hornig B, Drexler H. 2004. Endothelial function: a critical determinant in atherosclerosis?. *Circ*, 110(1):27-33.

Lavault M and Richomme P. 2004. Constituents of *Helichrysum stoechas* variety *olonense*. *Chem Nat Compounds*, 40: 118–121.

Lavie L. 2014. Oxidative stress in obstructive sleep apnea and intermittent hypoxia - Revisited - the bad ugly and good: Implications to the heart and brain. *Sleep Med Rev.* (In press). doi: 10.1016/j.smrv.2014.07.003.

Lee S, Shin D S, Oh K B, Shin KH. 2003. Antibacterial compounds from the leaves of *Acanthopanax senticosus*. *Arch Pharmacol Res*, 26:40–42.

Lemhadri A, Zeggwagh NA, Maghrani M, Jouad H, Eddouks M. 2004. Anti-hyperglycaemic activity of the aqueous extract of *Origanum vulgare* growing wild in Tafilalet region. *J Ethnopharmacol*, 92(2-3): 251-256.

- Li HB, Cheng K W C, Fan K W F, Jiang Y.** 2007. Evaluation of antioxidant capacity and total phenolic content of different fraction of selected microalgae. *Food chem*, 102: 771-776.
- Li Y H, Jiang B, Zhang T, Mu WM, Liu J.**2008. Antioxidant and free radical scavenging activities of chickpea protein hydrolysate (CPH).*Food Chem*, 106: 444–450.
- Li H, Horke S, Fo"rstermann U.** 2013. Oxidative stress in vascular disease and its pharmacological prevention.*Trends Pharmacol Sci*, 6 : 313-319.
- Li Z, Huc J, Ya-ling Li , Xue F, Zhang L, Xie J, Liu Z , Hua L , Yi D , Jin-cheng L, Wang S.** 2013. The effect of hyperoside on the functional recovery of the ischemic/reperfused isolated rat heart: Potential involvement of the extracellular signal-regulated kinase1/2 signaling pathway .*Free Radic Biol Med*, 57: 132–140.
- Liang C H, Chou T H , Ding H Y.** 2010. Inhibition of melanogenesis by a novel origanoside from *Origanum vulgare*.*J Dermatol Sci*, 57: 170-177.
- Lin Y L, Wang C N, Shiao Y J, Liu T Y, Wang W Y.** 2003. Benzolignanoid and polyphenols from *Origanum vulgare*. *J Chin Chem Soc*, 50:1079–1083.
- Ling S.** 2007. Effects of four medicinal herbs on human vascular endothelial cells in culture. Lipid status in healthy women.*Int J Cardio*, 111:34–38.
- Liu T, Sun Y , Laura T , Liang X, Ye H, Zeng X.** 2009. Determination of polyphenolic content and antioxidant activity of kudingcha made from *Ilex kudingcha*.*Food Chem*, 112: 35–41.
- Liu Q and Yao H.** 2007. Antioxidant activities of barley seeds extracts.*Food Chem*, 102(3): 732-737.
- Liu T , Cao Y, Zhao M.**2010. Extraction optimization, purification and antioxidant activity of procyanidins from hawthorn (*C. pinnatifida* Bge. var. *major*) fruits. *Food Chem*, 119:1656–1662.
- Ljubuncic P, Portnaya I, Cogan U , Azaizeh H, Bomzon A.**2005. Antioxidant activity of *Crataegus aronia* aqueous extract used in traditional Arab medicine in Israel. *J Ethnopharmacol*, 101: 153–161.
- Loizzo M R, Menichini F, Conforti F, Tundis R, Bonesi M, Saab A M, Statti GA, de Cindio B, Houghton PJ, Menichini F, Frega NG.** 2009. Chemical analysis, antioxidant, anti-inflammatory and anticholinesterase activities of *Origanum ehrenbergii* Boiss and *Origanum syriacum* L. essential oils. *Food Chem*, 117:174-180.
- Long S R, Carey R A, Crofoot K M, Proteau P J, Filtz T M.** 2006. Effect of hawthorn (*Crataegus oxycantha*) crude extract and chromatographic fractions on multiple activities in a cultured cardiomyocyte assay. *Phytomed*,13: 643-650.
- Lotito S B and Frei B.**2006. Consumption of flavonoid-rich foods and increased plasma antioxidant capacity in humans: Cause, consequence, or epiphenomenon?.*Free Radic Biol Med*, 41: 1727–1746.
- Maghrani M, Zeggwagh N A, Michel J P, Eddouks M.** 2005. Antihypertensive effect of *Lepidium sativum* L. in spontaneously hypertensive rats. *J Ethnopharmacol*, 100: 193–197.
- Malik Z A and Siddiqui S.** 1981. Hypotensive effect of freeze-dried garlic (*Allium sativum*) sap in dogs. *J Pakistan Med Assoc*, 13: 12–13.

- Manach C, Williamson G, Morand C, Scalbert, A, Remesy C.** 2005. Bioavailability and bioefficacy of polyphenols in humans: Review of bioavailability studies. *Am J Clin Nutr*, 81:230–242.
- Mansouri S, Foroumadi A, Ghaneie T, Najar A G.** 2001. Antibacterial activity of the crude extracts and fractionated constituents of *Myrtus communis*. *Pharm Biol*,39: 399–401.
- Markham K R.**1982. Techniques of Flavonoid Identification (Chap. 1 and 2). London: Academic Press.pp.1- 113.
- Mathew S and Abraham T E.**2006. *In vitro* antioxidant activity and scavenging effects of *Cinnamomum verum* leaf extract assayed by different methodologies. *Food Chem Toxicol*, 44: 198–206.
- Mazza G, Kay C D, Cottrell T, Holub B J.** 2002. Absorption of anthocyanins from blueberries and serum antioxidant status in human subjects. *J Agr Food Chem*, 50:7731–7737.
- Medhin D G, Hadhazy Bakos P, Verzar Petri G.** 1986. Hypotensive effects of *Lupinus termis* and *Coriandrum sativum* in anaesthetized rats. A preliminary study. *Acta Pharm Hung*, 56: 59–63.
- Medini H, Elaissi A, Khouja M A, Chemli R.** 2013. Phytochemical screening and antioxidant activity of *Juniperus phoenicea* ssp. *phoenicea* L. extracts from two Tunisian locations. *J Exp Bio Agr Sci*,1 (2) :77-82.
- Meera K S and Marcus S M.** 2012. Endothelial dysfunction and oxidative stress in hypertension. *Asian J Med Res*, 1 (3): 84-89.
- Mekonnen T, Urga K, Engidawork E.** 2010.Evaluation of the diuretic and analgesic activities of the rhizomes of *Rumex abyssinicus* Jacq in mice. *J Ethnopharmacol*,127: 433–439.
- Mezzouga N, Elhadri A, Dallouh A, Amkiss S, Skali NS, Abrini J, Zhiri A, Baudouxb D, Diallo B, El- Jaziri M, Idaomar M.** 2007. Investigation of the mutagenic and antimutagenic effects of *Origanum compactum* essential oil and some of its constituents. *Mutat Res*,629:100-110.
- Middleton E, Kandaswami C, Theoharides TC.** 2000. The effects of plant flavonoids on mammalian cells: implications for inflammation. Heart disease and cancer. *Pharmacol Rev*, 52: 4673–4751.
- Mimica-Dukić N, Bugarin D, Slavenko Grbović S, Mitić-Ćulafić D, Vuković-Gačić B, Orčić D, Jovin E, Couladis M.** 2010. Essential Oil of *Myrtus communis* L. as a potential antioxidant and antimutagenic agents. *Molecules*, 15: 2759-2770.
- 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: 1113–1122.
- Moghrani H and Maachi R.** 2008.Valorization of *Myrtus communis* essential oil obtained by steam driving distillation. *Asian J Sci Res*,1(5): 518-524.
- Mohamed N A and Nassier OA.** 2013. The antihyperglycaemic effect of the aqueous extract of *Origanum vulgare* leaves in streptozotocin-induced diabetic Rats. *Jordan J Biol Sci*, 6(1): 31 – 38.
- Montejano-Rodríguez J R, Almaguer-Vargas G, Gayosso-De-Lucio J A, Ocharan Hernández M E, Moreno Martínez R E, Hernández Caballero M E, Torres-Valencia J JM, Sierra**

- Rami rez J A.** 2013. Evaluation of the diuretic activity of the ethanolic extract of *Geranium seemannii* Peyr. in Wistar rats. *J Pharm Res*,6 : 709 -713.
- Montezano AC and Touyz RM.**2012. Oxidative stress, Noxs, and hypertension: experimental evidence and clinical controversies”. *Annu Med*, 44: 2-16.
- Montoro P, Tuberoso CIG, Piacente S, Perrone A, De Feo V, Cabras P, Pizza C.** 2006. Stability and antioxidant activity of polyphenols in extracts of *Myrtus communis* L. berries used for the preparation of myrtle liqueur. *J Pharm Biom Anal*, 41 : 1614–1619.
- Montoro P, Tuberoso CIG, Perrone A, Piacente S, Cabras P, Pizza C.**2008. Characterisation by liquid chromatography–electrospray tandem mass spectrometry of anthocyanins in extracts of *Myrtus communis* L. berries used for the preparation of myrtle liqueur. *J Chromatogr A*,1112:232–240.
- Moyo B, Oyedemi S, Masika P J, Muchenje V.** 2012. Polyphenolic content and antioxidant properties of *Moringa oleifera* leaf extracts and enzymatic activity of liver from goats supplemented with *Moringa oleifera* leaves/sunflower seed cake. *Meat Sci*,91: 441–447.
- Munzel T, Gori T, Bruno R M, Taddei S.** 2010. Is oxidative stress a therapeutic target in cardiovascular disease? *Eur Heart J*, 31: 2741-2748.
- Nabavi S M, Ebrahimzadeh M A, Nabavi S F, Jafari M.** 2008. Free radical scavenging activity and antioxidant capacity of *Eryngium caucasicum* and *Froripia subpinata*. *Pharmacologyonline*, 3:19-25.
- Najeeb Q, Bhaskar N, Masood I, Wadhwa S, Kaur H, Ishaq S.** 2012. Malondialdehyde (MDA) superoxide dismutase (SOD) levels-distinguishing parameters between benign malignant pleural effusions.*Free Radic Antiox*, 2: 8–11.
- Nakatani N and Kikuzaki H.** 1987. A new antioxidative glucoside isolated from oregano (*Origanum vulgare* L.). *J Agr Biol Chem*,51:2727–2732.
- 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):56-64
- Nakiboglu M, Urek RO, Kayali HA, Tarhan L.** 2007. Antioxidant capacities of endemic *Sideritis sipylea* and *Origanum sipyleum* from Turkey.*Food Chem*,104: 630-635.
- 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): 45-54.
- Nassar M I, Abou tabl SA, Ahmed R F, El-Khrisy E D A, Ibrahim K M, Sleem A A.** 2010. Secondary metabolites and bioactivities of *Myrtus communis*. *Pharmacogn Res*, 2:235-242.
- Navarro-Yepes J, Zavala-Flores L, Anandhan A, Wang F, Skotak M, Chandra N, Li M, Pappa A, Martinez-Fong D, Del Razo L M, Quintanilla-Vega B, Franco R.** 2014. Antioxidant gene therapy against neuronal cell death. *Pharmacol Ther*, 142: 206–230.
- Neuhaus-Carlisle K, Vierling W, Wagner H.** 1993. Calcium-antagonistic activity of extracts and constituents of *Petroselinum crispum* and other phenylpropane derivatives.*Planta Medica*, 59: 582-583.

- Ni'ciforovi' c N, Mihailovi' c V, Ma'skovi' c P, Soluji' c S, Stojkovi' c A, Muratspahi'c DP.** 2010. Antioxidant activity of selected plant species; potential new sources of natural antioxidants. *Food Chem Toxicol*, 48:3125–3130.
- Nilveses N, Wammanachinda W, Wanverakul B, Pidech P.** 1989. Diuretic effect of *Pluchea indica*. *Thai J Pharmacol*, 11: 1–7.
- Nistala R, Whaley-Connell A, Sowers J R.** 2008. Redox control of renal function and hypertension. *Antiox Redox Signal*, 10: 2047-2089.
- Norhaizan M E, Norashareena M S, Abdah M A.** 2011. Antioxidant and cytotoxicity effect of rice brain phytic acid as an anticancer agent on ovarian, breast and liver cancer cell lines. *Mal J Nutr*, 17(3): 367 -375.
- Nugroho A E, Malik A, Pramono S.** 2013. Total phenolic and flavonoid contents, and *in vitro* antihypertension activity of purified extract of Indonesian cashew leaves (*Anacardium occidentale* L.) *Int Food Res J*, 20(1): 299-305.
- Nunes XP, Silva F S, Almeida G J, Tolentino de Lima J, Ribeiro L A A, Quintans Júnior L G, Filho G M B.** 2012. Biological oxidations and antioxidant activity of natural products in : Phytochemicals as nutraceuticals – global approaches to their role in nutrition and health. Dr Venketeshwer Rao (Ed.), InTech, ISBN: 978-953-51-0203-8, pp:1-20.
- Ojeda D, Jiménez-Ferrer E, Zamilpa A, Tortoriello J, Alvarez L.** 2010. Inhibition of angiotensin converting enzyme (ACE) activity by anthocyanins dephinidin- and cyanidin-3-O-sambubiosides from *Hibiscus sabdariffa*. *J Ethnopharmacol*, 128: 7- 10.
- Olatunde F E, Ogundipe OO, Uhunwangho E S, Adeyanju MA, Olarenwaju M J.** 2003. Antioxidant properties of extracts from *Alchornea laxiflora* (Benth) Pax and Hoffman. *Phytother Res*, 17:713-716.
- Oliveira C, Amaro L F, Pinho O, Ferreira I M.** 2010. Cooked blueberries: Anthocyanin and anthocyanidin degradation and their radical-scavenging activity. *J Agr Food Chem*, 58: 9006–9012.
- 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*, 10:475–484.
- Orhan I, Oezcelik B, Kartal M, Karaoglu T, Bilge F.** 2007. HPLC quantification of vitexine-2"-O-rhamnoside and hyperoside in three *Crataegus* species and their antimicrobial and antiviral activities. *Chromatographia*, 66: S153- S157.
- Othman A, Ismail A, Ghani NA, Adenan I.** 2007. Antioxidant capacity and phenolic content of cocoa beans. *Food Chem*, 100:1523–1530.
- Oukil N, Bedjou F, Maiza-Benabdesselam F, Chibane M.** 2011. Antioxidant activity and separation of phenolic compounds of *Origanum glandulosum* from north Algeria by high performance liquid chromatography (HPLC). *African J Biotechnol*, 10(17): 3451-3454.
- Ozsoy N, Can A, Yanardag R, Akev N.** 2008. Antioxidant activity of *Smilax excels* L. leaf extracts. *Food Chem*, 110: 571–583.
- Özyürek M, Bener M, Güçlü1 K, Dönmez A A, Süzgeç-Selçuk S, Pırıldar S, Meriçli A H, and Apak R.** 2012. Evaluation of antioxidant activity of *Crataegus* species collected from different regions of Turkey. *Rec Nat Prod*, 6(3):263-277.

- Pandanaboina S C, Kondeti S R, Rajbanshi S L, Kunala P N, Pandanaboina S, Pandanaboina M M, Wudayagiri R.**2012. Alterations in antioxidant enzyme activities and oxidative damage in alcoholic rat tissues: Protective role of *Thespesia populnea*. *Food Chem*, 132: 150–159.
- Parejo I, Viladomat F, Bastida J, Rosas-Romero A, Flerlage N, Burillo J, Codina F.** 2002. Comparison between the radical scavenging activity and antioxidant activity of six distilled and nondistilled Mediterranean herbs and aromatic plants. *J Agr Food Chem*, 50:6882-6890.
- Patel B D, Kamariya Y H, Patel M B.**2011. Free radical scavenging potential of ethanolic extract of *Uraria picta* linn. *Pharmacologyonline*, 2: 134-145.
- Patel U, Kulkarni M, Undale V, Bhosale A.**2009. Evaluation of diuretic activity of aqueous and methanol extracts of *Lepidium sativum* Garden Cress (Cruciferae) in rats. *Trop J Pharm Res*,8 (3): 215-219.
- Patrick-Iwuanyanwu K C, Wegwu M O, Makhmoor T.** 2011. Hepatoprotective effect of crude methanolic extract and fractions of ring worm plant *Senna alata* (L. Roxb) leaves from Nigeria against carbon tetrachloride –induced hepatic damage in rats. *Eur J Exp Biol*, 1 (1): 128-138.
- Pendyala L K, Li J, Shinke T, Geva S, Yin X, Chen J P, King III S B, Robinson K A, Chronos N F, Hou D.**2009. Endothelium-dependent vasomotor dysfunction in pig coronary arteries with paclitaxel-eluting stents is associated with Inflammation and oxidative stress. *Cardiovas Interv*, 2 (3):253-258.
- Pereira P, Cebola M J, Bernardo-Gil MG.** 2012. Comparison of antioxidant activity in extracts of *Myrtus communis* L., obtained by SFE vs. solvent extraction. *J Env Sci EngA*,1:115–220.
- Perez-Vizcaino F and Duarte J.**2010. Flavonols and cardiovascular disease. *Mol Aspects Med*, 31: 478–494.
- Perron N R and Brumaghim J L.** 2009. A review of the antioxidant mechanisms of polyphenol compounds related to iron binding. *Cell Biochem Biophys*, 53: 75–100.
- Perusquia M, Mendoza S, Bye R, Linares E, Mata R.** 1995. Vasoactive effects of aqueous extracts from five Mexican medicinal plants on isolated rat aorta. *J Ethnopharmacol*, 46: 63–69.
- Petlevski R, Flajs D, Kalodera Z, Zovko Končić M.** 2013. Composition and antioxidant activity of aqueous and ethanolic *Pelargonium radula* extracts. *S Afr J of Bot*, 85:17–22.
- Pham-Huy L A, He H, Pham-Huy C.** 2008. Free radicals, antioxidants in disease and health. *Int J Biomed Sci*,4: 89–96.
- Phillips O A, Mathew K T, Oriowo M A.**2006. Antihypertensive and vasodilator effects of methanolic and aqueous extracts of *Tribulus terrestris* in rats. *J Ethnopharmacol*, 104: 351–355.
- Piccoli C, Quarato G, D’Aprile A, Montemurno E, Scrima R, Ripoli M, Gomaraschi M.** 2009. Native LDL-induced oxidative stress in human proximal tubular cells: multiple players involved. *J Cell Mol Med*,15(2): 375-395.
- Prinz S, Ringl A, Huefner A, Enne P, Brigitte K.** 2007. 4 "-acetylvitexin-2 "-O-rhamnoside, isoorientin, orientin, and 8-methoxykaempferol-3-O-glucoside as markers for the differentiation of *Crataegus monogyna* and *Crataegus pentagyna* from *Crataegus laevigata* (Rosaceae). *Chem Biodiv*, 4 (12): 2920-2931.

- Prior R L, Wu X, Schaich, K.** 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J Agr Food Chem*, 53: 4290–4302.
- Priyadharshini D and Sujatha V.** 2012. Antioxidant assessment for various solvent fractions of *Cassia fistula* Linn.Flowers. *Int J Pharm Tech Res*, 4 (1): 510-517.
- Procházková D, Boušová I, Wilhelmová N.** 2011. Antioxidant and prooxidant properties of flavonoids. *Fitoterapia*, 82: 513–523.
- Quezel P and Santa S.** 1963. Nouvelle flore de l'Algérie et des régions désertiques méridionales, Tome 11. Ed. CNRS, Paris, pp 804-806.
- Quiñones M, Muguerza B, Miguel M, Aleixandre A.** 2011. Evidence that nitric oxide mediates the blood pressure lowering effect of a polyphenol-rich cocoa powder in spontaneously hypertensive rats. *Pharmacol Res*, 64: 478– 481.
- Rah D K, Han D W, Sook Baek H, Hyon S Y, Park J C.** 2005. Prevention of reactive oxygen species-induced oxidative stress in human microvascular endothelial cells by green tea polyphenol. *Toxicol Let*, 155: 269–275.
- Rahal A, Kumar A, Singh V, Yadav B, Tiwari R, Chakraborty S, Dhama K.** 2014. **Oxidative stress, pro-oxidants, and antioxidants: The Interplay.** *Biomed Res Int*. doi: 10.1155/2014/761264.
- Rahmat A, Kumar V, Mei L F, Endrini S , Abdullah Sani H.** 2003. Determination of total antioxidant activity in three types of local vegetables shoots and the cytotoxic effect of their ethanolic extracts against different cancer cell lines. *Asian Pac J Clin Nutr*, 12 (3):308-311.
- Rajapakse N, Mendis E , Jung W K, Je J Y, Kim S K.** 2005. Purification of a radical scavenging peptide from fermented mussel sauce and its antioxidant properties. *Food Res Int*, 38: 175–182.
- Rattanachaikunsopon P and Phumkhachorn P.** 2010. Assessment of factors influencing antimicrobial activity of carvacrol and cymene against *Vibrio cholerae* in food. *J Biosci Bio eng*, 110:614–619.
- Rayese A, Raja V, Sharma M.** 2013. Hepatoprotective activity of ethyl acetate extract of *adhatoda vasica* in swiss albino rats. *Int J Cur Res Rev*, 5(6): 16-21.
- Re R.** 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med*, 26:1231–1237.
- Refaie A A, Ramadan A, Mossa AT.** 2014. Oxidative damage and nephrotoxicity induced by prallethrin in rat and the protective effect of *Origanum majorana* essential oil. *Asian Pac J Trop Med*, 7(1): 506-513.
- Rein D, Lotito S, Holt R R, Keen C L, Schmitz H H, Fraga C G.** 2000. Epicatechin in human plasma: *in vivo* determination and effect of chocolate consumption on plasma oxidation status. *J Nutr*, 130:2109–2114.
- Rezaeizadeh A, Zuki ABZ, Abdollahi M, Goh YM, Noordin MM, Hamid M, Azmi T.** Determination of antioxidant activity in methanolic and chloroformic extracts of *Momordica charantia*. *African J Biotechnol*, 10(24): 4932-4940.
- Rhiouani H, Lyoussi B, Settaf A, Cherrah Y, Hassar M.** 2001. Antihypertensive effect of *Herniaria glabra* saponins in the spontaneously hypertensive rat. *Annales Pharmaceutiques Francaises*, 59: 211–214.

- Rice-Evans C.** 2004. Flavonoids and isoflavones: absorption, metabolism and bioactivity. *Free Radic Biol Med*, 36: 827–828.
- Rivero D, Perez-Magarino S, Gonzalez-SanJose M L, Valls-Belles V, Codoner P, Muniz, P.** 2005. Inhibition of induced DNA oxidative damage by beers: correlation with the content of polyphenols and melanoidins. *J Agr Food Chem*, 53:3637–3642.
- Rodrigo R, Rivera G.** 2002. Renal damage mediated by oxidative stress: a hypothesis of protective effects of red wine. *Free Radic Biol Med*, 33(3):409–422.
- Rodrigo R, Gil D, Miranda-Merchak A, Kalantzidis G.** 2012. Antihypertensive role of polyphenols. *Adv Clin Chem*, 58:225–254.
- Romani A, Pinelli P, Mulinacci N, Vincieri F F, Tattini M.** 1999. Identification and quantification of polyphenols in leaves of *Myrtus communis*. *Chromatographia*, 49:17–20.
- Romani A, Coinu R, Carta S, Pinelli P, Galardi C, Vincieri F F, Franconi F.** 2004. Evaluation of antioxidant effect of different extracts of *Myrtus communis* L. *Free Radic Res*, 38:97–103.
- Rosa A, Deiana M, Casu V, Corona G, Appendino G, Bianchi F, Ballero M, Dessì MA.** 2003. Antioxidant activity of oligomeric acylphloroglucinols from *Myrtus communis* L. *Free Radic Res*, 9: 1013–1019.
- Sahin A, Gulluce M, Daferera D, Sokmen A, Sokmen M, Polissiou M, Agar G, Ozer H.** 2004. Biological activities of the essential oils and methanol extract of *Origanum vulgare* ssp. *Vulgare* in the Eastern Anatolia region of Turkey. *Food Control*, 15: 549–557.
- Saadoudi M, Hamebaba Le, Abdeddaim M.** 2012. Study of the glucidic fraction of *Celtis Australis* L, *Crataegus Azarolus* L, *Crataegus Monogyna* Jacq., *Elaeagnus Angustifolia* L. and *Zizyphus Lotus* L. *Fruits .World Acad Sci Eng Technol*, 71:1126–1129.
- Sachse A and Wolf G.** 2007. Angiotensin II-induced reactive oxygen species and the kidney. *J Am Soc Nephrol*, 18(9):2439–2446.
- Sadki C, Hacht B, Souliman A, Atmani F.** 2010. Acute diuretic activity of aqueous *Erica multiflora* flowers and *Cynodon dactylon* rhizomes extracts in rats. *J Ethnopharmacol*, 128: 352–356.
- Saha K, Lajis N H, Israfi D A, Hamzah A S, Khozira S, Khamis S, Syahida A.** 2004. Evaluation of antioxidant and nitric oxide inhibitory activities of selected Malaysian medicinal plants. *J Ethnopharmacol*, 92: 263–267.
- Samochowiec L, Kokot F, Kleinrok Z, Gachowski Z.** 1955. Investigations on hypotensive properties of some galenical preparations of *Viscum album* genus (Loranthaceae). *Farm Pol*, 11(6):128–132.
- Sandoo A, van Zanten V, Metsios G S, Carroll D, Kitas G D.** 2010. The endothelium and its role in regulating vascular tone. *Open Cardiovasc Med J*, 4: 302–312.
- Sari M, Biondi D M, Kaâbeche M, Mandalari G, D'Arrigo M, Bisignano G, Saija A, Daquino C, Ruberto G.** 2006. Chemical composition, antimicrobial and antioxidant activities of the essential oil of several populations of Algerian *Origanum glandulosum* Desf. *Flav Fragr J*, 21, 890–898.
- Sarikurkcu C, Arisoy K, Tepe B, Cakir A, Abali G, Mete E.** 2009. Studies on the antioxidant activity of essential oil and different solvent extracts of *Vitex agnuscastus* L. fruits from Turkey. *Food Chem Toxicol*, 47: 2479–2483.

- Schildknecht S and Ullrich V.** 2009. Peroxynitrite as regulator of vascular prostanoid synthesis. *Arch Biochem Biophys*, 484: 183–189.
- Schmitt CA and Dirsch V M.** 2009. Modulation of endothelial nitric oxide by plant derived products. *Nitric Oxide*, 21: 77–91.
- Schroder D, Weiser M, Klein P.** 2003. Efficacy of a homeopathic *Crataegus* preparation compared with usual therapy for mild (NYHA II) cardiac insufficiency: results of an observational cohort study. *Eur J Heart Fail*, 5: 319–26.
- Schroeter H, Heiss C, Balzer J, Kleinbongard P, Keen CL, Hollenberg N K, Sies H, Kwik-Urbe C, Schmitz H H, Kelm M.** 2006. (–)-Epicatechin mediates beneficial effects of flavanol-rich cocoa on vascular function in humans. *Proc Nat Acad Sci*, 103:1024–1029.
- Sen S and Chakraborty R.** 2011. The role of antioxidants in human health. In: Oxidative stress: Diagnostics, prevention, and therapy (ACS symposium series). Silvana A, Hepel M, editors. Washington DC: American Chemical Society, Chap1, pp. 1–37.
- Sen S, Biplab D, Devanna N, Chakraborty R.** 2013. Total phenolic, total flavonoid content, and antioxidant capacity of the leaves of *Meyna spinosa* Roxb., an Indian medicinal plant. *Chin J Nat Med*, 11(2): 0149–0157.
- Senejoux F, Girard C, Aisa H A, Bakri M, Kerram P, Berthelot A, Bévalot F, Demougeot C.** 2012. Vasorelaxant and hypotensive effects of a hydroalcoholic extract from the fruits of *Nitraria sibirica* Pall. (Nitrariaceae). *J Ethnopharmacol*, 141: 629–634.
- Shan B, Cai Y Z, Brooks J D, Corke H.** 2007. The *in vitro* antibacterial activity of dietary spice and medicinal herb extracts. *Int J Food Microbiol*, 117:112–119.
- Shatoor A S.** 2011. Acute and sub-acute toxicity of *crataegus aronia* Syn. *azarolus* (L.) whole Plant aqueous extract in wistar Rats. *Am J Pharmacol Toxicol*, 6 (2): 37–45.
- Shatoor A S, Soliman H, Al-Hashem F, El- Gamal B, Othman A, El-Menshawy N.** 2012. Effect of Hawthorn (*Crataegus aronia*) on platelet function in albino wistar rats. *Thrombosis Res*, 130: 75–80.
- Shatoor A S.** 2013. *In vivo* hemodynamic and electrocardiographic changes following *Crataegus aronia* (L) administration to normotensive Wistar rats. *Saudi Med J*, 34 (2): 123–134.
- Shihab Z A.** 2013. Study the effects of drenching aqueous extract of *origanum vulgare* on some hematological characteristics of mature male domestic rabbits. *Bas j Vet Res*, 12 (2): 212–220.
- Shiogai Y, Stefanovska A, McClintock P V E.** 2010. Nonlinear dynamics of cardiovascular ageing. *Physics Reports*, 488: 51–110.
- Silva B A, Ferreres F, Malva J O, Dias AC.** 2005. Phytochemical and antioxidant characterization of *Hypericum perforatum* alcoholic extracts. *Food Chem*, 90: 157–167.
- Simic D, Mimic-Oka J, Pljesa-Ercegovac M, Savic-Radojevic A, Opacic M, Matic D, Ivanovic B, Simic T.** 2005. Byproducts of oxidative protein damage and antioxidant enzyme activities in plasma of patients with different degrees of essential hypertension. *J hum hypertens*, 20 (2): 149–155.
- Singh M, Kaur M, Silakari O.** 2014. Flavones: An important scaffold for medicinal chemistry. *Eur J Med Chem*, 84: 206–239.

- Skaltsa H, Chatzopoulou A, Karioti A, Gousiadou C, Vivancos VL, Kyriazopoulos P, Golegou S.** 2010. Depsides and other polar constituents from *Origanum dictamnus* L. and their *in vitro* antimicrobial activity in clinical strains. *J Agr Food Chem*, 58:6064–6068.
- Skotti E, Anastasaki E, Kanellou G, Polissiou M, Tarantilis P A.** 2014. Total phenolic content, antioxidant activity and toxicity of aqueous extracts from selected Greek medicinal and aromatic plants. *Ind Crops Prod*, 53: 46- 54.
- Skoula M, Grayer R J, Kite G C, Veitch N C.** 2008. Exudate flavones and flavanones in *Origanum* species and their interspecific variation. *Biochem Syst Ecol*, 36: 646–654.
- Sonu S S, Sharma G, Sharma V, Rohilla A, Kushnoor A.** 2013. An emerging role of natural antioxidants in hypertension. *Int J Pharm Phytopharmacol Res*, 3 (1): 13-16.
- Sousa T, Afonso J, Carvalho F and Albino-Teixeira A.** 2012. Lipid peroxidation and antioxidants in arterial hypertension. In: *Lipid Peroxidation*. Intech, Open Access Publisher, ISBN: 980-953-307-143-0, Academic Press, Volume 1, Chap: 17, pp: 345-392
- Sreenivasamurthy B, Banji D, Banji O J F.** 2012. Investigation on antioxidant and hepatoprotective activity of ethanolic leaf extract of *Polygonum glabrum* Willd. on carbon tetrachloride-induced hepatotoxicity in rats. *Spatula*, 2(4):199-205.
- Srivastava A and Shivanandappa A.** 2010. Hepatoprotective effect of the root extract of *Decalepis hamiltonii* against carbon tetrachloride-induced oxidative stress in rats. *Food Chem*, 118: 411–417.
- Sugamura K and Keaney J F.** 2011. Reactive oxygen species in cardiovascular disease. *Free Radic Biol Med*, 51: 978-992.
- Sumbul S, Ahmad M A, Asif M, Saud I, Akhtar M.** 2010. Evaluation of *Myrtus communis* Linn. berries (common myrtle) in experimental ulcer models in rats. *Hum Exp Toxicol*, 29(11):935–44.
- Sumbul S, Ahmad M A, Asif M, Akhtar M.** 2011. *Myrtus communis* Linn. A review. *Indian J Nat Prod Resourc*, 2(4): 395-402.
- Sun T and Ho C T.** 2005. Antioxidant activities of buckwheat extracts. *Food Chem*, 90: 743–749.
- Suri S, Liu XH, Rayment S, Hughes DA, Kroon PA, Needs PW, Taylor MA, Tribolo S, Wilson VG.** 2010. Quercetin and its major metabolites selectively modulate cyclic GMP-dependent relaxations and associated tolerance in pig isolated coronary artery. *Br J Pharmacol*, 159: 566–575.
- Svedstroma U, Vuorela H, Kostianen R, Laakso I, Hiltunen R.** 2006. Fractionation of polyphenols in hawthorn into polymeric procyanidins, phenolic acids and flavonoids prior to high-performance liquid chromatographic analysis. *J Chromatography A*, 11(12):103–111.
- Swaminathan J K, Khan M, Mohan I K, Selvendiran K, Niranjali D S, Rivera B K, Kuppusamy P.** 2010. Cardioprotective properties of *Crataegus oxycantha* extract against ischemia-reperfusion injury. *Phytomed*, 17:744–752.
- Tadic V M, Dobric S, Markovic GM, Sofija M, Tanja S.** 2008. Anti-inflammatory, gastroprotective, free-radicalscavenging and antimicrobial activities of hawthorn berries ethanol extract. *J Agr Food Chem*, 56: 7700- 7709.
- Taheri A, Seyfan A, Jalalinezhad S, Nasery F.** 2013. Antibacterial effect of *Myrtus communis* hydro-alcoholic extract on pathogenic bacteria. *Zahedan J Res Med Sci*, 15(6):19–24.

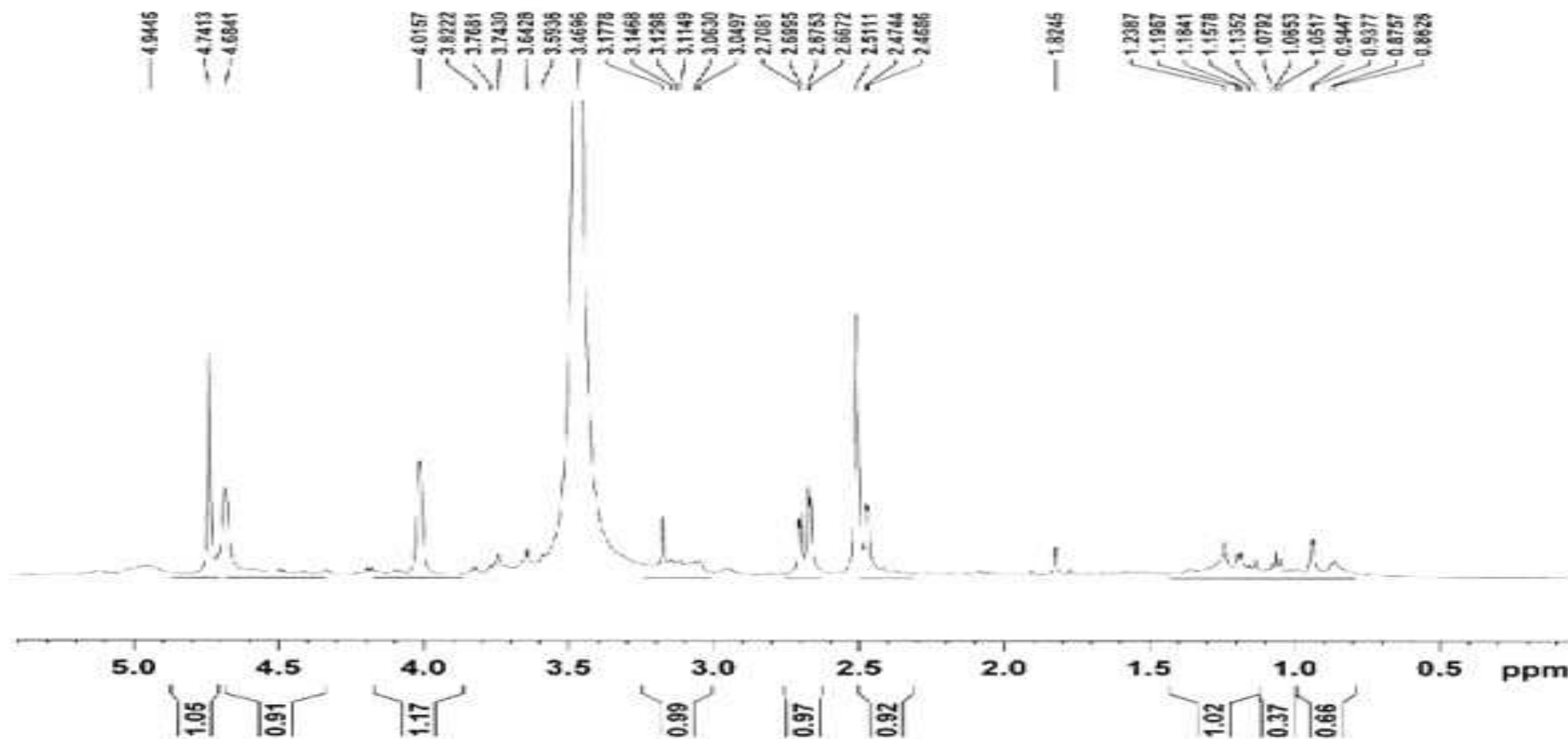
- Tahraoui A J, El-Hilaly E , Israili Z H, Lyoussi, B.** 2007. Ethnopharmacological survey of plants used in the traditional treatment of hypertension and diabetes in south-eastern Morocco. *J Ethnopharmacol*, 110: 105–117.
- Tahri A , Yamani S, Legssyer A , Aziz M, Mekhfi H , Bnouham M, Ziyat A.** 2000. Acute diuretic, natriuretic and hypotensive effects of a continuous perfusion of aqueous extract of *Urtica dioica* in the rat. *J Ethnopharmacol*, 73: 95–100.
- Tanito M, Nakamura H, Kwon YW, Teratani A, Masutani H, Shioji K, Kishimoto C, Ohira A, Horie R, Yodoi J.** 2004. Enhanced oxidative stress and impaired thioredoxin expression in spontaneously hypertensive rats. *Antioxid Redox Signal*, 6:89–97.
- Taniyama Y, Griending K K.** 2003. Reactive oxygen species in the vasculature: molecular and cellular mechanisms. *Hypertens*, 42(6):1075–1081.
- Tanumihardjo SA.** 2013. Carotenoids and Human Health. Humana Press, Springer. USA.
- Tanzadehpanah H, Asoodeh A, Chamani J.** 2012. An antioxidant peptide derived from Ostrich (*Struthio camelus*) egg white protein hydrolysates. *Food Res Int*, 49:105–111.
- Torres-Piedra M, Figueroa M, Hernandez-Abreu O, Ibarra-Barajas M, Navarrete-Vazquez G, Estrada-Soto S.** 2011. Vasorelaxant effect of flavonoids through calmodulin inhibition: *ex vivo*, *in vitro*, and *in silico* approaches. *Bioorg Med Chem*, 19: 542–546.
- Tretiakova I, Blaesius D, Maxia L, Wesselborg S, Schulze- Osthoff K, Cinatl J Jr, Michaelis M, Werz O.** 2008. Myrtucommulone from *Myrtus communis* induces apoptosis in cancer cells via the mitochondrial pathway involving caspase-9. *Apoptosis*, 13(1):119–131.
- Tuberoso C I G, Rosa A, Bifulco E, Melis MP, Atzeri A, Pirisi F M, Dessì M A.** 2010. Chemical composition and antioxidant activities of *Myrtus communis* L. berries extracts. *Food Chem*, 123:1242–1250.
- Valentova K, Vrba J, Bancir̃ova M, Ulrichova J, Kr̃en V.** 2014. Isoquercitrin: Pharmacology, toxicology, and metabolism. *Food Chem Toxicol*, 68 : 267–282.
- Valko M, Leibfritz D, Moncol J, Cronin M T D, Mazur M, Telser J.** 2007. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol*, 39: 44–84.
- Vekiari S A, Oreopoulou V, Tzia C, Thomopoulos CD.** 1993. Oregano flavonoids as lipid antioxidants. *J Am. Oil Chem Soc*, 70: 483–487.
- Veskoukis A S, Kyparos A, Nikolaidis MG, Stagos D, Aligiannis N, Halabalaki M, Chronis K, Goutzourelas N, Skaltsounis L, Kouretas D.** 2012. The antioxidant effects of a polyphenol-rich grape pomace extract *in vitro* do not correspond *in vivo* using exercise as an oxidant stimulus. *Oxid Med Cell Longev*. doi: 10.1155/2012/185867.
- 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: 116–122.
- Viel EC, Lemarié CA, Benkirane K, Paradis P, Schiffrin EL.** 2010. Immune regulation and vascular inflammation in genetic hypertension. *Am J Physiol Heart Circ Physiol*, 298(3):938–944.
- Vinson JA, Yong H, Xuchi S, Zubik L.** 1998. Phenol antioxidant quantity and quality in foods: Vegetables. *J Agr Food Chem*, 46: 3630–3634.

- Wali U, Binji A A, Ahmad K and Usman A.**2012. Lipid peroxidation and lipid profile in hypertensive patients in Sokoto, Nigeria. *Nig J Basic Appl Sci*, 20(3): 199-204.
- Walker A F, Marakis G, Simpson E, Hope J L, Robinson P A , Hassanein M, Simpson H C R.** 2006. Hypotensive effects of hawthorn for patients with diabetes taking prescription drugs: a randomised controlled trial. *British J General Practice*, 56(527): 437–443.
- Wang J, Yuan X, Jin Z, Tian Y, Song H.** 2007. Free radical and reactive oxygen species scavenging activities of peanut skins extract. *Food Chem*, 104: 242–250.
- Wang A N, Yi Y X, Yu H F, Dong B and Qiao S Y.** 2009. Free radical scavenging activity of *Lactobacillus fermentum in vitro* and its antioxidative effect on growing–finishing pigs. *J Appl Microbiol*; 107, 1140–1148.
- Wang S Y and Chen C T.** 2010. Effect of allyl isothiocyanate on antioxidant enzyme activities, flavonoids and post-harvest fruit quality of blueberries (*Vaccinium corymbosum* L., cv. Duke). *Food Chem*, 122: 1153–1158.
- Wang H P, Lu G F, Zhang G L, Li X Y, Peng H Y, Lu H, Zhao L, Ye Z J, Bruce I C, Xia Q, Qian L B.** 2014. Endothelium-dependent and –independent vasorelaxant actions and mechanisms induced by total flavonoids of *Elsholtzia splendens* in rat aortas. *Env Toxicol Pharmacol*, 38:453–459.
- Ward N C and Croft K D.** 2006. Hypertension and oxidative stress. *Clin Exp Pharmacol Physiol*, 33:872–876.
- Webb R C.** 2003. Smooth muscle contraction and relaxation. *Advan in Physiol Edu*, 27:201-206.
- Whitney E N and Rolfes S R.** 2008. Understanding nutrition. (10thed), Wadsworth Publishing. pp.632.
- Wilson D P, Sutherland C Walsh M P.** 2002. Ca²⁺ Activation of smooth muscle contraction, evidence for the involvement of calmodulin that is bound to the triton-insoluble fraction even in the absence of Ca²⁺. *J Biol Chem*, 277(3), 2186–2192.
- Wong D Z H, Abdul Kadir H, Ling S K.** 2012. Bioassay-guided isolation of neuroprotective compounds from *Loranthus parasiticus* against H₂O₂-induced oxidative damage in NG108-15 cells. *J Ethnopharmacol*, 139: 256– 264.
- Wu P, Ma G, Li N, Deng Q, Yin , Huang R.** 2015. Investigation of *in vitro* and *in vivo* antioxidant activities of flavonoids rich extract from the berries of *Rhodomyrtus tomentosa* (Ait.) Hassk. *Food Chem*, 173: 194–202.
- Xiao J, Chen T, Cao H.** 2014. Flavonoid glycosylation and biological benefits. *Biotechnol Adv.*(In press). doi:10.1016/j.biotechadv.2014.05.004
- Xu Y C., Leung S W S, Yeung D K Y, Hu L H, Chen G H, Che CM, Man R Y.** 2007. Structure -activity relationships of flavonoids for vascular relaxation in porcine coronary artery, *Phytochem*, 68: 1179-1181.
- Yadegarinia D, Gachkar L, Rezaei M B, Taghizadeh M, Astaneh S A, Rasooli I.**2006. Biochemical activities of Iranian *Mentha piperita* L. and *Myrtus communis* L. essential oils. *Phytochem*, 67: 1249–1255.

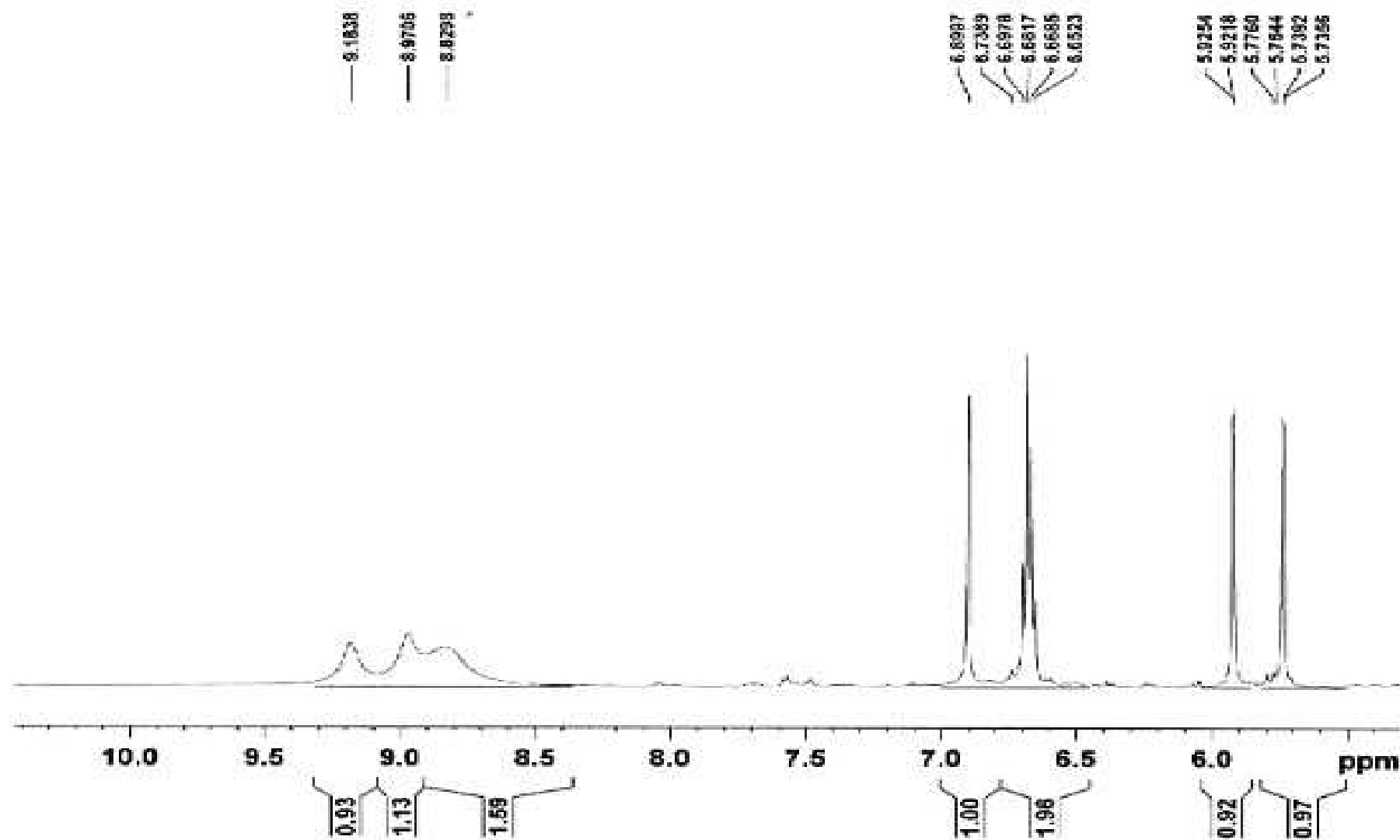
- Yang Q, Sun H Y, Zhang L, Xu L, Hu M Y, Liu X Y, Shi F Y, Gu Z Y.** 2014. Antihypertensive effects of extract from flower buds of *Coreopsis tinctoria* on spontaneously hypertensive rats. *Chin Herbal Med*, 6(2): 103-109.
- Ye Z W, Zhang J, Townsend D M, Tew K D.** 2014. Oxidative stress, redox regulation and diseases of cellular differentiation. *Biochim Biophys Acta*. (In press). doi: 10.1016/j.bbagen.2014.11.010
- Yen G C, Chang Y C, Su S W.** 2003. Antioxidant activity and active compounds of rice koji fermented with *Aspergillus Candidus*. *Food Chem*, 83:49–54.
- Yu L L.** 2001. Free radical scavenging properties of conjugated linoleic acids. *J Agr Food Chem.*, 49(7): 3452-3456.
- Zhang G, Zeng X, Han L, Wei J, Huang H.** 2010. Diuretic activity and kidney medulla AQP1, AQP2, AQP3, V2R expression of the aqueous extract of sclerotia of *Polyporus umbellatus* FRIES in normal rats. *J Ethnopharmacol*, 128:433–437.
- Zhang C, Hu J J, Xia M, Boini K M, Brimson C, Li P L.** 2010. Redox signaling via lipid raft clustering in homo-cysteine-induced injury of podocytes. *Biochim Biophys Acta*, 1803(4):482-491.
- Zhou R and Li S.** 2009. *In vitro* antioxidant analysis and characterisation of antler velvet extract. *Food Chem*, 114: 1321–1327.
- Zimmerman M C, Lazartigues E, Sharma R V, Davisson R L.** 2004. Hypertension caused by angiotensin II infusion involves increased superoxide production in the central nervous system. *Circ Res*, 95(2):210-216.
- Zin Z M, Abdul-Hamid A, Osman A.** 2002. Antioxidative activity of extracts from Mengkudu (*Morinda citrifolia* L.) root, fruit and leaf. *Food Chem*, 78:227–231.
- Ziyyat A, Legssyer A, Mekhfi H, Dassouli A, Serhrouchni M, Benjelloun W.** 1997. Phytotherapy of hypertension and diabetes in oriental Morocco. *J Ethnopharmacol*, 58: 45–54.

Appendixes

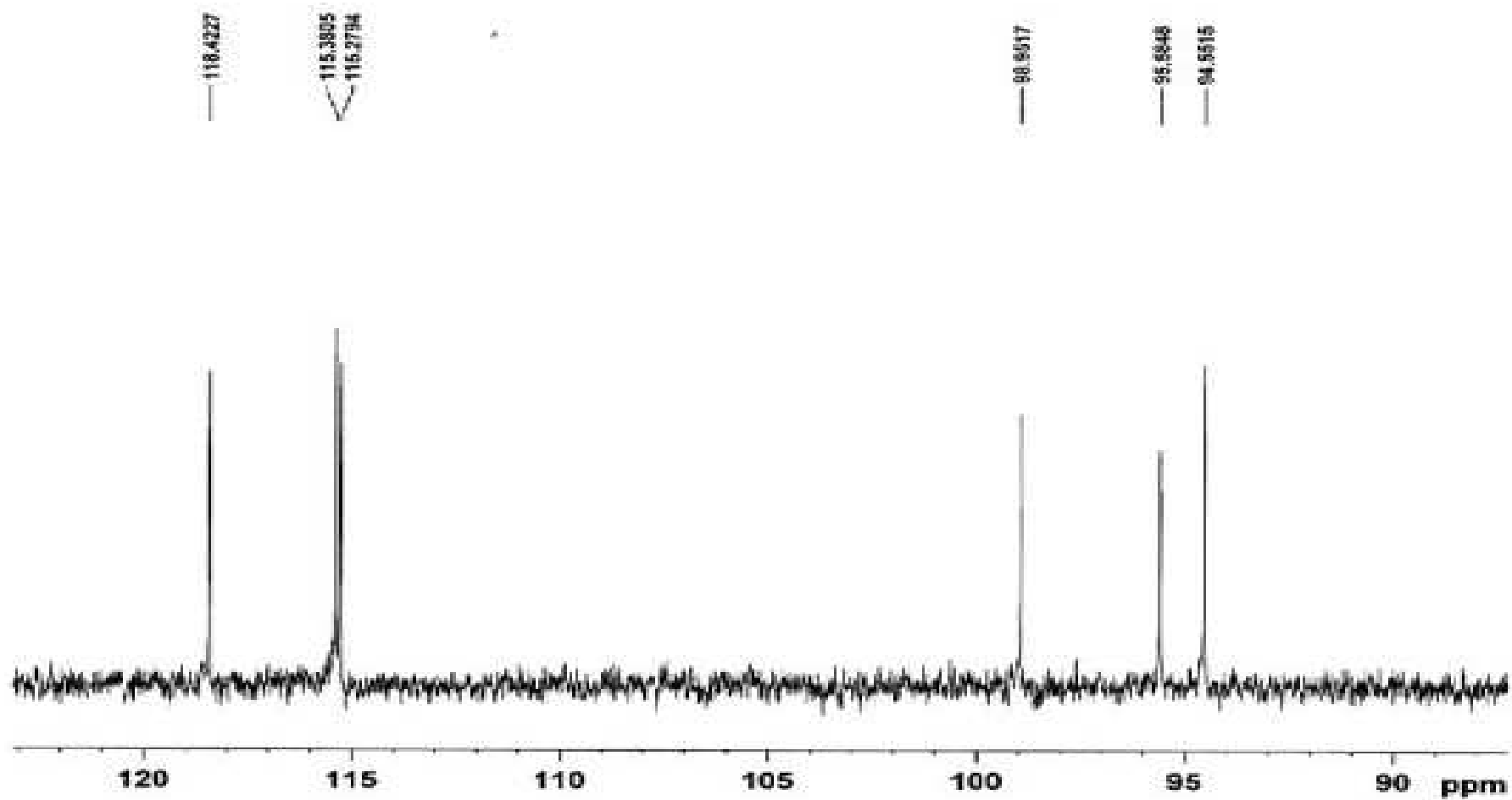
Appendix A



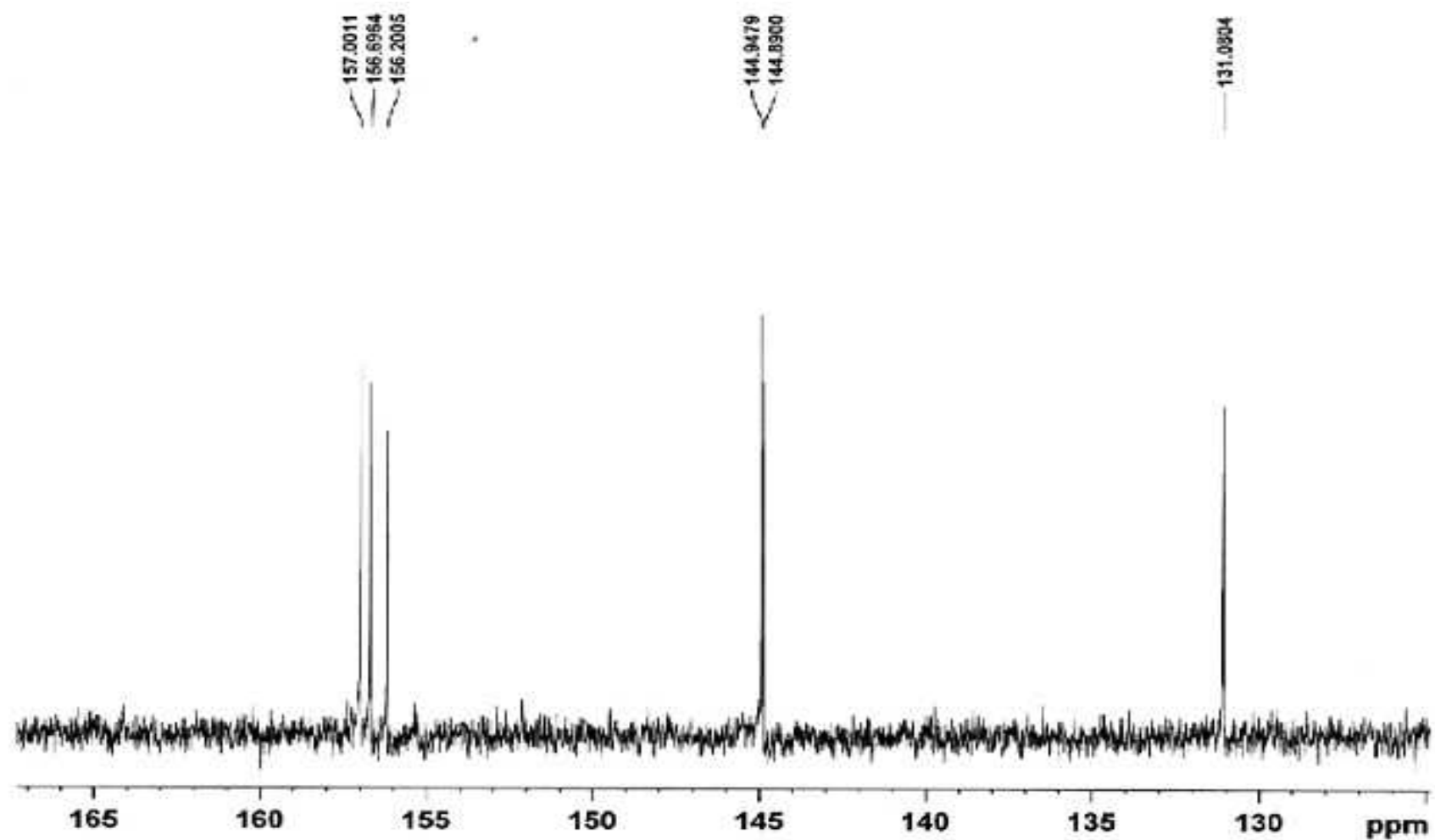
¹H-NMR(500MHz,DMSO) spectrum of (+)- Catechin(0.5-5 ppm).



^1H -NMR (500MHz, DMSO) spectrum of (+)- Catechin(5-10 ppm).

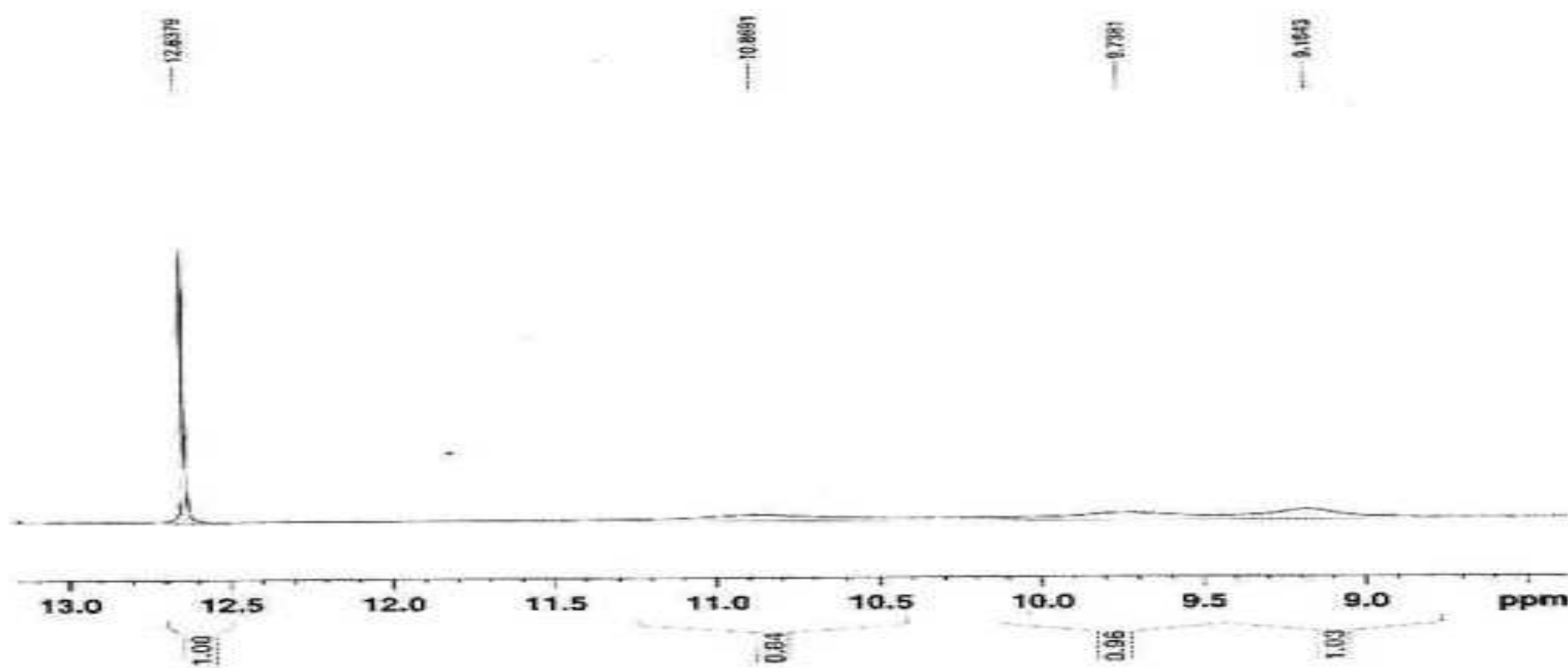


^{13}C -NMR(500MHz, DMSO) spectrum of (+)- Catechin (90-120ppm).

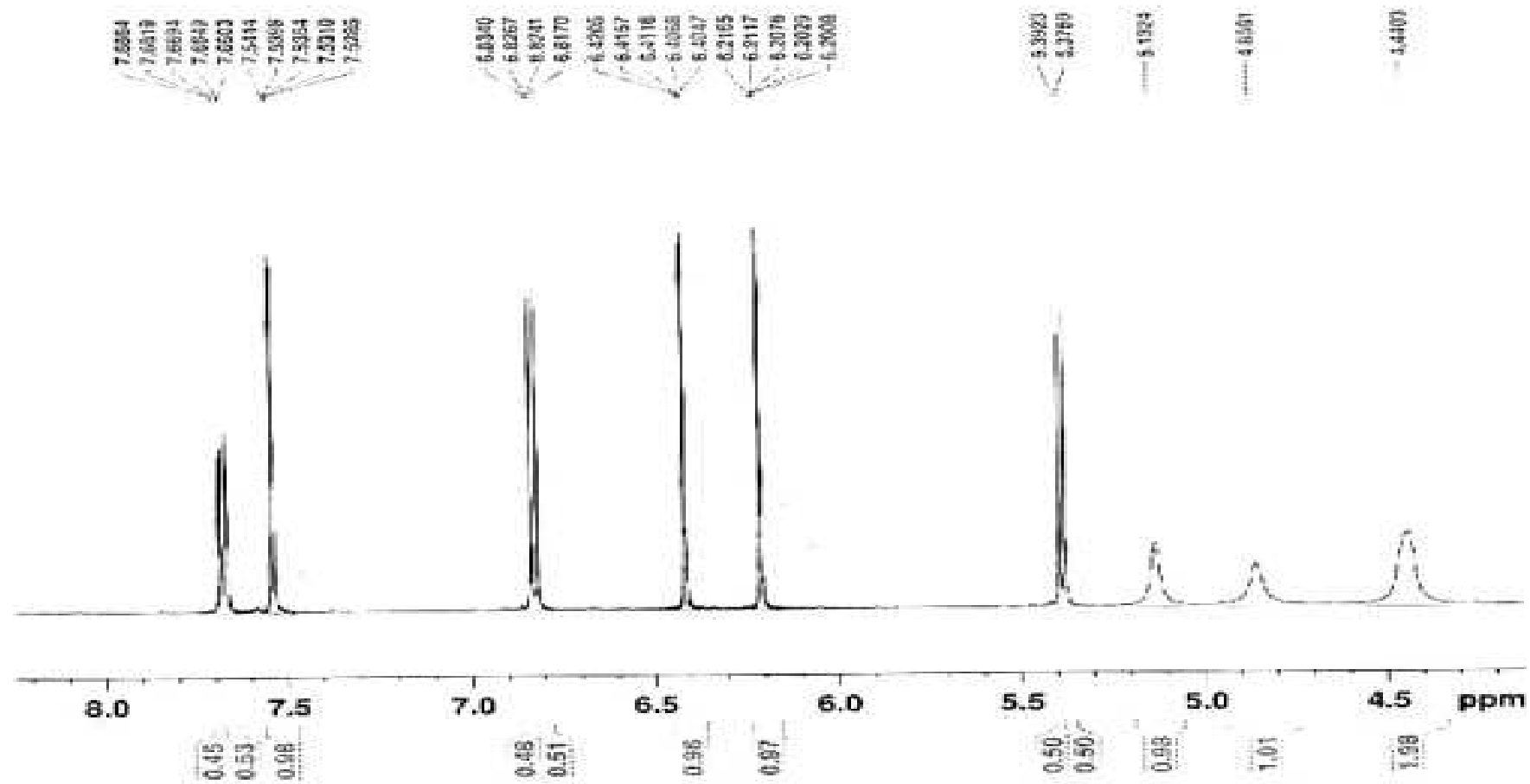


^{13}C -NMR(500MHz, $\text{DMSO}-d_6$) spectrum of (+)- Catechin(130-165ppm)

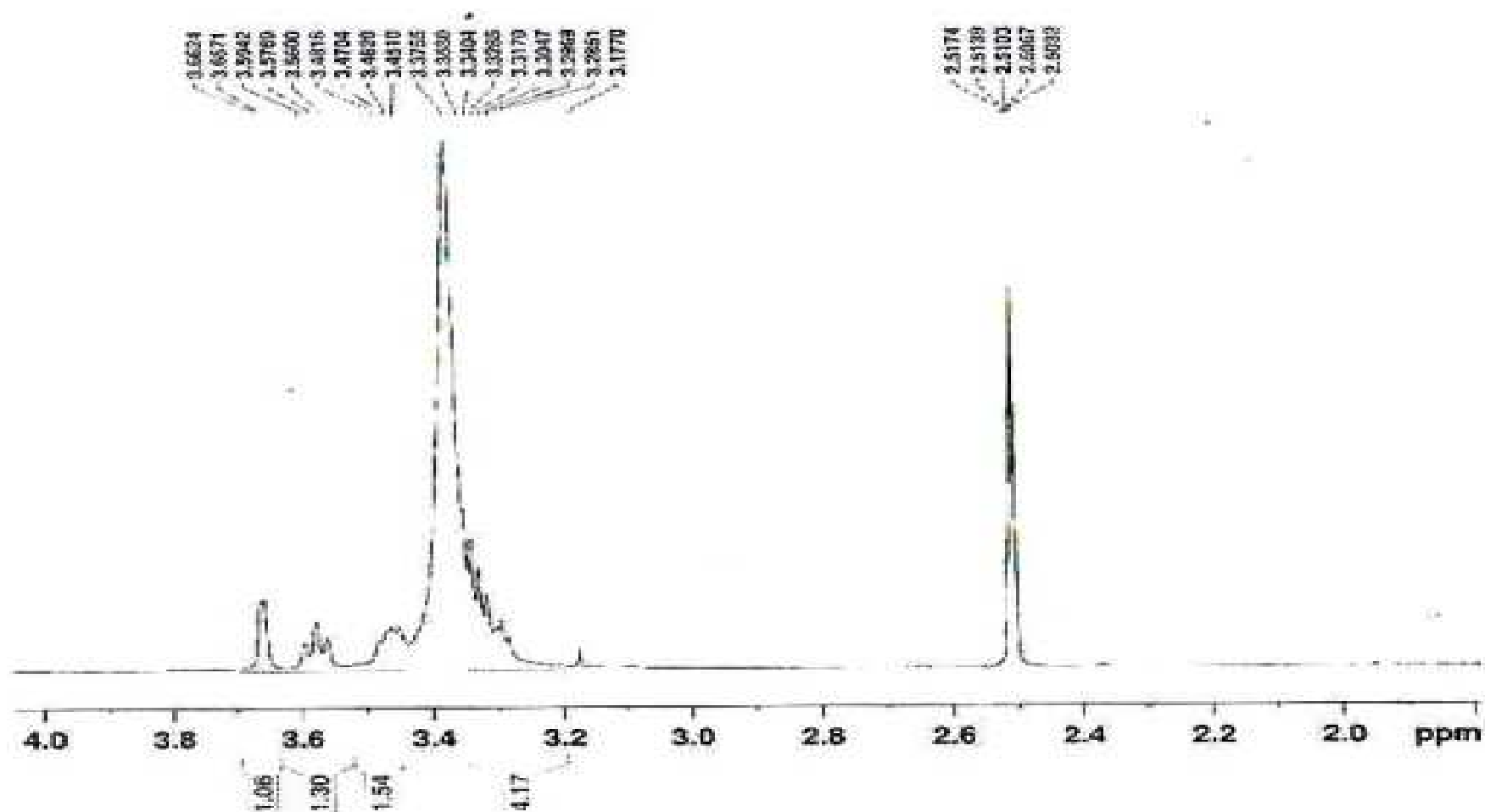
Appendix B



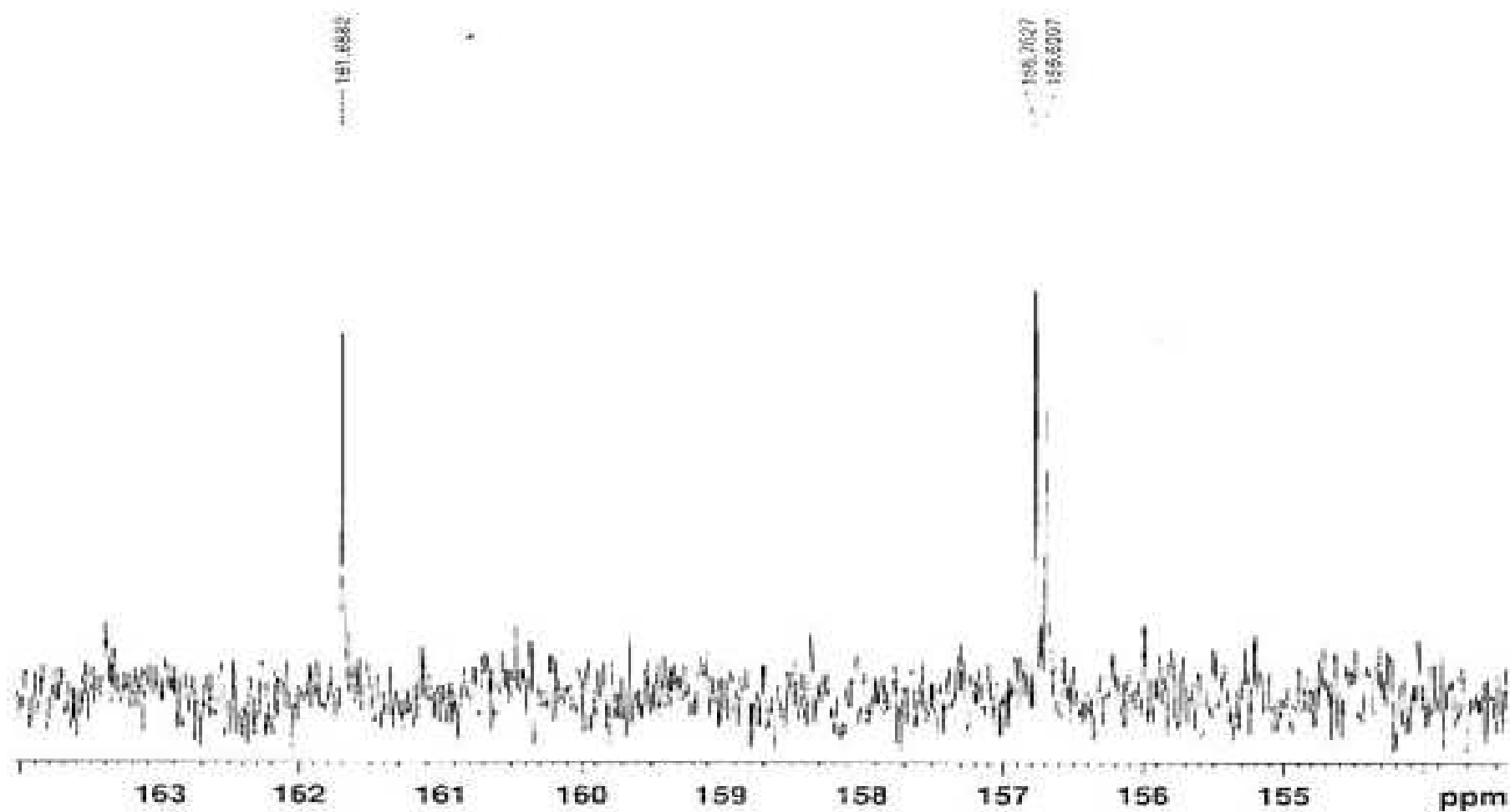
^1H -NMR (500MHz, DMSO) spectrum of quercetin-3-O-galactoside(9- 13 ppm).



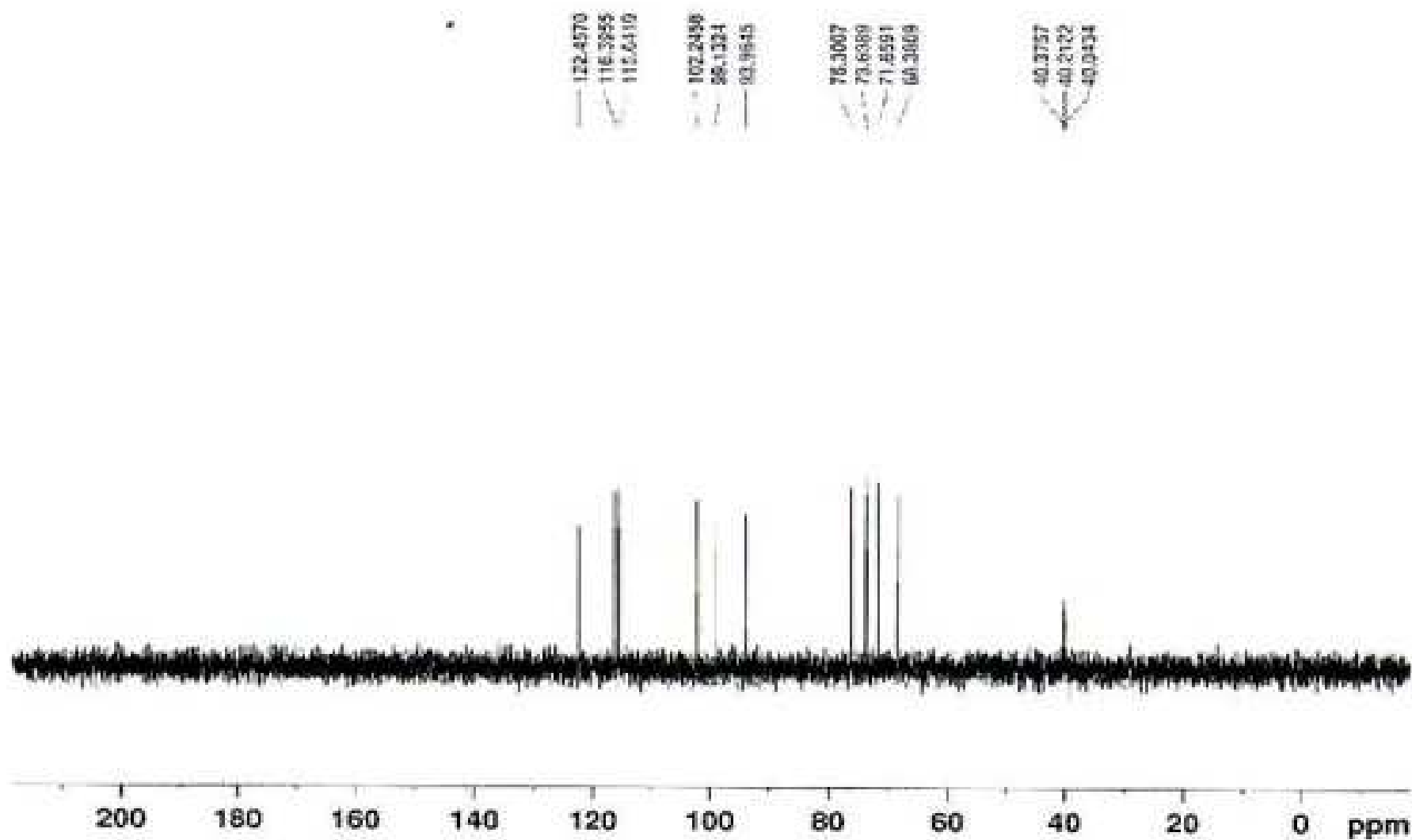
^1H -NMR (500MHz, DMSO) spectrum of quercetin-3-O-galactoside (4.5-8 ppm).



^1H -NMR (500MHz, DMSO) spectrum of quercetin-3-O-galactoside (2-4 ppm).

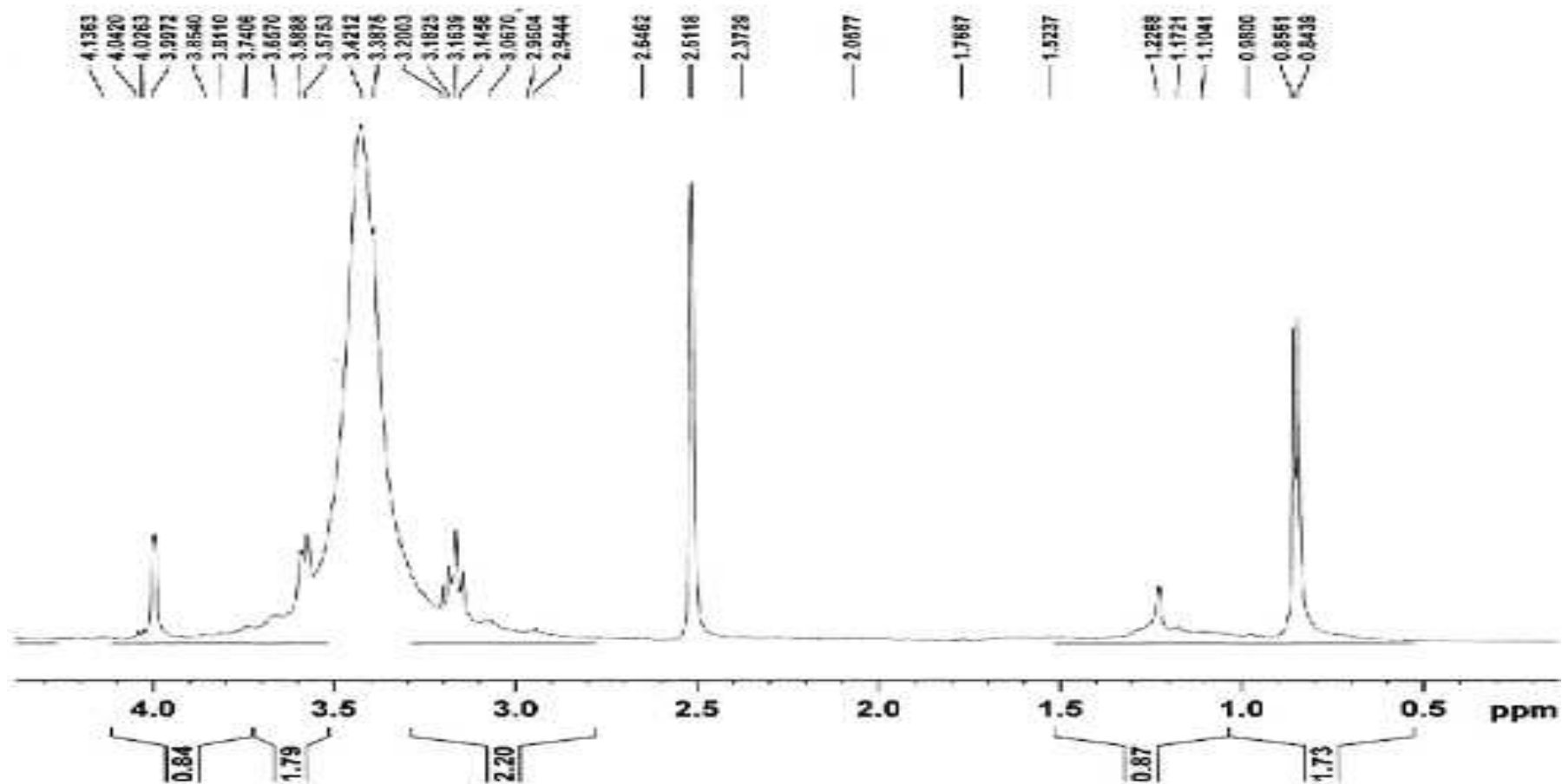


^{13}C -NMR (500MHz, DMSO) spectrum of quercetin-3-O-galactoside(155-163 ppm).

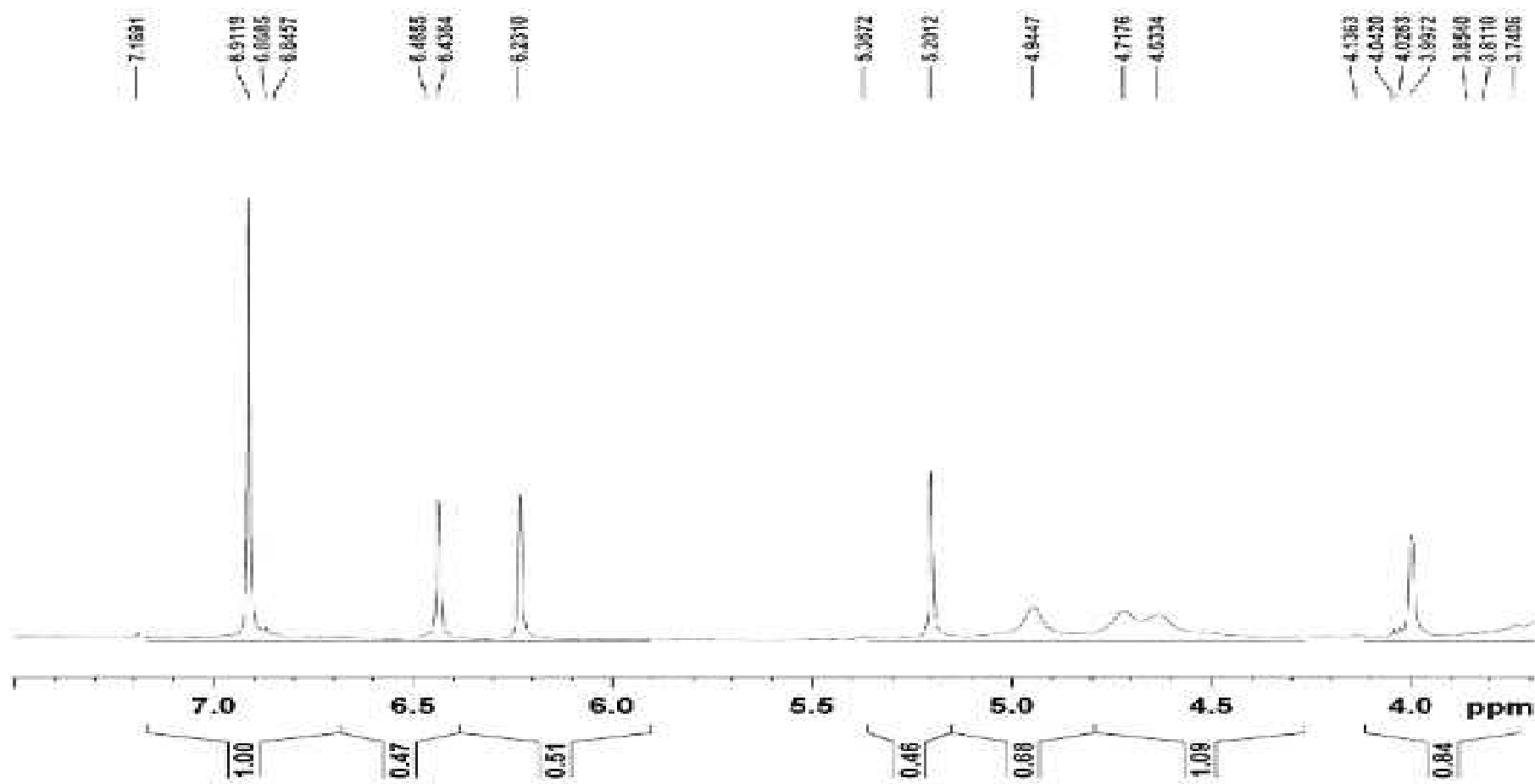


DEPT 90 (500MHz, DMSO) spectrum of quercetin-3-O-galactoside.

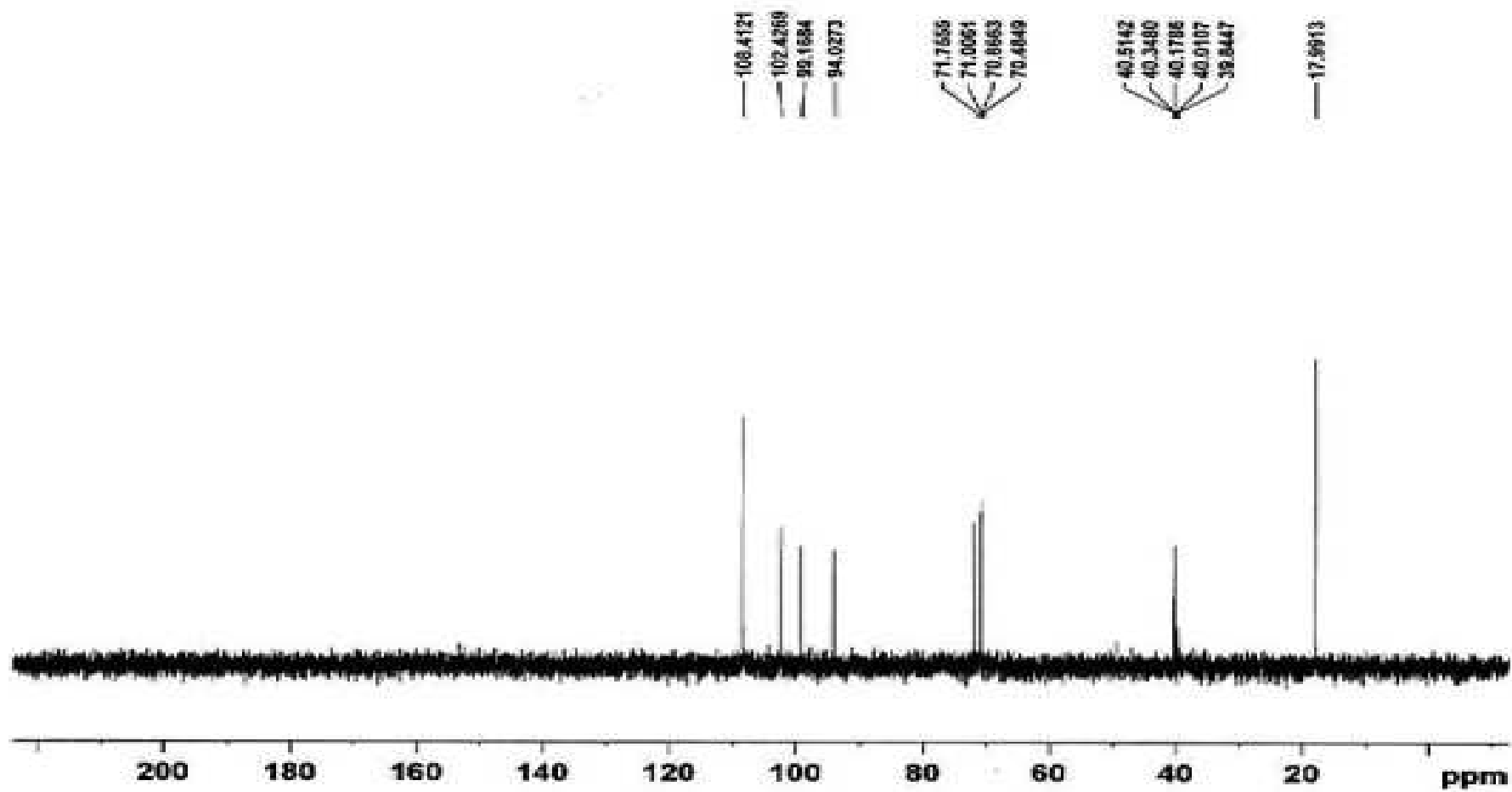
Appendix C



¹H-NMR (500MHz, DMSO) spectrum of myricetin – 3- O- α - rhamnoside (0.5-4ppm).

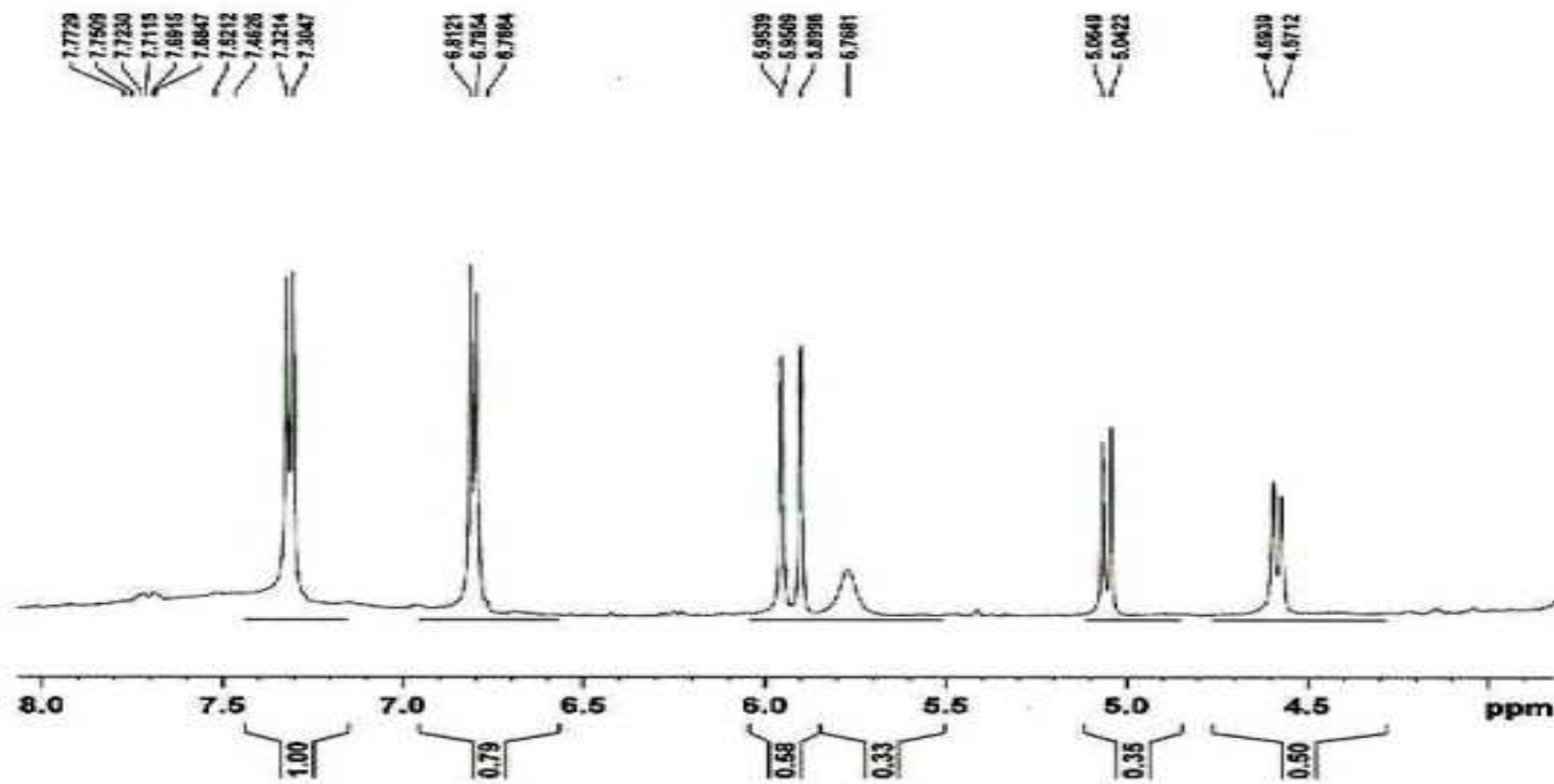


^1H -NMR (500MHz, DMSO) spectrum of myricetin – 3- O- α - rhamnoside (4 -7 ppm).



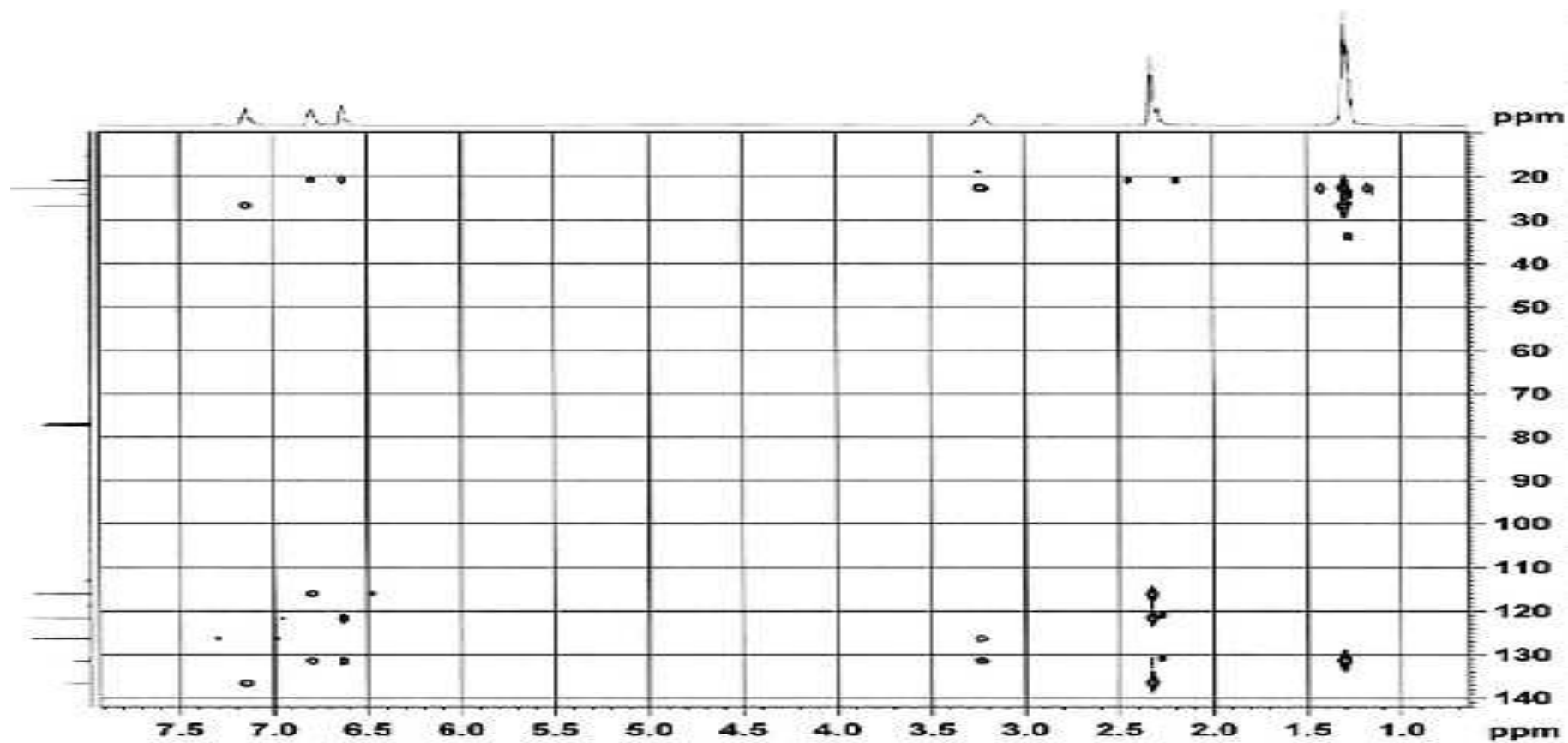
DEPT 90 (500MHz, DMSO) spectrum of myricetin – 3- O- α - rhamnoside.

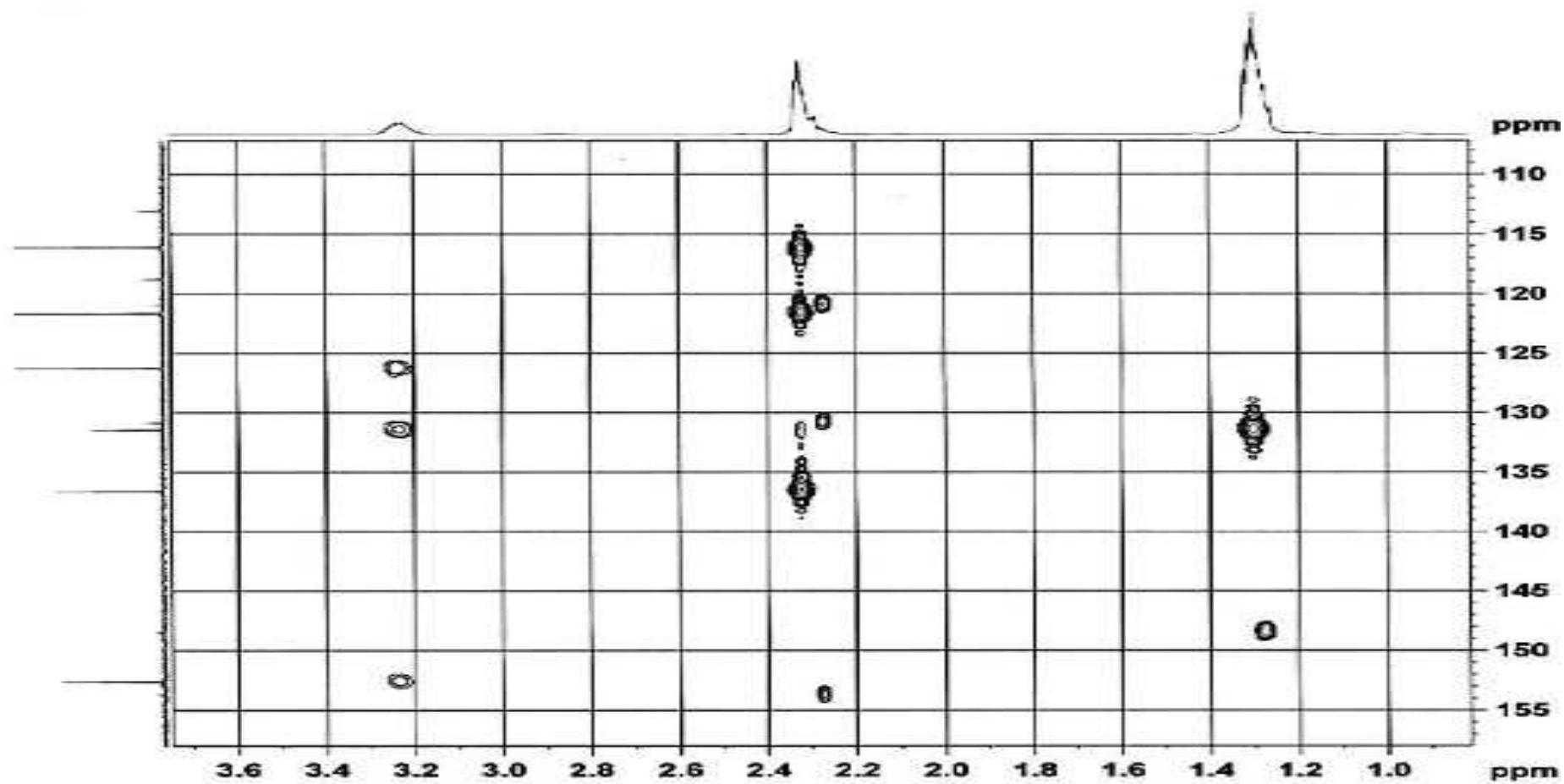
Appendix D



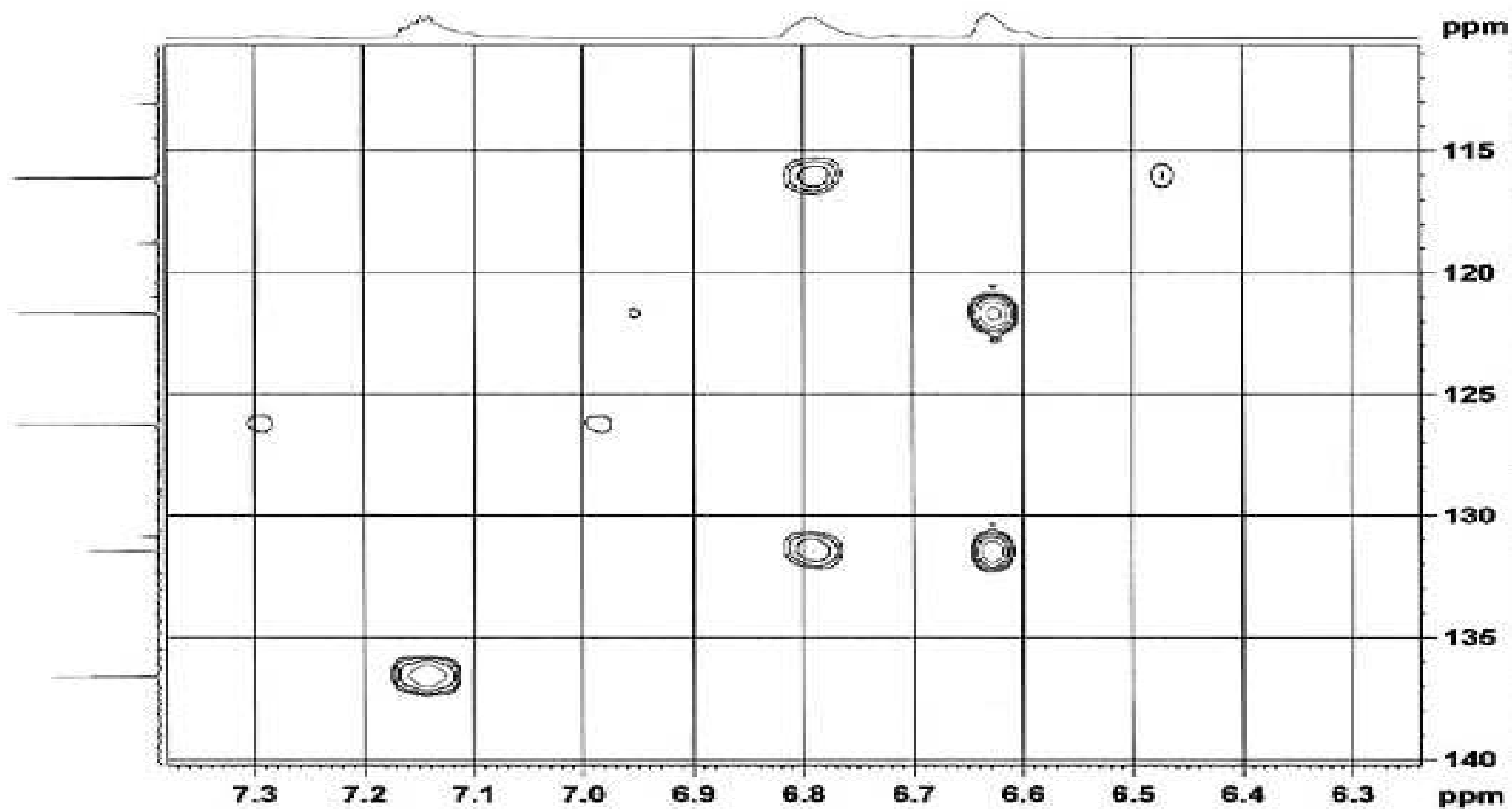
^1H -NMR (500MHz, DMSO) spectrum of katuranin (4.5-8 ppm).

Appendix E

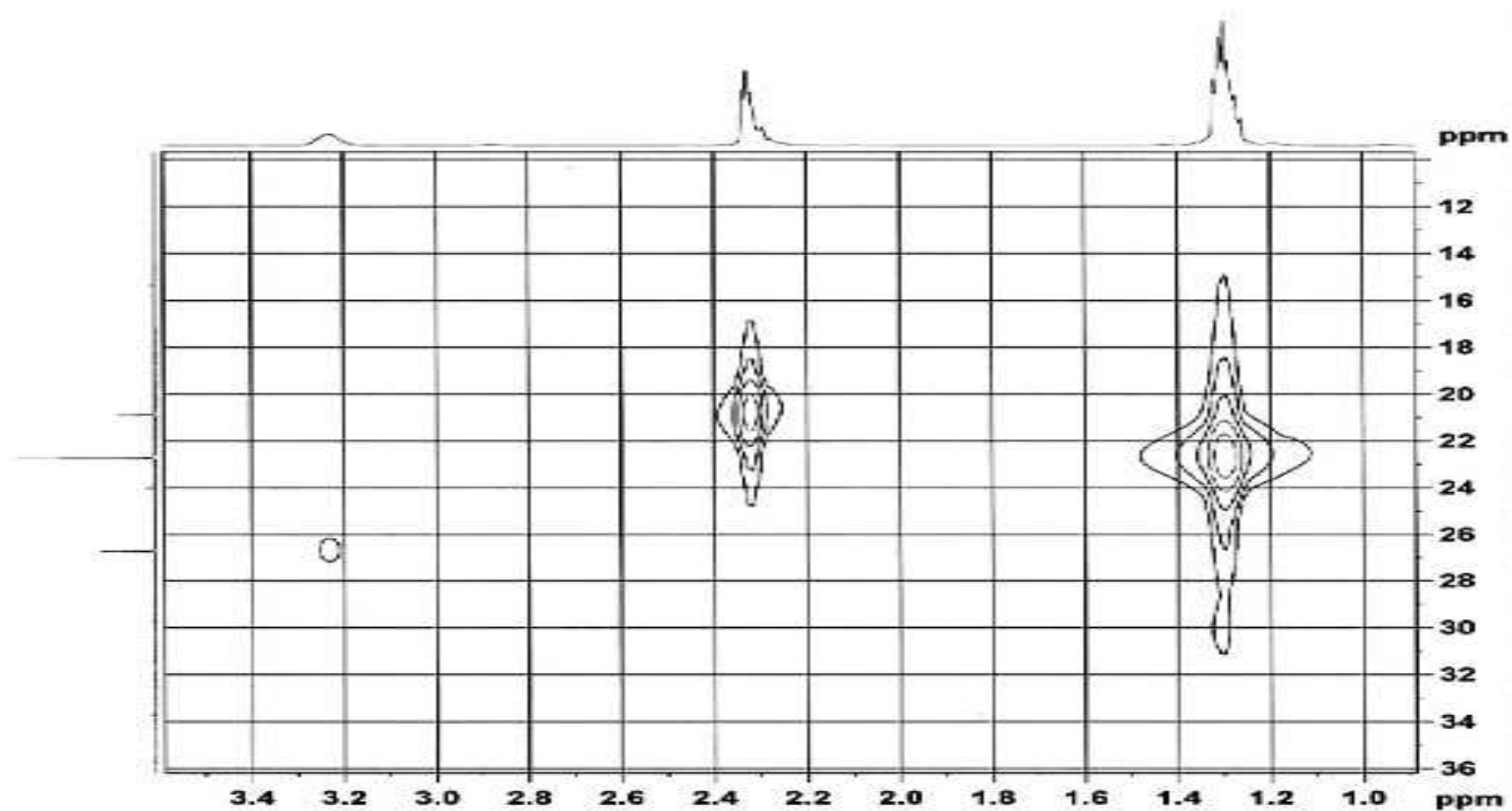
HMBC(500MHz, CDCl₃) spectrum of 5-isopropyl-3-methylphenol



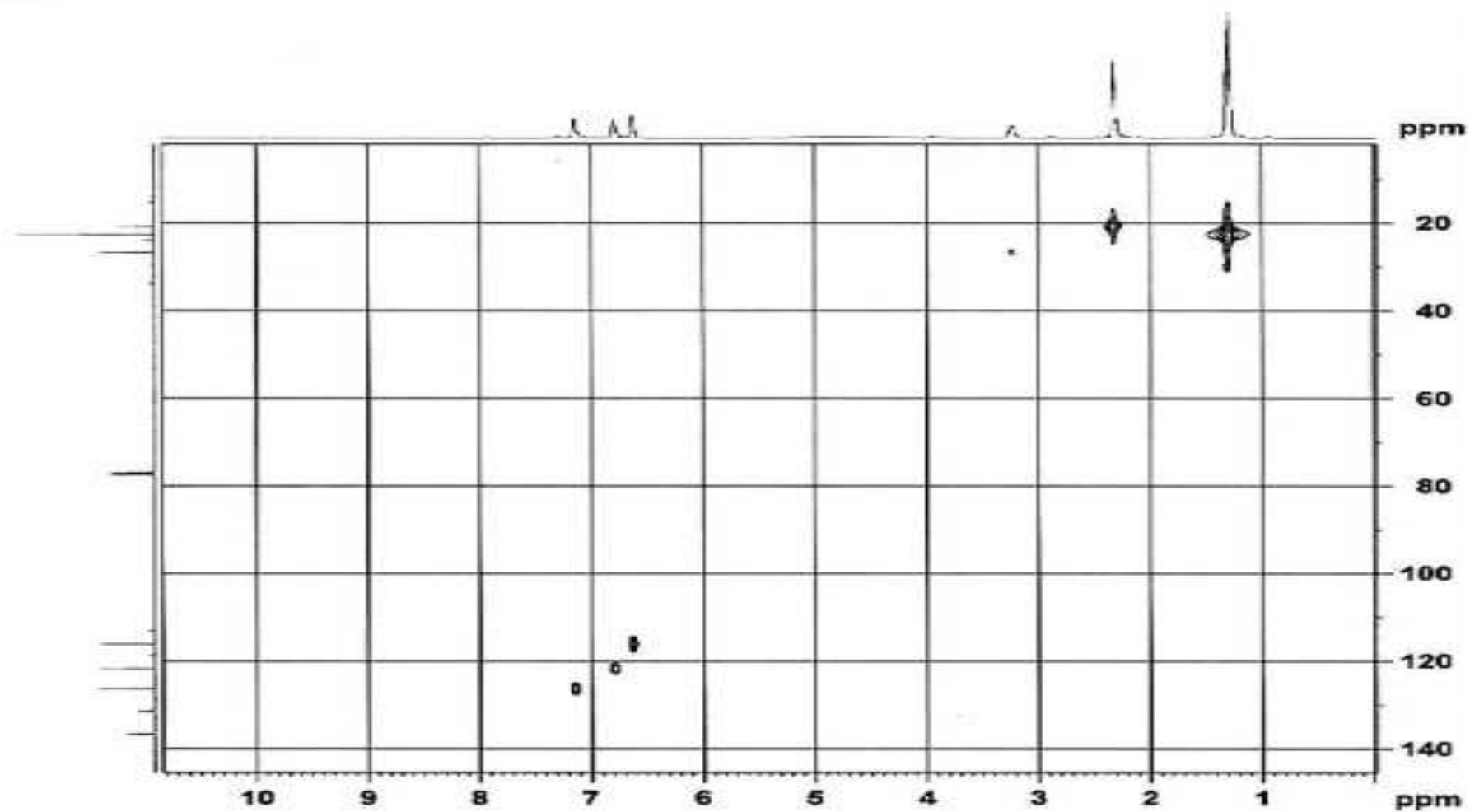
HMBC (500MHz, CDCl₃) spectrum of 5-isopropyl-3-methylphenol



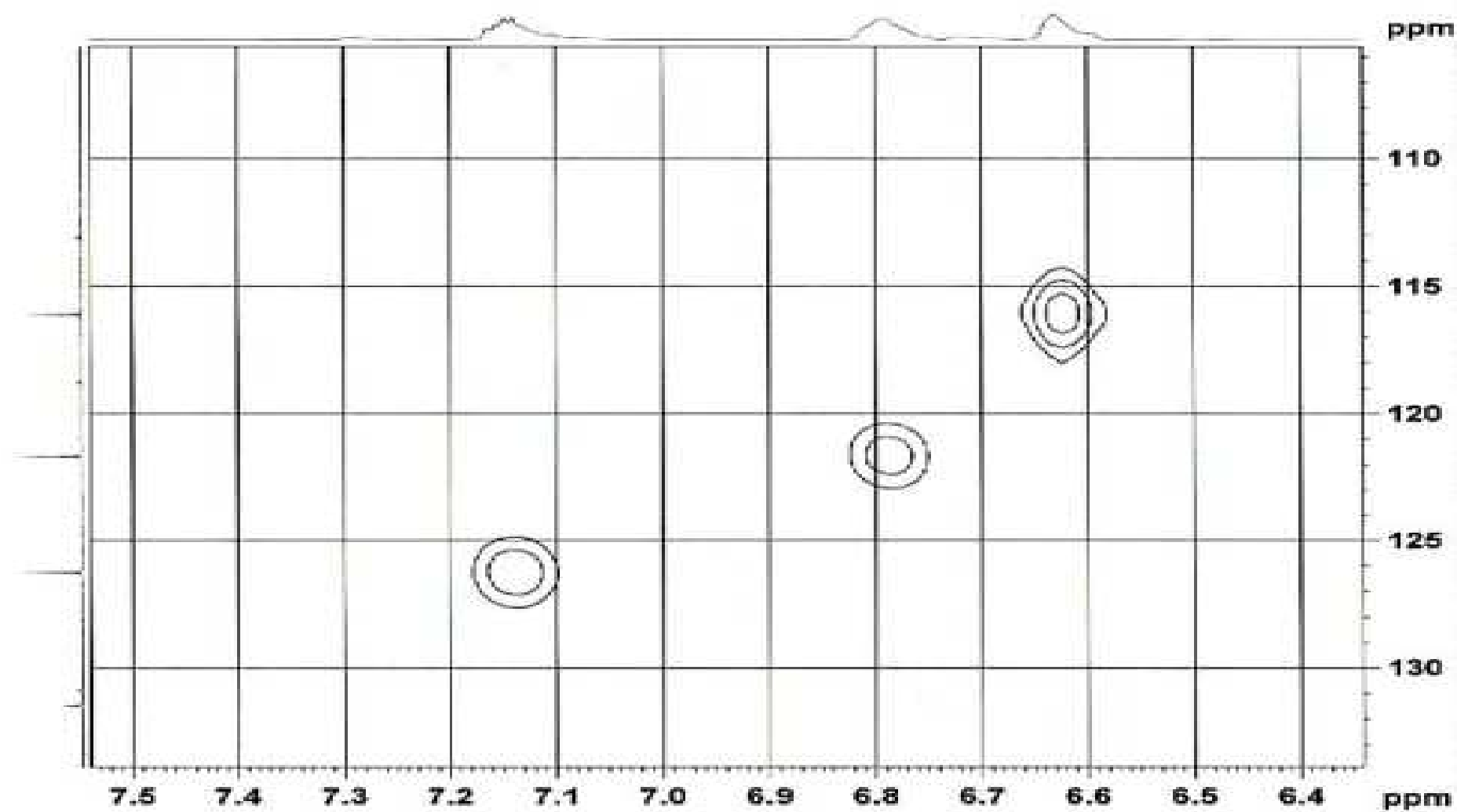
HMBC (500MHz, CDCl_3) spectrum of 5-isopropyl-3-methylphenol



HMQC (500MHz, CDCl_3) spectrum of 5-isopropyl-3-methylphenol

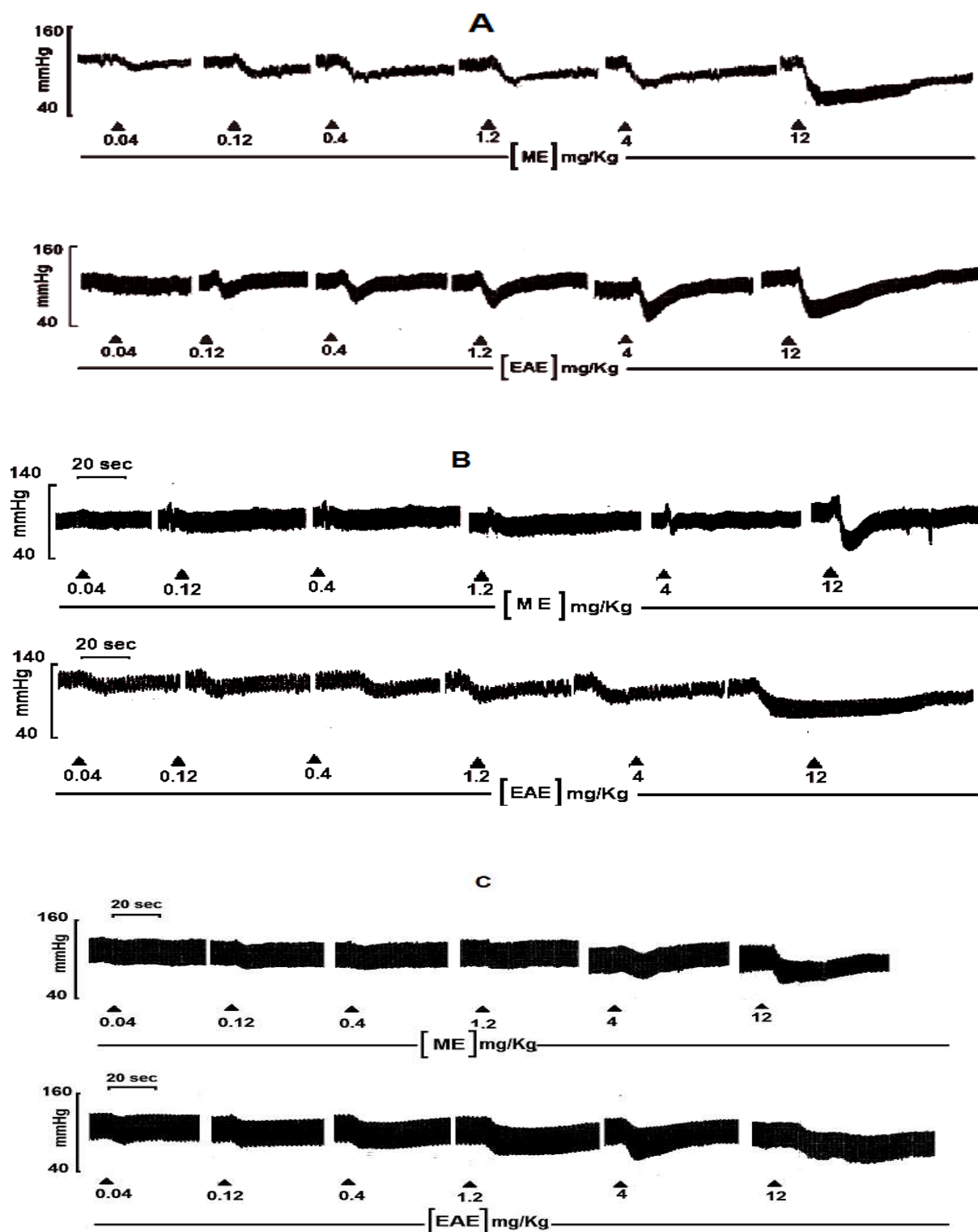


HMQC (500MHz, CDCl_3) spectrum of 5-isopropyl-3-methylphenol



HMQC (500MHz, CDCl_3) spectrum of 5-isopropyl-3-methylphenol

Appendix F



Typical tracing showing the blood pressure lowering effect of ME and EAE in anaesthetized rats. (A): *C. azarolus*; (B); *M. communis*; (C): *O. glandulosum*. Values are means $\pm$ SEM (n=6). Small triangles show the times at which the extracts were administered.

Abstract

The present study aimed to analyze chemically and evaluate the hypotensive, diuretic and the antioxidant activities of *Crataegus azarolus* (CA), *Myrtus communis* (MC) and *Origanum glandulosum* (OG) which are widely used as natural remedy for hypertension in the Algerian folk medicine. *In vitro* antioxidant potential of methanolic extract (ME) and its three fractions: Chloroform (CHE), ethyl acetate (EAE) and water (AqE) extracts has been investigated using several assays. Chemical analysis of EAE of selected plants revealed the isolation of five compounds; two flavonoids from CA ((+) - catechin and hyperoside), one flavonol glycoside from MC (myricetin -3-O-rhamnoside), one flavanone (katuranin), and one monoterpene (5-isopropyl-3-methylphenol) from OG. The quantification of polyphenols and flavonoids showed that EAE from the three studied plants contained the highest amount of polyphenols. However, CHE-MC, CHE-OG and EAE-CA contained the highest amount of flavonoids. EAE from CA, MC and OG exhibited the highest activity in scavenging DPPH, ABTS and hydroxyl radicals and reducing power test; whereas, ME and AqE exhibited higher chelating activity than CHE and EAE. Inhibition of lipid peroxidation was performed using the β -carotene bleaching assay, ferric thiocyanate method and thiobarburic acid method. CHE-MC, CHE-OG and EAE-CA were found to be the strongest inhibitors of lipid peroxidation in the three methods. Intravenous administration of ME and EAE decreased the mean arterial blood pressure in anesthetized rats dose-dependently, at the dose range of 0.4 to 12 mg/kg, and the strongest hypotensive effect was noticed for CA. The diuretic activity of ME (100, 300 and 600mg/kg) was assessed orally in rats. The results showed that only CA and OG produced an increase in urine output and the urinary excretion of Na^+ and Cl^- . The present study also assessed the effect of administration of ME (100 and 200mg/kg) in rats for 14 days on plasma antioxidant capacity (PAC) and oxidative stress biomarkers in liver and kidney. It was found that extracts caused elevation of PAC and enhancement of antioxidant status in liver and kidney. These results clearly indicate that these plants can be used as a hypotensive agent and as a protective shield against numerous free-radical mediated diseases.

Key words: *Crataegus azarolus*, *Myrtus communis*, *Origanum glandulosum*, phytochemistry, polyphenols, antioxidant, hypertension.

Résumé

L'objectif de cette étude est l'analyse phytochimique et l'évaluation des effets hypotensifs, diurétiques et l'activité antioxydante de *Crataegus azarolus* (CA), *Myrtus communis* (MC) et *Origanum glandulosum* (OG) qui sont largement utilisées en médecine traditionnelle Algérienne dans le traitement de l'hypertension. Le potentiel antioxydant de l'extrait méthanolique (EM) et ses trois fractions; l'extrait de chloroforme (ECH), l'extrait d'acétate d'éthyle (EAE) et l'extrait aqueux (EAQ) a été étudié *in vitro* en utilisant plusieurs techniques. L'analyse phytochimique d'EAE a permis d'identifier 5 molécules; deux flavonoïdes ont été isolés de CA ((+) - catéchine et l'hyperoside), un flavonole glycosylé (myricétine -3-O-rhamnoside) a été isolé de MC, un flavanone (katuranine), et un monoterpène (5-isopropyl-3-méthylphénol) ont été isolés d'OG. La quantification des polyphénols et des flavonoïdes a montré que l'extrait EAE de trois plantes étudiées est le plus riche en polyphénols. Cependant, les extraits ECH-MC, ECH-OG et EAE-CA ont présenté le contenu le plus élevé des flavonoïdes. L'EAE présente une activité antioxydante très importante en utilisant le test de piégeage des radicaux de DPPH, d'ABTS et d'hydroxyle, et le test du pouvoir réducteur. Cependant, l'EM et l'EAQ présentent une activité chélatrice plus élevée que celle de l'ECH et l'EAE. L'inhibition de la peroxydation lipidique a été évaluée par les tests du blanchissement de β -carotène, de thiocyanate ferrique et d'acide thiobarburique. Les extraits ECH-MC, ECH-OG et EAE-CA ont révélé l'inhibition la plus puissante dans ces trois méthodes. L'administration intraveineuse de l'EM et l'EAE (0.4 à 12 mg/kg) a diminué la pression artérielle de manière dose dépendante chez les rats anesthésiés, mais l'effet hypotensif le plus fort a été noté pour la plante CA. L'activité diurétique de l'EM (100, 300 et 600 mg/kg) évaluée chez les rats a prouvé que seulement CA et OG qui ont produit une augmentation du volume d'urine et de l'excrétion urinaire de Na^+ et Cl^- . En outre, l'activité antioxydante des plantes a été évaluée *in vivo* par l'administration de l'EM (100 et 200mg/kg) chez les rats pendant 14 jours. Les extraits ont entraîné une augmentation de la capacité antioxydante plasmatique et l'amélioration du statut antioxydant dans le foie et le rein. Ces résultats fournissent une base scientifique pour l'usage de ces plantes comme un agent hypotensif et protecteur contre de nombreuses maladies associées au stress oxydant.

Mots clés : *Crataegus azarolus*, *Myrtus communis*, *Origanum glandulosum*, phytochimie, polyphénols, antioxydant, hypertension.

ملخص

تهدف هذه الدراسة إلى التحليل الكيميائي وتقييم التأثير المخفض للضغط الدموي والمدر للبول والنشاطية المضادة للأكسدة لكل من مستخلصات *Crataegus azarolus* (CA)، *Myrtus communis* (MC) و *Origanum glandulosum* (OG) التي تستخدم على نطاق واسع في الطب الشعبي الجزائري لعلاج ارتفاع ضغط الدم. أسفر التحليل الكيميائي لمستخلص خلالات الإيثيل لهذه النباتات إلى التعرف على خمسة مركبات. فقد تم عزل فلافونويدين (catechin - (+) و hyperoside) من CA و فلافونول سكري (myricetin -3-O-rhamnoside) من MC و فلافانون (katuranin) و تاريخين أحادي (5-isopropyl-3-methylphenol) من OG. تم تقدير النشاطية المضادة للأكسدة مخبريا (*in vitro*) لكل من المستخلص الميثانولي (ME)، والكلوروفورمي (CHE) ومستخلص خلالات الإيثيل (EAE) والمستخلص المائي (AqE) باستخدام عدة تقنيات. أظهر التقدير الكمي لعديدات الفينول والفلافونويدات أن مستخلص EAE للنباتات الثلاثة يحتوي على أكبر كمية من عديدة الفينول بينما يحتوي كل من CHE-MC و CHE-OG و EAE-CA على أكبر كمية من الفلافونويدات. أظهر مستخلص EAE نشاطية مضادة للأكسدة معتبرة جدا باستخدام التأثير الإزاحي اتجاه كل من جذر DPPH و ABTS والهيدروكسيل وتقنية القدرة الازجعية، بينما تبين أن كل من ME و AqE يملكان قدرة عالية في استخلاص المعادن مقارنة بمستخلص CHE و EAE. تم تقييم تثبيط فوق أكسدة الليبيدات عن طريق اختبار تبييض β -كاروتين، وثيوسانات الحديد وحامض ثيobarبريك. وقد تبين أن كل من المستخلصات EAE-CA و CHE-MC و CHE-OG تملك قدرة تثبيطية عالية. أدت معالجة جرذان مخدرة بجرعات من 0.4 إلى 12مغ/كغ للمستخلصات ME و EAE محقونة وريديا إلى تخفيض الضغط الشرياني بشكل يتناسب مع الزيادة في التركيز، وقد سجل التأثير الأقوى عند حقن مستخلصات CA. كما أدى تقييم النشاط المدر للبول لجرعات (100 و 300 و 600مغ/كغ) من المستخلص ME المعطى شفويا لدى الجرذان، أن المعاملة بنباتي CA و OG أدت إلى زيادة معنوية في حجم البول وإطراح الصوديوم والكلور. درست النشاطية المضادة للأكسدة للمستخلصات حيويًا (*in vivo*) بجرعات 100 و 200 مغ/كغ من المستخلص الميثانولي عند الجرذان لمدة 14 يوما. حيث أدت المعاملة بهذه المستخلصات إلى زيادة في القدرة المضادة للأكسدة للبالازما وتحسين النشاطية المضادة للأكسدة للكبد والكلية. يمكن لهذه النتائج أن توفر أساسا علميا في استخدام هذه النباتات كعوامل خافض للضغط وواقى ضد العديد من الأمراض المرتبطة بالاجهاد التأكسدي.

الكلمات المفاتيح : *Crataegus azarolus*، *Myrtus communis*، *Origanum glandulosum*، التحليل الكيميائي، عديدة الفينول، ارتفاع الضغط الشرياني.