Chapter

Potential Antioxidant Activity of Terpenes

Bechir Baccouri and Imen Rajhi

Abstract

Terpenes play a key part in the metabolic processes of a wide variety of animals, plants and microorganisms in which they are produced. In nature, terpenoids serve a variety of purposes including defense, signaling and as key agents in metabolic processes. Terpenes have been used in perfumery, cosmetics and medicine for thousands of years and are still extracted from natural sources for these uses. Terpenes antioxidant activities may sometimes explain their capacity to adjust inflammation, immunological effects and neural signal transmission. They offer pertinent protection under oxidative stress situations including renal, liver, cancer, cardiovascular diseases, neurodegenerative and diabetes as well as in ageing mechanisms.

Keywords: terpenes, terpenoids, antioxydant, ROS, health

1. Introduction

Terpenes occur widely in nature. They are a large and varied class of hydrocarbons that are produced by varied plants and some animals. Thus, terpenes defend plants against pathogens like bacteria, fungus and can attract pollinating insects or repel herbivores [1]. Numerous plants produce volatile terpenes in order to attract specific insects for pollination or otherwise to expel certain animals using these plants as food [1].

They are also abundantly found in fruits and flowers. In plants, they function as infochemicals, attractants or repellents, as they are responsible for the typical perfume of many plants [2]. Last, but not least, terpenes play an important role as signal compounds and growth regulators (phytohormones) of plants, as shown by some studies [1]. Thousands of terpenes have been found across the *plantae*, but only a small percentage of all terpenes have been known. Terpenes are biosynthetically derived from isoprene units with the molecular formula C5H8 [1]. The basic formula of all terpenes is (C5H8)n, where n is the number of linked isoprene units [1].

Terpenes presented over 25,000 well defined compounds isolated from all biological kingdoms [3]. The numerous terpene synthases in plants are primarily responsible for terpene diversity; some of them produce different products from a single substrate [4].

The nomenclature of terpenes is based on the number of isoprene structures that they contain. Accordingly, these compounds are classified as sesquiterpenes, monoterpenes, diterpenes, triterpenes, tetraterpenes, and polyterpenes [5]. Monoterpenes, sesquiterpenes, and diterpenes are considered secondary metabolites as they are not essential for viability [5].

Including neurodegenerative diseases (Alzheimer's and Parkinson's diseases), cancer, cardiovascular diseases, liver diseases, diabetes, and other diseases; oxidative stress is involved in the pathological development of many diseases. Antioxidant therapy, via direct and indirect mechanisms, has become one of the main and promising strategies to face oxidative stress-induced cellular damage [1, 6]. Studies have shown that both natural terpenes and their synthetic derivatives enjoy diverse pharmacological properties, including antioxidant, antifungal, anti-inflammatory, antiviral, anticancer, antibacterial, antinociceptive, antiarrhythmic, antispasmodic, antiaggregating, local anesthetic and antihistaminic activities [6, 7]. These interesting characteristics were used in pharmaceuticals and cosmetic industries. In this context, the search for antioxidant compounds among natural terpene products has significantly increased in the last recent years. As shown throughout this chapter, terpenes can function as antioxidant compounds through modulating the endogenous antioxidant system and direct ROS scavenging pathway.

2. Oxidative stress

Reactive oxygen species (ROS) comprise a series of chemical molecules derived from molecular oxygen whose reactivity is much greater than that of this element in its basal state [8]. Intracellular ROS can oxydize lipids, proteins and DNA thus damaging many cellular components and even causing genetic damage and cell death, mainly by apoptosis [1, 8, 9]. These species include oxygen ions as atomic oxygen (O), ozone (O_3) and singlet oxygen ($1O_2$) free radicals such as superoxide radical (O_2• -), hydroxyl radical (OH •), the alkoxy radical (RO •) and peroxyl radical (ROO •), and peroxides such as hydrogen peroxide (H_2O_2) and peroxynitrite (ONOO-) [1, 8].

Molecules such as, ascorbic acid (vitamin C), α tocoferol (vitamin E), bilirubin, selenium and glutathione, between many others, proceed as ROS scavengers, preventing oxidative cellular damage [10, 11]. Among these, glutathione, the antioxidant compound, plays an important role in protecting vital functions [8, 11].

In addition to nonenzymatic compounds, during the antioxidant enzymes action including superoxyde dismutase (SOD), catalase (CAT), glutathione peroxydase (GPx) and heme oxygenase-1 (HO-1), ROS can be detoxyfied or converted into nontoxic forms [8]. Catalase, located principally in peroxisomes, efficiently converts hydrogen peroxide to water and oxygen [12]. The efficiency of this enzyme is such that one CAT molecule is able to turn 6 million of hydrogen peroxide molecules into water and oxygen per minute. In addition, this enzyme cannot be saturated at any hydrogen peroxide concentration [13]. Superoxyde dismutase produces molecular oxygen and hydrogen peroxide through dismutation of superoxide anion and this reaction is over 4 times faster than the non-enzymatic reaction [12]. GPx reduces hydroperoxides using glutathione (GSH) as substrate. The resulting GSSG is reduced back to GSH by the action of GR. The gene expression of all of these antioxidant proteins is regulated by nuclear factor erythroid-2 (Nrf2), through its binding to a specific DNA sequence called antioxidant response element (ARE) [14].

Nevertheless, under various pathological conditions, this endogenous cellular antioxyant defense system cannot remove excessive amounts of ROS, resulting in an oxidant-antioxidant imbalance called oxidative stress [1].

3. Terpenes antioxidants potential

The main triterpenes present in EVOO are two hydroxyl pentacyclic triterpene acids (oleanolic and maslinic acid) and two dialcohols (uvaol and erythrodiol)

(**Figure 1**), whose concentrations oscillate between 8.90 and 112.36 mg kg^{-1} [15]. Terpenes compounds are mostly found in the epicarp, then, pomace olive oil generally contains 10-fold elevated concentrations than EVOO [15].

In the incessant search for new bioactive natural products against oxidation and inflammation, terpenes are emerging as a rich source of these compounds. Some monoterpenes possess both anti-inflammatory and antioxidant properties [16, 17]. (+)-limonene, and 1,8-cineole demonstrated strong antioxidant, anti-inflammatory and anticancer properties in assays using DPPH method, pleural cell migration, and U251, UACC-62, MCF-7, NCI-ADR/RES, OVCAR-3 human cancer cell lines, respectively [16, 17].

Menthol is present in the aroma oil of numerous species of mint plants, such as cornmint oil from *M. arvensis* (wild mint) and peppermint oil derived from *Mentha piperita* (peppermint). Menthol and 1,8-Cineole ([11] had antioxidant characteristics in the ABTS-radical caption scavenging assay [18]. Cornmint and peppermint oils contain 70 and 50%, respectively, of menthol. Menthol can be extracted from other essential oils, such as citronella, eucalyptus and Indian turpentine oils.

Previous works have demonstrated that the antioxidant and prooxidant behaviour of a particular terpene depend most of all on it amount: at high concentrations, terpenes can act as a prooxidant compounds whereas at low concentrations, they can act as antioxidant compounds [19].

Ruberto and Baratta [20] studied the antioxidant activity of monoterpene and sesquiterpene compounds found abundantly in essential oils Two lipid model systems were used: one for evaluating the formation of thiobarbituric acid reactive species (TBARS), utilizing egg yolk as lipid oxydable substrate and the other one for evaluating the peroxides that are formed during linoleic acid oxydation in a micellar system. Among monoterpene hydrocarbons, such as terpinolene, α-terpinene, γ-terpinene and sabinene were the most active [19]. Among oxygenated monoterpenes the order of antioxidant activity effectiveness was monoterpene phenols (thymol and carvacrol > allylic alcoholes (nerol), perillyl alcohol, geraniol and cisverbenol > monoterpenes aldehydes and ketones. Concerning the sesquiterpene group [1], the radical

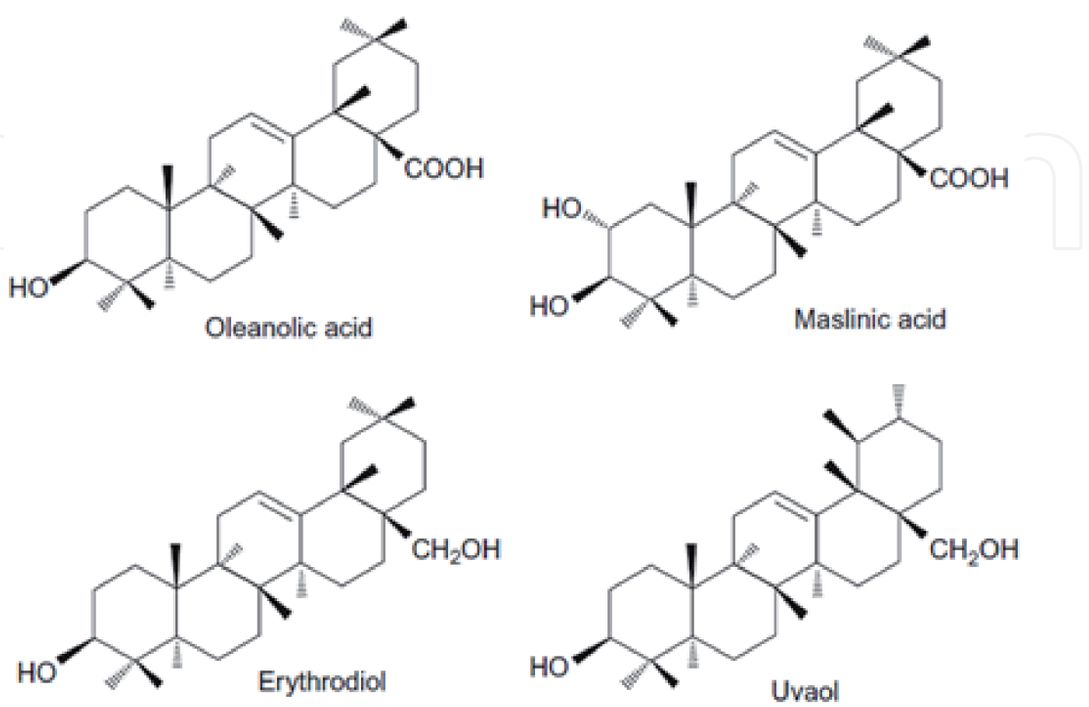

Figure 1.
Chemical structure of EVOO triterpenes.

scavenging properties of the hydrocarbons-type were quite low and lower than that of the monoterpene hydrocarbons cluster, but between the oxygenated type, mainly allylic alcohols (i.e. farnesol, guaiol, (+)-8- (15)-cedren-9-ol showed good scavenging properties, similar to those of oxygenated monoterpenes [1, 19].

Several terpenes display also a protective effect against the oxidative stress induced by heavy metals. Pretreatments with the triterpene arjunolic acid recovered almost completely from reduced antioxidant protection (SOD, CAT, GR, GPx GST, GSH) and increased oxidative damage (lipid peroxydation and protein carbonyl content), mainly via radical scavenging, in murine brain treated with arsenic. El-Missiry and Shalaby [21] indicated that treatments with the ß-carotene (tetra-terpene) protected against cadmium oxidative stress in brain with an associated increase in SOD, GST and non-enzymatic (GSH) antioxidant status [1], a decline in LDH activity and lipid peroxydation and an rise of ATPase activity [1, 22].

The oxidative pathway is also one of the described mechanisms to clarify glutamate toxicity. This excitatory neurotransmitter depletes intracellular GSH, produces ROS and augments lipid peroxydation levels. Koo et al. [23] identified the diterpene 15- methoxypinusolidic acid, obtained from the leaves of *Biota orientalis* L., as protective neuroagent with antioxidant activity in primary cultured cortical rat cells. Moreover, the monoterpenes from *Scrophularia buergeriana* Miq. were capable to ameliorate the antioxidant defense system in primary cultures of rat cerebral cortical cells in glutamate-mediated oxidative stress conditions [1].

Kim et al. [22] focused on the search for antioxidant compounds that delay or prevent oxidant/antioxidant imbalances and its harmful consequences, since oxidative stress is associated with Parkinson's disease pathology. The monoterpene catalpol, isolated from the roots of *Rehmannia glutinosa*, has demonstrated to protect cultured mesencephalic neurons against MPP+-induced toxicity by preventing the inhibition of the mitochondrial complex I, and thus avoiding mitochondrial dysfunction, and by diminishing the level of MDA content and increasing the activity of the antioxidant enzymes (SOD and GPx) [22]. The exogenous neurotoxins 1-methyl-4- phenylpyridinium (MPP+) and 6-hydroxydopamine (6-OHDA) are frequently used in experimental Parkinson models since these chemical compounds induce selectively oxidative stress in nigrostriatal dopaminergic neurons [24].

Its beneficial effects may be partly due to ROS scavenging and enhancement of endogenous antioxidants. Concerning fungi-derived terpenes, the labdane diterpenes, obtained from the fruiting body of the parasitic fungus *Antrodia camphorate* [24]. On the other hand, the carotene astaxanthin resulted to be a potent mitochondria-targeted antioxidant in dopaminergic SH-SY5Y cells treated with 6-OHDA [25].

Naval and Gómez-Serranillos [26] reviewed the neuroprotective activity of ginseng constituents, based on their antioxidant activities. Herein, we highlight some examples. *In vitro* studies concerning the neuroprotective activity of the isolated ginsenosides Rb1, Rb2, Rc, Rd., Re and Rg1 under hydrogen peroxide-induced oxidative stress in astrocytes revealed that the triterpene compound Re was the most effective among all tested ones since this compound could decrease cell death, improve SOD, GR and GPx activities and inhibit ROS production [27]. In addition, oxidative stress markers such as high ROS and MDA levels, low amounts of GHS and decreased antioxidant enzyme (SOD, CATand GPx) activity have been detected during oxygen–glucose privation and reoxygenation processes on hippocampal neurons. The ginsenoside Rd. lets return all these oxidant parameters to basal levels [27, 28].

Pretreatments with arjunolic acid isolated from the bark of *Terminalia arjuna* (Roxb.). Wight and Arn. prevented cardiac tissues from arsenic-induced oxidative

stress by restoring antioxidant status and inhibiting lipid peroxydation and protein carbonyl accumulation. There is evidence supporting a link between oxidative stress and cardiovascular tissue injury. Some studies have been conducted on the cardioprotective impacts of terpenes in response to cardiovascular pathological situations oxidative stress-related including hypertension and atherosclerosis, among others [29].

Moreover, antihypertensive beneficial effects through antioxidant actions have been also observed for astaxanthin. In another study, the endothelial function of resistance of arteries was improved in those experimental animals that had been during eight weeks on an astaxanthinenriched diet. Astaxanthin decreased NADPH enhanced O2·- production by direct ROS scavenging and improved NO bioavailability [30].

As it has been previously demonstrated, excessive cigarette smoking and alcohol drinking are both risk factors for triggering atherosclerosis. In a randomized double-bind placebo-controlled study undertaken in over 100 habitual cigarette smokers and alcohol consumers, men 22–57-aged, the possible protective effect of lycopene against heart disease was evaluated in these oxidative stress conditions (smoke and alcohol) [30].

Additionally, Bansal et al. [31] confirmed that the carotenoid lycopene acts as a myocardial protective agent for the prevention of oxidative stress caused after ischemia reperfusion in the heart of rats through lipid peroxydation reduction and antioxiydant capacity enhancement [31]. Through antioxidant mechanisms, particularly scavenging of oxygen free radicals, prevention of lipid peroxydation and upregulation of the Bcl-2/Bax ratio, the diterpene tanshinone IIA also exhibited a protective role on cardiomyocytes against ischemic injury [32].

ROS formation and subsequent oxidative stress events are one of the mechanisms of liver injury with hepatotoxic chemicals injury [32]. Several terpenes have shown hepatoprotective activity against this toxic chemical compound [27]. The kaurane diterpenes kahweol and cafestol, found in coffee beans, inhibited the production of superoxide anion radicals, reduced the level of the lipid peroxydation product malondialdehyde (MDA) and prevented the depletion of intracellular glutathione (GSH) injury [27, 32]. The labdane diterpenes neoandrographolide and andrographiside isolated from the plant species *Andrographis paniculata* (Burm.f.).

Moroever, *in vivo* studies demonstrated an increase in the concentration of reduced glutathione (GSH) in the liver of those rats after chronic alcohol consumption but fed with a diet containing the carotenoid ß-carotene [33].

Several environmental pollutants are able to induce oxidative stress, liver being the organ mostly affected [30]. Also, the ß-carotene (tetraterpene), behaving as an antioxidant, protected from liver damage associated with oxidative stress caused by bile acid or as a side effect of chemotherapy with methotrexate [34].

Among the monoterpene class, catalpol may hold promising protective actions against encephalopathy under hyperglycemic conditions [22].

Moreover, the protective effect of terpenes as antioxidants against excessive ROS production, it is worth to indicate the role of these compounds as chemoprotective agents against tumor cells. Many papers have demonstrated that terpenes could have a very efficient activity in different cancer types [1]. Anticancer therapy of terpenes targeting the apoptotic pathway rather than the antioxidant pathway [1, 35].

The protective effect of carotenoids was attributed to its capacity to inhibit lipid peroxydation, restore GSH levels and improve the activities of the enzymes superoxide dismutase, catalase and glutathione S-transferase (**Figure 2**) [36].

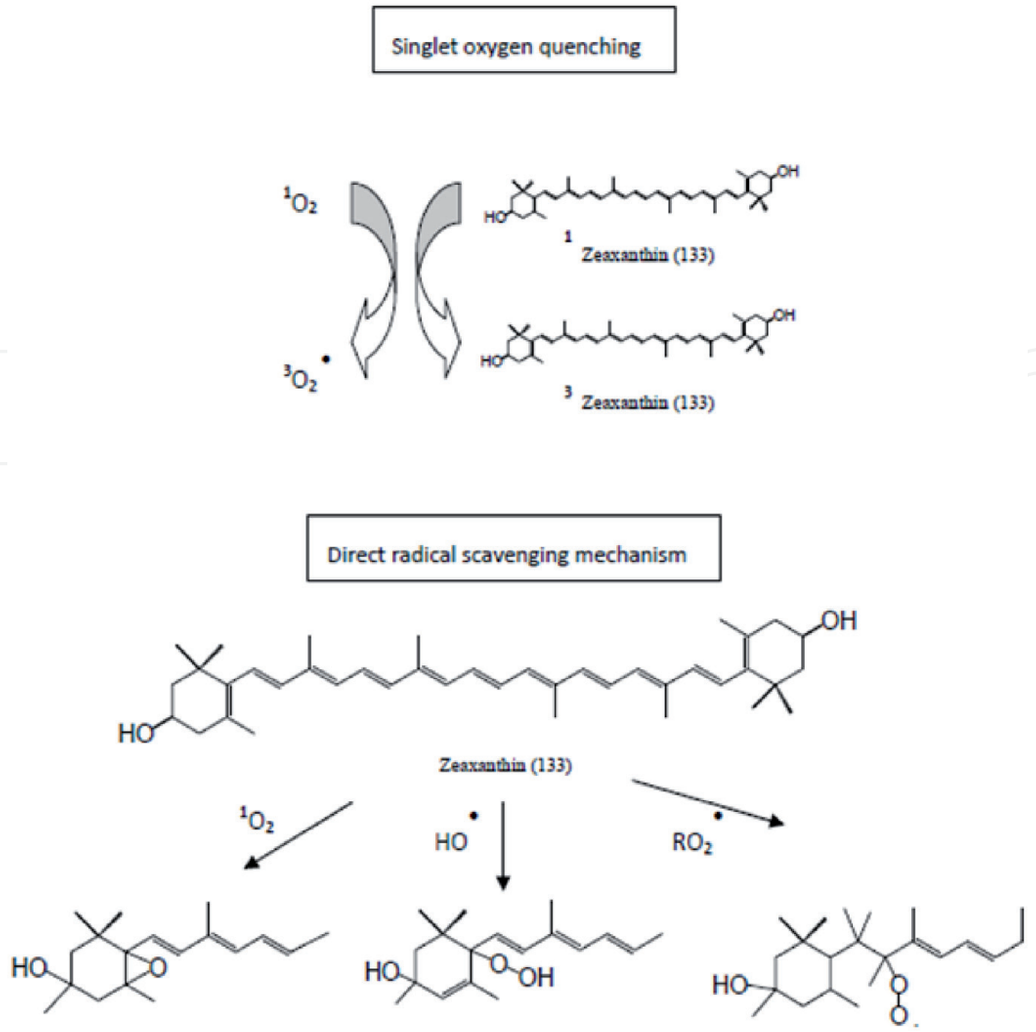

Figure 2.
Interaction of zeaxanthin With ROS.

4. Conclusions

Concerning the number of *in vivo* and *in vitro* studies that have evaluated the terpenes antioxidant activities it is relatively little when compared to the enormous number of identified Terpenes in nature. They have a ample biological activities including anti-inflammatory, anticancer, antimicrobial, antioxidant etc.. Several other as yet undiscovered compounds can exist with immense antioxidant potentials.

Conflict of interest

The authors declare that they have no conflict of interests.

"Reviewed by Pr. Mokhtar Zarrouk, Centre of Biotechnology of Borj Cédria, Tunisia".

Author details

Bechir Baccouri[1*] and Imen Rajhi[2]

1 Laboratory of Olive Biotechnology, Center of Biotechnology of Borj-Cédria, Tunisia

2 Laboratory of Legumes Center of Biotechnology of Borj-Cédria, Tunisia

*Address all correspondence to: bechirbaccouri@yahoo.fr

References

[1] Wang, S.Y.; Wu, J.H.; Shyur, L.F.; Kuo, Y.H.; Chang, S. Antioxidant activity of abietane-type diterpenes from heartwood of *Taiwania cryptomerioides* Hayata. Holzforschung, 2002, *56*, 487-492.

[2] Schulz, G.E.; Schirmer, R.H.; Sachsenheimer, W.; Pai, E.F. The structure of the flavoenzyme glutathione reductase. Nature. 1978, *273*, 120-124.

[3] Buckingham, J. *Dictionary of natural products on CD-ROM*, version 6.1; Chapman & Hall: London, 1998. antioxidant polyoxygenated triterpenes from *Salsola baryosma*, by 1D and 2D NMR spectroscopy.

[4] Ruzlcka, L. The isoprene rule and the biogenesis of terpenic compounds. Experientia, 1953, *9*, 357-367.

[5] Flesh, G.; Rohmer, M. Prokaryotic hopanoids: the biosynthesis of the bacteriohopane skeleton. Formation of isoprenic units from two distinct acetate pools and a novel type of carbon/ carbon linkage between a triterpene and Dribose. Eur. J. Biochem., 1988, *175*, 405-411.

[6] Avery, S.V. Molecular targets of oxidative stress. Biochem. J., 2011, *434*, 201-210.

[7] Valko, M.; Morris, H.; Cronin, M.T. Metals, toxicity and oxidative stress. Curr. Med. Chem., 2005, *12*, 1161-1208.

[8] D'Autreaux, B.; Toledano, M.B. ROS as signalling molecules: mechanisms that generate specificity in ROS homeostasis. Nat. Rev. Mol. Cell Biol., 2007, *8*, 813-824.

[9] Stadtman, E.R.; Moskovitz, J.; Levine, R.L. Forum Mini Review, Oxidation of methionine residues of proteins: Biological consequences. Antioxidatns & Redox Signaling, 2003, *5,* 577-582.

[10] Li, Y.; Cao, Z.; Zhu, H. Upregulation of endogenous antioxidants and phase 2 enzymes by the red wine polyphenol, resveratrol in cultured and aortic smooth muscle cells leads to cytoprotection against oxidative and electrophilic stress. Pharmacol. Res., 2006, *53*, 6-15.

[11] Townsend, D.M.; Tew, K.D.; Tapiero, H. The importance of glutathione in human disease. Biomed. Pharmacother., 2003, *57*, 145-155.

[12] Valko, M.; Rhodes, C. J.; Moncol, J., Izakovic, M.; Mazur, M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem. Biol. Interact., 2006, *160*, 1-40.

[13] Mates, M. Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. Toxicol., 2000, *153*, 83-104.

[14] Lee, J.M.; Johnson, J.A. An important role of Nrf2-ARE pathway in the cellular defense mechanism. J. Biochem. Mol. Biol., 2004, *37*, 139-143.

[15] Baccouri, B., Manaia, H., Casas, J.S., Osorio, E. & Zarrouk, M., (2018). Tunisian wild olive (Olea europaea L. subsp. oleaster) oils: Sterolic and triterpenic dialcohol compounds. Ind. Crops Prod. 120, 11-15.

[16] Luis, J.C.; Johnson, C.B. Seasonal variations of rosmarinic and carnosic acids in rosemary extracts. Analysis of their *in vitro* antiradical activity. *Spanish J. Agr. Res*., 2005a, *3*, 106.

[17] Sinha, M.; Manna, P.; Sil, P.C. Protective effect of arjunolic acid against arsenic-induced oxidative stress in mouse brain. J Biochem. Mol. Toxicol., 2008, *22*, 15-26.

[18] Qiu, L.H.; Xie, X.J.; Zhang, B.Q. Astragaloside IV improves homocysteineinduced acute phase endothelial dysfunction via antioxidation. Biol. Pharm. Bull., 2010, *33*, 641-646.

[19] Maleknia, S.D.; Adams, M.A. Reactions of oxygen-containing terpenes with peptides and proteins. *Proc. 4th Intl. Peptide Symp*., 2007, 334-335.

[20] Ruberto, G.; Baratta, M.T. Antioxidant activity of selected essential oil components in two lipid model systems. Food Chem., 2000, *69*, 167-174.

[21] El-Missiry, M.A.; Shalaby, F. Role of beta-carotene in ameliorating the cadmium-induced oxidative stress in rat brain and testis. J. Biochem. Mol. Toxicol., 2000, *14*, 238-243.

[22] Kim, S.R.; Koo, K.A.; Sung, S.H.; Ma, C.J.; Yoon, J.S.; Kim, Y.C. Iridoids from *Scrophularia buergeriana* attenuate glutamate-induced neurotoxicity in rat cortical cultures. *J. Neurosci. Re*s., 2003, *74*, 948-955.

[23] Koo, K.A.; Kim, S.H.; Lee, M.K.; Kim, Y.C. 15-Methoxypinusolidic acid from *Biota orientalis* attenuates glutamate-induced neurotoxicity in primary cultured rat cortical cells. Toxicol. In Vitro. 2006, *20*, 936-941.

[24] Tian, Y.Y.; Jiang, B.; An, L.J.; Bao, Y.M. Neuroprotective effect of catalpol against MPP(+)-induced oxidative stress in mesencephalic neurons. Eur. J. Pharmacol., 2007, *568*, 142-148.

[25] Liu, X.; Osawa, T. Astaxanthin protects neuronal cells against oxidative damage and is a potent candidate for brain food. Forum Nutr., 2009, *61*, 129-135.

[26] Naval-López, M.V.; Gómez-Serranillos, M.P. In: Ginseng research in the era of systems biology; Yan J, Ed; Int. J. Curr. Biomed. Pharmaceut. Res., 2012, pp. 1-10.

[27] López, M.V.; Cuadrado, M.P.; Ruiz-Poveda, O.M.; Del Fresno, A.M.; Accame, M.E. Neuroprotective effect of individual ginsenosides on astrocytes primary culture. Biochim. Biophys. Acta. 2007, *1770*, 1308-1316.

[28] Ye, R.; Li, N.; Han, J.; Kong, X.; Cao, R.; Rao, Z.; Zhao, G. Neuroprotective effects of ginsenoside Rd against oxygen-glucose deprivation in cultured hippocampal neurons. Neurosci. Res., 2009, *64*, 306-310.

[29] Lakshmi, S.V.; Padmaja, G.; Kuppusamy, P.; Kutala, V.K. Oxidative stress in cardiovascular disease. Indian J. Biochem. Biophys., 2009, *46*, 421-440.

[30] Sudhahar, V.; Kumar, S.A.; Varalakshmi, P. Role of lupeol and lupeol linoleate on lipemic-oxidative stress in experimental hypercholesterolemia. Life Sci., 2006, *78*, 1329-1335.

[31] Bansal, P.; Gupta, S.K.; Ojha, S.K.; Nandave, M.; Mittal, R.; Kumari, S.; Arya, D.S. Cardioprotective effect of lycopene in the experimental model of myocardial ischemia-reperfusion injury. Mol. Cell. Biochem., 2006, *289*, 1-9.

[32] Fu, J.; Huang, H.; Liu, J.; Pi, R.; Chen, J.; Liu, P. Tanshinone IIA protects cardiac myocytes against oxidative stress-triggered damage and apoptosis. Eur. J. Pharmacol., 2007, *568*, 213-221.

[33] Lin, W.T.; Huang, C.C.; Lin, T.J.; Chen, J.R.; Shieh, M.J.; Peng, H.C.; Yang, S.C., Huang, C.Y. Effects of beta-carotene on antioxidant status in rats with chronic alcohol consumption. Cell Biochem. Funct., 2009, 27, 344-350.

[34] Vardi, N.; Parlakpinar, H.; Cetin, A.; Erdogan, A.; Cetin Ozturk, I. Protective effect of beta-carotene on

methotrexate-induced oxidative liver damage. *Toxicol. Pathol*., 2010, *38*, 592-597.Vqrdi et al., 2010.

[35] Aune, D., Chan, D.S., Vieira, A.R., Navarro Rosenblatt, D.A., Vieira, R., Greenwood, D.C., Norat, T. Dietary compared with blood concentrations of carotenoids and breast cancer risk: a systematic review and meta-analysis of prospective studies. Am. J. Clin. Nutr. 2012, *96*, 356-373.

[36] Gupta, S.K.; Trivedi, D.; Srivastava, S.; Joshi, S.; Halder, N.; Verma, S.D. Lycopene attenuates oxidative stress induced experimental cataract development: an *in vitro* and *in vivo* study. Nutrition. 2003, *19*, 794-799.