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Xanthine Oxidase Inhibition and Antioxidant Effects of *Peaganum harmala* Seed Extracts

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Research Article

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ABSTRACT

In the present study, seeds of *Peaganum harmala* were extracted with solvent of varying polarity allowed their separation into four subfractions. Total polyphenols and flavonoids contents of *Peaganum harmala* seeds extracts (PHSE) were determined as gallic acid equivalent and Quercetin equivalent and were in the following order: chloroform extract (CHE)>ethyl acetate extract (EAE)>crude extract (CE). The superoxide radical scavenging properties of PHSE were determined by either production of uric acid and reduction of Cytochrom c (Cyt c). The inhibition ratios of the Xanthine Oxidase (XO) by CHE and EAE were effective followed by CE. Results for the Cyt c assay were in the same order. The antioxidant potential was confirmed with the non enzymatic methods. The highest DPPH radical scavenging activity was observed with CE followed by EAE and CHE. The β-carotene/linoleic acid bleaching assay revealed that PHSE have a very important antioxidant activity. Results showed that EAE has the highest antioxidant activity (94.21%), followed by CE and CHE respectively. Ferrous ion chelating capacity assay showed that CE, EAE and CE are very active with IC_{50} of 0.088, 2.256 and 5.286 mg/ml, respectively. *Peaganum harmala* extracts have strong free radicals scavenging and antioxidant activities and may prove to be of potential health benefit. In addition, the ability of PHSE to inhibit XO can be of great use in some diseases where XO is incriminated.

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Keywords: Xanthine Oxidase; Peaganum harmala; antioxidant; free radicals; scavengers; polyphenols.

1. INTRODUCTION

Free radicals are produced in normal and/or pathological cell metabolism. Oxidation is essential to many living organisms for the production of energy to fuel biological processes. However, the uncontrolled production of oxygen-derived free radicals is involved in the onset of many diseases such as cancer, rheumatoid arthritis, cirrhosis and arteriosclerosis as well as in degenerative processes associated with ageing. Exogenous chemical and endogenous metabolic processes in the human body or in the food system might produce highly reactive free radicals, especially oxygen derived radicals, which are capable of oxidizing biomolecules, resulting in cell death and tissue damage (Halliwell and Gutteridge, 2003).

Among the endogenous source of free radicals is Xanthine Oxidase (XO), a complex molybdenum iron–sulphur flavoprotein enzyme that is generally recognized as the terminal enzyme of purine catabolism in man and in a few other uricotelic species. Xanthine Oxidase catalyses the hydroxylation of hypoxanthine to xanthine and xanthine to uric acid (Bray, 1975). The capacity of XO to generate Reactive Oxygen Species (ROS) has led to widespread interest in the enzyme as an initiator of tissue damage in a range of pathological states. Hydrogen peroxide (H_2O_2) and superoxide anion radicals ($O_2^{\bullet -}$) can interact in the presence of certain transition metal ions to yield a highly reactive oxidizing species, the hydroxyl radical ($OH^{\bullet}$) (Halliwell, 1997).

All organisms are well protected against free radical damage by oxidative enzymes or chemical compounds such as a-tocopherol, ascorbic acid, carotenoids, polyphenol compounds and glutathione (Niki et al., 1994). When the mechanism of antioxidant protection becomes unbalanced by factors such as ageing, deterioration of physiological functions may occur, resulting in diseases and accelerated ageing. However, antioxidant supplements or antioxidant containing foods may be used to help the human body to reduce oxidative damage (Halliwell and Gutteridge, 2003). Natural antioxidants are being extensively studied for their capacity to protect organisms and cells from damage brought on by oxidative stress, the latter being considered a cause of degenerative diseases (Cazzi et al., 1997).

These natural antioxidants not only protect food lipids from oxidation, but may also provide health benefits associated with preventing damages due to biological degeneration (Hu and Kitts, 2005). As part of our efforts to find antioxidants from edible herbs, we have investigated the antioxidant potential of *Peganum harmala*, a plant which belongs to the Zygophyllaceae (Hilal and Young ken, 1983) commonly known as '*Harmal*' grows spontaneously in semiarid and predesertic regions of south-east Algeria and distributed in North Africa and the Middle East (El-Bahri and Chemli, 1991). The Harmel is largely used in traditional medicine in Algeria and the Maghreb to treat different gastrointestinal disorders (Goel et al., 2009), Skin (Monsif et al., 2004), infections (Idrissi Hassani and Hermas, 2008). It is also used for treating rheumatic pain and painful joint (Bellakhdar, 1997).

The present study was undertaken to investigate the inhibition of XO by *Peganum harmala* seeds extracts (PHSE) and their antioxidant effects using established *in vitro* assays.

2. MATERIALS AND METHODS

2.1 Materials

Peganum harmala L. was collected from N'gaouss, Batna, Algeria in July 2010 and identified by Pr. Laouar H (Department of Ecology and Vegetal Biology, University of Setif). Bovine milk was obtained from a local farm. All other reagents were purchased from Sigma Chemicals (Germany), Fluka and Prolabo.

2.2 Extraction Procedure

The extractions were carried out using various polar and non-polar solvents (Markham, 1982). According to the method, the powdered plant material (100g) was extracted with methanol (MOH) (85% and 50%), at room temperature overnight. The MOH extracts were combined and concentrated under reduced pressure on a rotary evaporator. MOH extract (CE) 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 (R-H_2O) extracts.

2.2.1 Determination of total flavonoid contents

The total flavonoid content of 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 aluminium chloride ($AlCl_3$) solution (2%) and allowed to stand for 15 min. Absorbance of the mixture was then determined at 430 nm versus prepared methanol blank. Results were expressed as equivalent quercetin and rutin (mg quercetin or rutin/g dried extract).

2.2.2 Determination of total polyphenols

Total polyphenols were measured using Prussian blue assay described by Price and Butler (1977) modified by Graham (1992). Phenolics were expressed as gallic acid equivalents. Briefly 0.1 mL of each sample, were dissolved in methanol, 3 mL of distilled water was added and mixed, then 1 mL of K_3Fe (CN) 6 (0.016 M) was added to each sample followed by the addition of 1 mL of $FeCl_3$ (0.02 M dissolved in 0.1 M HCl), and 5 mL stabilizer (30 mL gum Arabic, 1%; 30 mL H_3PO_4, 85% and 90 mL of distilled water) were added to the sample and mixed. The absorbance was measured at 700 nm. Phenols were expressed as gallic acid equivalents (mg gallic acid/g dried extract) ranging from 0.00 to 200 µg/mL.

2.3 Purification of Bovin Milk XOR

Xanthine oxidoreductase was purified from fresh Bovin milk by ammonium sulphate fractionation, followed by affinity chromatography on heparin and ion exchange fast protein liquid chromatography, essentially as described for bovine (Baghiani et al., 2002, 2003).

Concentration of enzyme was determined from the UV-Visible spectrum by using an absorption coefficient of 36 000 M^{-1} cm^{-1} at 450nm (Bray, 1975). The oxidase content of XOR was determined by measuring the rate of oxidation of xanthine to uric acid spectrophotometrically at 295 nm, using an absorption coefficient of 96 00 M^{-1} cm^{-1} (Avis et al., 1956). Assays were performed at room temperature in air-saturated 50 mM phosphate buffer, pH 7.4 supplemented with 0.1 mM EDTA.

2.4 SDS-Page

SDS-PAGE is carried out with a discontinuous buffer system (Laemmli, 1970). It consists of a stacking gel poured over a resolving gel. The protein samples and stacking gel are prepared using Tris-HCl (pH 6.8), whereas the resolving gel is made in Tris-HCl (pH 8.8). However, for running the gel, the buffer reservoirs are filled with Tris-glycine buffer (pH 8.3). In order to prepare the gel, first, resolving gel 10% is poured between the glass plates. After polymerization, stacking gel of larger pore size (5%) is poured. Constituents of the sample loading buffer are: Tris-HCl (pH 6.8) glycerol, SDS, Glycerol and bromophenol blue. Generally, five minutes of boil is given to the samples and then allowed to cool then loaded carefully into the wells. While loading the protein samples in the gel, protein molecular weight markers (MW-SDS-200 Kit, Sigma) are also loaded in a separate well. After the gel has run 100 V (stacking gel) then 200 V (running gel), the gel running apparatus is de-assembled. The gel is taken out carefully. For visualizing the proteins, gel is stained with Coomassie Brilliant Blue R-250 (CBB).

2.5 Effects of *Peganum harmala* Extracts on XOR Activity

The effect of PHSE on Xanthine Oxidation was examined spectrophotometrically at 295 nm following the production of uric acid using an absorption coefficient of 9600 M^{-1} cm^{-1} (Avis et al., 1956). Different concentrations of tested compounds were added to samples before the enzyme had been added and their effect on the generation of uric acid was used to calculate regression lines and IC_{50} values. The reaction was started by the addition of XO (1227 nmol of urate/min/mg protein for BXO). The enzyme activity of the control sample was set to 100% activity. Allopurinol was used as reference compound.

2.6 Measurement of Superoxide Anion Scavenging Activity

Free radical scavenging activity was assayed spectrophotometrically by the reduction of cytochrome c method as described by Robak and Gryglewski, (1988). In brief, 50 µl of various concentrations of PHSE extracts was dissolved in 50 mmol/l phosphate buffer (pH 7.4), then 10 µl of 1227 nmol/min/mg Xanthine Oxidase, 100 µl of 100 µmol/l xanthine and 100 µl of 25 µmol/l cytochrome c were added to these samples. They were vigorously mixed and followed by spectrophotometric determination of the ferricytochrome c reduction at 550 nm. The sensibility of the reaction was determined using bovine erythrocytes superoxide dismutase (SOD) (330 U/mL final concentrations).

2.7 DPPH Radical Scavenging Activity

Hydrogen atom or electron-donation ability of the corresponding extracts was measured from the bleaching of the purple-coloured methanol solution of 2, 2 -diphenyl-1-picrylhydrazyl (DPPH). This spectrophotometric assay uses stable DPPH radical as a reagent (Brand-Williams et al., 1995). Fifty microliters of various concentrations of the samples in methanol were added to 5 ml of a 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm.

Inhibition of DPPH free radical in percent (I%) was calculated in the following way:

$$I\% = (A_{blank} - A_{sample}) \times 100 / A_{blank}$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. Sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration. Tests were carried out in triplicate.

2.8 Beta-Carotene Bleaching Test

The antioxidant activity of PHSE extracts was evaluated by the β-carotene linoleate model system (Dapkevicius et al., 1998). A solution of b-carotene was prepared by dissolving 2 mg of β-carotene in 1ml of chloroform. One millilitre of this solution was pipetted into a 100 ml round-bottom flask. After that, the chloroform was removed at 40 C° under vacuum, 25 µl of linoleic acid, 200 mg of Tween 40 emulsifier, and 100 ml of distilled water were added to the flask with vigorous shaking. Aliquots (2.5 ml) of this emulsion were transferred into different test tubes containing 350µl of different concentrations of the PHSE. Absorbance readings at 490 nm using a spectrophotometer in deferent time 0h, 1h, 2h, 3h, 4h, 6h, 24h, 48h, respectively. Lipid peroxidation inhibition was calculated using the following equation:

$$RAA\ \% = A_{sample} / A_{BHT} \times 100$$

Where A_{sample} is the absorbance of the test compound. A_{BHT} is the absorbance of the BHT. BHT was used as standard. Sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage against extract concentration.

2.9 Ferrous Ion Chelating Activity

To evaluate compound-iron interaction, we performed the ferrozin test, which assesses the capability to interact with iron (II), preventing the formation of the complex between the reduced metal form and the specific Fe^{2+} colorimetric detector ferrozin. The chelation of ferrous ions by PHSE and by the standard molecule was carried out according to Decker and Welch (1990). Briefly, different concentrations of test compounds (0.5 ml) were added to a solution of 0.6 mM of $FeCl_2$ (0.1 ml) and 0.9ml of methanol. The reaction was initiated by the addition of ferrozin 5 mM (0.1 ml) and the mixture was shaken. Absorbance of the solution was then measured spectrophotometrically at 562 nm in a spectrophotometer. EDTA was used as a positive control. An IC_{50} value defined as the inhibitive concentration of test material which produces 50% of maximal scavenging effect. All tests were run in triplicate.

3. RESULTS AND DISCUSSION

3.1 Total Phenolics and Flavonoid Content

Determination of total phenolic and flavonoid contents of different extracts of *P. harmala* (PHSE) were carried out using Prussian bleu assay and $AlCl_3$ methods, separately. EAE extract of PHSE contain high amounts of phenolics and flavonoids compounds.

The total phenolic content of each PHSE was reported as mg gallic acid equivalent per gram dried extract. The results showed that PHSE fractions contained phenolic compounds in the following order: EAE>CHE>CE. The total flavonoid contents of different PHSE fractions were reported as mg quercetin and rutin equivalent per g dried extract. The results, presented in Table 1, show that the flavonoid contents of the PHSE fractions have the following order: EAE > CE > CHE.

Table 1. Total polyphenol and flavonoid contents of *P. harmala* seed extracts

Extracts	%Yield (W/W)	Total polyphenols mg gallic acid/g	Total flavonoids mg quercetin/g
Crud	1.65 ± 0.27	27.91 ± 0.98	7.39 ± 0.68
Chloroform	2.19 ± 0.16	66.29 ± 1.58	13.88 ± 0.13
Ethyl acetate	2.41 ± 0.31	58.1 ± 2.87	12.18 ± 0.08

Value are expressed as mean ± SEM, n =5

3.2 Xanthine Oxidase Purification

The Bovine freshly purified milk XO showed an ultraviolet/visible spectrum with three major peaks at 280, 325, 450 nm, with A280/A450 (protein to flavin, PFR) ratio of 5.3, indicating a high degree of purity (Bray, 1975). Enzyme purity was confirmed by SDS-PAGE, which showed a single major protein band with Mr 150 KDa. The XO specific activity of purified bovine milk was 1988.55 nmol of urate/min/mg protein. This result is analogous to the well-studied bovine milk XO (Baghiani et al., 2002, 2003).

3.3 Effects of *Peganum harmala* on XO Activity

The enzyme XO plays a crucial role in the production of uric acid, catalyzing the oxidation of hypoxanthine and xanthine. During the reoxidation of XO, molecular oxygen acts as an electron acceptor, producing superoxide radical and hydrogen peroxide. Consequently, XO is considered to be an important biological source of superoxide radicals. Inhibition of XO results in a decreased production of uric acid, which can be measured spectrophotometrically, and a decreased production of superoxide. The present results showed that all the extracts were effective in inhibiting the uric acid production in a dose-dependent manner ($p < 0.05$), (Fig.1).

The IC_{50} values of different extracts (Fig. 2) show that both extracts; CHE and EAE seem to have the strongest inhibitory effect ($p < 0.001$), with an IC_{50} of 0.055 ± 0.0053 and 0.057 ± 0.0141 mg / ml, respectively, followed by CE with an IC_{50} of 0.252 ± 0.0051 mg / ml. The CHE and EAE have an effect almost six times lower than the allopurinol, whereas the CE is almost 30 times lower. Numerous studies have evaluated the inhibitory effect of various plants on the activity of XO (Ferraz Filha et al., 2006; Umamaheswari et al., 2007). This inhibitory activity may be attributed to the presence of various bioactive compounds such as polyphenols and flavonoids (Da Silva et al., 2004).

Cos et al., (1998) determined the relationship between the chemical structure of flavonoids and their inhibitory activities of XO. The richness of the CHE and EAE in flavonoids may be the cause of their powerful inhibitory effect.

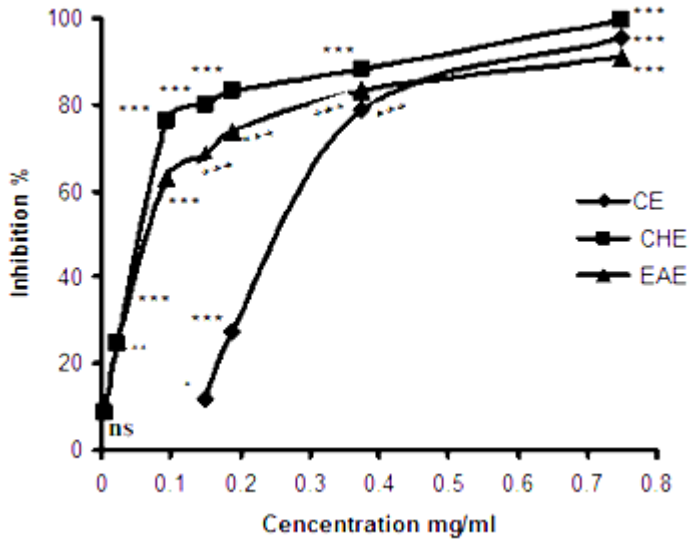

Fig. 1. Inhibitory actions of *P. harmala* extracts on xanthine oxidase.

Results are expressed as percentage of control where no inhibitor was added. Values are means ± SD (n= 3). Significantly different; ns: not significant. CE: crude extract, CHE: chloroform extract, EAE: ethyl acetate extract. Significant differences (*: p<0.05, **: p<0.01 and *** p<0.001).*

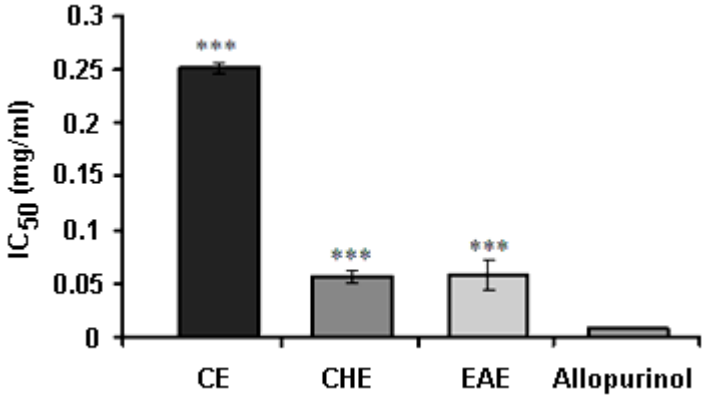

Fig. 2. IC_{50} (Inhibitory concentration of PHSE for 50 %) of BXOR activity.

*The concentration of extracts is expressed as mg/ml equivalent of extract in the reaction mixture. CE: crude (methanol) extract; CHE: chloroform extract; EAE: ethyl acetate extract. Values are means ± SD (n = 3). ***: p<0.001*

3.4 Effects of *Peganum harmala* Seeds Extracts on the Generation of Superoxide Anion Radicals

Cytochrome c^{3+} (Cyt c^{+3}) has been extensively used for the detection of $O_2^{\bullet-}$ produced in biological systems due to its fast superoxide-mediated reduction to Cytochrome c^{2+} (Cyt c^{+2}) (McCord and Fridovich, 1968). The effect of PHSE at different concentrations were studied for their ability to scavenge $O_2^{\bullet-}$ generated by the xanthine/xanthine oxidase system. The amount of generated $O_2^{\bullet-}$ was determined by measuring the reduction of Cyt c. Under our

experimental conditions, the activity of Cyt c, in the absence of extracts, reduced by $O_2^{\bullet-}$ generated from XO was 2135.91 nmols/min/mg protein. The reduction of Cyt c^{+3} was almost totally inhibited by SOD (330 U/mL). All extracts of *Peganum harmala* seeds inhibit Cyt c reduction significantly ($p < 0.01$) in a dose-dependent manner (Fig. 3).

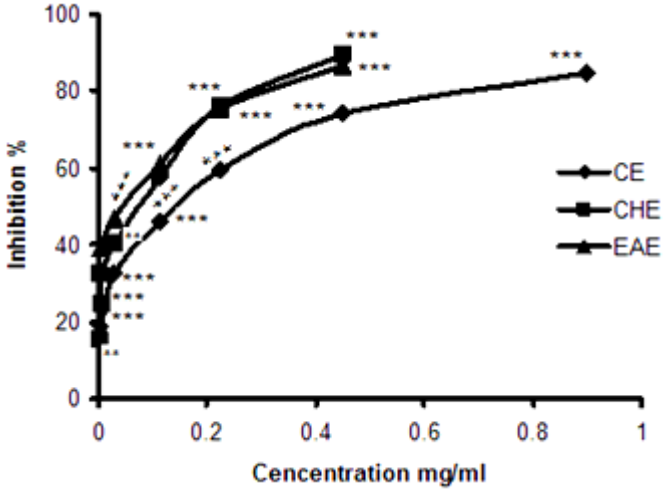

Fig. 3. Scavenging effects of *P. harmala* extracts on superoxide anion radicals.
*CE: crude (methano)l extract; CHE: chloroform extract; EAE: ethyl acetate extract. Values are means ± SD (n = 3). **: p<0.01 and *** p<0.001*

For each extract tested, two IC_{50} values (50% inhibitory concentrations) can be calculated by linear regression analysis: 50% inhibition of XO (50% decrease of uric acid production) and 50% reduction of the superoxide level (Fig. 4).

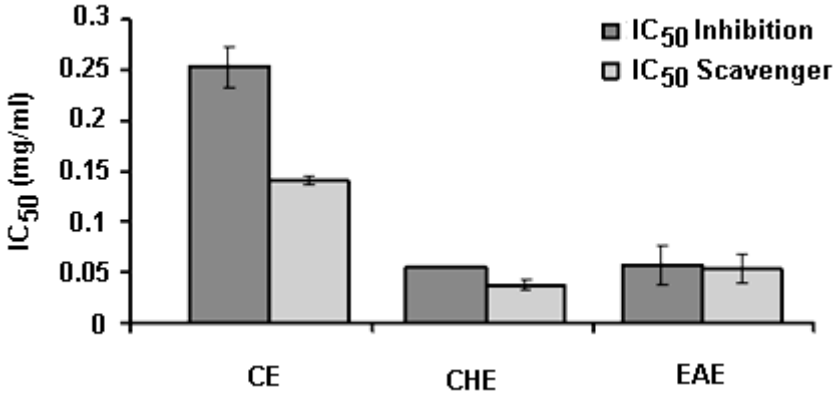

Fig. 4. IC_{50} (Inhibitory concentration of PHSE for 50 %), IC_{50} (Scavenging effects of PHSE on superoxide anion radicals) of BXOR activity.
*The concentration of extracts is expressed as mg/ml equivalent of extract in the reaction mixture. Values are means ± SD (n = 3).CE: crude extract, CHE: chloroform extract, EAE: ethyl acetate extract. *: p<0.05, **: p<0.01 and ***: p<0.001*

The different extracts of *Peganum harmala* seeds have an inhibitory effect on the XO, which makes it difficult to know, in the test of Cyt-c 3, if these extracts have a scavenger effect on

$O_2^{\bullet-}$ or an inhibitory effect on the enzyme. The most powerful inhibitory effect of reduced Cyt c was shown with CHE (IC_{50}=0.037 ± 0.0008 mg/ml) ($p<0.001$), followed by EAE and CE with IC_{50} = 0.053 ± 0.0141 mg/ml and 0.14 ± 0.0051 mg/ml, respectively.

The IC_{50} of CE for the production of uric acid from the oxidation of xanthine by XO is nearly 2 times lower than that inhibiting the reduction of Cyt c. CHE has an IC_{50} of reduced Cyt c presents almost two folds of the inhibition of XO and the EAE has an IC_{50} of reduced Cyt c almost identical to that inhibiting XO. Cos et al. (1998) showed that if the values of IC_{50} for the scavenger of $O_2^{\bullet-}$ are below those of inhibiting the production of uric acid, this indicates that the extracts have inhibitory effect on XO activity and scavenger of $O_2^{\bullet-}$. The scavenger effect of free radicals by the various constituents of plants may be due to phenolic acids and flavonoids (Boumerfeg et al., 2009; Baghiani et al., 2010). The inhibition of Cyt c reduction is due to the inhibitory effect of XO and / or the scavenger effect on the $O_2^{\bullet-}$ produced by this enzyme (Valentao et al., 2002).

3.5 Diphenyl-2-Picryl-Hydrazil (DPPH) Scavenging Activity by Extracts

The discoloration degree of the deep violet colour of DPPH indicates the free radical scavenging potentials of the sample/antioxidant and it has been found that known antioxidant such as cysteine, glutathione, ascorbic acid, tocopherol, polyhydroxy aromatic compounds (hydroquinone, pyrogallol etc.) reduce and decolorize the DPPH by their hydrogen donating ability (Blois, 1958). The three PHSE extracts showed a significant ($p<0.05$) scavenging effect on the DPPH radical in a dose-dependent manner (Fig. 5).

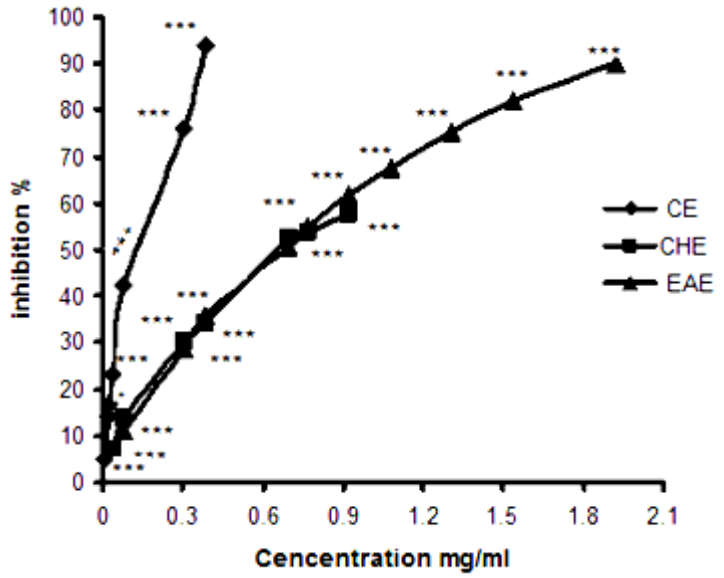

Fig. 5. The percentage inhibition of free DPPH radical in the presence of different concentrations of *Peaganum harmala* extracts.

*CE: crude extract, CHE: chloroform extract, EAE: ethyl acetate extract. Values are means of three assays. *** p<0.001*

Based on the calculated IC_{50} values presented in Figure 6, the samples are ordered for their scavenging activity as follows: gallic acid (0.56 ± 0.034 µg/ml)>quercetin (3.49 ± 0.012

µg/ml)>Rutin (4.1 ± 0.089 µg/ml)>BHT (32.55 ±18.6)>CE (100 ± 6.9 µg/ml) > EAE (711 ± 29.8 µg/ml) > CHE (744 ± 19.8 µg/ml). The lowest IC_{50} indicates the strongest ability of the extracts to act as DPPH scavengers.

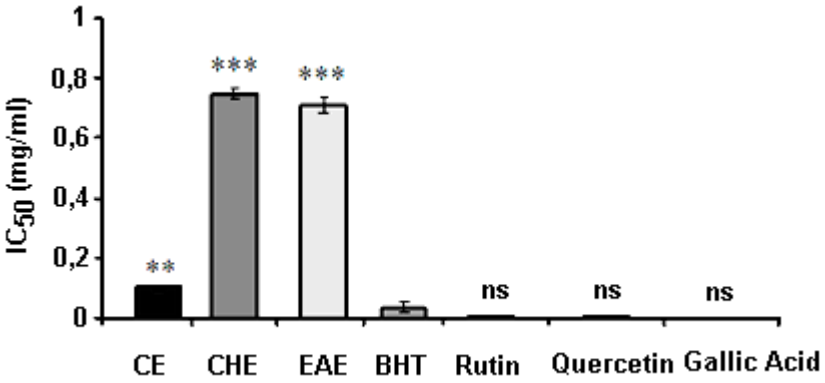

Fig. 6. DPPH radical scavenging activity of different *P. harmala* extracts.
CE: Crude extract; EAE: ethyl acetate extract; CHE: chloroform extract.
*Data are presented as IC_{50} mean ± SD (n = 3). (**: p<0.01 and *** p<0.001*

According to the results shown in Fig. 6, the extract, which had a strong antioxidant activity, contain high flavonoid contents. This result was confirmed previously. Sun and Ho (2005) reported a significant correlation between total phenolics and scavenging ability of buckwheat extracts on DPPH radicals. The mechanism of the reaction between antioxidant and DPPH depends on the structural conformation of the antioxidant (Williams et al., 2004).

3.6 β-Carotene–Linoleic Acid Bleaching Assay

In β-carotene /linoleic acid model system, β-carotene undergoes rapid discoloration in the absence of an antioxidant. The linoleic acid free radical formed upon the abstraction of a hydrogen atom from one of its diallylic methylene group attacks the highly unsaturated b-carotene molecules. As a result, b-carotene is oxidized and broken down in part; subsequently the system loses its chromophore and characteristic orange colour, which is monitored spectrophotometrically at 470 nm. All PHSE inhibit the oxidation of β-carotene in a very important level (Fig. 7). 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).

EAE is the best inhibitor of the oxidation of β-carotene in 24h (94.21 ± 0.003%), followed by CE (86.24 ± 0.027%) and finally the lowest effect was seen with CHE (77.35 ± 0.032%). Several studies have shown that the antioxidant effect of natural sources is due to their phenolic compounds (Nagai et al., 2003). In this study, the most powerful extract is the one containing the glycones compounds.

The high antioxidant activity exhibited by the PHSE in the current assay suggests that it has a potential for use in foods containing emulsified oils. The antioxidant effect of the extract may also be of biological relevance as it may prevent oxidation of lipid components within cell membranes. Therefore, the plant extract may prove to be of potential health benefit.

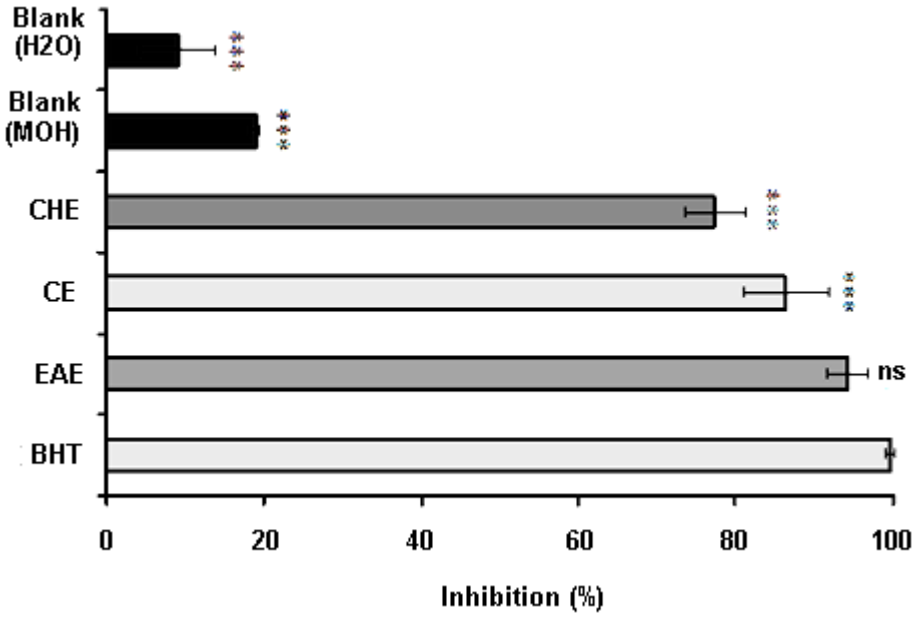

Fig. 7. Percentage inhibition of the linoleic acid oxidation by the *P. harmala* extracts.
*(CE: crude extract; CHE: chloroform extract; EAE: ethyl acetate extract), BHT and blanks (H_2O and Methanol) after 24h. Results are means of three different experiments. *** p<0.001*

3.7 Ferrous Ion Chelating Activity

This method is based on the inhibition of the training (formation) of complex Fe-Ferrozine by following the discoloration of colour purple at 562 nm, the obtained results showed that the various extracts of the *Peganum harmala* seeds as well as the standards interfere in a dose-dependent manner with the formation of the complex (Fe^{2+}- Ferrozine), suggesting that they have a chelating activity while capturing the ferrous ion before being complexed with the Ferrozine. The values of IC_{50} of the various tested extracts show a big variety; the CE expresses the most powerful effect with IC_{50} of 0.088 ± 0.0008 mg / ml followed by the EAE and CHE with 2.256 ± 0.0398 mg / ml and 5.286 ± 0.0053 mg / ml, respectively (Fig. 8).

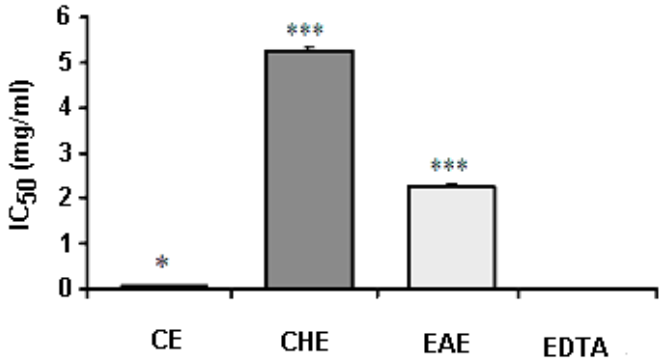

Fig. 8. Metal chelating activity of different *Peganum harmala* extracts.
*(CE: crude extract, CHE: chloroform extract, EAE: ethyl acetate extract). Data were presented as IC_{50}. Values are means ± SEM (n = 3). *: p<0.05, *** p<0.001*

In this assay, both the PHSE and the standard EDTA interfered with the formation of ferrous-ferrozine complex, suggesting that they have chelating activity, capturing the ferrous ion before it reacts to form a complex with ferrozine. 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 also accelerates peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation (Elmastaş et al., 2006). Phenolic compounds have been reported to be chelators of free metal ions (Brown, 1998). But in this study a low and no significant correlation was observed between the chelating extracts of *Peganum harmala* seeds and their contents in phenolic compounds. Similar results have been reported in other recent work, for example in a study on extracts of fourteen varieties of barley, Zhao and colleagues (2008) have found a very weak correlation between the chelating activity of these extracts and their contents in phenolic compounds. However, a poor correlation of iron chelating activity with total phenolic compounds of all extracts might indicate that phenolic compounds might not be the main chelators of ions (Wong et al., 2006). In addition, the ability for phenolic compounds to chelate metal ions depends on the availability of properly oriented functional groups (van Acker et al., 1996). Therefore, a sample high in polyphenols might not chelate metal if the polyphenols present did not have suitable groups that could chelate the cation. Also, when a phenolic group is conjugated with a carbohydrate group, as in naturally-occurring phenolic glycosides, it can no longer bind metals (Wong et al., 2006).

In conclusion, the present study has demonstrated that *P. harmala* seed extracts possess significant inhibition of XOR, scavenging of superoxide and high antioxidant activity. These results suggest that this plant could be used in many applications to treat many disorders, where the inhibition of XO and/or the antioxidant activities are warranted.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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