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Study of Antioxidant Capacity and Stability of Phenolic Compounds from the Seeds of *Peganum harmala*

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ABSTRACT

Peganum harmala (harmala) is a plant native to arid regions of the eastern Mediterranean to northern India. In vitro studies of antibacterial extracts for removing microbes have been used in traditional medicine. The antioxidant properties and total phenolic compounds extracted from *Peganum harmala* using water, ethanol and ethanol - water was evaluated. The highest amount of phenolic compounds from water extract, ethanol - water and then ethanol was obtained. Antioxidant activity of extracts tests to trap free radicals DPPH & β-caroten are investigated and compared with the synthetic TBHQ antioxidant. Water and ethanol- water extract had the highest antioxidant activity in all the tests were done. The results showed that harmala seeds have high antioxidant activity, is a rich source of antioxidant compounds.

KEYWORDS: *Peganum harmla*, Antioxidant activity, Extract, Phenplic compound

INTRODUCTION

Phenolic compounds are classified to simple phenols, phenolic acids, and flavonoids derivatives. Many phenolic compounds function as potent antioxidants have been reported by researchers. In recent decades, researchers have focused on finding antioxidants from natural sources. The health properties of natural antioxidants and their role in disease prevention are the main reasons for this increase. The antioxidants compounds commonly used to prevent lipid peroxidation, as some products have been added to increase the shelf life will be (1). Phenolic compounds and antioxidant rich herbs, good examples in medicine, food and perfumery since ancient times been considered, antifungal, anti- bacterial, and because they are more applications(2). The extract of this plant, the first key step is very important for the extraction of antioxidant compounds. Selected solvents and plants can affect the quantity and type compounds isolated from the experiences of several optimization techniques for extracting and comparing antioxidant plant extract has been tested (3,4,5).

Peganum Harmala (harmala) is a plant native to arid regions of the eastern Mediterranean to northern India. The plant originated in Central Asia. Harmala has been existed since ancient times in India, Egypt, Iran, Spain, the Mediterranean and also known to have medicinal uses (6, 7).

Ordinary people on different occasion's harmala seeds in fire and smoke poured from the wound to be immune enemies, many antibacterial predecessors as warm poultice to reinforce and strengthen the body and used to have black hair. In traditional medicine , herbal extracts Espand to increase milk secretion , excretion of milk secretion , excretion treating rheumatism, Increase sexual power, housing, sweating, hypnotic, sedative, anti- parasitic, bacterial and fungal binding rule, abortion, the fetus, anti- cancer and nervous system stimulant is used(8, 9, 10). In the laboratory of antibacterial extract is used to kill germs. Callus extracts from antimicrobial properties against microbes such as *Staphylococcus aurous*, *Escherichia coli* and *Candida albicans* shown (11, 12, 13, and 14).

The main compounds of flavonoids and alkaloids of Peganum contain antimicrobial substances that are in different parts of the material (seeds, seedlings and callus) found high (15, 16). Seeds of this plant are rich in carbohydrates , lipids , proteins , minerals , and amino acids are alkaloids, grain , liquid antibacterial fatty acids include stearic acid , linoleic acid , palmitic acid , linolenic acid , and so on. The steroid antibacterial agents, including Beta Sitostrol, and finally Lanosterol harmala alkaloids in the seeds which make up about 4% of the dry weight of grain and numerous industrial and medical importance of these compounds can be Harmalyn ,harmine, harmalol and vazsyn named(8,17).

In relation to the evaluation on the antioxidant properties of this plant , in accordance with the function of the antioxidant system and lipid or aqueous two-phase systems is different, the use of a method for activity antioxidant , may not be effective in the exact amount of antioxidant power plant , would , by reason of the B-carotene linoleic acid peroxidation system of investigation of the test (determines the polarity of the substances

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in the extracts) and DPPH free radical scavenging capacity was used that Hydrophilicity or hydrophobicity properties can predict antioxidant substances(18). The main objective of the study population and the researchers draw attention to provide valuable scientific and documentary evidence and commercialization of products from them.

MATERIALS AND METHODS

Materials

Chemicals and solvents used in this study were analytical grade and were purchased from Merck and Sigma.

Extraction

Amol harmala seeds collected from nearby mountains and transported immediately to the laboratory in dry shade and by crusher(Molinex 684- French) was thoroughly dried , and the solvent ratio of 1 to 10 with water , ethanol and ethanol - water mixture away from light for 48 h in a shaker (LABTRON Ls-100) with the speed rpm 200 been Then centrifuged three times (10 min each time with 300HERMLE z200A -Germany) (rpm) and the aqueous phase (top phase)Were collected , the sediment at the bottom of the tube to be seen. The aqueous phases were collected; with Whatman No. 1 paper was smooth. Followed by the evaporator (maximum temperature 50 ° C) (TAM 2times- Iran) and evaporation of the solvent extracts were obtained. Extracts were stored until testing at 18 ° C (4).

Measurement of total phenolic compounds:

The total amount of phenolic compounds in the extracts was measured using Folin- Ciocalteu. In this method, the presence of phenolic compounds dissolves in alkaline reagent Fulin, rehabilitation and blue is produced in solution (18, 19).

50 ml of sample with 1.5 ml most Folin- Ciocalteu represents the ratio of 10: 1 was diluted with distilled water, was mixed. Then 2 mL of sodium carbonate (7.5 % weight by volume) was added to the Then for 15 minutes at 45 ° C incubate the aqueous phase at 764 nm is expanded and absorbed by UV-Vis spectrophotometer was read, the total amount of phenolic compounds using the equation for a line is drawn on the basis of gallic acid and gallic acid, gallic acid is heated in a kilogram of dry matter was reported The linear equation relating the absorbance of the solution at 764 nm with a concentration gallic acid show as follows. Read the 764 nm absorption wavelength of the X and Y values of phenolic compounds in milligrams per ml.

$$Y = 1/0766\ X^2 + 0/2644\ X + 0/0099$$

$$R^2 = 0/9922$$

Inhibitory power of free radical DPPH:

DPPH assay is one of the most widely used method is to estimate the antioxidant content. 2,2diphenyl-1-picrilhydrazyl a stable radical , which reacts with the hydrogen atoms of the test compounds on inhibition of DPPH radical species or by adding antioxidants cause discoloration DPPH solution was determined. 2, 2diphenyl-1-picrilhydrazyl purple color combination, due to the structure easily phenyl radical and is, in fact, is a source of free radicals. This combined with an electron capture antioxidant compounds, from purple to yellow color. absorbance at 517 nm in DPPH free radicals that follow the law Bier Lambert decrease the absorption of antioxidant linearly, The antioxidant is added to the amount of DPPH was used more and more purple color tends to yellow(20). In this method, the composition of the stable radical DPPH was used as a reagent. Thus, 50 ml of different concentrations of 20, 40, 60, 80 and 100 micrograms per ml of methanol extract

s of 0/004 percent to 5 mM solution of DPPH in methanol was added, After 30 min incubation at room temperature, absorbance of the samples was read at a wavelength nm 517 vs. control. In this test the ability of a hydrogen atom or an electron by a variety of compounds and extracts of soluble purple discolorations 2, 2diphenyl-1-picrilhydrazyl (DPPH) was measured in methanol.

β-caroten - Linoleic acid test

Unsaturated fatty acids such as linoleic acid, the process is very sensitive to oxidation. The oxidation this material as a valuable method for the determination of antioxidant activity can be used. In this method, the antioxidant activity by inhibiting the oxidation of linoleic acid and conjugated avoids volatile compounds and hydro peroxides, is examined (21).To perform the test, a basic solution of Beta caroten - linoleic acid (sigma-

Aldrich) was prepared as follows:

Half milligrams of beta-carotene was dissolved in 1 ml of chloroform and 25 microliter of linoleic acid and 200 mg Tween 40 was added and thoroughly mixed. Then isolated by evaporation of chloroform and 100 ml of distilled water saturated of oxygen (100 ml per minute for 30 minutes under pressure) were added with vigorous stirring. 2.5 ml of the solution prepared above was transferred to a test tube and 350 micro liter of extract (2 g per liter of ethanol) was added to a test tube After 48 h incubation at room temperature, absorbance at 490 nm was read samples and antioxidant activity Comparing the absorbance of the sample at zero time and the stability of the yellow carotene content was measured.

Statistical analysis

Comparison of results from three replicates with Duncan's test (P <0/05) in a completely randomized design was used .Data analysis with SPSS software version 12 and graphs were plotted using Excel software. All experiments were performed in triplicates.

RESULTS AND DISCUSSION

Table 1 shows the results of measuring the amount of total phenolic compounds in the extracts show As you can see, Extracts were significant differences (P <0/05) together. The maximum amount of phenolic compounds was extracted by solvent water.

Table1. The total amount of phenolic compounds in the extracts from seeds of Peganum

Type of extraction	The amount of total phenolic compounds of the extract (mg Gallic acid / g dry weight)
The aqueous extract	348.82±2.79^{ab}
Ethanol extract	318.41±28.49^{b}
Ethanol - water extract	329.09±27.48^{ab}

Researcher's differences between different extractions solvents used to the difference in polarity of opinion. Extraction of antioxidant compounds from plant material is dependent on the solubility of these compounds in various solvents. The polarity of solvent used plays a key role in increasing the solubility of these compounds (22, 13, and 23).

And solvents such as ethanol and methanol mixed with water (40-80 %) than in the pure state in the ability of phenolic compounds extracted from the plant tissues (24). The use of water as solvent extraction, a highly polar environment is created in which some degree of phenolic compounds of low polarity, are less. Add water to Relatively polar organic solvents to form a mobile environment, so the extraction of phenolic compounds in the amounts and more types of conditions will ensure In addition, the extract contained large amounts of impurities, such as organic acids, soluble proteins and sugars that can be used in the diagnosis and quantification of phenolic compounds and antioxidant activity They interfered with the antioxidant , on the other hand the presence of adequate amounts of water in organic solvents, the desired increase The swelling of plant tissue swelling, which Increase the contact area between the plant and the solvent matrix and thus increase the extraction rate(25).

DPPH free radical trap:

The method is based on DPPH bleaching solution is performed by the antioxidants present in the extract by inhibiting the action of free radicals occurs. Testing inhibition of DPPH in harmala extracts were analyzed by various methods. Figure 1 shows the DPPH extracts in different ways. Statistical analysis of the results of this study suggest that according to Duncan test at 5% level between the DPPH ethanol and aqueous extracts showed no significant difference (P> 0.05) and the highest DPPH radical scavenging activity have and the difference will be in a range of low power and radical scavenging DPPH, based on TBHQ.

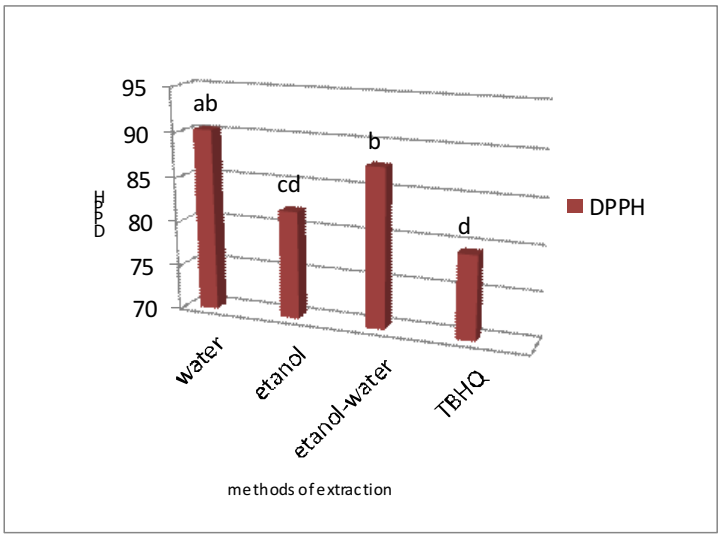

Fig1. Comparison of antiradical activity of various seed extracts and TBHQ using DPPH

Anti-radical activity of beta-carotene bleaching test (beta-carotene-linoleic acid):

Linoleic acid inhibition of antibacterial activity of plant extracts were analyzed by different methods, the results of the antioxidants beta-carotene model system shown in Figure 2. Statistical analysis according to Duncan's test at 5% level indicates that the degree of inhibition of linoleic acid in the extracts and the synthetic antioxidant TBHQ, water extract and ethanol-water extract and ethanol extract had the greatest amount of inhibition and inhibition of linoleic acid TBHQ was the least power.

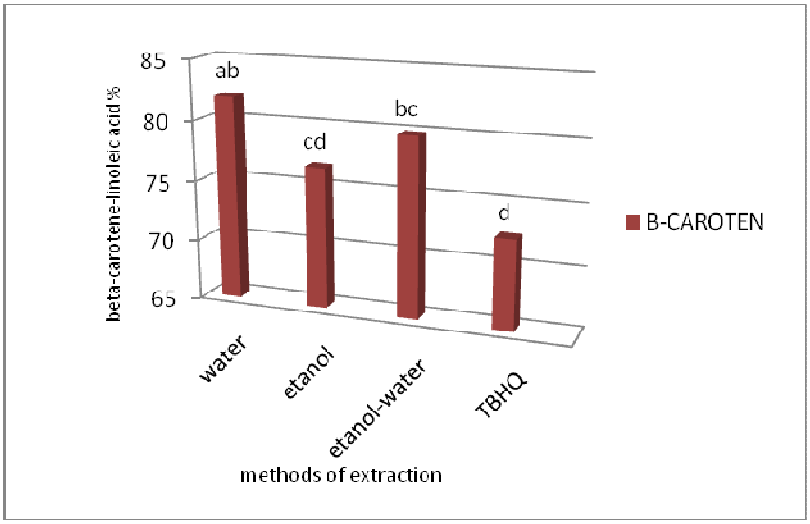

Figure2. Comparison of antiradical activity of various seed extracts and TBHQ with beta-carotene bleaching test (β-caroten - linoleic acid)

The ability of beta-carotene, an antioxidant to prevent oxidation of lipids and fatty acids is investigated for corruption. High activity of beta-carotene antioxidants in model systems indicate the potential antioxidant activity at the interface between the water phase and fat (26).Thus, the antioxidant activity of phenolic constituents of Peganum sign of biological activity of this extract in preventing oxidative decomposition of lipids in the membrane.

Conclusions

Among the extracts, the highest amount of phenolic compounds extracted from the water extract and ethanol-water and ethanol extracts, respectively were the next steps. DPPH radical scavenging test indicate that the water and alcoholic extracts of the highest DPPH radical scavenging power are and almost in a statistical range and are generally DPPH radical scavenging power when we compare these extracts with TBHQ, We see that the extracts in DPPH radical disable located at a much higher level. The high antioxidant activity of beta-caroten antioxidants in model systems is the fact that phenolic compounds in the extracts can react with radicals generated as a result of lipid oxidation of lipids in cell membranes, resulting in injury to prevent oxidative degradation of lipids is inhibited. Based on these results, we can say harmala seed due to a large amount of phenolic compounds with high antioxidant potential and hence can as a rich source of natural antioxidants in the manufacture of various products such as jam, marmalade, pickles and other food products in which the presence of lipid oxidation is likely to be used effectively.

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