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Original Article

Autecology, Phytochemical and Antioxidant Activity of *Peganum harmala* L. Seed Extract in North of Iran (Tash Mountains)

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Abstract

Peganum harmala L. (Zygophyllaceae) is one of the most important medicinal plants had been used in traditional medicine in North of Iran as anti parasite, anti inflammatiom, anti tumor and anti infection. The aim of this study was to evaluate of ecological factors , ethno pharmacology and record of the relationship between secondary metabolites content and their antioxidant activity in seeds extract of plant. Ecological factors and ethnopharmacological data were obtained in many field observation. The seeds were collected in Tash Mountainous region (2750m) in August 2011, dried and were extracted by ethanol solvent. Total phenolic (TP) and total flavonoids (TF) content were determined by spectrophotometry method. Total antioxidant capacity (TAC), 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay and reducing power assay (RP) methods were applied to measure antioxidant activity. Field observation showed that *Peganum harmala* is a perennial plant which can grow 30 to 70 cm tall, somewhere with annual average rainfall about 197mm , in sandy clay loam soil . Flowers appeared in mid to late of May and fruit maturation occurred in June to July. The results demonstrated that the TP was 61.55±0.84 mg GAE g -1 and TF content 42.21±0.66 mg QUE g-1. IC50 was measured 53.64±0.5 mg/ml in DPPH 17.34±0.71 in TAC and 84.75±0.89 in RP method. Analyses of results showed a positive correlation between antioxidant activity and the most important secondary metabolites, which explains and confirmed the application of plant in traditional medicine as an antiseptic, anti-tumor and disinfectant agent.

Key words: *Peganum harmala* L., Aut ecology, Secondary metabolites, Antioxidant activity, Ethnopharmacology, Iran.

Introduction

Plants show versatility in synthesizing complex materials, which refer to as secondary metabolites. Plants secondary metabolites act as defense mechanisms against many ecological stresses, predation by herbivores, microorganisms and insects; also, plants can produce similar substances as a part of their normal growth and development or in response to stress. Phytochemicals are naturally occurring and biologically active plant compounds that have potential disease inhibiting capabilities. It is believed that phytochemicals may be effective in combating or preventing disease due to their antioxidant effect [1]. Free radicals and oxidative stress are involved in variety of disorders including atherosclerosis, chronic renal failure, diabetes mellitus, cancer, immune dysfunction and aging [2]. Antioxidants have been presented for the treatment or prophylaxis of disorders attributed to free radical oxidative [2-4]. Natural antioxidants can protect the body against free radicals, which may cause chronic inflammatory diseases [5]. In this regards, the interest in natural antioxidants studies greatly increased recent years [6,7]. Antioxidant activity of many phenolic compounds like flavonoids has attracted considerable attention to be more powerful antioxidants than vitamins C, E and β-carotene [2,8].

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Peganum harmala L. is a perennial, bushy and wild-growing flowering plant with short creeping root is known as "Espand'' in Iran ,"Harmel" in North Africa and "African Rue", "Mexican Rue" or "Turkish Rue" in United States [9, 1]. It is native to semi-arid rangeland, steppe area and sandy soils. This plant is widely distributed in North Africa, Mediterranean, the Middle East, Pakistan, India and Iran. *P. harmala* traditionally has been used in Iran as an antiseptic and disinfectant agent by burning its seeds [10,11]. This plant has been considered for the treatment of a variety of human disease such as lumbago, asthma, colic, jaundice. In India, it is used in syphilis treatment, in north of Africa is used for the treatment of fever [12].

Its roots are boiled and used to kill lice and the extract is useful in wound healing [13]. Red dye is also obtained from the seeds of *Peganum* [14]. Moreover, this plant had different pharmacological activities such as malaria, rheumatism, Parkinson, hysteria, uterus prolapse, eye infection [6] and have antiplasmodial, antileishmanial, anthelmintic, analgesic and anti-inflammatory, insecticidal, anti-tumor, anti-histaminic, anti-oxidant activity [15-20]. Furthermore, it has been reported that this plant had antibacterial, antifungal and antiviral activity [21].

Several alkaloids of *P. harmala* L. have an extensive variety of pharmacological actions in various scales especially in the seeds and the roots. The β-carboline alkaloids such as harmine, harmaline,harmol and harmalol, quinazoline derivatives like vasicine and vasicinone have been reported in this plant. Harmaline, the main alkaloid of *Peganum* seeds, induces blood pressure drop and toxic for protozoa. Previous studies were proven the abortificant, narcotics, aphrodisiac, stimulant, sedative, emetic, vermifuge and soporific application [1,14]. Considering structural and biological diversity of terrestrial plants, we offer a unique renewable resource for the discovery of potential new drugs and modern medicine. The main purpose of this study was to evaluate secondary metabolic contents, antioxidant activity in seed part of *Peganum harmala* L.

Materials and Methods

Field observation

Ecological factors, phenological characteristics and ethnopharmacological data of plant were obtained in many field observations and to talk from rural peoples or healers in this region (45-67 years old). Plant seeds were collected from south east of Golestan province (2750 m – Tash Mountains) in during august to September 2011.

Plant materials

The voucher specimen of plant was identified and deposited in the herbarium museum of the Gorgan University. The seeds were separated, dried in shade and grinded into fine powder and maintained at room temperature (21–23°C). The prepared powder was kept in tight containers protected completely from light to perform the extraction of the secondary metabolites.

Preparation of plant extract

The dried seeds of plant (5 g) were extracted overnight in 100 ml of ethanol, in a mechanical shaker at room temperature. Plant extract was filtered through Whatman filter paper (No. 1) and stored at 4°C [22].

Antioxidant activity

A 45 g of seed plant were extracted in 300 ml of methanol solvent with mechanical shaker at room temperature (RT). Extract was filtered through Whatman filter paper (No. 1). The filtrates obtained from extract were evaporated into dry at 40°C in a rotary from evaporator and stored at 4°C [22].

Determination of total phenolic content

Total phenolic content of extracts were estimated based on Folin-Ciocalteu reagent, according to the suggested procedure of [23]. Then 0.5 ml of plant extract or Gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (5 ml) and aqueous Na_2CO_3 (4 ml, 1 M). The mixtures were allowed to stand for 15 min and the total phenols were determined by colorimetric at 765 nm. Gallic acid was used as a standard for calibration curve. Total phenol values were expressed in terms of mg equal Gallic acid in 1 gr powder dry plant.

Determination of total flavonoids content

Total flavonoids content were determined by aluminum chloride method [23]. Seed extract (0.5 ml) were separately mixed with 1.5 ml of solvent, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. They were kept at room temperature for 30 min; the absorbance of the reaction mixture was determined spectrophotometrically at 415 nm. Quercetin was used as a standard for calibration curve. Total flavonoid values were expressed in terms of mg equal quercetin in 1 gr powder dry plant.

Antioxidant and free radical scavenging potential Determination (Reducing Power assay)

The reducing power of extracts was determined as per the Arabshahi-Delouee method. The dried extract (12.5–1000 µg) in 1 ml of the corresponding solvent was combined with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (K3Fe (CN) 6; 10 g l^{-1}), after the mixture was incubated at 50 °C for 30 min. 2.5 ml of trichloroacetic acid (100 g l^{-1}) was added and the mixture centrifuged at 1650g for 10 min. Then, 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (1 g l^{-1}), and the samples absorbance was measured at 700 nm [22].

Total Antioxidant Capacity (TAC)

Total antioxidant capacity of extracts was estimated as described by Arabshahi-Delouee method, which is based on the reduction of Mo (VI) to Mo (V) by the sample and observation of a green phosphate/Mo (V) complex at acidic pH. An aliquot of 0.1 ml of sample solution, containing 12.5-1000µg of dried extract in corresponding solvent, was combined in a tube with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). They were incubated in a thermal block at 95 ^{oC} for 90 min. Then we got cold the samples and measured their absorbance at 695 nm. A typical blank solution contained 1 ml of reagent solution and the appropriate volume of the same solvent was used for the sample, and was incubated under the same conditions as the rest of the samples [22].

1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging Assay

Scavenging activity on DPPH was assessed according to the method suggested by [22]. Briefly, 1ml of a 1mM methanolic solution of DPPH was mixed with 3ml of extract solution in methanol (containing 12.5–1000 µg of dried extract). The mixture was shaken well and incubated for 30 min at room temperature in the darkness. The absorbance was measured at 517 nm spectrophotometercally and activity was expressed as percent DPPH scavenging relative from control using the following equation:

DPPH scavenging activity (%) =[($A_{control} - A_{sample}$) / $A_{control}$] ×100

Statistical analysis

The data were expressed as. Mean ± standard deviation (SD) and differences at $P < 0.05$ were considered statistically significant.

Results

Autecology, Phenology and ethnopharmacology

In this region, one natural population of *Peganum harmala* L. was found in cold arid (Tash Mountain) regionin South east of Golestan province, results showed that this perennial plant which can grow to about 30-70 cm tall, roots can reach a depth of up to 40cm in semi-arid rangeland, steppe area and sandy soils. Dormant plants commenced growth in mid of April, grew through May and June ,but shoot development was formed through April to September. White flower appeared in mid to late of May and fruits normally one month after the first flowers were formed (June to July). This natural habitat where have annual average rainfall is about 197mm , which preferred pH range 7.3, EC in 0.39 in Sandy clay loam soil.

Ethnopharmacological results showed, it has been used by the people and rural healers as strong anti evil, anti-air infection, sedative, anti-bacterial ,anti parasite, anti tumor and anti inflammation to treatment of fungal, gastro intestinal infection and dysmenorrheal. They were believed that the smoke of seeds can kills the air pathogen, bacteria and intestinal parasites, despite have used the fragrant smoke of seeds that is wafted around the head to exposed the eyes of strangers, another the uses of 7-10 *peganum* seeds with flowers of *Achillea micrantha* and *Cuminum ciminum* to treat of dismenorrhae, but in combination of powder of *Provskia abrotanoides* to treat leishmaniasis infection,

Total phenolics, flavonoids and anthocyanin determination

Amount of phenol and flavonoids compounds in seed extract of *Peganum harmala* L. are shown in Table 1. Results indicated that the TP content of various extract was 61.55 mg GAEgr^{-1} dry weight and TF content 42.21 mg QUEg^{-1} (Table 1, Fig 1). Antioxidant capacity of the seed extract in TAC, DPPH and RP ranged from 17.34 to 84.75 mg ml^{-1}.

IC_{50} was measured 53.64 mg/ml in DPPH, 17.34 in TAC and 84.75 in RP method (Fig 2).

Table 1 Comparison of secondary metabolites and antioxidant activity of *P. harmala* L.

Test / Part	Secondary metabolites		Antioxidant activity		IC_{50} (mg/ml)
	Phenol (mg GAEgr^{-1})	Flavonoid (mg QUE g^{-1})	TAC	DPPH	RP
Seed	61.55±0.84	42.21±0.66	17.34±0.71	53.64±0.5	84.75±0.89

The finding in Table and figure 1, indicated that the TP content of seeds extract had significant more than TF content ., whereas this findings showed that the seeds of plant with the highest content of TP and TF compounds could be used as an important part with high potency in scavenging of free radicals.

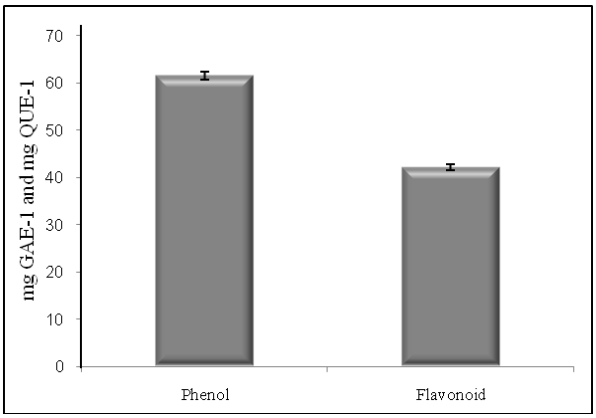

Fig 1 Secondary metabolites content in seed extract of *P. harmala* L.

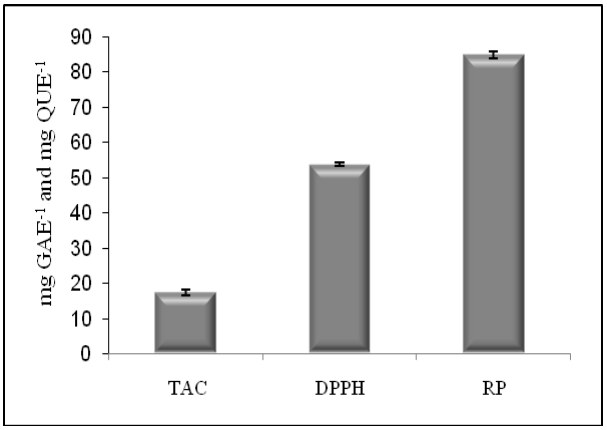

Fig 2 IC_{50} values of *P. harmala* seed extract in various methods

Discussion

According to similar research [12], our results showed that *Peganum harmala* L. is a perennial, herbaceous plant native to arid and semi-arid regions of the Mediterranean region, mainly grows in dry stepic to mountain grasslands and saline waste areas commonly along roadsides, field edges and run-down pastures, ,which has resistant to many ecological region ,in sea level to dry cold arid in Mountainous region . it was often grow to 70 cm tall, deep roots , shoot development was formed through April to September, flowers appeared in mid to late of May and fruits were formed in June to July and so preferred pH range 7.3, EC in 0.39 in Sandy clay loam soil.

This plant is known as “Esfand” , which has been used in traditional medicinal of this region as an antiseptic, anthelmintic, narcotic, given for fever and colic and disinfectant agent by burning its seeds and according in similar work it is used in syphilis treatment, in north of Africa is used for the treatment of fever [12].

Its roots are boiled and used to kill lice, and the extract is useful in wound healing [13], also its antiseptic property it is applied during the epidemic by using the smoke of the seeds, obtain by placing it on burning coal. Red dye is also obtained from the seeds of *Peganum* [14]. *P. haramala* was shown to possess antihelmitic, lactogogue, abortifient and emetic properties [6].

According to our results in Table 1, phenols (61.55 mg GAEg^{-1}) and flavonoids (42.21 mg QUEgr^{-1} -are well known for their antioxidant activity due to their redox properties especially in TAC method with IC50 17.34 mg/ml.

The high content of secondary metabolites in *P. harmala* could explain its high radical scavenging activity. Previous studies demonstrated that, the direct relationship between amount of total phenol and flavonoid contents and antioxidant activity in many medicinal plants [11, 23-25, 27-29].

Previous studies indicated that the antioxidant activity of phenolic and flavonoid compounds is attributed to their redox properties, despited the high antioxidant activity of extracts of some medicinal plants (*Onosma dichloroanthum, Artemisia annua, Sylibum marianum and Heracleum gorganicum*)

were directly depended on amount of TP and TF contents [29-31].

Mazandarani *et al.,* (2011) reported that the antioxidant activity of root extract of *Onosma dichroanthum* Boiss., was directly depended on amount of TP and TF and in another research. They were showed that even antibacterial activity of *Onosma dichroanthum* were depended on their TP and TF [29, 31].

In previous studies, the phenolic contents in aerial part of *P. harmala* were higher than the phenolic content in the leaves [6]. Harman alkaloids (0.2%) ,harmine and 1.5 % harmaline per total weight) reported of *Peganum harmala* seeds [14]. The amount of total phenolic compounds in methanolic extract of *P. harmala* L. aerial parts was 98 mg/g equivalent to Gallic acid [12].

In other research ethyl acetate, chloroform, butanol and methanol extracts of the leaves of *Peganum harmala* L. were tested for antioxidant activity, the results showed that the methanol extract had the highest antioxidant activity (75.90) in comparison to other solvent, also indicated that among the tested extracts, methanolic and butanolic extracts had the highest content (112.5; 63 mg CEg-1) and ethyl acetate extract had the lowest total phenolic content (25.7 mg CEg-1) [6]. Fazal *et al., (*2011), showed that DPPH scavenging antioxidant activity of ethanol extract in seed of *P. harmala* [13]. In Souri et al findings (2004) the amount of IC_{50} in methanol extract of *P. harmala* L. aerial parts indicated 1.98 µg [2].

Conclusion

The results of this study demonstrate that due to high amount of TP, TF compounds, and antioxidant activity, the seeds of *Peganum harmala* L. could be an important part of this plant, which shows a high potency in scavenging of free radical. High amount of antioxidant activity may be related to the high amount of secondary metabolites in this organ. Demonstration of the antioxidant potential of the herbs could provide natural sources of antioxidant compounds and confirmed some scientific evidence for the traditional usage of these plants as an antiseptic and disinfectant agent in Persian ethnical home remedies. Further activity guided isolation and characterization of the extract is in progress to identify the full composition of the extract and the exact compounds responsible for its bioactivity.

These results suggested that the phenolic compounds contributed significantly to the antioxidant capacity of the investigated plant species. which findings of many research groups who reported such positive correlation between total phenolic content and antioxidant activity .

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