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Research Article

Antioxidant Potential and Total Phenolic Compounds of Extracts and Fractions of *Pistasia atlantica*

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ABSTRACT

Different parts of *Pistasia atlantica* have been used in traditional medicine for various purposes in Iran. The aim of this study was to measurement and compare antioxidant activity and polyphenolic compounds of crude ethyl alcohol extract and four fractions of *P. atlantica* leaf. Crude ethyl alcohol extract of *P. atlantica* leaf was prepared using maceration method and subjected to fractionation with different polarity. The antioxidant potential of all these fractions was evaluated by the 2,2 diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity method. The total phenolic, flavonoid, and flavonol components were measured with Folin-Ciocaltiue and Chlorid Aluminum methods. According to the radical scavenging capacity, the ethyl acetate fraction exhibited the highest antioxidant activity with IC50 value 1.54±0.12 µg/ml, followed by the chloroform fraction with higher percent inhibition of the DPPH with 3.4±0.11 µg/ml. The results are represented relative to a reference standard, butylated hydroxytoluene (BHT), with IC50 value of 33.5±3.67µg/ml. Among these fractions, the ethyl acetate fraction and chloroform fraction had the highest amount of total phenolic compounds with value of 532.73 and 355.14 mg GAE/g, respectively. The results of this study showed that some fractions of *P. atlantica* leaf extract could be used as easily accessible source of natural antioxidants.

Keywords*:* Antioxidant, Total phenolic, Medicinal plants, *Pistacia atlantica*.

INTRODUCTION

Free radicals or highly reactive oxygen species are capable of oxidizing bio-molecules and can initiate different degenerative diseases like cancer, neurological disorders, cirrhosis, emphysema, atherosclerosis, arthritis etc[1-7]. Antioxidants are the compounds which wind up the attack of free radicals and thus decrease the risk of these disorders[8,9]. Recent attention has been paid to directly evaluating the antioxidant properties of plant extracts as the source of natural antioxidants[10]. Many antioxidants have been found to be free radical or active oxygen scavengers and thus plants have been investigated for their biological activities and antioxidant properties[4-7,11]. Recent reports about antioxidant activity and polyphenol contents of herbals showed that polyphenols, i.e. flavonoids, tannins, and phenolic acids, have antioxidant properties.

Species of Pistacia genus are important species in Western Asian areas including Syria, Jordan, Iran and Afghanistan[13]. Various types of compounds like terpenoids, phenolic compounds, fatty acids, and sterols have been identified from different parts of Pistacia species[14]. According to previous researches, wide pharmacological activities had been showed from various parts of Pistacia species such as anti-inflammatory, antitumor, antioxidant, antimicrobial, antiviral and their effects in gastrointestinal disorders improvement. *Pistacia atlantica* (*P. atlantica*) belonging to the family Anacardiaceae is a species of flowering plants. Traditionally some indigenous peoples used the resin and fruits of *P. atlantica* for stomach aches, dyspepsia and throat infections[14].

Based to our knowledge until now any studies have been not reported on the antioxidant activity and phenolic of different fraction of *P. atlantica* leaves. The aims of this study was to measurement and compare the antioxidant potential and total phenolic compounds of crude ethyl alcohol extract and four correspond fractions of *P. atlantica* leaf.

MATERIAL AND METHOD

Plant collection and extraction

P. atlantica leaves was gathered from southwest region of Iran. Then, in herbarium of Medical Plants Research Center of Shahrekord University of Medical Sciences (Iran), genus and species of the plant were identified and confirmed. The dried *P. atlantica* leaves were separately ground to obtain uniform powders. The leaf powder (100 g) was dissolved in 80% ethyl alcohol (400 ml) and kept at room temperature for 96 hours. Subsequently, the mixture was filtered and concentrated under nearly vacuum pressure and at 40°C using rotary evaporator.

Fractionation of plant material

Four fractions of the crude extract, with different polarity through in-solution isolation and using the difference in

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various secondary metabolites' polarity were prepared. To isolate the hexane fraction, the extract concentrated, suspended in 80% ethyl alcohol, and mixed with equal volume of normal hexane (Merck, Germany) with shaking vigorously. The remaining solution from which the ethyl alcohol was removed was mixed with distilled water and with chloroform (Merck, Germany) in equal volume, shaken, and hydrated using sodium sulfate and used as the Chloroform fraction. Subsequently, remaining solution was mixed with ethyl acetate (Merck, Germany) in equal volume, shaken and used as the ethyl acetate fraction. To prepare n-Butanol fraction, equal volume of n-Butanol (Merck, Germany) was added to the remaining aqueous phase of the material, shaken and concentrated at 40°C and in vacuum condition. The remaining aqueous phase was concentrated, under the similar condition, as mentioned above and used as aqueous fraction. The extract/fractions were kept in sterile bottles, under refrigerated conditions, until further use.

Determination of the free-radical scavenging activity

The free-radical scavenging activity was measured by the 2,2 diphenyl-1-picrylhydrazyl (DPPH) method described by Moon and Terao, with some modification[15]. Different amounts of the extract in methanol 100% were added to a solution of 0.3 mg/ml methanolic solution of DPPH to make up a total volume of 2.0 ml. After standing for 15 min at room temperature, the absorbance was measured at 517 nm using UV–Vis spectrophotometer (UNICO 2100: USA). High absorbance of the reaction mixture indicated low free radical scavenging activity. Butylated hydroxytoluene (BHT) was used as positive control. Inhibition of free radical by DPPH was calculated as follows:

Antiradical activity (%) = [(A control – A sample)/A control]×100.

The IC_{50} value, defined as the amount of antioxidant necessary to decrease the initial DPPH concentration by 50% and was calculated based on linear regression of plots of the percentage antiradical activity against the concentration of the tested compounds[16]. The experiment was carried out in triplicate and the results are average values.

Determination of total phenolic content

The total phenolic content of the extract was determined using Folin Ciocalteu method with slight modifications[17]. Briefly, 0.2 ml of the diluted sample (1 mg/ml in methanol 60%) was added to 1 ml of 10% (v/v) Folin–Ciocalteu reagent and kept at room temperature for 3-8 min. Subsequently, 0.8 ml of 7.5% (w/v) sodium carbonate solution was added to the mixture. After being kept in total darkness for 30 min, the absorbance of the reaction mixture was measured at 765 nm using a UV–Vis spectrophotometer (UNICO 2100: USA). Amounts of total phenolic were calculated using a gallic acid calibration curve. The results were expressed as gallic acid equivalents (GAE) mg/g of dry plant matter.

Determination of total flavonoids

The amount of total flavonoids in the extract/fractions was determined calorimetrically assay as previously reported method[18]. Briefly, 0.2 ml of diluted plant material (1 mg/ml in methanol 60%), 0.2 ml of 2% (w/v) aluminum chloride and 1.2 ml of 5% potassium acetate. Following incubation at room temperature (RT) for 40min, the absorbance of the reaction mixture was read at 415 nm using a UV–Vis spectrophotometer (UNICO 2100: USA). The results were expressed in terms of rutin equivalents of dry plant matter (mg RUT/g) by comparison with the standard curve, which was made in the same condition.

Determination of total flavonols content

The total flavonol content of the extract/fractions was measured as previously reported method (19). Briefly, 0.2 ml of diluted plant material (1 mg/ml in methanol 60%), 0.2 ml of 2% (w/v) aluminum chloride and 1.2 ml of 5% sodium acetate. Following incubation at room temperature (RT) for 2.5 hours, the absorbance of the reaction mixture was read at 440 nm using a UV–Vis spectrophotometer (UNICO 2100: USA). The results were expressed in terms of rutin equivalent (mg RUT/g), which is a common reference

Statistical analysis

All experiences were carried out in triplicate. The IC_{50} values were calculated using dose-response analyses and related models with Probit procedure using SPSS program.

RESULT AND DISCUSSION

Free radical scavenging activity

The scavenging activity of the *P. atlantica* extract/fractions increases as the concentration increases. The crud extract and four fractions of *P. atlantica* leaves reduced the DPPH significantly (Table 1). The ethyl acetate fraction exhibited the highest DPPH scavenging with IC50 (50% inhibitory concentration) value (1.54±0.12µg ml) as compared to the other fractions of *P. atlantica*. Chloroform fraction, n-butanol and crude extract had IC50 values of 3.4±0.11, 3.66±1.52 and 4.6±0.66 µg/ml, respectively. The n-hexane fraction showed lowest antioxidant activity with IC50 value 14.84±2.07 µg/ml. The results are represented relative to butylated hydroxytoluene (BHT), a reference standard with IC50 value of 33.5±3.67 µg/ml. The IC50 values of the chloroform fraction, the n-butanol fraction, the crude extract, and the n-hexane fraction were found to be significant ($p<0.05$) while that of the aqueous soluble fraction non significant ($p>0.05$) as compared with BHT (Table 2).

Total Phenolic Content

The total phenolic content of the *P. atlantica* leaf extract and its fractions were expressed as gallic acid equivalent. Among the *P. atlantica* fractions, the ethyl acetate fraction and the n-hexane had the highest and lowest amount of total phenolic content with value 532.73 and 18.84 mg Gallic Acid Equivalent (GAE)/g, respectively. The total phenolic contents of the chloroform fraction, the crude extract and the n-butanol fraction were 355.14, 269 and 188.5 mg GAE/g (Table 2).

Flavonoids and flavonol content

The flavonoids and flavonol contents of the crude extract and the four fractions of *P. atlantica* were expressed as mg Rutin equivalent. Among the *P. atlantica* fractions, the chloroform fraction and the n-butanol had the highest and

Table 1: DPPH radical-scavenging activity of the *Pistasia atlantica* leaves extract and their different fractions.

Samples	Concentration (µg/ml)	Scavenging of DPPH radical activity inhibition (%)	DPPH-radical scavenging activity IC50 (µg/ml)
Crud extract	12.5	89.8±1.8	4.6±0.66
	6.25	57.5±2.2	
	3.125	29±2.6	
	1.56	15±2.3	
	0.78	3.9±1.9	
n-Butanol fraction	15	69.6±1.78	3.66±1.52
	7.5	64.5±2.63	
	3.75	57±3.25	
	1.87	46.8±2.5	
	0.93	34.2±1.34	
	0.47	29.1±2.6	
Ethyl acetate fraction	3.75	92.2±2.36	1.54±0.12
	2.5	79.3±1.49	
	1.25	42.8±0.87	
	0.625	2.7±2.38	
Chloroform fraction	10	90.4±2.63	3.4±0.11
	7.5	80.5±1.45	
	5	68.3±2.1	
	3.75	44.3±1.69	
	2.5	42.2±0.47	
	1.25	12.9±1.2	
n-Hexane fraction	30	65.8±2.4	14.84±2.07
	15	50.6±1.3	
	7.5	34±1.24	
	3.75	21.5±0.85	
BHT	200	90.2±1.2	33.5±3.67
	100	78.2±3.5	
	50	55.6±0.99	
	25	37±2.35	
	12.5	22.9±1.63	
	6.25	15.2±2	
	3.125	12.3±1.76	

BHT: Butylated hydroxytoluene; DPPH: 1,1-Diphenyl-2-picrylhydrazyl.

lowest amount of flavonoids with value 57.2, 38.5 mg RUT/g and the ethyl acetate and the n-butanol had the highest and lowest flavonols content with value 141.6 and 29.8 mg RUT/g, respectively ($P<0.05$, Table 2).

In recent years, many studies have focused on native plants to search for a new source of natural compound with medical properties[19-29]. In this study, hydro alcohol crude extract of *P. atlantica* leaf and its fractions with different polarities were prepared through in-solution isolation using the difference polarity. Also, the ability of these plant fractions to scavenging free radicals was assessed using DPPH. DPPH stable free radical method is a sensitive way to determine the antioxidant activity of plant extracts[30].

According to the radical scavenging capacity, the ethyl acetate fraction exhibited the highest antioxidant activity with IC50 value of 1.54±0.12 µg/ml, followed by the chloroform fraction with higher percent inhibition of the DPPH with 3.4±0.11 µg/ml. Free radicals are involved in many disorders like cancers, neurodegenerative, inflammatory, and viral diseases[31]. Natural antioxidants due to their scavenging activity are useful for the management of various diseases[32-37].

Among the fractions, the ethyl acetate fraction and chloroform fraction had the highest amount of total phenolic compounds. From the results can be concluded that there is proper correlation between total phenol content. Actually when the total phenol content increases, the ability to scavenging free radicals will increase.

Highlighting pharmacological studies on crude plant parts, extracts, and some pure metabolites has provided scientific evidence for traditional uses and has revealed genus *Pistacia* can be a valuable source for important molecules. So many studies were carried out on antioxidant activity of this genus considering their flavonoids, anthocyanins, and other phenolic compounds.

Similarly, some other studies have illustrated a linear relation between the antioxidant capacity of some plant extracts and their total phenolic content[4]. Therefore, the high antioxidant potential of ethyl acetate fraction of *P. atlantica* probably is due to presence of phenolic compounds.

Our findings that based on the DPPH method showed

Table 2: Total phenol, flavonoids and flavones of the Pistasia atlantica leaves extract and its fractions.

Samples	Total Phenol (mg Gallic Acid /g)	Flavonoids (mg Rutin/g)	Flavonols (mg Rutin/g)
Crude extract	269	40.7	88.12
Ethyl acetate fraction	532.73	51.2	141.6
n-Butanol fraction	188.5	38.5	29.8
Chloroform fraction	355.14	57.2	126.99
n-Hexane fraction	18.84	41.25	74.15

increase the polarity of the fractions of *p. atlantica* leaf, their radical scavenging activity will increase. Similar results were reported on the rate of antioxidant property of *Quercus brantii* fractions[4]. In this study, the crude extract of *P. atlantica* leaf and some of the fractions indicated antioxidant activity related to their total phenolic content and increasing the polarity.

CONCLUSION

This study reveals that *P. atlantica* extract and fractions have significant free radical scavenging activity. The result of present study suggests that the ethyl acetate fraction of *p. atlantica* extract can be used as a source of antioxidants for pharmacological preparations which is very well evidenced by the present work.

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