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Total phenolic content, antioxidant, and antibacterial activities of seed and pod of *Prosopis farcta* from Sistan region, Iran

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ABSTRACT

In this study, antioxidant and antibacterial activities of *Prosopis farcta* (*Fabaceae*) were investigated. The extracts were derived from seed and fruit pod parts by Ethanol, Methanol, Octanol and n-heptan solvents. High performance liquid chromatography analysis was used to measure antioxidant activities. Among the extracts, the seed Octanol extract (IC_{50}=0.95 µg/ml) showed the best antioxidant activity. Antioxidant activities in various solvents showed different trends that may be as a result of differences in polarity and H-bonding ability of each solvent. The highest phenolic content of *P. farcta* was also obtained by the Octanol extraction in both seed and pod organs. Antibacterial properties of extracts were only recorded by methanol and ethanol extracts of the fruit pods which inhibited the growth of *Staphylococcus* and *Escherichia coli*. The results of the present work indicated that the selective extraction of *Prosopis farcta* by appropriate solvents could be very important to obtain bioactive fractions.

INTRODUCTION

Production of chemical medications in recent decades has been greatly influenced by utilization of herbal medicines due to the presence of active chemical substances in plants possessing a strong safety profile. Nowadays, it has been reported that synthetic additives serve as antibacterial agents in food industries has a noticeable contribution in causing potential problems leads to various disease conditions. Therefore, to decrease the risk of free radical production from chemical substances entering into the human body, exploiting the plant extracts has attracted a great attention as sources of antibacterial and antioxidant agents (Škrovánková et al. 2012).

Free radicals and reactive oxygen species (ROS), the natural byproducts of cellular metabolic pathways, are mainly unstable molecules and are readily scavenged by antioxidants and antibacterial agents. The damages induced by high concentration of ROS which are due to peroxidation of bio-membranes and nucleic acids and lead to tissue damage, has been extensively studied (Gupta and Sharma 2006). They delay the oxidation process, inhibit the polymerization chain initiated by free radicals and other subsequent oxidizing reactions (Halliwell and Gutteridge 2006).

Various herbs and spices have been reported to exhibit antioxidant activities including *Lannea alata* (Okoth et al. 2013), *Mentha spicata* (Scherer et al. 2013), *Lactuca sativa* (Edziri et al. 2011), *Allium sativum* Linn., *Terminalia bellerica, Camellia sinensis* Linn. (Aqil et al. 2006). *Trigonella foenum graecum* Linn., *Elettaria cardamomum* Linn. (Khalaf et al. 2008). The majority of the antioxidant activity is due to the flavones, isoflavones cartenoids, glycosides, flavonoids, anthocyanin, coumarin lignans, L-ascorbic acid and isocatechins (Gupta and Sharma 2006).

One of the popular plants which is found commonly in Northern Africa, Southwestern Asia, West to the Middle East, and in the USA is a woody perennial dwarf legume shrub namely *Prosopis farcta (Syrian mesquite*) from *Fabaceae* family. Its height is about 0.4 – 1m, rarely up to 3 m but it can attain more than 2 m in height in

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certain places where weed control is absent. It is well adapted to drought and warm weather (Qasem 2007). It consists of leave, spine, pod, and seed. Leaves and beans of *P. farcta* have been used in folklore medicine for treatment of some disorders. Numbers of its beneficial effects have been proven by researchers. Recently, the physiological properties of this plant have opened up new strategies toward treatment of neurological disorders (Mollashahi et al. 2013). A recent study showed that the beans of *Prosopis farcta* increases HDL level and induce a decrease of LDL cholesterol in ostriches (Omidi et al. 2013).

The antioxidant and antibacterial properties have already been investigated in some of the *Prosopis* species. The results obtained from the antioxidant capacity of honeybee-collected pollen extract from *Prosopis juliflora* suggested that it is an important source of flavonoids which can be considered as natural antioxidants (Almaraz-Abarca et al. 2007). In another study, it was shown that *P. cineraria* bark possess significant antihyperglycemic, antihyperlipidemic and antioxidative properties (Sharma et al. 2010). Also the analysis of South American *Prosopis* pod's syrup showed that the phenolic constituents of the syrups including C-glycosyl flavonoids have anti-inflammatory, antioxidant and other nutraceutical properties (Quispe et al. 2014).

In this study, the antioxidant and antibacterial properties of *Prosopis farcta* endemic to Sistan, Iran was investigated for the first time.

MATERIALS AND METHODS

Preparation of plant extracts

The herb was collected from Sistan region (latitude of 30º54′ N and longitude of 61º41′ E with an elevation of 481m), located in south-eastern part of Iran. Taxonomic classification of the collected plant samples was confirmed by expertise in botany and plant taxonomy sciences. The samples were dried, grinded, and the plant extracts were prepared by maceration method as follows: 2.5 g of dried powder of herb was mixed with 50 ml of each solvent *viz*., Ethanol, Methanol, Octanol and n-hepthan. The suspension was stirred on a magnetic stirrer for 48h with 400 rpm at room temperature while the container was being capped to prevent solvent evaporation. After the extraction, the solutions were filtered and stored at 4°C.

DPPH assay

The antioxidant properties of the extracts were measured by determining the radical scavenging capacity (RSC) according to the method of Yamaguchi et al. (1998). The capacity of herbal extracts to scavenge the lipid-soluble radical, 2,2-Diphenyl-1-pykryl-Hydrazyl (DPPH), which results in the bleaching of the purple color exhibited by the stable DPPH radical, was monitored at 517 nm. The aliquots of the different concentrations (1-10 µg/ml) of the extract were added to 3 ml of a 0.004% w/v DPPH solution. For determining the antioxidant properties, the 1:1 mixture of plant extract: DPPH was shaken for 20 minutes in dark and then 100 µl of solution was injected to the high-performance liquid chromatography (HPLC-JASCO automatic injection system). HPLC parameters were adjusted as follows: mobile phase flow rate: 1 ml/min, wavelength detector: 517 nm, mobile phase: water/ methanol (70:30, v/v), column: Finepak sil C18-10 (4.6 * 250 mm, Jasco, Tokyo, Japan), injection value: 100 µl automatic and 25°C temperature. These parameters were maintained uniformly during performance of all experiments.

To determine the IC_{50} value of each extract, interpolation from linear regression analysis was employed. The IC_{50} representing the inhibitory concentration (mg extract/ml) at which 50% of DPPH radicals were scavenged.

Total phenolic contents

The total phenolic compound (TPC) content of the extracts was determined by Folin–Ciocalteu colorimetric method (García-Andrade et al. 2013). 1000 µg/ml aqueous solution of each extract was prepared and aliquots of 1 ml were mixed with 500 µl of water and 125 µ of Folin-Ciocalteu reagent (2 N). Solutions were stirred and then left for 6 min. Then 1250 µl of Na_2CO_3 (7%) and 1 ml of water were added into the solution and incubated in darkness for 90 min. Afterwards, the absorbance was measured at 765 nm by a UV- RAYLEIGH UV-2100 spectrophotometer. Calibration curves with gallic acid were prepared and results expressed as gallic acid equivalents (GAE) per gram of dry extract (mg $GAE/g_{d.e}$).

Antioxidant activity

In this study, the plant extracts were obtained from two various parts of the herb i.e. pods and seeds. Four different solvents including ethanol, methanol, octanol, and n-heptane were selected to investigate the effect of solvent on antioxidant properties of the extracts. The antioxidant properties of *P. farcta* are based on its phenolic content which is already shown in previous reports (Harzallah-Skhiri and Ben Jannet 2005; Almaraz-Abarca et al. 2007). The DPPH radical scavenging activity was evaluated by analyzing the differences in graph peaks reflecting decrease of the DPPH radicals compared between blank and the extracts (Yamaguchi, 1998). The following formula was used: Inhibition (%) = $[(A_{Control} - A_{Sample}) / A_{Control}) \times 100]$ where in $A_{Control}$ is the peak area for DPPH standard solution and A_{Sample} is the peak area for

the DPPH solution after reaction with plant extract (Figure1).

Antibacterial activity

The antibacterial activity was determined by the diffusion method and determination of minimum inhibitory concentration (MIC) (Pelczar et al. 1993). First of all, few colonies of two different types of bacteria (*Escherichia coli* and *Staphylococcus*) were cultured in TSB (Trypticase Soy Broth) medium for 24 h. Those bacteria grown on the media were harvested and dissolved in 1 ml sterilized water. Inoculums (10, 20, 40 and 80) were spread evenly onto 20 ml Mueller-Hinton agar set in Petri dishes using a sterile cotton swab. Then 10 µl of the plant extract was injected in the center of each well using a micropipette. Then the plate was placed onto the inoculated agar ensuring even distribution to avoid overlapping of zones. After overnight incubation at 37°C, the MIC was noted. Whereas ethanolic and methanolic extracts of *Prosopis farcta* exhibited remarkable antioxidant activities at greater level than other extracts, they were selected for assessment of the antibacterial tests.

Statistical analyses

The obtained data were statistically analyzed making use of SPSS vs.11.5 (IBM SPSS, New York, USA) software.

RESULTS and DISCUSSION

Antioxidant activity

A comparison between the antioxidant activities of the pod and seed extracts in four solvents are graphically represented in Figure 2 and Figure 3. The IC_{50} values are shown in Table 1. It is observed that at the concentration of 10 µg/ml, the IC_{50} of the various extracts are in the following orders for pod and seed respectively:

Pod extracts: Ethanol>Octanol>Methanol> n-heptane

Seed extracts: Octanol>Methanol>Ethanol>n-heptane

The obtained orders can be related to diverse factors. According to Harzallah-Skhiri and Ben Jannet (2005), the phenolic content in pericarps and flowers of *prosopis farcta* are very similar. Here, we assume that the flavonoids found in flower are similar to those in the seed. Thus, it can be concluded that the slightly higher antioxidant activity of seed extracts can be related to flavonoids such as Vitexin which are found in seed but not in pod. In both the cases, it is observed that the solvents having higher polarity index (PI) such as methanol (PI= 0.762), ethanol (PI=0.654), and octanol (PI=0.537) show better antioxidant activities against DPPH. This is probably due to the polar solvents capacity to solubilize flavonoid components (Edziri et al. 2011). However, by considering the polarity indices of the solvents, apart from the PI, hydrogen-bonding ability would be another factor affecting the antioxidant activities of the extracts especially in the case of seed extracts where n-heptane (a nonpolar solvent) results in higher antioxidant activity (Pedrielli et al. 2001). The hydrogen-bond recipient solvents affecting the flavonoids not only through the energy of the phenolic antioxidants but also via the energy of the intermediate radical. Therefore, a substantial decrease was observed in the free radical inhibition rate of flavonoids by changing the solvent from the non-hydrogen-bonding solvent to the hydrogen bond-recipient solvent. In this work, the irregular trends of the antioxidant activities in different extract solvents are attributed to both polarity and H-bonding ability of the

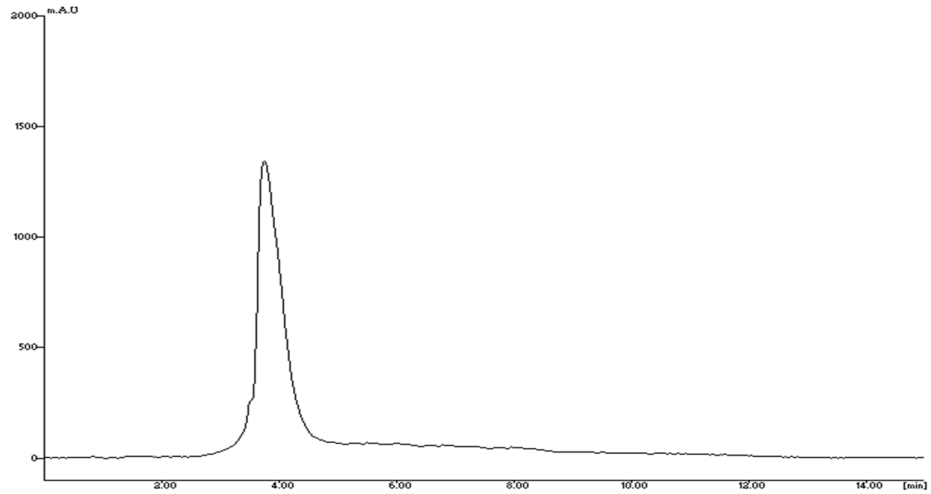

Fig1. Ethanol chromatogram of *P.farcta* pod

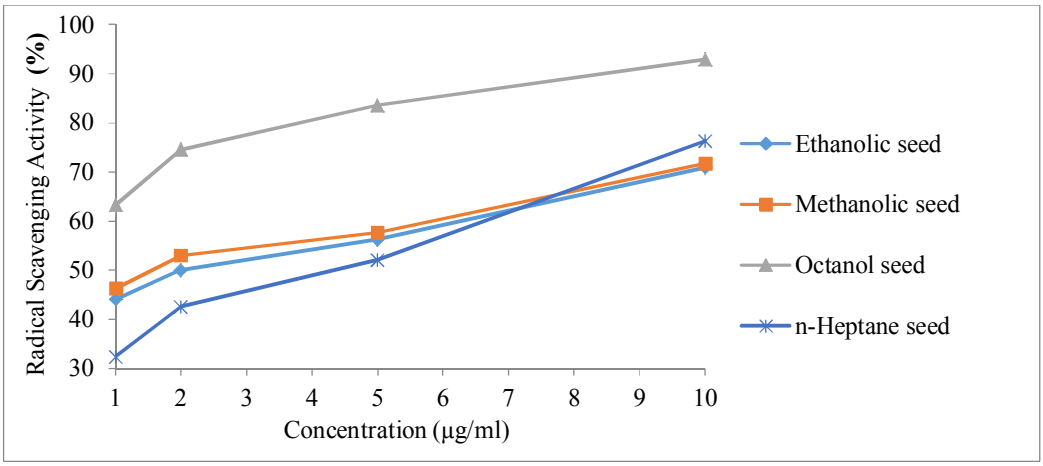

Fig. 2 DPPH scavenging assay of seed extracts in four solvents.

solvents.

Comparing the results obtained from seed and pod extracts showed that seed octanolic extract is the strongest antioxidant with IC_{50} value of 0.95 µg/ml

Antibacterial activity

The results showed that there is no antibacterial activity the seed extracts while certain concentrations of the pod extracts exhibited inhibitory properties suggesting the lack of antibacterial substances in seed extract. The data dealt with antibacterial activities of the methanol and ethanol pod extracts are compiled in Table 2 and Table 3, respectively. A perusal of data revealed that there is no antibacterial activity against *Staphylococcus* at 80 µl concentration of the extracts. In case of *E. coil*, 40 µl of the methanol extract is sufficient to inhibit bacterial growth ring by 15 mm.

The ethanolic extract of the pod shows better antibacterial activity on *Staphylococcus* culture compared to *E. coli* bacteria. This fact indicates that extraction solvents affect dissolving process of various bioactive compounds responsible for antibacterial activities. Generally, depends on several factors such as nature and content of secondary metabolite present in each part of plant and also genus and the family of the plant, type of solvent and extraction method, different results could be obtained

Total phenolic content

Presence of phenolic compounds possessing ability of hydrogen donation to the free radicals is extremely important for antioxidant properties of a plant extract. The obtained results for total phenolic compound (TPC) content is shown in Figure 4 and the results are more or less in agreement with radical scavenging activity of the extracts. The slight difference in antioxidant activity, especially in the case of n-heptan could be attributed to the participation of non-phenolic antioxidants (Ahumada-Santos et al. 2013). As it is shown in Figure 2 and 3, it is evident that octanolic extract of both the seed and pod show the highest radical scavenging profile at high concentration by means of DPPH assay. The *P. farcta* octanolic extract showed the higher TPC values for both seed and pods (Figure 4). It seems that octanol is the most polyphenol containing solvent which is suitable for

Table 1. DPPH radical-scavenging activity of *P. farcta* extracts

Specimen	Extract solvent	IC_{50} (µg/ml)
Pod	Ethanol	1.00
	Methanol	6.15
	Octanol	3.55
	n-heptane	7.75
Seed	Ethanol	2.00
	Methanol	1.51
	Octanol	0.95
	n-heptane	4.45

Table 2. Antibacterial activity of the methanol pod extract of *prosopis farcta*

Extract concentration	Zone of inhibition(mm)	
	Staphylococcus	E.coli
10***µl***	-	-
20 ***µl***	-	-
40 ***µl***	-	15
80 ***µl***	12	20

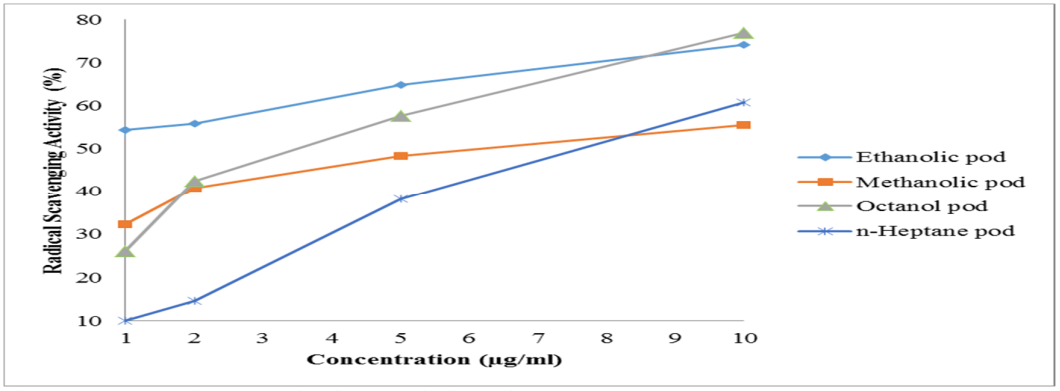

Fig.3 DPPH scavenging assay of pod extracts in four solvents.

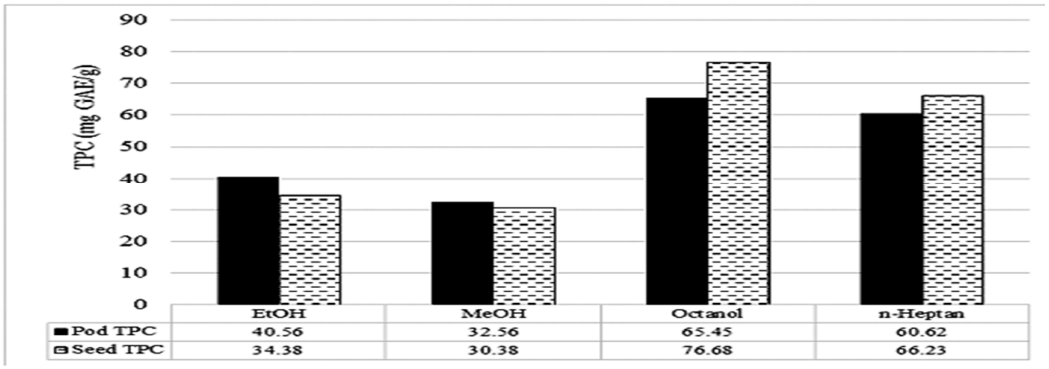

Fig.4 Total phenolic content of seed and pod extracts in four solvents.

Table 3. Antimicrobial activity of the ethanol pod extract of *prosopis farcta*

Extract concentration	Zone of inhibition(mm)	
	Staphylococcus	E.coli
10***µl***	-	-
20 ***µl***	-	-
40 ***µl***	14	-
80 ***µl***	14	10

our purpose.

TPC values for both seed and pod organs, as well. It seems that octanol is the most polyphenol selective and significant solvent for our purpose.

CONCLUSION

A comparative study of various extracts of *Prosopis farcta* collected from Sistan, Iran in four different solvents showed that the antioxidant activities of pod and seed extracts had a slightly distinct trend. The irregular trends of the antioxidant activities in various solvents for two plant organs were attributed to both polarity index and H-bonding ability of each solvent. The antibacterial properties of each extract were verified employing analysis of zone of inhibition for *Staphylococcus* and *E. coil* which showed that both the methanolic and ethanolic extracts of the fruit pod could extensively inhibit the bacterial growth. The results of the present work indicate the selective extraction of antibacterial and radical-scavenging factions from natural sources by appropriate solvents are crucial for obtaining bioactive fractions.

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