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Phytochemical Analysis and Antioxidant Activity of *Salvadora persica* extracts

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Abstract: Reactive oxygen species (ROS) are founded in many health diseases and often generated from biological reactions or external factors. Natural antioxidant based on the prevention or treatment of complicated diseases has attracted an important deal of research interest. Phytochemical screening, total phenolic and flavonoid contents and hydrogen peroxide radical scavenging assays for evaluation of *Salvadora persica* (SP) *in vitro* antioxidant analysis of aqueous, methanolic and ethanolic extracts of roots were determined. It was found that the aqueous water gave the highest total extract yield followed by ethanol and methanol. Qualitative phytochemical screening showed the presence of different phytochemicals in water, methanol and ethanol extracts. Aqueous extract dispalayed the largest total phenolic contents followed by ethanolic and methanolic extracts while methanolic extract showed the higher flavonoids content than the ethanolic and aqueous extracts. Hydrogen peroxide scavenging method revealed that the aqueous and ethanolic extracts have good scavenging ability compared to gallic acid which used as positive control. there were strong negative significant correlations between hydrogen scavenging and phenolic contents (-0.369), but not with the contents of flavonoids. Also, the results revealed that there was a strong negative correlation between flavonoids and total phenolics. The results clearly indicate that root aqueous extract of *Salvadora persica* is having effective antioxidant activity.

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1. INTRODUCTION

Reactive oxygen species (ROS) includes free radicals and non-free radicals cause DNA damage and initiate the lipid peroxidation of membrane (Hallliwell and Gutteridge, 1985; Ames et al., 1993). These damages may results in many diseases like cancer, hepatic disorders, cardiovascular diseases and decline of immunity (Malila et al., 2002).

Antioxidants are compounds that act as free radical scavengers, electron donors and can form innocuous end products such as water. So, antioxidants can protect cells from oxidative stress and damage (Canadanovic-Brunet et al., 2005). Many artificial antioxidant compounds have shown toxic and carcinogenic effects. Therefore focusing on naturally products antioxidants and identification of effective free radical scavengers from natural plants becomes new standard strategy for drug development.

Salvadora persica (SP) also known as miswak, distributed mainly in tropical and sub tropical Asia. SP has been used commonly as toothbrush to strengthen the gums (Sofrata et al., 2008). The fresh root barks and leaves have been used in folk medicine for the treatment of a wide range of medical problems and liver diseases (Kirtikar et al., 1975). Essential roots oil of SP contain benzyl isothiocyanate with other constituents such as α-pinene, camphene, benzaldehyde, β-pinene, myrcene, δ-3-carene, limonene, terpinolene, benzyl nitrile, umbellulone, β-elemene, γ-muurolene, myristicin, β-caryophyllene and longifolene (Bader and Flamini, 2002). The aqueous extract of SP leaves possesses analgesic activity and decreases carrageenan induced inflammation in rat paw (Parihar et al., 2007). Aqueous and alcoholic extract from leaves of SP ameliorate elevated urinary oxalate levels and deposition of stone-forming constituents in the kidneys of calculogenic rats (Geetha et al., 2010). In present study we aimed to explore the phytochemical constituents and the antioxidant potential of SP root extract using solvents of different polarity (ethanol, methanol, aqueous).

2. MATERIALS AND METHODS

2.1. Collection and Authentication of Plant.

Salvadora persica L. (SP) was purchased from local market in Jeddah, Kingdom of Saudi Arabia, and authenticated by Herbarium, King Abdulaziz University.

2.2. Preparation of Extracts.

The fresh Miswak root sticks were cut into small pieces and allowed to dry at room temperature for one week. Then they were ground in grinding machine to fine powder. 30 g of the dry powder was weighed and was used for extract preparation. Extracts for the plant roots were prepared using ethanol, methanol and distilled water. 150 ml of the respective solvents were added and extracted for 24 h at 150 rpm at 25 ^{0}C in a shaker. The mixture was then centrifuged at 3000 rpm for 20 min. The supernatants were subsequently filtered through Whatman No. 1 filter paper and the filtrate was concentrated in rotary evaporator (Buchi Rotavapor R-200) for 10 min at 100 °C (for aqueous extraction) and 60 °C (for alcoholic extraction) and was lyophilized. The resulting powder was packed in a glass bottle and stored at 4∘C until needed (Kandil et al., 1994). The extract was suitably diluted for use. The total yield of water, methanol and ethanol extracts were 20.54 %, 4.56 % and 6.16 % respectively.

2.3. Preliminary Phytochemical Screening

The presence or absence of the phytochemical constituents in all extracts were analyzed using standard procedures for carbohydrates, saponins, flavonoids, steroids, phenols, tannins and glycosides as prescribed by Goyal et al., 2010.

2.4. Quantitative Analysis of Antioxidant Compounds

2.4. 1. Estimation of total phenol contents (TPC)

The total phenol contents were determined by Folin- Ciocalteu reagent method (McDonald et al., 2001). 0.5 ml of extract (1:5 dilution) and 0.1 ml of Folin-Ciocalteu reagent (0.5N) were mixed and incubated at room temperature for 15 min. 2.5 ml saturated sodium carbonate was added, incubated for 30 min at room temperature and absorbance was measured at 760 nm. The total phenol content was expressed in terms of gallic acid equivalent (mg/g) (Chanda and Dave, 2009).

2.4.2. Estimation of total flavonoids contents (TFC)

The total flavonoid contents were determined by Aluminum chloride method (Chang et al., 2002). The reaction mixture (3.0 ml) that comprised of 1.0 ml of extract (1:10 dilution), 0.5 ml of aluminum chloride (1.2%) and 0.5 ml of potassium acetate (120 mM) was incubated at room temperature for 30 min and absorbance was measured at 415 nm. The total flavonoid contents were expressed in terms of ascorbic acid equivalent (mg/g) (Mervat et al., 2009).

2.5. Assessment of Antioxidant Activity

The antioxidant activities of Miswak root extract were measured in vitro using the hydrogen peroxide scavenging activity. The assays were carried out in triplicate and the average value was obtained. All determinations were made spectrophotometrically using UV–VIS spectrophotometer (Jenway, Japan).

2.5.1. Hydrogen Peroxide scavenging capacity

The ability of the SP extracts to scavenge hydrogen peroxide was determined according to Goyal et al., 2010. A solution of hydrogen peroxide (40 mM) is prepared in phosphate buffer (50 mM, pH 7.4) and 2 ml of the solution is added to 1 ml extract (1:20 dilution). The absorbance at 230 nm is determined after 10 mins. H_2O_2 radical scavenging activity was expressed in terms of gallic acid equivalent (mg/g). The percentage of H_2O_2 scavenging ability of the extract or positive control was calculated as follows:

The H_2O_2 scavenging ability of sample (%) = $[(A\,b - A\,s)/A\,b] \times 100$, where Ab is the absorbance of the blank and As is the absorbance of the sample solution.

3. RESULT AND DISCUSSION

3.1. Effect of Extraction Procedures of Salvador persica (SP)

In the present study, solvents with different polarities water, methanol and ethanol were used to extract antioxidants from SP root extract. Results for the total extract yields reported as percentage of g of extract per 100g *Salvadora persica* (SP) on dry basis indicated that the SP extracted with water gave the highest total extract yield (20.54±0.802), followed by ethanol (6.16 ± 0.49) and methanol (4.56±0.327) when the extractions were done with the ratio of solvent/sample of 10:1 (w/w) at 25 °C for 24 h (Table 1). It should be noted that, because of polarity differences between solvents, the solubility of the solute into the solvent is expected to be different.

Table 1: Effect of extraction solvents on the percentage yields of *Salvadora persica*.

Solvents	Extraction yield (gm)	Total extraction yield (w/w %)
Water extract	1.027	20.54 ± 0.8
Methanol extract	0.228	4.56 ± 0.32
Ethanol extract	0.308	6.16 ± 0.49

Each value represents the mean ± SD (n = 3).

3.2. Phytochemical Analysis.

The phytochemical analysis conducted on SP extracts revealed the presence carbohydrates, flavonoids, steroids, total phenols, tannins and glycosides whereas saponins were absent in methanol and ethanol extracts (Table 2).These phytochemical compounds are known to support

bioactive activities in medicinal plants (Hamid et al., 1997) and thus responsible for the antioxidant activities of this plant extract used in the study.

Table 2: Preliminary qualitative tests on various root extracts of *Salvadora persica*

Sr. no	Test for	Water Extract	Methanolic Extract	Ethanolic extract
1	**Carbohydrates**	+	+	+
2	**Saponins**	+	-	-
3	**Flavonoids**	+	+	+
4	**Steroids**	+	+	+
5	**Phenols and Tannins**	+	+	+
6	**Glycosides**	+	+	+

-Absent +Presence

3.3. Phenolic and Flavonoid Concentration.

In the last decade there are numerous publications proving the antioxidant activity of many plant extract, due to the presence of the phenolic compounds (Almas and Al-Zeid, 2002). However the results are incomparable since they were tested through various methods. Therefore the results of our research shows that values of total phenolic compounds in aqueous, methanolic and ethanolic extracts are 15.4 ± 0.76, 12.4 ± 0.61 and 13.6 ± 0.62 mg gallic acid equivalent /gm dry weight extract, respectively (Table 3). Aqueous extract exhibited the highest total phenolic contents followed by ethanolic and methanolic extracts, Fig 1.

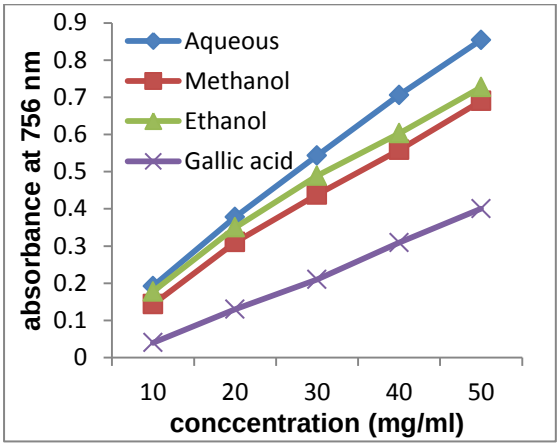

Fig 1: The effect of different solvents on the yield of total phenolics of *Salvadora persica*.

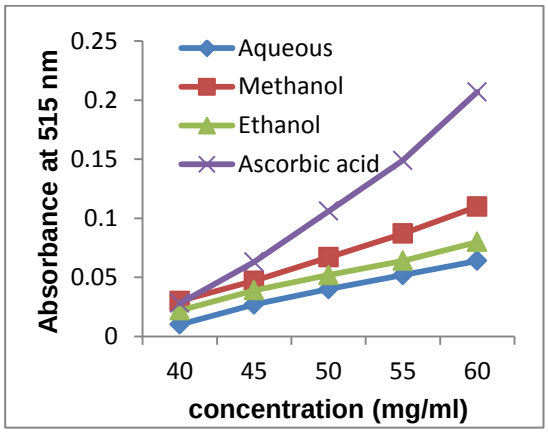

Fig 2: The effect of different solvents on the yield of flavonoids contents of *Salvadora persica* using ascorbic acid as standards.

Besides any other secondary metabolites, mostly plants show their antioxidant activities due to phenylpropanoid derivatives, such as polyphenols. The antioxidant activities of SP may also be due to its polyphenol content (Hajimahmoodi et al., 2008). Phenolic compounds are the prime antioxidant components of natural products and are composed of phenolic acids and flavonoids, which are potent radical terminators. They donate an electron to radicals and break the reaction of lipid oxidation at the initiation step (Yen and Chen, 1995; Singh et al., 2002).

The total flavonoid contents of the aqueous. methanolic and ethanolic extracts were 3.82 ± 0.12, 6.04 ±0.26 and 4.8 ± 0.21 mg ascorbic acid equivalent/g extract, respectively. On the other hand, the ratio of total flavonoids/total phenolics (0.24–0.48) in the present samples indicates high proportions of flavonoids (Table 3, Fig 2). The flavonoids might be the major ones responsible for this biological activity, because flavonoids, especially those having hydroxyl groups, which are potent hydrogen donators and consequently can neutralize free radical activity easily (Duh et al., 2001).

Table 3. Total phenolic, flavonoid contents and total flavonoids/ phenolics present in different extracts of *Salvadora persica*

Extracts	**Total phenolic contents (mg GAE/g extract)**	**Total flavonoid contents (mg AAE/g extract)**	**Total flavonoids/phenolics**
Aqueous extract	15.4 ± 0.76	3.82 ± 0.12	0.248
Methanolic extract	12.4 ± 0.61	6.04 ±0.26	0.48
Ethanolic extract	13.6 ±0.62	4.8 ± 0.21	0.35

Each value represents the mean ± SD (n = 3).

Table 4. Hydrogen peroxide scavenging activity.

Concentration (µg/ml)	Percentage Inhibition (%)			
	Aqueous Extract	Methanol Extract	Ethanol Extract	Gallic acid
10	17 ± 0.2	9.5 ± 0.3	9.5 ± 0.45	42.4± 0.11
20	38 ±0.12	16.1 ± 0.23	11.2 ± 0.43	45.8± 0.22
30	40 ± 0.23	24.4 ± 0.33	20.01± 0.33	46.1 ± 0.33
40	41 ± 0.33	33.9 ± 0.34	28.1 ± 0.54	52.2± 0.65
50	43.9 ± 0.15	43.9 ± 0.32	45.3 ± 0.34	59.01 ± 0.55

Data are presented as mean ± SD.

3.4. Hydrogen Peroxide Scavenging.

Figure 3 shows that the aqueous, methanolic and ethanolic extracts are good scavenger of H_2O_2 (% inhibition at 50 µg/ml) = 43.9 ± 0.15, 43.9 ± 0.32 and 45.3 ± 0.34 µg/mL, respectively) compared with standard gallic acid (59.01 ± 0.55 µg/mL). The percentage inhibition value of the extracts was lesser than that of the standard. Hydrogen peroxide occurs naturally at low concentration levels in the air, water, human body, plants, microorganisms, food and beverages. The H_2O_2 scavenging activity in SP roots was estimated to be higher in the ethanolic extract (Table 4). This can be correlated to the presence of total phenols in the extract (Elmastas et al., 2006).

The antioxidative properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, like iron and copper, and inhibition of enzymes responsible for free-radical generation. Depending on their structure, flavonoids are able to scavenge practically all known ROS (Chandha, Dave, 2009). In the current investigation, all extracts from SP roots gave good results indicating that it possesses significant amount of phytochemicals and *in vitro* antioxidant activity. Highest content of phenolic and flavonoids in aqueous extract in comparison to other solvents used, make this extract used an ideal and selective when the toxicity and cost aspects are also considered in animal studies. Also, the ethanolic extract of SP showed 45.3 % inhibition in the superoxide scavenging model at the concentration of 50 µg/ml. Activity decreased for aqueous and methanolic extract; 43.9 % compared to the ethanolic extract at the same concentration. The correlation coefficient (R2) between methanol and ethanol extracts and H_2O_2 scavenging activity was found to be 0.99 and 0.91, respectively, indicating the strong correlation (Fig 4).

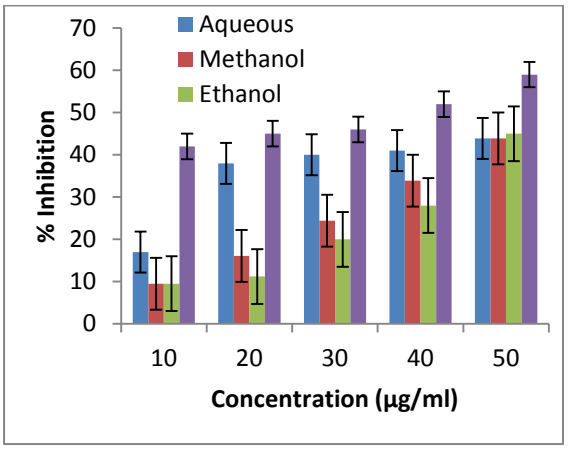

Fig3. Hydrogen peroxide (H_2O_2) scavenging activity of *Salvadora persica* extracts and gallic acid. Data are presented as the percentage of H_2O_2 radical scavenging.

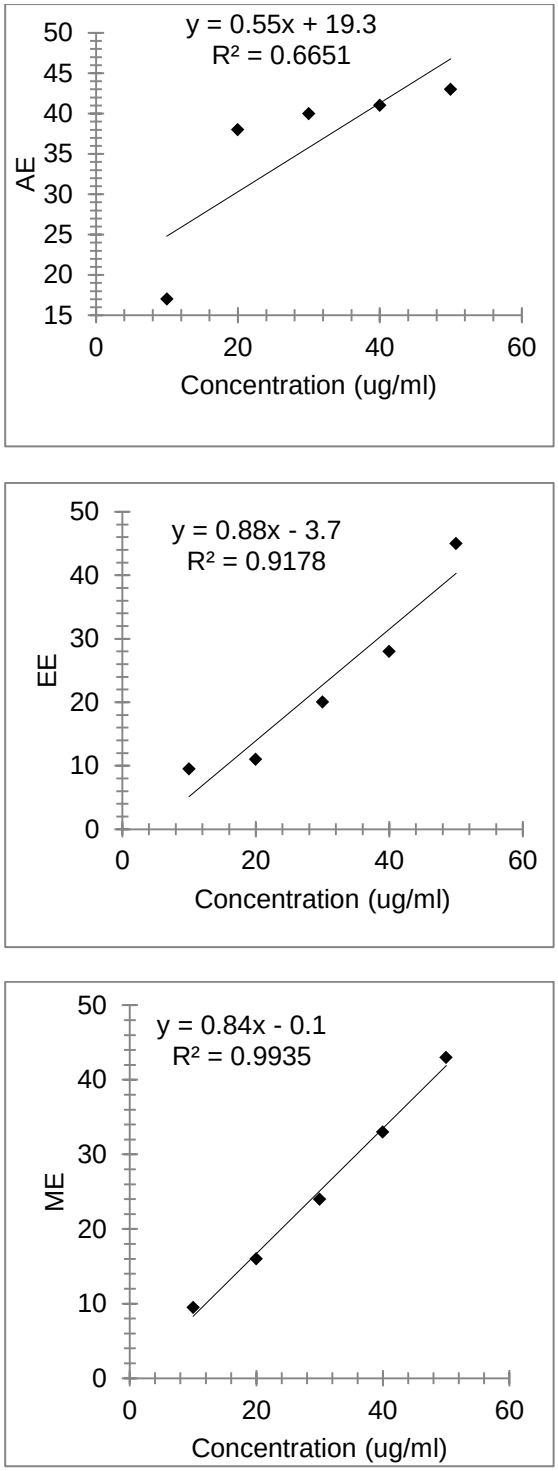

Fig 4. Correlation between different concentrations of SP extracts and their antioxidant capacity as determined by the Hydrogen peroxide scavenging method.

Phenolic compounds and other phytochemicals appear to be responsible for the *in vitro* antioxidant activity of the extracts and may contribute to the therapeutic activity observed. On the basis of the results obtained, SP root extracts are rich sources of natural antioxidants and could be developed into functional food or drug against diseases and for a variety of beneficial chemo-preventive effects. Our previous reports have also revealed

antidiabetic potential of SP (Ramadan and Alshamarani, 2015). Further studies are in progress in our laboratory to evaluate the *in vivo* antioxidant potential of SP roots in various animal models. Phytochemical studies required to establish the types of compounds responsible for the bioactivity are also currently being pursued.

3.5. Correlation studies

The Pearson's correlation coefficients between the variables are presented in Table 5. As shown in the table, there were strong negative significant correlations between hydrogen scavenging and phenolic contents (-0.369), but not with the contents of flavonoids. Also, the results revealed that there was a strong negative correlation between flavonoids and total phenolics ($r = -0.99$, $p < 0.01$).

Table 5. Correlation coefficient between parameters (*P< 0.01).

	TP	TF	H_2O_2
TP	-	-0.99*	-0.369*
TF	-0.99*	-	NS

4. CONCLUSION

The study therefore concluded that the aqueous extract of *Salvadora persica* (SP) has potential antioxidant activity when compared to other extracts. Further studies are required to better understand these compounds and their effects on cellular function. Antioxidant properties of Salvadora persica could be beneficial in pathological condition involving oxidative stress.

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