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ORIGINAL ARTICLE

Assessment of antioxidant activities in roots of Miswak (*Salvadora persica*) plants grown at two different locations in Saudi Arabia

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Abstract Traditionally, in Middle Eastern countries, many cultures use chewing sticks of arak for medicinal purposes especially, for oral cleanliness care. It was used by Muslims for treatment of teeth and highly recommended to be used by Muslims during the whole day. Therefore, the present work aimed to determine the total phenolic content and total flavonoids in two Miswak extracts obtained from arak roots collected from two different localities in Saudi Arabia. They were extracted with aqueous ethanol (80%) and used to estimate in vitro their antioxidative abilities. The new findings showed that the two tested extracts contained significantly different amounts of both total phenolic content and total flavonoids. According to the increase of total phenolic contents and total flavonoids obtained from the two extracts, Miswak collected from the southern region was found to contain more contents than those collected from the middle region. The results of antioxidant activities of Miswak root extract obtained by using different in vitro methods were varied depending on the technique used. According to the malondialdehyde (MDA) method, hydrogen peroxide (H_2O_2) scavenging ability and 1,1-diphenyl-2-picrylhydrazyl (DPPH) methods, the two Miswak extracts exhibited to have high to very high antioxidant activities. Mostly, the values of antioxidant activities of Southern region have been shown to be always the highest.

1. Introduction

The World Health Organization has recommended and encouraged the use of chewing sticks as an effective tool for oral hygiene in areas where such use is customary (WHO, 1987). *Salvadora persica* is known to contain several biologically active chemical constituents such as volatile oils, flavonoids, alkaloids, steroids, terpenoids, saponins, and

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carbohydrates (Abdillahi and Finnie, 2010; Garboui et al., 2009; Kamil et al., 1999). Roots of *S. persica* were found to contain salvadourea, a urea derivative (Al-Quran, 2008). *S. persica* and other related plants are reported to be effective against bacteria that are imperative for the development of dental plaque.

Free radicals are highly reactive particles also highly reactive by products with an unpaired electron, produced when cells are exposed to stress(s). They initiate chain reactions, which lead to disintegration of cell membranes and cell compounds, including lipids, proteins, and nucleic acids (Leong and Shui, 2002). Free radical oxidative stress has been implicated in the pathogenesis of a wide variety of clinical disorders, such as cancer, cardiovascular disease, Alzheimer's disease, autoimmune disease, diabetes, multiple sclerosis and arthritis (Halliwell and Gutteridge, 1999). Biological systems protect themselves against the damaging effects of activated species by several means (e.g. Such as production of antioxidants). Antioxidants are substances that markedly delay or prevent the oxidation of oxidizable substrate when present in foods or body at low concentrations. There are two types of antioxidants, (1) enzymatic antioxidants (e.g., superoxide dismutase, ascorbic peroxidase, polyphenoloxidase and catalase) and (2) non-enzymatic antioxidants (e.g., ascorbic acid (vitamin C), α-tocopherol (vitamin E), glutathione, carotenoids, and flavonoids) (Krishnaiah et al., 2011). Antioxidants may help the body to protect itself against various types of oxidative damage caused by reactive oxygen species, which are linked to a variety of diseases including cardiovascular diseases, cancers (Gerber et al., 2002), neurodegenerative diseases and Alzheimer's disease (Di Matteo and Esposito, 2003). The natural plant antioxidants can therefore serve as a type of preventive medicine. Some researchers suggest that two-thirds of the world's plant species have medicinal value; in particular, many medicinal plants have great antioxidant potential (Krishnaiah et al., 2011). If human disease is believed to be due to the imbalance between oxidative stress and antioxidative defense, it is possible to limit oxidative tissue damage and hence prevent disease progression by antioxidant defense supplements (Bhattacharya et al., 1999). In other words, if the balance sways in the direction of pro-oxidants, oxidative stress can arise, which under normal circumstances is controlled by a broad range of antioxidant enzymes, proteins and antioxidants provided by the diet.

The protection offered by fruits and vegetables against oxidative stress in several diseases has been attributed to various antioxidants and vitamins. Dietary phenolic compounds and flavonoids have generally been considered, as non-nutrients and their possible beneficial effect on human health has only recently been recognized. Flavonoids are known to possess anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, neuroprotective, and anticarcinogenic activities (Araceli et al., 2003). Therefore, the search for natural antioxidants of plant origin has gained momentum in recent years. The phenolic compounds may contribute directly to the antioxidant action due to the presence of hydroxyl functional groups around the nuclear structure that are potent hydrogen donators. These phenolic compounds of plant origin show their antioxidant effect by various mechanisms including their ability to scavenge free radicals, chelate metal ions that serve as the catalysts for production of free radicals or activate various antioxidant enzymes and inhibit oxidases (Kulkarni et al., 2004).

The goal of the current research is to determine the total phenolic and flavonoid contents in two arak tree roots collected from two localities in Saudi Arabia. Furthermore, to estimate the antioxidative properties of arak root extract in vitro by applying different methods, subsequently these stick roots could be natural and cheap source of antioxidant compounds.

2. Materials and methods

2.1. Plant materials

Roots of Miswak (*S. persica* L.) are used as publicly available herbarium. Miswak roots were collected from two sites, the first one was collected from Asir Mountains and the other from the middle region near Riyadh, Kingdom of Saudi Arabia. The roots of *S. persica* were cut into small pieces, dried in shade according to the standard procedure and crushed. The crushed *S. persica* root powder was extracted in ethanol solvents for 24 h. The obtained extracts were transferred to sterile labeled bottles and kept at −20 °C till used (Kandil et al., 1994).

2.2. Chemicals reagents

Thiobarbituric acid, D-catechin, quercetin, DPPH and 1,1,3,3-tetraethoxypropane were obtained from Sigma. All other reagents used in this experiment were obtained from BDH.

2.3. Extraction

All roots were washed with tap water, and the seeds were removed. A portion of each sample was weighed (300–400 g) and 1500 ml of the extracting solvent (80% ethanol) was added. The extraction was carried out at 80 °C for about 30 min, and the extract was filtered through cotton wool. The residue was extracted again by 1000 ml of the same extracting solvent for about 5 min on a boiling water bath and left overnight in the fridge and filtered through a cotton wool plug in the neck of filter funnel. The two extracts were combined and evaporated by using a rotary evaporator apparatus under vacuum at 40 °C until no more water can be distilled. The obtained heavy extracts were weighed and stored at −80 °C to be used for further studies.

2.4. Determination of total phenolic compounds and total flavonoids

Measurement of total phenolic compounds in Miswak root extracts expressed as D-catechin equivalent (mg CE/100 g arak root extract). Five grams of each extract was initially dissolved in some amount of distilled water and the volume was then adjusted to 25 ml. The total phenolic contents were measured according to the method described by Singleton and Rossi (1965). Briefly, 0.1 ml of the solution was added to 0.5 ml Folin–Ciocalteu reagent, mixed for 1 min and 1 ml of sodium carbonate solution (0.08 g/ml) was added. The volume was then adjusted to 2 ml with distilled water and mixed. The

mixture was left for 1 h at room temperature in a dark place and the absorbance was measured at 760 nm using UV/VIS spectrophotometer (Shimadzu, UV-1601). Measurements were made in triplicates. The calibration curve of D-catechin was prepared by using concentration from 50 to 400 lg/100 ml. The concentration of each sample was calculated from the D-catechin standard curve.

2.5. Determination of total flavonoids in Miswak root extract

Expressed as quercetin equivalent (mg/100 g Miswak root extract) Aluminum chloride colorimetric method was used for flavonoid determination (Chang et al., 2002). Therefore, 0.1 ml of each extract (10 mg/ml) in methanol was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% $AlCl_3$, 0.1 ml of 1 M CH_3COOK and 2.8 ml of distilled water and kept at room temperature for 30 min. The Abs. of the reaction mixture was measured at 415 nm. A calibration curve of quercetin was prepared by using concentration from 12.5 to 100 µg/ml in methanol, and the total flavonoids were expressed as quercetin equivalent (mg QE/100 g arak root extract).

2.6. Determination of root oxidizability

Root oxidizability was determined as described by Singh et al. (2007) and measured as the amount of red triphenyl formazan and the absorbance was read at 485 nm.

2.7. Antioxidant enzyme assay

The antioxidant activities of Miswak root extract were measured in vitro using seven complementary different methods, namely: the DPPH free radical scavenging assay, the total reducing power method, the TBARS method, the nitric oxide (NO) scavenging activity, the chelation of Fe^{2+} ions, the hydrogen peroxide scavenging activity, and the hydroxyl radical scavenging activity. All assays were carried out in triplicate and the average value was obtained. All determinations were made spectrophotometrically using UV–VIS spectrophotometer (Jenway, Japan).

2.8. Total antioxidant activity of Miswak root extracts using TBARS method

The thiobarbituric acid reactive species method was used as described by Duh et al. (2001), which measures the total antioxidant activity with slight modifications. Briefly, this method was carried out using homogenate (10%) in phosphate buffer (pH 7.4) as lipid rich media. A stock solution of each extract in methanol (1 mg/ml) was prepared and different levels (100, 200, 500, and 1000 µl) from each stock solution were transferred into different test tubes and volumes were adjusted to 1 ml with the same solvent. To each test tube 3.0 ml of 10% liver homogenate was added and incubated for 30 min. Lipid peroxidation was initiated by adding 4.0 ml of ferric chloride (400 mM) and 40ll l-ascorbic acid (200 mM) and incubated for 1 h at 37 °C. After incubation, 3 ml of 0.25 N HCl containing 15% trichloroacetic acid and 0.375% thiobarbituric acid was added. The reaction mixture was boiled for 30 min, then cooled, and centrifuged at 2000*g* for 5 min. A blank was prepared with the same reagents without sample extract, and using Vit. C as a positive control (100 µg/1 ml). The absorbance was measured at 532 nm and the decrease of absorbance indicates an increase of antioxidant activity. The values of antioxidant activity were expressed as the percentage inhibition of lipid peroxidation in liver homogenate as follows: The total antioxidant activity (% Inhibition of lipid peroxidation) = $[(A_b - A_s)/A_b] \times 100$; where A_b is the absorbance of blank and A_s is the absorbance of sample or positive control.

2.9. The total reducing power ability (TRPA) of Miswak roots extract

The total reducing power of samples was determined according to the method described by Oyaizu (1986). A stock solution of each extract in methanol (1 mg/ml) was prepared and different levels (100, 200, 500, and 1000 µl) from each stock solution were transferred to different test tubes and the volume in each test tube was adjusted to 1 ml with the same solvent. Then, 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6), and 2.5 ml of 1% potassium ferricyanide were added to each test tube and incubated at 50 °C for 20 min. After incubation, 2.5 ml of 10% trichloroacetic acid was added and centrifuged at 2000*g* for 10 min. The upper layer in each tube (2.5 ml) was mixed with 2.5 ml of deionized water and 0.5 ml of 0.1% ferric chloride. The absorbance was measured at 700 nm against a blank. The reducing power increases with the increase of absorbance. The total reducing power ability of each arak root extract at different concentrations was compared to Vit. C as a positive control and the results were expressed as Vit. C equivalent (lM).

2.10. Chelation of Fe^{2+} ions

Concentration of free iron ions (Fe^{2+}) was estimated using chelating agent 2,2′-bipyridyl as described by Harris and Livingstone (1964). Briefly, a stock solution of each extract containing 1 mg/ml in methanol was prepared and different levels (100, 200, 500, and 1000 µl) from each stock solution were transferred to different test tubes and the volume in each test tube was adjusted to 1 ml with the same solvent. To each tube 1 ml of a solution containing 50 µM $FeSO_4$, 50 µM NaCl (pH 7.0) was added. A blank solution was prepared using 1 ml of methanol instead of sample. Samples were incubated for 30 min, at the end of which 2 ml of 2,2′-bipyridyl (1 mM) was added. Absorbance of ferrous–bipyridyl complex was measured at 525 nm against a solution devoid of ferrous sulfate. The results were expressed as percentage of inhibition of 2,2′-bipyridyl–Fe^{2+} complex formation and calculated as follows:

$$\text{The inhibition of } 2,2'\text{-bipyridyl–}Fe^{2+} \text{ complex formation } (\%) = 1 - [(A_b - A_s)/A_b] \times 100,$$

where A_b is the absorbance of the 2,2′-bipyridyl–Fe^{2+} complex in the absence of extract sample (or a blank) and A_s is the absorbance in the presence of the extract sample.

2.11. Determination of H_2O_2

Hydrogen peroxide scavenging ability of Miswak root extract was obtained by using the method described by Jayaprakasha et al. (2004). A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). A stock of aqueous solution of palm date extract containing 1 mg/ml was prepared. Different amounts (100, 200, 500, and 1000 μl) of the stock solution were transferred into different test tubes. 3 ml of phosphate buffer solution (PBS) was added to each test tube and the volume adjusted to 4 ml with PBS. One milliliter of H_2O_2 solution (40 mM) was added and the reaction mixtures were incubated for 10 min and the absorbance was recorded at 230 nm. A blank solution was prepared in the same way without a sample. 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 A_b is the absorbance of the blank and A_s is the absorbance of the sample solution.

2.12. Hydroxyl radical scavenging assay

The hydroxyl radicals (·OH) in aqueous media were generated through the Fenton system (Strlic et al., 2002). The ·OH scavenging activity of palm date extract was determined according to the method described by Li et al. (2007). An extract stock solution was prepared with DMF (1 mg/ml). Different amounts (100, 200, 500, and 1000 μl) of the stock solution were transferred into different test tubes. To each test tube 1 ml of safranin solution (1.14 mM) in PBS (67 mM, pH 7.4), 0.5 ml of EDTA solution (0.04 M) in PBS, 0.5 ml of Fe^{2+} solution (0.04 M) in PBS, and 2 ml of H_2O_2 solution (3%) were added and adjusted the volume to 5 ml with PBS. The assay mixtures were incubated at 37 °C for 30 min in a water-bath. After which, the absorbance was measured at 520 nm. The suppression ratio for ·OH radical was calculated from the following expression:

Hydroxyl radical scavenging assay (%)

$$= [(A_i - A_0)/(A_c - A_0)] \times 100,$$

where A_i is the absorbance in the presence of the tested compound, A_0 is the absorbance in the absence of the tested compound, and A_c is the absorbance in the absence of the tested compound, EDTA-Fe(II) and H_2O_2.

2.13. Nitric oxide (NO) scavenging activity

Nitric oxide was generated from spontaneous decomposition of sodium nitroprusside (20 mM) in phosphate buffer (pH 7.4). Once NO is generated, it interacts with oxygen to produce nitrite ions, which were measured by the Griess reaction. The nitric oxide scavenging activity of extract was determined as described by Shirwaikar et al. (2006) with a slight modification. Briefly, a stock solution of each extract, was prepared to contain 1 mg/ml. Different amounts (100, 200, 500, and 1000 μl) of the stock solution were transferred to different test tubes and the volume was adjusted to 1 ml by the same solvent. 0.2 ml of sodium nitroprusside (20 mM) in phosphate buffer solution (pH 7.4), and 1.8 ml of PBS solution was added and incubated at 37 °C for 3 h. 1 ml of each solution was taken and diluted with 1 ml of Griess reagent [1% sulfanilamide, 2% H_3PO_4 and 0.1% N-(1-anphthyl) ethylenediamine]. Similarly, a blank was prepared containing equivalent amount of reagents (only sodium nitroprusside and vehicle), but without the extract. The absorbances of these solutions were measured at 540 nm against the corresponding blank solutions. Ascorbic acid was used as a positive control (100 μg/1 ml). The percentage inhibition of nitric oxide was calculated as follows:

The NO scavenging activity (%) of Miswak root extract

$$= 1 - [(A_b - A_s)/A_b] \times 100,$$

where A_b is the absorbance of the blank and A_s is the absorbance in the presence of sample extract or positive control.

2.14. DPPH radical scavenging activity

Herein, the antioxidant activities of extract were measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH (Brand-Williams et al., 1995). A methanolic stock solution of each sample was prepared to contain 1 mg/ml. Different amounts (100, 200, 500, and 1000 μl) of the stock solution were transferred to different test tubes and the volume was adjusted to 1 ml by the same solvent. Two milliliters of DPPH (0.06 M in methanol) was added to each test. A positive control (Vit. C, 100 μg/1 ml) was prepared in the same way as samples. Finally, a solution containing only 1 ml methanol and 2 ml of DPPH solution was prepared and used as a blank. All test tubes were incubated in a dark place at room temperature for 1 h. The spectrophotometer was set at 517 nm and the absorbance was adjusted at zero for methanol. The absorbance of blank, positive control, and samples were recorded. The disappearance of DPPH was recorded and the percent inhibition of the DPPH radical by samples and positive control, was calculated as follows:

% Inhibition (or % radical scavenging activity)

$$= [(A_b - A_s)/A_b] \times 100,$$

where A_b is the absorbance of blank (has the highest value) and A_s is the absorbance of sample or positive control (Vit. C).

3. Results

The total phenolic content for *S. persica* collected from the southern region was 794.6 mg as D-catechin equiv./100 g Miswak, and the total flavonoids for the same Miswak samples was 503.8 as quercetin equiv./100 g Miswak. However, *S. persica* were collected from the middle region, their contents of total phenolic compounds and total flavonoids were 569.7 and 387 mg D-catechin equiv./100 g Miswak. However, the present study dealt with two samples of Miswak roots, one of them was obtained from Asir Mountains, while the other collected from the middle region, near to Riyadh. The present results (Table 1) showed that the extract of Miswak collected from the southern region had the highest amounts of both total phenolics and total flavonoids among the two Miswak extracts. On the other hand, the ratio of total flavonoids/total phenolics (0.63–0.68) in the present samples indicates high proportions of flavonoids (Table 1).

Table 1 Total phenolics, total flavonoids, total flavonoids/total phenolics and root oxidizability in Miswak *Salvadora persica* roots collected from two different localities in Saudi Arabia. Values are the mean of 3 replicates ± SD.

Sample	Total phenolic content (mg/100 g)	Total flavonoid content (mg/100 g)	Total flavonoids/ phenolics	Root oxidizability (g^{-1} h^{-1})
Southern region	794.6 ± 6.54	503.8 ± 6.44	0.63	0.06 ± 0.002
Middle region	569.7 ± 3.67	387 ± 3.18	0.68	0.08 ± 0.003

Table 2 Antioxidant activities of two different Miswak root extracts using different methods.

Sample (μg)	TBARS (% inhibition)	Fe^{2+}-Chel. (% inhibition)	Sc. A. of H_2O_2 (% inhibition)	Sc. A. of (·OH) (% inhibition)	Sc. A. of (NO) (% inhibition)	Sc. A. of (DPPH) (% inhibition)
Southern region						
100	59.23 ± 0.96	9.45 ± 0.63	33.21 ± 1.15	16.28 ± 0.32	8.33 ± 0.46	69.45 ± 1.46
200	78.32 ± 1.12	18.78 ± 0.89	42.52 ± 0.82	29.46 ± 1.21	19.33 ± 0.79	83.76 ± 0.34
500	93.29 ± 1.33	43.24 ± 2.24	73.62 ± 1.33	44.49 ± 2.03	33.43 ± 1.58	89.22 ± 1.15
1000	96.21 ± 1.54	58.29 ± 3.45	92.56 ± 2.54	69.42 ± 3.25	41.35 ± 2.46	94.61 ± 0.52
Middle region						
100	48.22 ± 1.01	8.10 ± 0.45	21.71 ± 1.04	12.32 ± 0.11	14.78 ± 0.66	60.22 ± 1.26
200	68.88 ± 1.13	14.21 ± 0.77	29.25 ± 1.17	20.97 ± 0.18	27.31 ± 1.43	77.53 ± 1.47
500	88.26 ± 1.22	31.28 ± 1.93	49.82 ± 1.93	33.65 ± 0.21	34.56 ± 1.67	83.55 ± 0.93
1000	93.83 ± 1.37	47.76 ± 2.65	84.63 ± 3.21	53.54 ± 1.23	97.33 ± 0.64	90.91 ± 1.75
Vit. C (100 μg)	93.65 ± 3.34	42.38 ± 3.87	83.00 ± 4.54	71.08 ± 2.23	14.78 ± 0.66	95.02 ± 3.13

The observed values of root oxidizability further recommended the efficient antioxidant activity in Miswak roots. The values indicate more efficient antioxidant activity in samples collected from the southern region than those collected from the middle region.

Due to the chemical complexity of Miswak roots, different methods are required to assess their antioxidant activity. In the present study, therefore, eight complementary methods were followed to evaluate the ability to scavenge free radicals and the capacity to inhibit lipid peroxidation. In addition, the correlation of total phenolic contents and total flavonoids with antioxidant activity of Miswak extract has been studied. The values of total antioxidant activity of different types of Miswak extract on lipid peroxidation were expressed as percentage inhibition of TBARS formation (Table 2). The data showed that both the Miswak extracts had antioxidant activity as they inhibited lipid peroxidation in a dose-dependent manner. Thus, the results of TBARS obtained from the lowest and the second levels of extract have shown that the Southern Miswak extract has significantly higher activity in preventing lipid peroxidation in comparison with other extract. However, at higher levels both the extracts and the positive control showed almost the same ability to prevent lipid peroxidation.

In the present study, the percentage inhibition of 2,2′-bipyridyl–Fe^{2+} complex formation was obtained in the presence and absence of Miswak extract (Table 2). These findings showed that the Miswak extract has a low to intermediate iron binding capacity at the tested levels, which means that the extract can act as peroxidation protectors. However, the southern extract still has significantly higher ability to chelate Fe^{2+} than others as observed from the results of the lowest and second levels. The effect of the extract on the inhibition of Fe^{2+} and 2,2′-bipyridyl complex formation is shown to be in a dose-dependent mode (Table 2). However, at the highest concentration, the southern Miswak extract showed significantly higher ferrous chelating efficacy than Vit. C. The present results showed that Miswak extract has low to very high H_2O_2 scavenging ability and observed to be in a concentration-dependent manner (Table 2). The present results showed that the southern Miswak extract at the highest level (1000 μg) indicating the greatest activity to remove H_2O_2 from the reaction media in comparison with other extract and the positive control, while the scavenging abilities of the middle Miswak extract at the same level were not significantly different from that of the positive control. However, none of the concentrations of Miswak extract assayed could completely remove H_2O_2 from the assay medium.

Regarding the hydroxyl radical scavenging activities of Miswak extract, they were noticed to be low to intermediate and increased in a dose-dependent manner (Table 2). In addition, these results showed that the ·OH scavenging ability of Miswak extract was almost similar to their chelating the metal ions (Fe^{2+}), which are involved in hydroxyl radical generation. This study, therefore, confirmed that Miswak extracts were active scavengers of hydroxyl radicals, which cause damage to DNA. This study showed that, both the extracts had significantly lower ·OH radical scavenging activity than the positive control, but at the highest concentrations the southern Miswak extract showed a significantly higher ·OH radical scavenging activity than the middle Miswak extract. According to the present results, the extract showed a low ability to scavenge NO at their tested concentrations (Table 2), and may be of considerable interest in preventing the negative effects of excessive NO generation in the human body. The new findings showed that both extracts had a significantly lower ability than Vit. C to scavenge nitric oxide from the reaction media. However, at higher levels the southern Miswak extract had significantly greater ability than the middle Miswak extract, which almost has similar NO scavenging activity.

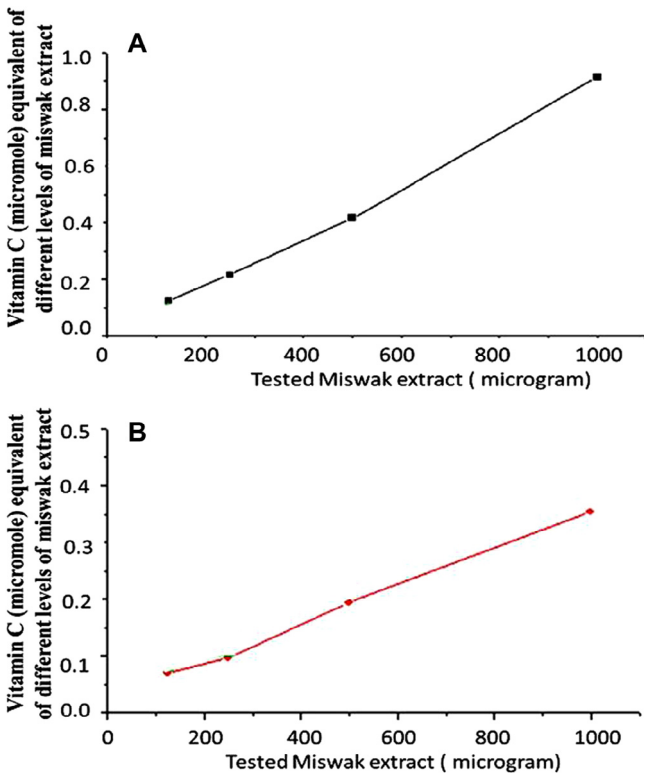

Figure 1 Total reducing power ability (TRPA) of two Miswak extracts (A) roots collected from the southern region, (B) roots collected from the middle region; expressed as μm Vit. C.

The present work has shown that the both Miswak extracts exhibited a marked DPPH scavenging activity (Table 2). As a result, the new findings (Table 2) showed that the increase of extract concentration caused a significant decrease in the concentration of DPPH due to the free radical scavenging effect of Miswak extract. Since the hydrogen donating of the tested extract was comparable to Vit. C, it was evident that the extract could serve as a hydrogen donor consequently terminating the radical chain reaction. The reducing ability of Miswak extract was measured on the basis of the oxidizability of their chemical constituents, such as phenolic and polyphenolic compounds, which could reduce Fe^{3+} to Fe^{2+} ions.

The total reducing ability of each Miswak extract at different concentrations was compared to Vit. C (a positive control), and the results were expressed as Vit. C equivalent (lM) (Fig. 1). These data showed that the reducing power ability of the two Miswak extracts appeared in a dose dependent tendency where the southern Miswak extract has shown to have the highest values.

4. Discussion

Ethno-pharmaceutical studies and alternative medicine have become increasingly valuable in the recent years and medicinal plants are now part of the health care system. The medicinal plant conservation programs and their sustained supply are part of global health strategy (Sher et al., 2010a). The present findings were in agreement with another study carried out by other researchers Sher et al. (2010a,b) and Abd EL Rahman et al. (2003) who carried out the ethnomedicinal and ecological evaluation of *S. persica*. Miswak roots have been used for medicinal purposes in Middle Eastern countries. They are used in folk medicine for teeth treatment. It is known that Miswak roots can be collected and marketed for consumption at different regions of Saudi Arabia mainly at the southern and western regions.

The significant differences in total phenolics and total flavonoids in Miswak extract could be ascribed to many reasons, such as the development of roots and the environmental conditions. The significantly higher contents of total phenolics and total flavonoids in the southern type than in others reflected greater ability to prevent lipid peroxidation. However, at higher levels (500 and 1000 μg) both the extracts showed almost complete inhibition of lipid peroxidation and they were not significantly different from the result of the positive control. These results are in agreement with many studies carried out by other researchers who attributed the antioxidant activities to the presence of phenolic and polyphenolic compounds in many vegetables (Gazzani et al., 1998), fruits (Meyer et al., 1998), and medicinal plants (Vinson et al., 1995). The oxidation of lipid peroxides leads to the formation of alkoxy and proxy radicals, which in turn produce numerous carbonyl products, such as malondialdehyde (MDA). The carbonyl products are responsible for DNA damage, generation of cancer and aging related diseases (Shinmoto et al., 1992). Thus, the decrease in the MDA level with the increase in the concentration of the Miswak extract indicates the role of the extract as antioxidants. Thus, former studies showed that Miswak contained flavonoids, such as luteolin, quercetin, and apigenin (Sher et al., 2010b), and p-coumaric, ferulic, and sinapic acid, and cinnamic acid derivatives (Abd EL Rahman et al., 2003). These compounds have been shown to be strong antioxidants. In addition, this study has shown that the ratio of the total flavonoids to the total phenolics was in the range of 63–68%, which means that the flavonoids might be the major ones responsible for this biological activity, because flavonoids, especially those having hydroxyl groups, which are potent hydrogen donators (‘H) and consequently can neutralize free radical activity easily. The effect of the extract on the inhibition of Fe^{2+} and 2,2′-bipyridyl complex formation is shown to be in a dose-dependent manner. However, at the highest Miswak extract concentration, a higher ferrous chelating efficacy has been significantly obtained and is shown to be more than Vitamin C. In fact, iron is essential for life, because it is required for oxygen transport, respiration, and the activity of many enzymes (Duh et al., 2001). However, iron is well-known as an initiator of unwanted oxidative reactions in lipids, proteins and other cellular components. In other words, iron is capable of generating free radicals from peroxides by Fenton reaction, so the production of these radicals can lead to lipid peroxidation, protein modification and DNA damage (Leong and Shui, 2002). Therefore, lowering of the Fe^{2+} concentration in the Fenton reaction affords protection against oxidative damage (Rival et al., 2001). Thus, chelating agents inactivate metal ions (Fe^{2+}) and potentially inhibit the metal-dependent processes (Finefrock et al., 2003). The natural phenolic and polyphenolic compounds in Miswak extract could be responsible for the interference with the formation of the 2,2′-bipyridyl–Fe^{2+} complex, suggesting that chelating activity and capture of ferrous ions by phenolic and polyphenolic compounds dose occur before chelating and capture by 2,2′-bipyridyl. Transition metal ions have a great importance

in the generation of oxygen free radicals in living organisms. The present results showed that the Miswak extract at the highest level (1000 μg) has the greatest activity to remove H_2O_2 from the reaction media in comparison with other extract and the positive control, while the scavenging abilities of the two Miswak extracts at the same level were not significantly different from those of the positive control. However, none of the concentrations of the Miswak extract assayed could completely remove H_2O_2 from the assay medium. Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (–SH) groups. However, hydrogen peroxide can cross cell membranes rapidly, once inside the cell, it may react with Fe^{2+},and possibly Cu^{2+}, ions to form hydroxyl radical and this may be the origin of its toxic effects. Therefore, it is important for cells to avoid the accumulation of H_2O_2.

This study showed that both the extracts had a significantly lower ·OH radical scavenging activity than the positive control, but at the highest concentrations the southern Miswak extract showed significantly higher ·OH radical scavenging activity than the middle region extract. The ·OH is an extremely reactive free radical formed in biological systems, which may lead to serious damage, such as damaging the biomolecules of living cells. The ·OH has the capacity to break DNA strands, which contributes to carcinogenesis, mutagenesis, and cytotoxicity. In addition, this radical species is thought to be one of the quick initiators of lipid peroxidation process, abstracting hydrogen atoms from unsaturated fatty acids (Bloknina et al., 2003).

Our results showed that both Miswak extracts had significantly lower ability than Vit. C to scavenge nitric oxide from the reaction media. However, at higher levels the Miswak collected from the southern region had significantly greater ability than the Miswak collected from middle region, which has almost similar ·NO scavenging activity. The mechanism of the antioxidant activity of phenolic compounds is widely known to act as free radical scavengers, leading to the formation of phenoxyl radicals. However, there are two possible mechanisms for the production of phenoxyls from the reaction of ·NO (nitric oxide, NO, is also called nitric oxide radical, ·NO, because nitric oxide has an unpaired electron on the nitrogen atom) with a phenol moiety. The first mechanism, involves H-atom abstraction to produce HNO and phenoxyl radicals. The second mechanism, is also possible in which phenols reduce ·NO by single electron transfer to produce the phenol radical cation, with subsequent loss of a proton to form phenoxyl radical. Reaction of ·NO with phenolic groups may prevent accumulation of ·NO in the reaction system (Janzen et al., 1993). Furthermore, the scavenging activity may also help to arrest the chain of reactions initiated by excess generation of NO that are detrimental to human health (Moncada and Higgs, 2006). In the present study, the extracts might be involved in competition with oxygen to react with nitric oxide and thus inhibit generation of the mentioned anions (NO_2^- and ONO_2^-). As a result, the present data suggest that the extracts might be potent and novel therapeutic agents for scavenging of NO and the regulation of pathological conditions caused by excessive generation of NO and its oxidation products, nitrite (NO_2^-) and peroxynitrite ($ONOO^-$).

The present work has shown that both the examined Miswak extracts exhibited a marked DPPH scavenging activity (Table 2). As a result, the new findings showed that the increase of extract concentration caused a significant decrease in the concentration of DPPH due to the free radical scavenging effect of Miswak extracts. Since the hydrogen donating of the tested extracts was comparable to Vit. C, it was evident that the extracts could serve as hydrogen donors, and consequently terminating the radical chain reaction. This means that the new data are indicative of the hydrogen donating ability of the extracts antioxidants, such as phenolic and polyphenolic compounds. They can be explained on the bases of other studies (Conforti et al., 2005) which relate the hydrogen donating ability using DPPH method to the presence of phenolic and polyphenolic compounds. In the presence of hydrogen donors, DPPH· is oxidized and a stable free radical is formed from the scavenger. Fukumoto and Mazza (2000) noted that the position and degree of hydroxylation of flavonoids, especially of the B-ring, play a major role in antioxidant activity with all flavonoids having 3-0,40, dihydroxy configuration. As a result, the new data (Table 1) showed that the increase of extract concentration caused a significant decrease in the concentration of DPPH· due to the scavenging effect of palm date extracts. These data showed that the reducing power ability of Miswak extracts appeared in a dose dependent fashion where the southern collected samples has shown to have the highest values and these results were in agreement with those obtained by Halawany (2012). The reducing power of extracts as measured by this method could be related to phenolic and polyphenolic compounds, which reduced ferricyanide ions ($[Fe(CN)_6]^{3-}$) to ferrocyanide ions ($[Fe(CN)_6]^{4-}$) and the latter reacts with Fe^{3+} ions to give what is called the Prussian blue colored complex (i.e., ferric ferrocyanide, $Fe_4[Fe(CN)_6)]_3$). This reduction does occur due to the electron (or ·H) donating ability of Miswak extract containing phenolic and polyphenolic compounds having more number of hydrolysable OH groups. These OH groups act as more powerful reducing agents, because they have more electron (or ·H) donating ability, which results in the termination of free radical chain reactions.

5. Conclusion

The scientific results obtained from this study signify that the Miswak roots are very important source of natural antioxidants. In addition, the new data support the folk medicine, which uses Miswak roots for treatment of teeth. Moreover, according to the new results, the Miswak roots collected from the southern region are highly recommended to be consumed in comparison with the other type.

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