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Full Length Research Paper

Ethnobotanical and antibacterial potential of *Salvadora persica* I: A well known medicinal plant in Arab and Unani system of medicine

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The current study includes ethnobotanical and antibacterial importance of *Salvadora persica* L. roots which are commonly used by different Muslim communities worldwide. Various medicinal properties are attributed to *S. persica* in traditional system of medicine. A detailed survey was conducted in different parts of Saudi Arabia to obtain information about the folklore uses, knowledge of local people and traditional healers about this popular plant. Based on the results obtained, it was concluded that *S. persica* is a versatile medicinal plant used to treat different human and livestock ailments. It is used for dental care, antiulcer, and possesses anti-inflammatory properties. In addition, various parts of *S. persica* are being used as food, fruits and fodder. *S. persica* aqueous extract and methyl alcohol extract were prepared and tested against selected pathogenic microbes: *Staphylococcus aureus, S. mutans, Lactobacillus acifophilus*, and *Pseudomonas aeruginosa,* by standard protocol. The aqueous extract showed significant inhibition in the growth of all pathogens tested in the current study. However, *S. persica* water extract was found to possess profound inhibitory activity against *Staphylococcus* species as compared to other extracts. Methyl alcohol extract was more active against *L. acifophilus* and *P. aeruginosa.* The results indicate promising antibacterial activity of *S. persica* root extract and recommend further study on its efficacy and safety.

Key words: *Salvadora persica* L., antibacterial activity, oral pathogen, ethnobotanical uses.

INTRODUCTION

Man has long been using various plants to avert pain, cure diseases and to provide relief from health problems. Literature review revealed that ancient people and old cultures over ages knew the use of several medicinal plants to help in curing a disease state. In primitive medical history based on human efforts, trials, and errors, some natural toxic products were also recorded (Sher and Hussain, 2009; Sher et al., 2010b). It is interesting to notice that world-wide opinion has not yet fully changed and people with low resources and living in remote areas, still make use of those herbal remedies as a part of their traditional medicinal systems (Sher et al., 2010a). Needless to say that medicinal plant use has persisted as a long standing tradition in all cultures of the world. Moreover, the activity of different medicinal plant extracts against microorganisms and bacteria supported the scientists in developing pharmaceutical industry and global elevation of human health standards. Several medicinal plants used in Chinese, African, Arab, German, and local Asian systems of medicine are part of herbal pharmacopoeias (Noumi et al., 2010; Suffredini et al., 2004). Miswak (*Salvadora persica*) is one of the most

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commonly used medicinal plants for oral hygiene among global Muslim community (Sher et al., 2010c).

In the world today, an herbal drug is defined as a remedy derived from plants (roots, leaves, flowers, fruits, seeds) and/or other natural sources which can be used for therapeutic purposes and its active chemical constituents provide the basis for pharmaceutical synthesis. Thus confirming the economic values of various medicinal plants used in different traditional systems of medicine (Sher et al., 2010a). Interestingly, in the recent years, there is a global trend for survival interests in traditional medicines which, being natural are believed to be without any side effects.

Screening of medicinal herbs and herbal drug products have become a potential source of biodynamic compounds of therapeutic value. Ethnopharmaceutical studies have become increasingly valuable in the recent past and medicinal plants are now part of health care system. The medicinal plants conservation programs and their sustained supply are part of global health strategy (Sher and Hussain, 2009; Sher et al., 2010c). Furthermore, recent reports suggested that in some cases, synthetic antibiotic are no more effective to treat some infectious diseases because of bacterial resistance. Some drug products were found to cause health hazards if used in higher dose range. The observed bacterial resistance and undesirable side effect of certain antibiotics, led scientific community to find new antibacterial compounds from medicinal plants or to prepare synthetic and semi-synthetic antibacterial drug products with low toxicity (Marchese and Shito, 2001; Poole,2001). In several studies, medicinal plant extracts, plant products, and isolated phytochemical constituents showed highly significant antimicrobial activity which added more to encourage research on such potential natural drugs (Amani et al., 1998; Salvat et al., 2001). *S. persica*, commonly called Miswak or Toothbrush tree, is one of the most popular medicinal plants that has proved to be effective in the prevention of tooth decay and mouth infections (Salehi et al., 2006). The traditional medicinal use of *S. persica* as antimicrobial toothbrush stick for oral hygiene, and to treat gum inflammation, is a centuries old practice and a part of Greeko-Arab system of medicine (Al-Otaibi et al., 2004; Zakaria et al., 1998; Sher et al., 2010c). *S. persica* is harvested as wild, rather than cultivated, and about 80% world population use it as a natural remedy for the treatment of different diseases (Noumi et al., 2010; Sofrata et al., 2007; Monforte et al., 2002).

S. persica is known to contain several biologically active chemical constituents such as volatile oils, flavonoids, alkaloids, steroids, terpenoids, saponins, and carbohydrates (Abdillahi et al., 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. Despite the wide use of miswak, in Saudi Arabia, the plant, has not received much attention and has not been fully evaluated with respect to tooth diseases. Therefore, the aim of the present study was to evaluate the antibacterial activity of Saudi miswak using disc diffusion and micro dilution assays. Besides medicinal claims, various parts of this plant are of great ethnobotanical importance and play a vital role in the livelihood of local communities (Ghazanfar, 2007; Abuzinada et al., 2005; Sher et al., 2004). Keeping in view the popular use of miswak in the whole Muslim world and Saudi Arabia in particular, the present endeavour was initiated with the aim to prepare an inventory of the ethnobotanical and ethnopharmaceutical importance of *S. persica*. The present study will also add important points to the disciplines of ethno pharmacy and medicine.

MATERIALS AND METHODS

Ethno-botanical survey

Detailed study on the ethnobotanical evaluation of *S. persica* was conducted during summer of 2010 in various parts of Saudi Arabia. A semi-structured questionnaire was devised to document the traditional knowledge of local people regarding *S. persica*. Generally, in Saudi culture, elder persons with practical knowledge are respected by others and such tribes men are more confident to respond. In addition, people practicing popular folk medicine were contacted and interviewed for their comments on *S. persica*. Information about the medicinal and other local uses, parts used, time of collection, processing and recipe preparation were recorded. Secondly, information on the market value of the plants was collected from local collectors, hakims and shopkeepers. A simple procedure was adopted as to how, from whom, and to whom the plants were sold. The nomenclature was confirmed from the available literature and a set of voucher specimen (No. Vg: 09-78) was deposited to herbarium of King Abdulaziz University.

Plant material

The roots of *S. persica* were collected from Asir Mountains of Saudi Arabia. The plant material was identified and kept on record in the herbarium of King Abdulaziz University, Jedda, Saudi Arabia. The plant root were cut into small pieces, dried in shade according to the standard procedure and crushed. The crushed *S. persica* root power (500 g) was extracted with water and methyl alcohol separately in soxhlet extractor. As a routine, the solvent after extraction was filtered through a filter paper (Whatman No. 1) to remove any gross particles. Buchi B-491 evaporator coupled with vacuum controller V-850 and vacuum pump V-700 was used to remove the solvent. The obtained semi-solid extract after freeze drying, was transferred to sterile labeled bottles and kept at -20°C till used (Chhabra et al. 1982; Kandil et al., 1994).

Preparation of microbial cultures and inoculums

Total of four species of bacteria: *Staphylococcus aureus, S. mutans, Lactobacillus acidophilus* and *Pseudomonas aeruginosa* were used to test the activity of *S. persica* root extracts. All microorganisms were isolated from patients of the general dental clinic of King Khalid Hospital, Riyadh, Saudi Arabia. These were identified by using standard microbiological procedures (Koneman

et al.,1988). From the isolated colonies of the pure bacterial cultures, five colonies were picked up using a sterile wire loop and then inoculated in a test tube containing the broth media, that is, tryptone soya broth for bacteria. The inoculated broth cultures were then incubated at 40°C for 9 to 16 h in an incubator. The following two assays were used for the antibacterial activities of *S. persica*.

The extracts of *S. persica* were evaluated for microbiological activity using slightly modified agar diffusion procedure. The nutrient agar was inoculated with microbial cell suspension, using 200 µl in 20 ml of medium and shifted to Petri dishes. In each case, filter paper discs with 6 mm diameter were impregnated with 20 µl of *S. persica* extracts. Throughout the experiment, sterile paper discs, sterile Petri dishes, and same solvent were used. The extract was dissolved and following concentration: 300, 150, 75, 37.5, and 18.75 mg/ml were prepared by using same solvent. All the preparations were sterilized by using pasteurization, or membrane filtration as required, and positioned on the inoculated agar surface. For making positive control, 6 mm standard discs containing streptomycin (25 µg/disc) and amphotericin B (10 µg/disc) were used. However, paper discs loaded with 20 µl of the solvent served as negative control. Pre-incubation of plates was effective for 4 h in refrigerator followed by overnight incubation for 15 to 21 h at 30°C.

Secondly, the micro dilution technique was used for 96 multi well micro titer plates as described earlier and minimal inhibitory concentration (MIC) was determined for the extracts of *S. persica* (Sarker, 2007). The highest dilution of *S. persica* was subjected to *in vitro* antibacterial activity testing against the selected oral infectious bacteria (18.75 mg/ml). Nutrient broth (50 µl each) was allocated between well-2 up to well-12. In addition, a 100 µl quantity of *S. persica* aqueous extract and methanol extract was pipetted to the first test wells of each micro titer line. Later, 50 µl quantities of different dilutions were assigned to 2^{nd} till 12^{th} well. Resazurin indicator (10 µl) was added to each well. (Resazurin indicator was prepared by dissolving a 270 mg tablet in 40 ml sterile distilled water). Lastly, bacterial suspension (10 µl) was added to each well. The extracts were further diluted from 18.75 mg/ml reaching 0.004 mg/ml and tested for possible antimicrobial effects.

In each plate, three columns were used as controls: Column 1 contained streptomycin in serial dilutions of 18.75 to 0.004 mg/ml while columns 2 and 3, contained water and methanol respectively to serve as negative control. All preparations were prepared in triplicates and the plates were wrapped in cling film to avoid any dehydration. The preparations were kept in incubator at 30°C for 15-21 h. Any change in colour was judged visually. The change of colour from pink to colorless was considered to be positive. The lowest concentration where color change was observed was taken as MIC value. In each case, mean of three values was taken as MIC of the material under study.

RESULTS AND DISCUSSION

Ethnobotanical Importance of *S. persica*

The current ethnobotanical evaluation of *S. persica* was conducted during summer of 2009 in various parts of the Kingdom of Saudi Arabia. The results showed that the fruits of *S. persica* are eaten in raw, cooked, or dried by the inhabitants of the sites where *S. persica* grow in abundance. Similarly, the local folks cook the leaves as a sauce and eat it with couscous or as green vegetables. The flowers of *S. persica* were found to be a good source of nectar; therefore, it is used as a honey bee plant. The local Bedouins were found to have strong belief that the honey of *S. persica* had high medicinal value as compared to honey from other plant regions. It is worth mentioning that the leaves and young shoots of *S. persica* are used as fodder for camels, cows, goats and sheep. *S. persica* leaves make good fodder because of the high water content (15 to 36%). Moreover, it was confirmed that the leaves of this popular medicinal plant were used in animals to increase lactation in cows and improve general body weight of all animals. The findings of our current studies are in full agreement with the earlier reports (Sofrata et al., 2007).

S. persica was found to be a multipurpose plant and posseses several agro-pharmaceutical applications. The current ethnobotanical evaluation of *S. persica* also revealed its significant pharmaceutical importance. The study described that toothbrushes made from roots and small branches have been in use for over thousands of years, and currently the use of *S. persica* is more popular among Islamic communities of the world. These findings are well supported by earlier researchers (Azaizeh et al., 2003), who reported toothbrushes prepared from the roots and small branches of *S. persica*, to be highly useful as maintainer of teeth (Noumi et al., 2010; Al-Khteeb et al., 1991). Teeth cleaning with *S. persica* sticks were found to provide relief from toothache and gum inflammation. Our findings were substantiated by an earlier investigation as well as reports of the use of tooth stick in cleaning teeth, treating gum infection and getting relief from toothache (Akhtar and Ajmal, 1981). The results of our present study further documented that in Arab traditional medicine, the decoctions of *S. persica* leaves and root were successfully used as mouthwash, and in the cure of tooth/gum problems and as a remedy for joint pain, thus supporting the reports of earlier researchers (Al-Sadhan et al., 1999). It was interesting to note that decoction of *S. persica* root besides using to treat epilepsy, were used to cure gonorrhea and skin diseases, spleen troubles, and stomach ulcer (Sanoqo et al., 1999).

The results of present study are supported by earlier reports where decoction of *S. persica* was shown to posses significant protective action against ethanol and stress-induced ulcers, and the elements of gastric mucosa reestablished normally in animal models (Sanoqo et al., 1999; Sher et al., 2010c). Another report suggested that crusted leaves of *S. persica* immersed in cow urine could facilitate the removal of hair with knife. *S. persica* is known to be a good source of volatile oil (also called essential oils). Generally, essential oils obtained from different plants were found to possess different pharmacological, microbiological, and insect repellent activities. Other findings in Sudan has supported our results and showed that *S. persica* possessed antiplas-modial activity and is used as part of remedies totreat malaria (Ali et al., 2002). During current study, it was also found that seeds of *S. persica* were believed to increase fertility, while seed oils were used to treat skin inflammation and rheumatism.

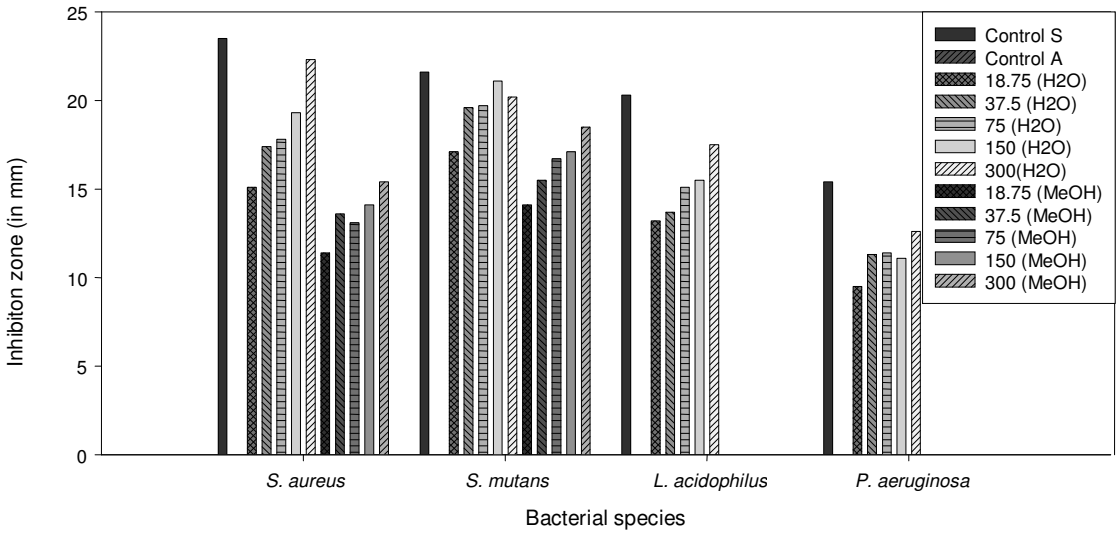

Figure 1. Antimicrobial activity of aqueous and methanol extracts of *S. persica* roots. S: streptomycin (25 µg); A: amphotericin B (10 µg).

The present information and medicinal claims recorded about different parts of *S. persica* are in agreement with the report of Ahmed et al. (2008) who stated that *S. persica* seed oil is useful for the treatment of some skin diseases and joint pain. Mansour et al. (1996) also reported that the plant extract itself has an analgesic effect against heat stimuli but not the chemical stimuli. In Greco-Arab system of medicine, the fermented juice prepared from the fresh fruits is a strong aphrodisiac agent and is also used as general body tonic (Ahmed et al., 2008; Darmani et al., 2003).

Antibacterial activities of *S. persica*

The present study included to explore antibacterial potential of *S. persica* against selected oral bacteria. A total of 45 microbial samples were collected from adult patients and investigated. It was found that 11.59% were *S. aureus*, 5.02% were *S. mutans*; 5.97% were identified to be *L. acidophilus*, and 3.2% *P. aeruginosa*. The results of the present study are depicted in Figure 1. *S. persica* aqueous extract was found active against all oral pathogens especially against the selected *Streptococcus aureus* and it showed relatively weak activity against *P. aeruginosa*. It was confirmed that *S. persica* root aqueous extract possessed higher and significant antimicrobial activity as compared to the methanol extract. It is worth specifying that *P. aeruginos* showed resistance to different methanol extract concentrations (Figure 2). The strongest activity was observed by the aqueous root extract against *S. aureus* (MIC: 2.49 mg/ml) followed by *L. acidophilus* (MIC: 7.34 mg/ml), and P. aeruginosa (MIC: 7.31 mg/ml).

The methanol extract of *S. persica* showed weaker activities against different microbes. The weakest activity was noticed in *S. aureus* (MIC: 5.13 mg/ml). However, the standard drug streptomycin showed noteworthy antimicrobial activity against all the microorganisms tested. These findings confirmed the validity of the current experiment. Streptomycin showed significant antibacterial activity, where zone of inhibition range was 15.4 to 23.5 mm and MIC range was found to be: 0.087 to 0.987 mg/ml. The results of the current study clearly demonstrated that both methanolic as well as aqueous extracts of *S. persica* could inhibit the growth of several pathogenic bacteria, however, the effectiveness varied against different bacteria tested. For aqueous extract the inhibition zone ranged between 12.6 to 22.3 mm, while methanol extract zone of inhibition ranged between 9.2 to 15.7 mm. The antimicrobial activity of the aqueous extract with promising MIC values against *S. mutans* and *S. aurens* as observed during present study, were in agreement with the findings of earlier researchers (Hussein, 1992; Saleh et al., 2006). The results of the current study are in agreement with the earlier investigations where *S. persica* exhibited significant antimicrobial activity against both aerobic as well as anaerobic bacteria collected from teeth with inflamed gums and necrotic pulps (Al-Sabawi et al., 2007).

Our results were further supported by other reports using the same disc diffusion and micro well assay method and established water extract of *S. persica* to be effective against *S. pyrogenis*, *S. faecalis*, *P. aeruginosa, Lactobacillus acidophilus*, and also against *Candida albicans* (Suffredini et al., 2004; Al-Sabawi et al., 2007; Al-Bayati et al. 2008). The methanol extract showed lower inhibitory activity against *P. aeruginosa* and *L. acidophilus*. The variation in results and resistance of microbes against plant extracts may be attributed to

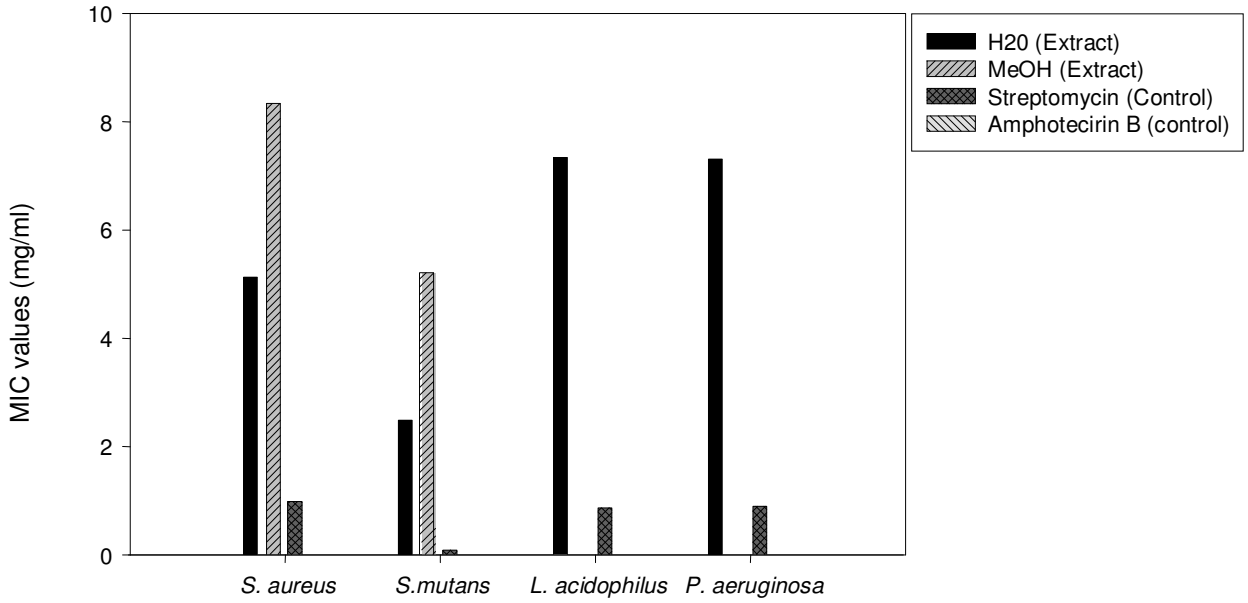

Figure 2. MIC values of *S. persica* aqueous and methanol extracts against isolated microorganisms.

different factors including: decrease in uptake, site modification, changed membrane permeability, enzymatic modifications or inactivation, and bypass of pathways, etc., as reported earlier (Coates et al., 2002). Furthermore, the chemical constituents of *S. persica* such as essential oils, flavonoids, salvadorine, cyanogenic glycosides, lignans, saponins, alkaloids, tannins, linoleic acid, steraric acid, salvadourea, vitamin C, silica and different salts are also known to possess significant antimicrobial activity (Kamel et al., 1992; Darout et al., 2000; Abd-Rahman et al., 2003), based on the results of present study supported by previous reports in the literature which provides great evidence of *S. persica* use for oral hygiene. The use of low price miswak stick or powder, is suggested to be promoted in developing countries where cost effectiveness plays a vital role.

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