Middle East Journal of Applied Sciences
ISSN 2077-4613

Volume : 08 | Issue :03 |July-Sept.| 2018
Pages: 768-775

Antimicrobial and Preliminary Phytochemical investigation of *Galenia asiatica* (Mustard bush)

Ahmed A. Aly[1] and Inas M. Khamis[1,2]

[1]*Medicinal and Aromatic Plant Department, Desert Research Center, Ministry of Agriculture and Land Reclamation, Egypt.*
[2]*Biology Department, the University College – Alkhafji, Hafr Albatiin University, Kigdom of Saudi Arabia*

Received: 21 March 2018 / Accepted: 28 June 2018 / Publication date: 26 July 2018

ABSTRACT

The current study includes antimicrobial importance of *Galenia asiatica* plant which are commonly used by different Muslim communities worldwide. Various medicinal properties are attributed to *Galenia asiatica* in traditional system of medicine. The preliminary phytochemical screening on three extracts (chloroform, methanol and water) of different plant parts (root, stem and leaf) showed that, it contains many active materials, namely flavonoids, steroids, triterpenoids, tannins, saponins and alkaloids in different plant parts. However, none of the root crude extracts showed any color change for the saponin test. The antimicrobial activity of chloroform, methanol and water of *Galenia asiatica* root, stem and leaf extracts against *Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Alcaligenes faecalis* were included in this work. The strongest activity was observed by the aqueous root extract against *S. aureus* followed by *P. aeruginosa*, while the weakest activity was demonstrated against *Staphylococcus epidermidis.* The methanol extract showed lower inhibitory activity against all bacteria strains under investigation. All extracts revealed did not antifungal activity against *Candida albicans.*

Keywords: *Galenia asiatica,* phytochemical screening, antibacterial activity.

Introduction

Recent studies have focused on the organization of food and the use of medicinal plants. In its international conferences, the World Health Organization (WHO) has devoted great attention to pharmaceutical food as one of the modern bases for avoiding many side effects of drugs. Its statistics confirm that about 70-80% of human communities around the world use these plants for their medicinal effects (WHO, 2008). The research has found at least 182 plants or trees of different factions, which use their sticks to prepare toothpicks. Of these trees, there are at least 158 plants in the continent of Africa alone and the most famous of these trees is the most common and used is the tree of the god *Galenia asiatica* (mustard bush) synonyms *Salvadora persica* which belongs to the Arakian family Salvadoraceae (Yadav *et al.,* 2005). It has been used as a brushing stick for more than 1,300 years. Nowadays chewing sticks is being used in Africa, South America, the Middle East and Asia. In Pakistan, more than the half of the rural population used chewing sticks as an oral hygiene tool. It is narrated that there are more than one hundred hadeeths narrated in Miswak. The Prophet (peace and blessings of Allaah be upon him) urged the imam to use it and to confirm this year in many of the hadiths mentioned in most of the Sahih (Khalid, 2002 and Al-Etebi, 2008).

Pharmacological studies indicated that *Galenia asiatica* plant possess anti-microbial, anti-plaque, aphrodisiac, alexiteric, analgesic, anti-inflammatory, anti-pyretic, astringent, diuretic and bitter stomachic activities. It has great medicinal use in the treatment of nose troubles, piles, scabies, leucoderma, scurvy, gonorrhea, boils and toothache, to treat hook worm, venereal diseases, for teeth cleaning, in rheumatism, cough and asthma, to lower cholesterol plasma levels, reestablishment of the components of gastric mucosa, and as a laxative (Noumi, *et al.,* 2011). It contains important phytoconstituents such as vitamin C, salvadorine, salvadourea, alkaloids, trimethylamine, cyanogenic glycosides, tannins, saponins and salts mostly as chlorides (Alali and Al-Lafi, 2004; Almas, 2002;

Corresponding Author: Ahmed A. Aly, Medicinal and Aromatic Plant Department, Desert Research Center, Ministry of Agriculture and Land Reclamation, Egypt.

Almas *et al.,* 2005; Rajesh *et al.,* 2009). Antifungal, antibacterial and antioxidant activities of this plant have also been described (Isanga and Zhang, 2007; Miraliakbari and Shahidi, 2008; Amaral *et al.,* 2003). Plant-derived products can also be used as antimicrobial agents, with phenolics and polyphenolic having major interest (Kapoor and Bansal, 2013). Flavonoids separated from the plant extract act as antimicrobials, antioxidants, antioxidants and infections (Goswami and Farnandes, 2002; Cushine and Lamp, 2005 and Arora and Gupta 2011).

Aqueous and methanol extracts of *S. persica* were investigated by AL-Bayati and Sulaiman, (2008) for its antimicrobial activities against seven isolated oral pathogens *Staphylococcus aureus*, *Streptococcus mutans*, *Strep. faecalis*, *Strep. pyogenis*, *Lactobacillus acidophilus*, *Pseudomonas aeruginosa*, and *Candida albicans*, the aqueous extract inhibited all isolated microorganisms, especially the *Streptococcus* sp., and was more efficient than the methanol extract, which was resisted by *L. acidophilus and P. aeruginosa*.

Miswak embedded in agar, or suspended above the agar plate, had strong antibacterial effects against all bacteria tested. The antibacterial effect of suspended miswak pieces suggested the presence of volatile active antibacterial compounds (Sofrata, *et al.,* 2007). Also, this plant extract inhibits the growth of some dental plaque bacteria, and antibacterial effect of the herbal toothpaste was significantly greater than that of the placebo (Poureslami, *et al.,* 2007).

Antimicrobial activity of eight commercially available mouth rinses and 50% miswak extract against seven microorganisms was compared by Almas and Ahmad in 2005. Corsodyl, Alprox, Oral-B advantage, Florosept, Sensodyne, Aquafresh Mint, Betadine, and Emoform mouthrinses were used, while 50% aqueous extract was used against *Strep. faecalis, Strep. pyogenis, Strep. mutans, C. albicans, Staph. aureus,* and *Staph. Epidermidis*. Also, antimicrobial activity of Arak chewing stick's aqueous extracts at various concentrations was compared by some research workers. Data suggested that both chewing stick extracts was effective at 50% concentration on *Strep. mutans* and *Strep. faecalis*. Arak extract was more effective at lower concentrations for *Strep. faecalis* (Almas, 1999).

Material and Methods:

1. Plant sample preparation:

The plant samples (root, stem and leaf) were collected and washed carefully with water to remove dust and foreign materials. Then each washed part was dried under shade at temperature (25°C) for 7 days. After drying the plant samples (150 gm of each) were ground into a powder form using a grinder for 30 s.

2. Preliminary phytochemicals screening:

The different plant extracts were obtained from the samples of the three plant parts (root, stem and leaf) of the *Galenia asiatica* plant, by soaking a quantity of each sample with ethyl alcohol by 70%. Each extract was then filtered and used for phytochemical screening according to well-established methods of Harborne and Kokate (Harborne, 1998 and Kokate, 1997).

2.1. Test for flavonoids:

The stock solution (1 ml) was taken in a test tube and added few drops of dilute NaOH solution. An intense yellow color was appeared in the test tube. It became colorless when on addition of a few drop of dilute acid that indicated the presence of flavonoids.

2.2. Test for steroids:

The crude plant extracts (1 mg) was taken in a test tube and dissolved with chloroform (10 ml), then added equal volume of concentrated sulphuric acid to the test tube by sides. The upper layer in the test tube was turns into red and sulphuric acid layer showed yellow with green fluorescence. It showed the presence of steroids.

2.3. Test for triterpenoids:

The dry crude plant extract (5 mg) was dissolved in chloroform (2 ml) and then acetic anhydride (1 ml) was added to it. Concentrated sulphuric acid (1 ml) was added to the solution. Formation of reddish violet colour shows the presence of triterpenoids.

2.4. Test for tannins:

The stock solution (3 ml) was taken in a test tube and diluted with chloroform and added acetic anhydride (1 ml). Finally, sulphuric acid (1 ml) was added carefully by the side of test tube to the solution. A green colour was formed which showed the presence of tannins.

2.5. Test for saponins:

The stock solution (1 ml) was taken in a test tube and diluted with 20 ml of distilled water. It was shaken by hand for 15 min. A foam layer was obtained on the top of the test tube. This foam layer indicated the presence of saponins.

2.6. Test for alkaloids:

Powder samples of *Galenia asiatica* plant (1 gm) taken in a conical flask and added ammonia solution (3 ml). It was allowed to stand for few minutes to evaluated free alkaloids. Chloroform (10 ml) was added to the conical flask shaken by hand and then filtered. The chloroform was evaporated from the crude extract by water bath and added Mayer's reagent (3 ml). A cream color precipitation was obtained immediately that showed the presence of alkaloids.

3. Antimicrobial activities:

3.1. Extraction procedure for antimicrobial activity:

The dry powder samples (150 gm for each) were extracted with methanol solvent (350 ml) for 3 days using Soxhlet extractor until complete extraction. After extraction, the sample was filtered with filter paper (Whatmann No. 1). The methanol solvent was evaporated using a rotary evaporator (Yamato, Rotary Evaporator, model-RE 801, Japan) under pressure for 30 min resulting in a semi solid crude extract. The crude extracts were transferred into a separatory funnel and finally extracted by different solvents with increasing polarities followed the sequence of chloroform, methanol and water to give chloroform, methanol and water fractions, respectively. After extraction all crude extracts were put inside the fume hood for the solvents to evaporate. After the solvent was completely evaporated the different crude extracts of the plant parts were obtained for preliminary phytochemical screening and estimate the effects of these extracts on some pathogenic bacterial strains.

3.2. Microorganisms:

The tested microorganisms included the following bacteria: *Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Alcaligenes faecalis* and one Fungai was *Candida albicans* were used in this study.

3.3. Disk-diffusion assay:

Antimicrobial activity was done according to the protocol described by Hajlaoui *et al.* (2009). For the experiments, a loopful of the microorganisms working stocks were enriched on a tube containing 9 ml of Mueller-Hinton broth then incubated at 37°C for 18 to 24 h. The overnight cultures were used for the antimicrobial activity of the extracts used in this study and the optical density was adjusted at 0.5 McFarland turbidity standards with a DENSIMAT (BioMérieux). The inoculate of the respective bacteria were streaked into MH agar plates using a sterile swab. A sterile filter disk

(diameter 6 mm, Whatman paper No. 3) were impregnated with 10 µl of each extract placed on the MH agar medium. Gentamicin (10 µg/disk) was used in this study as positive controls. After incubation at 37°C for 18 to 24 h, the diameter of the inhibition zone was measured with 1 mm flat rule and the diameters were interpreted according to the Comité de la Société Française de l'Antibiogramme (Cavallo *et al.,* 2006). The antifungal activity was achieved by the agar-well diffusion method. *Candida albicans* was inoculated into Sabouraud dextrose agar and incubated for 18 h at 37°C. The yeast cultures were harvested and then suspended in sterile saline (0.8 % NaCl) and the cell density was adjusted to 107 cells/ml (OD540= 0.5). For the antifungal activity of the plants extracts used in this study, three sterile 6 mm paper disks (Whatman paper N°3), impregnated with 30 mg of extract (10 µl/disk) at a final concentration of 300 mg/ml were placed on the inoculated surface. Plates were then incubated at 37°C for 18 to 24 h. The ATCC and CECT strains were used as a quality control strains. The diameter of the zones of inhibition around each disk were examined after 24 h, measured and recorded as the mean diameter (mm) of complete growth inhibition. As a positive control, 10 µg of amphotericin B. (Fungizone, Bio basic INC) was used. The diameter of the zones of inhibition around each of the disks was taken as measure of the antimicrobial activity. Each experiment was carried out in triplicate and the mean diameter of the inhibition zone was recorded.

Results and Discussion:

1. Preliminary phytochemicals screening:

The results recorded in table (1) showed that alkaloids, flavonoids, saponins, steroids and tannins were present in different polarities of crude root, stem and leaf extracts. It contains many active ingredients, namely flavonoids, steroids, triterpenoids, tannins, saponins and alkaloids in different plant parts. However, none of the root crude extracts showed any color change for the saponin test. The highest indication of active substances detected with plant root extracts were for flavonoids, steroids, triterpenoids, and tannins. While the highest indication of active substances detected with plant stem extracts were for flavonoids and tannins. On other hand, the highest indication of active substances detected with plant leaf extracts were for tannins, and alkaloids. These results are consistent with a number of researchers who confirmed that containment or plant on these compounds (Noumi *et al.,* 2011; Abdillahi, *et al.,* 2010 and Garboui *et al.,* 2009), In addition to other compounds where the plant contains vitamin C, salvadorine, salvadourea, alkaloids, trimethylamine, cyanogenic glycosides, tannins, saponins and salts mostly as chlorides (Ahmed & Rajagopal, 2013).

Table 1: Phytochemical Analysis of *Galenia asiatica* Extracts.

Test Plant parts	Flavonoids	Steroids	Triterpenoids	Tannins	Saponins	Alkaloids
Root	+++	+++	+++	+++	-	++
Stem	+++	++	++	+++	++	++
Leaf	++	++	++	+++	++	+++

2. Antimicrobial activities:

Medicinal plants are gifts of nature to cure number of diseases among human beings and a large number of plants in different location around the world have been extracted, semi-purified to investigate individually their antimicrobial activity (Vadlapudi and Chandrashekar, 2010).

The activity of plant extracts against bacteria have been studied for years, but in a more intensified way during the last three decades. During this period, numerous antimicrobial screening evaluations have been published based on the traditional use of Chinese, African, and Asian plant-based drugs (Suffredini*, et al.,* 2004). Miswak is a common name for *Galenia asiatica* (*S. persica*) which is commonly used in Saudi Arabia and the Arab world. Miswak wicks clean between the teeth

and do not break, regardless of the amount of pressure applied, as they are flexible and strong. The small wicks bend to the appropriate shape to clear plaque and left-over food in between teeth and do not damage the gums. The WHO recommended the use of miswak in 1986 and in 2000 an international consensus report on oral hygiene concluded that further research was needed to document the effect of miswak.

The antimicrobial activity of chloroform, methanol and water extracts of *Galenia asiatica* root, stem and leaf extracts included in this work was conducted by disk diffusion method. The results obtained for the zones of growth inhibition (mm) were summarized in Table 2.

Table 2: Antimicrobial activities of *Galenia asiatica* extracts.

Bacteria &fungi Names	**Extracts**	**Plant parts**		
		Root	**Stem**	**Leaf**
Pseudomonas aeruginosa	Chloroform	20	17	15
	Methanol	17	13	11
	Water	30	20	20
Alcaligenes faecalis	Chloroform	-ve	-ve	-ve
	Methanol	-ve	-ve	-ve
	Water	12	-ve	10
Staphylococcus aureus	Chloroform	27	18	18
	Methanol	20	16	14
	Water	33	25	26
Staphylococcus epidermidis	Chloroform	10	9	9
	Methanol	-ve	-ve	-ve
	Water	11	10	9
Candida albicans	Chloroform	-ve	-ve	-ve
	Methanol	-ve	-ve	-ve
	Water	-ve	-ve	-ve

It was confirmed that *Galenia asiatica* root aqueous extract possessed higher and significant antimicrobial activity followed by chloroform and finally methanol extract for all plant parts. The strongest activity was observed by the aqueous root extract against *S. aureus* followed by *P. aeruginosa*, while the weakest activity was demonstrated against *Staphylococcus epidermidis* and these results were in agreement with the findings of earlier researchers (Saleh *et al.,* 2006). The results of the current study are in agreement with the earlier investigations where *Galenia asiatica* 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 *Galenia asiatica* 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 and Al-Bayati *et al.* 2008). The methanol extract showed lower inhibitory activity against all microbes under investigation. The variation in results and resistance of microbes against plant extracts may be attributed to 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 the plant such as flavonoids, saponins, alkaloids and tannins are also known to possess significant antimicrobial activity (Kamel *et al.,* 1992; Darout *et al.,* 2000 and Abd-Rahman *et al.,* 2003), based on the results of present study supported by previous reports in the literature which provides great evidence of *Galenia asiatica* use used as antimicrobial activities. The use of low price miswak stick or powder, is suggested to be promoted in developing countries where cost effectiveness plays a vital role. On the other hand, the methanol extract of *Galenia asiatica* from all plant parts showed less inhibitory activity against the tested bacteria than did the aqueous and chloroform extract. *Staphylococcus*

epidermidis and *Alcaligenes faecalis* resisted all methanol extracts, and *Alcaligenes faecalis* also resisted all chloroform extracts.

All extracts revealed did not antifungal activity against *C. albicans*, although slight differences were seen using the disc diffusion method. The present study agrees with a previous report (Almas, 2001) *S. persica* had no antifungal activity against *C. albicans*. The resistance of bacteria towards different drugs can be due to modification of the target site, bypass of pathways, decreased uptake (reduced intracellular concentration of the antimicrobial agent, either reducing membrane permeability or by active efflux pump), enzymatic inactivation or modification of the drug, or overproduction of the target (Coates, *et al.* 2002). The antimicrobial and cleaning effects of miswak may be attributed to various chemicals contained in its extracts, such as sodium chloride and potassium chloride, as well as salvadourea and salvadorine, saponins, tannins, vitamin C, silica, and resin (Darout, *et al.,* 2000), in addition to cyanogenic or lignan glycosides (Kamel, *et al.,* 1992), alkaloids, terpenoids, and oleic, linoleic, and stearic acids (Abd El Rahman, *et al.,* 2003). It could be concluded that miswak and powdered miswak are excellent oral hygiene agents, and their use should be promoted based on scientific knowledge of their benefits and proper use. Because it is widely available in this part of the world and is inexpensive, miswak chewing sticks can be a great help in developing countries with financial constraints and limited oral health care facilities.

Conclusion

It is concluded that miswak (*Galenia asiatica*) have many secondary metabolites and act as antibacterial. The water extract possesses highest effect. The present review showed that it is useful in a number of diseases, such as anti-inflammatory, anticonvulsant, sedative, antiulcer, hypolipidemic, and hypoglycemic activities. Therefore, it is imperative that more clinical and pharmacological studies should be conducted to investigate unexploited potential of this plant. Nevertheless, further investigations are required to isolate and purify novel pharmacologically active and industrially important compounds.

References

Abd EL-Rahman, H.F., N. Skaug and A. Whyatt, 2003. Volatile compounds in crude *Salvadora persica* extracts. Pharmaceut Biol., 41: 399-404.

Abdillahi, H.S., G.I. Stafford, J.F. Finnie and J.V. Staden, 2010. Ethnobotany, phytochemistry and pharmacology of Podocarpus sensus latissimo (S.I) South Afr. J. Bot., 76(1): 1-24.

Ahmad, H. and K. Rajagopal, 2013. Biological Activities of *Salvadora persica* L. (Meswak). Med Aromat Plants 2: 129.

Alali, F. and T. Al-Lafi, 2004. GC-MS analysis and bioactivity testing of the volatile oil from the leaves of the toothbrush tree *Salvadora persica* L. Nat Prod Res, 17: 189-194.

AL-Bayati, F.A. and D.K. Sulaiman, 2008. *In vitro* antimicrobial activity of *Salvadora persica* L. extracts against some isolated oral pathogens in Iraq. Turk J Biol. 32:57–62.

Al-Bayati, F.A. and K.D. Sulaiman, 2008. *In vitro* antimicrobial activity of *Salvadora persica* L. extracts against some isolated oral pethogens in Iraq. Turk J. Biol., 32: 57-62.

Al-Etebi, M.F., 2008. The scientific signs of prophety sunnah in security of *salvadora persica* and effect on oral hyginene, immunity of human cells. The eighth world conference on scientific signs in Quran and sunnah.

Almas, 2002. The Antimicrobial effects of seven different types of Asian Chewing Sticks. Odonto-Stomatologie Tropical., Vol. 96. 17-20.

Almas, K., 1999. The antimicrobial effects of extracts of *Galenia asiatica* (Neem) and *Salvadora persica* (Arak) chewing sticks. Indian J Dent Res; 10:23–6.

Almas, K., 2001. The antimicrobial effects of seven different types of Asian chewing sticks. Odonto-Stomatologie Tropicale 96: 17-20.

Almas, K., N. Skaug and I. Ahmad, 2005. *In vitro* antimicrobial comparison of miswak extract with commercially available non-alcohol mouthrinses. Int J Dent Hyg; 3: 18-24.

Al-Sabawi, A.K., A.K.K. Al-Sheikh and M.Y.J. Taha, 2007. The antimicrobial activity of Salvadora persica solution (Miswak-Siwak) as root canal irrigant (A comparative study). Univ. Sharjah J. Pure Appl. Sci., 4(3): 69-91.

Amaral, J.S., S. Casal, J. Pereira, R. Seabra and B. Oliveira, 2003. Determination of sterol and fatty acid compositions, oxidative stability, and nutritional value of six walnut (*Juglans regia* L.) cultivars grown in Portugal. J. Agric. Food. Chem., 51: 7698-7702.

Arora, M. and V. Gupta, 2011. Phytochemical Biological Studies O-Salvadora Persica Wall: Review. Pharmacology online 1: 591-601.

Cavallo, D., H. Chardon, C. Chidiac, P. Choutet, P. Courvalin, H. Dabernat, H. Drugeon, L. Dubreuil, F. Goldstein, V. Jarlier, R. Leclerq, H. Nicolas, A. Philippon, C. Quentin, B. Rouveix, J. Sirot and J. Soussy, 2006. Comité de l'antibiogramme de la société Française de Microbiologie (Communiqué).

Coates, A., Y. Hu, and R. Bax, 2002. The future challenges facing the development of new antimicrobial drugs. Nat Rev Drug Discov., 1: 895-901.

Cushine, T.P.T. and A.J. Lamp, 2005. Antimicrobial activity of flavonoids. International journal of antimicrobial agents; 26(5): 343-356.

Darout, I., A. Christy, and N. Skaug, 2000. Identification and Quantification of Some Potentially Antimicrobial Anionic Components in Miswak Extract. Indian J Pharmacol 32: 11-14.

Garboui, S.S., A.K. Borg-Karlson and K. Palsson, 2009. Tick repellent properties of three Libyan plants. J. Med. Entomol., 46(6):1415-1419.

Goswami, U. and N. Farnandes, 2002. Muscarinic antagonist methyl palmitate obtained from amangrove plant *Salvadora persica*. U.S. Pat. Appl. Publ.

Hajlaoui, H., N. Trabelsi, E. Noumi, M. Snoussi, H. Fallah, R. Ksouri, and A. Bakhrouf, 2009. Biological activities of the essential oils and methanol extract of tow cultivated mint species (*Mentha longifolia* and *Mentha pulegium*) used in the Tunisian folkloric medicine. World. J. Microbiol. Biotechnol., 25: 2227-2238.

Harborne, J.B., 1998. Phytochemical methods: A guide to modern techniques of plant analysis. 2nd ed. London: Chapman and Hall., pp. 54–84.

Isanga, J. and G.N. Zhang, 2007. Biological active components and nutraceuticals in peanuts and related products: Review. Food Rev. Intl., 23: 123-140.

Kamel, M.S., K. Ohtani and M.H. Assaf, 1992. Lignan glycosides from stems of *Salvadora persica*. Phytochem 31: 2469-2471.

Kapoor, B.B.S. and R. Bansal, 2013. Flavonoid Contents from Some Medicinal Tree Species of Nagaur District Of Rajasthan. International Journal of Herbal Medicine, 1 (4): 14-15.

Khalid, A., 2002. The effect of *salvadora persica* extract (Miswak) and chlorahexidine Gluconate on human dentin:ASEM study,Tye journal of contemporary dental practice, Vo1.3,No.3,August 15.

Kokate, K.C., 1997. 4th ed. Delhi: Vallabh Prakashan; Practical pharmacognosy; p. 218.

Miraliakbari, H. and F. Shahidi, 2008. Oxidative stability of tree nut oils. J. Agric. Food. Chem., 56: 4751-4759.

Noumi, E., M. Snoussi, H. Hajlaoui, E. Valentin and A. Bakhrouf, 2011. Antifungal properties of *Salvadora persica* and Juglans regia L. extracts against oral Candida strains. Eur J Clin Microbiol Infect Dis., 29: 81-88.

Poureslami, H.R., A.Makarem and F. Mojab, 2007. Paraclinical effects of Miswak extract on dental plaque. Dent Res J., 4:106–10.

Rajesh, V., P. Suresh, B. Anil, K. Brijesh and P. Priyanka, 2009. *Salvadora persica* L, (Tooth Brush Tree): A Review. J PR 2: 1809-1812.

Salehi, P., C. Momeni and S.H. Danaie, 2006. Comparison of the antibacterial effects of persica mouthwash with chlorhexidine on streptococcus mutans in orthodontic patients. DARU 14: 178-182.

Sofrata, A., P. Lingstrom, M. Baljoon and A. Gustafsson, 2007. The effect of miswak extract on plaque pH: An in vivo study. Caries Res., 41(6): 451-454.

Sofrata, A.H., R.L. Claesson, P.K. Lingström and A.K. Gustafsson, 2008. Strong antibacterial effect of miswak against oral microorganisms associated with periodontitis and caries. J Periodontol, 79:1474–9.

Suffredini, I.B., H.S. Sarder and A.G. Goncalves, 2004. Screening of antibacterial extracts from plants native to the Brazilian Amazon Rain Forest and Atlantic Forest. Brazilian J Med Biol Res 37: 379-384.

Vadlapudi, V. and N.K. Chandrashekar, 2010. *In vitro* Bioactivity of Indian Medicinal Plant Calotropis Procera (Ait.). Journal of Global Pharma Technology; 2(2): 43-45.

World Health Organization (WHO), 2008. Traditional medicine. Fact sheet number,134(December).Retrievedfrom:"http://www.who.int/mediacentre/en.

Yadav, J.P., S. Saini, and A.N. Kalia, 2005. Botanical, cytological, phytochemical and pharmacognostical studies on *Salvadora* species. Journal of Medicinal and Plant Sciences; 28: 231-238.