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REVIEW ON CHEMICAL AND BIOLOGICALLY ACTIVE COMPONENTS OF THE TOOTHBRUSH TREE (*SALVADORA PERSICA*)

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ABSTRACT

Miswak stick for mechanical tooth cleaning has been prepared from *Salvadora persica* or Arak tree. Miswak use has a very long tradition more economical, ecological and can be used without dentifrices. Currently many of the communities in the developing world use miswak as the only method for tooth cleaning. This review highlights the chemical, antimicrobial and biologically active components of miswak sticks with beneficial effects on oral health promotion. Research papers considering the chemical, antimicrobial and biologically active components of miswak extracts have been revisited in addition to papers that evaluating oral promotion and antimicrobial effects of miswak different extracts. Potential antimicrobial anionic components including chloride, sulphate, nitrate and thiocyanate have been identified in miswak aqueous extracts. Chlorides and thiocyanate (pseudohalides) in miswak may be substrates for salivary peroxidase and/or the myeloperoxidase hydrogen peroxide antimicrobial system. In addition, benzylisothiocyanate is a component exhibiting antiviral and antimycotic activity other more biologically active components including resins in small amount, tannins (tannic acid), alkaloids in large amounts have been identified in different miswak extracts. The finding of these components of miswak may lead to better understanding the clinical effectiveness and antimicrobial properties of miswak as an alternative method to the modern toothbrush for oral health promotion.

KEYWORDS: Miswak chewing sticks; extracts; chemical components; bioactive components; antimicrobial effects.

INTRODUCTION

Miswak is an Arabic word meaning tooth cleaning stick. In English, miswak has been mentioned as the "natural toothbrush". In geographical regions in which the Arak (synonymous with Araak) tree grows, miswak is interpreted as tooth sticks prepared from the Arak tree. *Salvadora persica L (family: Salvadoracae*) is the botanical name of the Arak tree. Geographically, *S. persica* is widely distributed: from Rajasthan (India) in the east through Southern Arabia, Iran, Iraq, Israel, Egypt to Mauritania in the west, and from North Africa in the north through Sudan, Ethiopia, Central Africa to South West Africa in the south.[1]

Chemical active compounds

Chemical analysis of *S. persica* root extracts demonstrated the presence of β-sitosterol, *m*-anisic acid and elemental sulphur as well as substantial amount of silica.[2,3] Chlorides, salvadourea and high content of gypsum have been found in the stem.[4] Organic components identified in *S. persica* included pyrrolidine, pyrrole and piperidine derivatives; glycosides such as salvadoraside; flavonoids such kaempferol, rutin, and a quercetin glucoside; and sugar terpenoids, alkaloids, esters of fatty acids and of aromatic acids, and fats.[5,6,7]

An indole alkaloid being a urea derivative, salvadoricine, from the leaves, salvadourea and glucotropaeolin, from the roots, 1-triacontanol and 1-octacosanol from the stems and trimethylamine from the root bark have been isolated and characterised by chemical and spectral data.[8, 9] In these studies myristic, lauric and palmitic acids have been identified as the major components of the seeds. Trimethylamine was also isolated from the leaves and the bark and the presence of alkaloids has been reported.

Dried seeds contain 30% to 40 % non-edible oil, with 30% lauric and 50% myristic acid which is used as a substitute for coconut oil in soap-making and detergent-preparation.[10, 11, 12] Various Indian companies like Godrej Soaps Ltd, Bombay, Tata Oil Mills, Hindustan Lever Ltd, Aegis Chemicals, Lauric Oils/Seeds and Seedlings, and KVIC, Bombay are exploiting the oil of *Salvadora* spp. Five glycosides from the stem have been isolated. Two of them were new and identified as sodium 1-*O*-benzyl- β-D-glucopyranoside- 2-sulphate (salvadoside) and 5,5′-dimethoxylariciresinol, 4′-4-bis-*O*-β-D- glucopyranoside (salvadoraside), in addition to syringin, liriodendrin and sistosterol 3-*O*-glucopyranoside. This represents the first report of

syringin and lignan glycosides from the family Salvadoraceace. The leaves contain a flavonoid quercetin and many phenolic acids like vanillic, syringic, ferulic, *p*-hydroxybenzoic and salicylic acid.

Chemical characterisation of the branches of the (tooth-brush tree) was carried out using a pyrolysis / gas chromatography coupled to an ion-trap detector mass spectrometer. [12] Electron impact and chemical ionisation mass spectra, together with chromatographic profiles obtained by flame ionisation and nitrogen/phosphorous detectors, enabled the structure of some eighty pyrolysis fragments to be proposed. These were mainly lignin and polysaccharide derivatives of phenolic or furanic nature, respectively. However, when compared with the pyrograms of other well-studied ligocellulosic materials, *S. persica* showed several unique nitrogen-containing compounds. Some of these were tentatively identified as pyrrolidine, pyrrole and piperidine derivatives. Their identification was established on the basis of their mass spectra and chromatographic elution order. The presence of minerals, fluoride, silica, sulphur, sodium bicarbonate, chloride and calcium has been reported. Resins in small amount, tannins (tannic acid), alkaloids in large amounts, flavonoids, sterols, β-sitosterol, *m*-anisic acid, salvadourea [1,3-Bis-(3-methyoxy-benzyl)urea], essential volatile oils, e.g. mustard oil, vitamin C, and steam-distillable compound of 10% benzyl nitrate and 90% benzylisothiocyante have all been reported from the roots. Another studies showed that in addition to some of the above mentioned compounds, the root contains 27 % ash, large amounts of chlorine, trimethylamine, but negligible amount of tannins and saponins. It is believed that some of these components are useful in tooth cleaning. [13]

Results of capillary electrophoresis revealed that the anionic components present in the aqueous extract of *S. persica* were chloride, sulphate, nitrate and thiocyanate and the quantities varied with its different parts. [14] For instance, the chloride found was 4.64% in the roots, 6.84% in the twigs and 12.38% in the leaves. The sulphate content was 19.85% in the roots, 20.10% in the twigs and 3.35% in the leaves. The nitrate was found to be 0.05% in the roots and the twigs while in the leaves it was 0.03%. The concentration of thiocyanate was 0.28%, 0.38% and 0.60% in the roots, the twigs and the leaves, respectively. The values represent each compound as present of reconstituted extract (Fig. 1 and 2) showing electropherogram of a mixture of Br-, Cl -, So42, No3- and SCN- (ca40 ppm each and electropherogram of a solution of stem of *S. persica*). [14]

Miswak prepared from *S. persica* roots and stems was analysed for soluble and total content of fluoride, calcium, phosphorus and silica. The results showed that the fluoride released from miswak soaked in water was negligible (< 0, 07 μg/ml). Approximately 39% of the total fluoride in the sticks was in a form that could be leached out. The leached calcium and phosphorus averaged 582 μg/ml and 34 g/ml, respectively, representing 19.6% and 26.4% of their total content in the sticks. There was a substantial amount of silica in the ashes of miswak.

Samples of *S. persica* obtained from Saudi Arabia and Lebanon were prepared for analysis using scanning electron microscopy (SEM), X-ray fluorescence (XRF), electron probe microanalysis (EPMA) and wavelength dispersive spectrometer (WDX). [15] XRF study indicated the presence of calcium and trace amount of phosphorous and fluorine. EPMA supported these findings by showing that calcium was present within the circular objects. SEM revealed that circular objects ≤ 5 microns in diameter abound in the lignified cell structures in the *S. persica*. Rhombohedral crystals were found in the central portion of the branch.

Ideas on the influence of the environmental factors on the chemical compositions of organs in plants, animals and man have existed for a long time. [16] The content of different elements in the plants can give valuable information on the need for fertilisers in agricultural investigations. Great differences in chemical composition also occur between different plant organs. The age of tissues has an effect on the chemical composition of the plant and also the physical growth factors. No specific literature could be found on this topic with respect to *S. persica*

Biologically active compounds

The substantial amount of silica detected in *S. persica* ashes has been thought to contribute to miswak's mechanical action in dental plaque removal. Benzylisothiocyanate is a component exhibiting antiviral and antimycotic activity of miswak extracts but its mode of action was not clearly delineated. Moreover, it has been speculated that the high amount of NaCl and KCl, sulphur-containing organic substances (salvadourea and salvadorine), and an alkaloid yielding trimethylamine on hydrolytic cleavage might somehow be responsible for the observed antibacterial and gum stimulating effect. [17, 18] Decoction of *S. persica* has been used for the treatment of spleenomegaly, rheumatism, tumors, and renal stones in humans by folk medicine practitioners. [19] It was also shown to possess hypoglycemic effects and an incremented oral-glucose tolerance in normal rats, and to enhance plasma immune-reactive insulin level.[20]

A protective action of *S. persica* decoction against ethanol and stress-induced ulcers has been observed in rats. [21] Extracts from the roots and stems of *S. persica* have been used for treatment of oral infections in animals. In humans, miswak extracts have been shown to induce morphological changes in tissue culture using L929 cells and IgE-mediated allergic reactions in humans. [22] However, fresh plant materials from *S. persica* demonstrated no cytotoxic effect in a mammalian monolayer cell culture while cytotoxicity was

demonstrated after 24 hrs. [23] Recent studies showed that aqueous and ethanol extracts of *S. persica* were able to remove the smear layer from dentin surfaces and occlude dentine tubules. [24]

Antimicrobial activity

Some *in vitro* studies have shown that *S. persica* extracts inhibited growth of various oral aerobic and anaerobeic bacteria as well as *Candida albicans*. [25] Inhibition of *in vitro* plaque formation, growth and acid production of various cariogenic bacteria by such extracts has also been demonstrated. Moreover, it is found that aqueous extracts of *S. persica* bark, pulp as well as whole miswak were effective against various bacteria including *Streptococcus mutans*. Some differences were noted in antimicrobial activities between the pulp and bark extracts. *S. persica* alcoholic extracts were more antimicrobial potent than water extracts. Regarding the effect of storage on the activity of miswak extracts, there was no noticeable difference in antimicrobial effect between fresh and one-month-old miswak. [26] When extracts prepared from *S. persica* and *A. indica* (neem) chewing sticks were compared, both were able to suppress growth of *S. mutans* and *Streptococcus faecalis* at 50% concentration, only the *S. persica* extract was reported to be more effective at lower concentrations. Various *S. persica* extracts showed *in vitro* that root alcoholic extracts were more amtimicrobially potent against *S. mutans* whereas *Lactobacillus acidophilus* was the least susceptible one and that minmum inhibitory concentration values for the various *S. persica* extracts ranged from 100 mg/ml to 300 mg/ml indicating moderate to weak antimicrobial activity. Another recent study showed that renal transplant patients that used miswak had significantly lower prevalence of oral candidiasis than had such patients using modern toothbrush. [27, 28] For more information see Tables 1 and 2 which show extracts of different parts of *S. persica* and the antibacterial activities.

ORAL HEALTH BENEFITS OF MISWAK

Resources for oral health care are limited in many developing countries and the need to explore and test easily available and inexpensive traditional preventive tools is recognized and supported. [29, 30} In line with these, the present review was carried out to better understand the clinical effectiveness and antimicrobial and chemical properties of miswak as an alternative method to the modern toothbrush for oral health promotion and disease prevention. Recently, it has been demonstrated that the commercially available dentifrice (Whitening Toothpaste) which contains 10% of silica is efficacious for control of supragingival calculus formation. [31] Thus, it has been demonstrated that miswak contained substantial amount of silica which can benefit removal of calculus. Several of the anionic components detected in miswak are known to have antimicrobial effects. For instance, nitrite exerted *in vitro* inhibitory effects against cariogenic bacteria including *S. mutans*, *L. casei* and *A. naeslundii* at acidic pH. The authors also demonstrated that the ability of these bacteria to recover from nitrite exposure was markedly affected by nitrite concentrations. At acidity levels below pH 7, low concentrations of nitrite (0.2 mM) caused complete killing of the test bacteria. [32] Miswak significantly decreased salivary pH due to its high chloride content. This condition may increase the bactericidal effect of nitrite in the mouth. Moreover, the bactericidal effect of nitrite is significantly enhanced by SCN^-. Chemical analysis of miswak indicated that the root and stem are rich in SO_4^{2-}. The presence of SO_4^{2-} compounds in the root has previously been reported. Antibacterial and weak anti-inflammatory effects of miswak extracts have been attributed to their content of beta-sitosterol, SO_4^{2-} compounds and Cl^-. {33] In addition, Cl^- leaching into saliva from miswak while in the mouth may mediate the innate host defense systems in human saliva. Cl^-, I^- and SCN^- (pseudohalides) are substrates for salivary peroxidase and/or the myeloperoxidase hydrogen peroxide antimicrobial system. The peroxidase-hydrogen-peroxide-chloride system is a part of the innate host defense that is mediated by polymorphonuclear leukocytes in humans. [34] It has been shown that the latter system was more bactericidal against *A. actinomycetemcomitans* than with the myeloperoxidase-thiocyanate and hydrogen peroxide system. Recently, it has been indicated that the oxidation product of lactoperoxidase and myloperoxidase with I^- and/or Cl^- was bactericidal against *P. gingivalis, F. nucleatum* and *S. mutans*. [35] The lower levels of *P. intermedia* and *F. nucleatum* in the miswak users may be attributed to its Cl^- and SCN^- content.[36]

NO_3^- in miswak water extracts may be released from the residual nitrate ions taken up by the *S. persica* plant or from the oxidation of ammonia and other nitrogen compounds. Nitrate, nitrite and nitrosamines have been demonstrated to occur naturally in vegetables. The antimicrobial agent nitric oxide (NO) is formed in the mouth and its concentration is directly related to salivary nitrite, which in turn is related to dietary nitrate intake. Nitrite, upon its ingestion and mixture with gastric acid, is a potent bacteristatic and/or bactericidal agent. Acidified nitrite is bactericidal against gastrointestinal, oral, and skin pathogens. *In vitro* the acidified nitrite exhibited growth-inhibitory effect on *F. nucleatum, E. corrodens* and *P. gingivalis*. Furthermore, it has been shown that the salivary nitrite exerted bactericidal effect on several pathogens such as *Escherichia coli, Salmonella typhimurium, Yersinia entrocolitica, Shigella sonnei* and *C. albicans* at acidic pH. [37, 38]

The bactericidal effect of acidified nitrite is due to the production of NO and nitrogen dioxide (NO_2). However, the real mechanisms of the bactericidal action and the chemistry of acidified nitrite are still unclear. Another possibility is that nitrite, in synergy with acid in the stomach, mouth, or skin may be an element of innate immunity. [39]

The salivary generation of nitrite is accomplished by a symbiotic relationship involving nitrate-reducing bacteria on the tongue surface, which are designated to provide a host defense against microbial pathogens in the mouth and lower gut via NO production. Thus, the finding that miswak extract exhibited antimicrobial effects against *S. faecalis*, *P. aeruginosa*, *Actinomyces* spp. and *Staph*. *Aureus* may be due to its nitrate and high Cl^- content that creates acidified conditions for antimicrobial action of nitrate similar to that in the mouth and the stomach.

SCN^- leaching out from miswak while in the oral cavity may lead to an elevated level of salivary SCN^-. This in turn may enhance the efficacy of the salivary peroxidase-thiocyanate and hydrogen peroxide system, a known innate antimicrobial component of human saliva. There are data showing that the most susceptible bacteria to this antimicrobial system are the oral streptococci, whereas other anaerobic oral bacteria (*C. sputigena, C. gingivalis, P. gingivalis, E. corrodens, S. sputigena P. micros, T. denticola,* and *C. rectus*) were less affected. [34] SCN^- in human saliva acts as a substrate for salivary peroxidase to generate hypothiocyanite ($OSCN^-$) in the presence of hydrogen peroxide (H_2O_2) produced by oral bacteria and leukocytes. $OSCN^-$ has antibacterial properties and other important protective functions for the host such as oral mucosal cells from the toxic accumulation of H_2O_2 by converting it into non-toxic $OSCN^-$.

In saliva up to 5mM endogenous SCN^- is secreted by the salivary glands per day as the result of catabolism of thiols. [40] Rather high concentrations of SCN^- in aqueous extract of miswak were found. Thus, miswak exposed to saliva during use may provide saliva with adjunct SCN^- which may serve as an external source for the salivary peroxidase-thiocyanate and hydrogen peroxide antimicrobial system. It has been claimed that a dentifrice containing 0.5% SCN^- and 0.1% H_2O_2 inhibited dental plaque and gingivitis. [41]

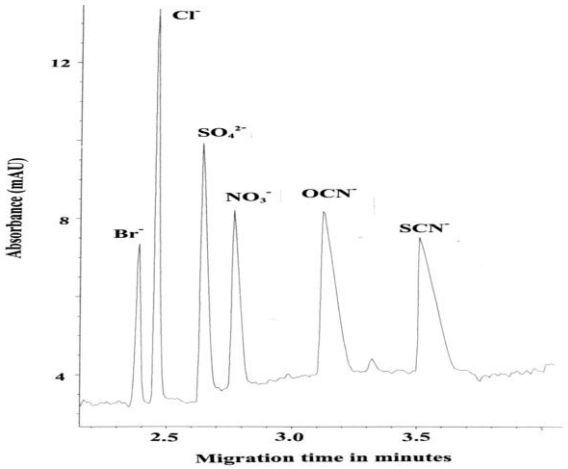

Fig. 1. Electropherogram of a mixture of Br-, Cl -, So42, No3- and SCN- (ca 40 ppm each).

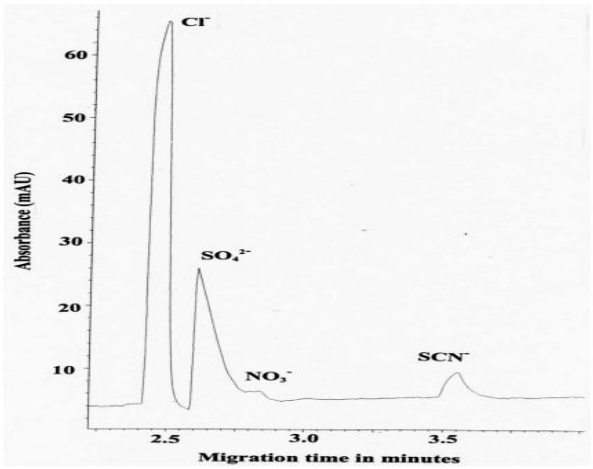

Fig. 2. Electropherogram of a solution of stem of *S. persica.*

***Table 1 Antimicrobial activity of different* S. persica exatracts**

Stem extracts	Incubation periods			Microorganisms
	24 hrs	**48 hrs**	**72 hrs**	
Ethanol (96%)	Reduction	Reduction	Not carried out	*C. albicans*
	Inhibition	Inhibition	Not carried out	*A. naeslundii* and *A. actinomycetemcomitans*
	Enhancement	Enhancement	Not carried out	*L. acidophilus*
	Enhancement	Inhibition	Not carried out	*S. mutans*
	Not carried out	Inhibition	Inhibition	*P. gingivalis*
Water	Reduction	Reduction only at highest concentration otherwise no effect	Not carried out	*C. albicans*
	Enhancement	Reduction	Not carried out	*A. naeslundii*
	Enhancement	Enhancement	Not carried out	*L. acidophilus*
	Enhancement	Reduction	Not carried out	*S. mutans*
	Enhancement	Enhancement	Not carried out	*A. actinomycetemcomitans*
	Not carried out	Inhibition	Inhibition	*P. gingivalis*
Ethyl acetate	Reduction	Enhancement	Not carried out	*C. albicans*
	Inhibition	Inhibition	Not carried out	*A. naeslundii, S. mutans* and *A. actinomycetemcomitans*
	Enhancement	Enhancement	Not carried out	*L. acidophilus*
Acetic acid (2%)	Inhibition	Inhibition	Not carried out	*C. albicans, A . naeslundii, S. mutans* and *A. actinomycetemcomitans*

Table 2 Antimicrobial activity of different *S. persica* exatracts continue.

Root Extracts	Incubation periods			Microorganisms
	24 hrs	48 hrs	72 hrs	
Ethanol (96%)	Inhibition	Inhibition	Not carried out	*C. albicans, A . naeslundii, S. mutans, L. acidophilus,* and *A. actinomycetemcomitans*
	Not carried out	Inhibition	Inhibition	*P. gingivalis*
Water	Enhancement	Reduction in the highest concentration otherwise no effect	Not carried out	*C. albicans*
	Inhibition	Inhibition	Not carried out	*A. naeslundii* and *A. actinomycetemcomitans*
	Enhancement	Enhancement	Not carried out	*L. acidophilus*
	No growth indicated	Inhibition	Not carried out	*S. mutans*
	Not carried out	Inhibition	Inhibition	*P. gingivalis*
Ethyl acetate	Enhancement	Enhancement	Not carried out	*C. albicans* and *L. acidophilus*
	Inhibition	Inhibition	Not carried out	*S. mutans, A. naeslundii* and *A. actinomycetmcomitans*
Acetic acid (2%)	Enhancement	Enhancement	Not carried out	*C. albicans* and *L. acidophilus*
	Inhibition	Inhibition	Not carried out	*S. mutans, A. naeslundii* and *A. actinomycetmcomitans*
	Not carried out	Enhancement	Enhancement	*P. gingivalis*

CONCLUSSIONS

1. Investigation of the chemical and biologically active components of miswak may lead to better understanding the clinical effectiveness and antimicrobial properties of miswak as an alternative method to the modern toothbrush for oral health promotion.
2. Miswak extracts contained potential antimicrobial components including chlorides, sulphates, thiocyanate, and nitrate oxides. Benzylisothiocyanate is a component exhibiting antiviral and antimycotic activity in miswak extract.
3. The free and bound thiocyanate exist in miswak root and stem extracts which may serve as an external source for the salivary peroxidase-thiocyanate and hydrogen peroxide antimicrobial system.
4. Miswak contains substantial amount of silica which can benefit removal of calculus.

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