Pharmaceutical Biology

ISSN: 1388-0209 (Print) 1744-5116 (Online) Journal homepage: informahealthcare.com/journals/iphb20

Volatile Compounds in Crude Salvadora persica Extracts

Howaida F. AbdELRahman, Nils Skaug, Ann Margot Whyatt & George W. Francis

To cite this article: Howaida F. AbdELRahman, Nils Skaug, Ann Margot Whyatt & George W. Francis (2003) Volatile Compounds in Crude Salvadora persica Extracts, Pharmaceutical Biology, 41:6, 399-404, DOI: 10.1076/phbi.41.6.399.17826

To link to this article: https://doi.org/10.1076/phbi.41.6.399.17826

Published online: 29 Sep 2008.

Pharmaceutical Biology
2003, Vol. 41, No. 6, pp. 399–404

1388-0209/03/4106-399$16.00

Volatile Compounds in Crude *Salvadora persica* Extracts

Howaida F. AbdELRahman[1], Nils Skaug[2], Ann Margot Whyatt[3] and George W. Francis[3]

[1]Centre for International Health, University of Bergen, Norway; [2]Institute of Odontology – Oral Microbiology, University of Bergen, Norway; [3]Department of Chemistry, University of Bergen, Norway

Abstract

Miswak is a widely used chewing stick in Middle Eastern and African cultures that is prepared from twigs and roots of the plant *Salvadora persica* L. It has been reported to inhibit dental diseases. Crude extracts of *S. persica* twigs and roots have demonstrated *in vitro* antimicrobial effects. The aim of the present study was to study the chemical composition of such extracts. Chloroform and ethanol Soxhlet extracts were investigated by gas chromatography-mass spectrometry in order to identify volatile organic components. This study is the first to report the presence of oleic, linolic and stearic acids in this plant. This work has led to the identification of a variety of low molecular weight compounds most of which are simple secondary metabolites. Among the compounds identified are esters of fatty acids and of aromatic acids, and some terpenoids. Storage of *S. persica* twigs for two years prior to extraction did not seem to affect the result of the chemical analysis greatly.

Keywords: Arak, chewing sticks, gas chromatography-mass spectrometry, miswak, phytochemistry, *Salvadora persica* L., siwak.

Introduction

Extracts and decoctions of the shrub *Salvadora persica* L. (Salvadoraceae), often called the arak tree, have shown a variety of biological effects and have been used therapeutically and prophylactically for many diseases (Saeed, 1988; Wu et al., 2001). A protective action of *S. persica* decoction against ethanol- and stress-induced ulcers was recently observed in rats (Sanogo et al., 1999). The use of chewing sticks (miswak) prepared from the twigs and roots of indigenous plants is widespread in Middle Eastern, some Asian and African cultures (Hardie & Ahmed, 1995; Wu et al., 2001). The utilisation of *S. persica* for this purpose is particularly prevalent and the resulting miswak has been reported to have beneficial effects on dental health (Hardie & Ahmed, 1995; Wu et al., 2001). The broad acceptance of miswak as such is founded to a great extent on the fact that Islam has incorporated dental hygiene as a part of religious practice and specifically recommends miswak for this purpose (Saeed, 1988; Ra'ed & Almas, 1999; http://www.islam.tc/Miswaak/ – 22-08-2003). The continuing use of and research on miswak find support in epidemiological and clinical studies (Wu et al., 2001), and recent international recommendations on dental hygiene (WHO, 1987; Anonymous, 2000).

The chemical constituents of *S. persica* have previously been investigated by a number of workers using different methods (Chan et al., 1987; Saeed, 1988; Kamel et al., 1992; Galletti et al., 1993; Darout et al., 2000). These studies have examined a variety of compounds, particularly those of a more polar nature. *In vitro* studies have shown various antimicrobial activities of *S. persica* extracts (Saeed, 1988; Abo AlSamh & Al-Bagieh, 1996; Al-Bagieh & Almas, 1997; Almas, 1999; Almas et al., 1997; Almas & Al-Bagieh, 1999; AbdELRahman et al., 2002; Al-Mohaya et al., 2002). A recent *in vivo* study (Al-Mohaya et al., 2002) showed that renal transplant patients who used miswak had a significantly lower prevalence of oral candidiasis than had such patients using modern toothbrushes.

The present work is based on our *in vitro* investigation on the efficacy of *S. persica* extracts in inhibiting growth of the oral pathogens *Candida albicans*, *Streptococcus mutans*, *Actinobacillus actinomycetemcomitans*, *Lactobacillus acidophilus*, *Actinomyces naeslundii* and *Prophyromonas gingivalis* (AbdELRahman et al., 2002). The results obtained were sufficiently promising as to encourage chemical investigation of the extracts used in order to search for the compounds responsible for the antimicrobial activity. The present report provides results relating to the somewhat less polar and more volatile components identified. This study focused

Accepted: April 9, 2003

Address correspondence to: Howaida Faisal AbdELRahman, Centre for International Health, University of Bergen, Armauer Hansens Hus, 5021 Bergen, Norway. Fax: 004755974689; E-mail: howaida.abdelrahman@cih.uib.no

mainly on twigs since they are the part of the plant usually used in Sudan. Roots and seeds were also included for the sake of determining which parts of the plant are richest in the active components and could thus provide natural material for use in further studies.

Materials and methods

Salvadora persica authenticity, collection, preparation and extraction

Roots, twigs and seeds of *S. persica* were collected at Khor Adeit, Sinkat in north-eastern Sudan in July 2001, with additional material being collected at the same location in July 1999. The location for collection was recommended by two plant taxonomists from the Department of Botany, University of Khartoum and from the School of Life Sciences, University of ElNeelen, Sudan on the basis that the plant is the dominant species in the area (ElAmin, 1990). An agriculturist stationed in the area and knowledgeable local inhabitants helped in collection. The plant material was then air-dried in a shaded place for 7 days and 1 month later air-shipped to the University of Bergen (UoB), Bergen, Norway.

Preparation of plant extracts

Soon after the air-dried material was received at UoB, it was cut into manageable pieces and ground or chopped to a fine powder.

Soxhlet extraction of twigs

The twig powder (12 g) contained in a paper thimble was extracted in either of the two solvents, 96% ethanol or chloroform (200 ml in each case), using a Soxhlet extraction procedure for 4 h. The solvents were reduced in volume under reduced pressure at 35–38 °C. After examination of the results of the above Soxhlet extracts, a new sample was examined using similar but consecutive extraction with chloroform and ethanol. Each extraction was carried out for a period of 4 h with intermediate air-drying.

Fractionation of twig and root extracts

Both the twigs and the roots in the powder form were fractionated separately as follows: 5 g of each plant powder was dissolved in 50 ml methanol : water (4 : 1) solution for 5 min using a magnetic stirrer (Harbone & Turner, 1984). The volume of each supernatant was then reduced to approximately 1/10 under vacuum, and then acidified with 2 M H_2SO_4. The concentrated supernatants were then extracted with $CHCl_3$ using three times the reduced volume. The chloroform layer (MeOH-$CHCl_3$) was separated and reduced in volume under reduced pressure as shown above.

The aqueous acidic layer of each sample was neutralised using concentrated ammonium hydroxide (NH_4OH), which was added dropwise until a dark precipitate was formed. These precipitates were then centrifuged at 15,800 × g for 10 min and washed with 1% NH_4OH. The residue was dissolved in a few drops of ethanol and $CHCl_3$ at a final ratio of (1 : 1). This fraction will be referred to as (MeOH-aq. acid) during this study.

Gas chromatographic-mass spectrometric analysis

The chemical investigation of *S. persica* was largely carried out on samples that were collected in July 2001. Twigs collected in 1999 were available and this provided raw material for extraction and identification of components retained in the plant after two years in storage. The dry residues of the Soxhlet extracts and the chloroform fraction (MeOH-$CHCl_3$) were then re-dissolved in the same solvent to give samples for gas chromatography-mass spectrometry (GC-MS) having a concentration of approximately 0.001 M. The re-dissolved precipitates from the (MeOH-aq. acid) layers were also examined using GC-MS in the manner described above.

The GC-MS analysis was performed using a Hewlett-Packard HP 5890 series II chromatograph interfaced with a Fisons VG-7070E mass spectrometer. The gas chromatography column was a Chrompack WCOT fused silica column (30 m × 0.25 mm) with a CP-Sil 8 CB Low Bleed MS film (thickness 0.25 µm). Helium was used as carrier gas with a flow rate of 0.7 ml/min and temperature programming employed during the analysis. An injector temperature of 290 °C was used, and the initial oven temperature of 50 °C was maintained for a period of 3 min after injection of each sample (1 µl). Thereafter a heating rate of 5 °C/min was applied until a temperature of 250 °C was reached, at which time the heating rate was increased to 20 °C/min to arrive at a temperature of 300 °C which was then maintained for 14.5 min.

The mass spectrometer was operated in the EI mode and mass spectra were obtained at 70 eV, 100 µA using a source temperature of 220 °C and a pressure of approximately 2×10^{-6} Torr throughout the analysis.

Compounds were identified by their characteristic mass spectrometric fragmentation patterns either by direct automatic comparison with library spectra, the instrument used has direct access to a NIST spectral library, or through comparison with established spectra available in the literature for similar compounds or through the following websites (http://webbook.nist.gov/chemistry/ and http://www.aist.go.jp/RIODB/SDBS/menu-e.html – 22-08-2003).

Results and discussion

Yields

Soxhlet extractions of twigs

Chloroform extract = 0.24 g (2%); ethanol extract = 0.71 g (5.9%)

Table 1. Comparison between the compounds identified in the Soxhlet chloroform-extract and Soxhlet ethanol extract of the twigs of *Salvadora persica*.

Compound name MF	MW	RT	Relative Abundance Chloroformic extract	 Ethanolic extract
cis-2-Methylcyclohexanol $C_7H_{14}O$	114	4:04	–	+
Benzaldehyde C_7H_6O	106	7:19	–	++
Chloromethylbezene C_7H_7Cl	147	8:49	–	+
Benzyl alcohol C_7H_8O	108	9:44	–	+
N-Acetylpiperidine $C_7H_{13}NO$	127	9:58	–	+
3-Methyl–2-furancarboxylic acid $C_6H_6O_3$	126	11:10	–	+
Nonanal $C_9H_{18}O$	142	11:39	+	–
(*E*)-2-Octenol $C_8H_{14}O$	126	12:04	–	+++
Indole C_8H_7N	117	12:49	–	++
2,3-Dihydro-3,5-dihydroxy-6 methyl-[4H]-pyran-4-one $C_6H_8O_4$	144	13:27	–	+
Tetrahydro-[4H]-pyran-4-one $C_5H_8O_2$	100	14:20	–	++
Benzoic acid pentyl ester $C_{12}H_{16}O_2$	192	15:36	–	++
Pyridine derivative	*	17:56	–	+++
Pyridine derivative	*	19:00	–	+
Tetradecene $C_{14}H_{28}$	196	19:59	–	+++
Unknown	*	22:19	–	+
Unknown	*	25:02	+	–
Octadecanol $C_{18}H_{38}O$	270	25:08	–	+++
3,4,5-Trimethoxyphenol $C_9H_{12}O_4$	184	26:19	–	++
Tetradecanoic acid $C_{14}H_{22}O_2$	228	29:19	+	–
(E)-9-Octadecenoic acid $C_{18}H_{34}O_2$	282	29:44	–	+++
9,12-Octadecadienoic acid $C_{18}H_{32}O_2$	280	30:40	+	++
Hexadecanoic acid $C_{16}H_{32}O_2$	256	33:52	+++	–
(*Z*)-9-Octadecenoic acid $C_{18}H_{34}O_2$	282	33:55	–	+++
(*Z*)-9-Octadecenoic acid methyl ester $C_{19}H_{36}O_2$	296	37:18	+++	+++
(*Z*)-9-Octadecenoic acid ethyl ester $C_{20}H_{38}O_2$	310	37:43	–	++
Eicosanol $C_{20}H_{42}O$	298	40:28	+	–

◆◆ Steroids observed after retention time 40 min were not identified.

Compound names (*indicates unknown compound and/or unknown molecular weights (MW), (molecular formula (MF), retention times (RT) and relative abundance (main component +++, moderately abundant component ++, minor component +, compound absent – in extract) are shown in each table. Gas-chromatography column: Chrompack WCOT fused silica (30 m × 0.25 mm) with a CP-Sil 8 CB Low Bleed MS film (thickness 0.25 µm). Carrier gas: Helium 0.7 ml/min. Temperature programme: 50 °C isothermal for 3 min; 5 °C/min from 50 °C to 250 °C (requires 40 min); 20 °C/min from 250 °C to 300 °C (requires 2.5 min); 300 °C isothermal for 14.5 min.

Table 2A. Compounds found in the aqueous acid fraction of the methanol-water extract of the seeds, twigs and roots of *Salvadora persica*.

Compound name MF	MW	RT	Seed	Stem 99	Stem 01	Root
Benzaldehyde C_7H_6O	106	7:21	+	–	+	–
Indole C_8H_7N	117	12:46	++	–	–	+
2,3-Dihydro-3,5-dihydroxy-6-methyl-[4H]-pyran-4-one $C_6H_8O_4$	144	13:18	+	–	–	–
Phenylethanoic acid methyl ester $C_9H_{10}O_2$	150	13:50	+++	–	–	+
5-Hydroxymethyl-2-furancarboxaldhyde $C_6H_6O_3$	126	19:07	++	–	–	–
Dodecene $C_{12}H_{24}$	168	14:08	–	+	+	+
Phenylethanoic acid $C_8H_8O_2$	136	17:04	+	–	–	–
Tetradecene $C_{14}H_{28}$	196	19:54	+	+	++	+
1-Hexadecene $C_{16}H_{32}$	224	25:02	–	+	++	+
Dodecanoic acid methyl ester $C_{13}H_{26}O_2$	214	23:29	+	–	–	–
Tetradecanoic acid methyl ester $C_{15}H_{30}O_2$	242	28:14	+	–	–	–
1-Octadecene $C_{18}H_{38}$	252	29:38	–	+	+	+
Hexadecanoic acid methyl ester $C_{17}H_{34}O_2$	270	32:31	++	–	+	+++
Unknown	*	33:59	–	–	+	–
(E)-9-Octadecenoic acid methyl ester $C_{19}H_{38}O_2$	296	35:57	+++	–	+	+++
Tetracosane $C_{14}H_{50}$	336	33:49	–	–	+	+

◆◆ Steroids observed after retention time 40 min were not identified. These are only abundant in the twigs collected in 2001.
Compound names (*indicates unknown compound and/or unknown molecular weights (MW), (molecular formula (MF), retention times (RT) and relative abundance (main component +++, moderately abundant component ++, minor component +, compound absent – in extract) are shown in each table. Gas-chromatography column: Chrompack WCOT fused silica (30 m × 0.25 mm) with a CP-Sil 8 CB Low Bleed MS film (thickness 0.25 µm). Carrier gas: Helium 0.7 ml/min. Temperature programme: 50 °C isothermal for 3 min; 5 °C/min from 50 °C to 250 °C (requires 40 min); 20 °C/min from 250 °C to 300 °C (requires 2.5 min); 300 °C isothermal for 14.5 min.

GC-MS analysis

The main results of the GS-MS analysis are given in tabular form where compounds are identified by name, molecular weights are given and retention times noted. Table 1 represents the comparison between the analysis of the chloroform and ethanol Soxhlet extracts of the twigs. The comparison of the MeOH-aq. acid layer and MeOH-$CHCl_3$ layer between twigs, which was collected 1999 and 2001, roots and seeds is shown in Table 2A and 2B, respectively. The relative amounts of compound present are indicated in a qualitative way, but absolute measures are not given. The present investigations have revealed that material collected in different years and from different plant parts vary somewhat and we are anxious to avoid the use of absolute measures under these circumstances. Nevertheless, we should emphasise that the variations tend to be in quantity rather than in kind. The compounds identified are unexceptional in that they are for the most part esters of aliphatic and aromatic acids commonly found in plants (Harborne & Turner, 1984). However, the abundance of ethyl esters was not expected and is an unusual feature. Repeated trials do not suggest that this is an artefact and the finding may be significant in terms of the proven though relatively low antimicrobial activity of the extracts (AbdELRahman et al., 2002).

There are some expected differences between the chemical compounds found in analysis of the twigs which had been preserved for two years and those analysed in the year of

Table 2B. Compounds indicated in the chloroform fraction of the methanol-water extract of the seeds, twigs and roots of *Salvadora persica*.

Compound name MF	MW	RT	Seed	Stem 99	Stem 01	Root
4-Ketopentanoic acid methyl ester $C_6H_{10}O_3$	130	8:09	+	–	–	–
1,4-Butanedioc acid dimethyl ester $C_6H_{10}O_4$	146	9:32	+	–	–	–
Unknown	*	12:06	+	–	–	–
Indole C_8H_7N	117	12:57	+	–	–	+
2,3-Dihydro-3,5-dihydroxy-6-methyl-[4H]-pyran-4-one $C_6H_8O_4$	144	13:26	+	–	–	–
Phenylethanoic acid methyl ester $C_9H_{10}O_2$	150	13:57	+	–	–	–
Dodecanoic acid methyl ester $C_{13}H_{26}O_2$	214	23:35	+	–	–	–
Heptadecene $C_{17}H_{34}$	238	27:26	–	+	–	–
Tetradecanoic acid methyl ester $C_{15}H_{30}O_2$	242	28:20	+	–	–	–
9,12-Octadecadienoic acid $C_{18}H_{32}O_2$	280	30:47	–	–	+	–
Isomer of 9,12-Octadecadienoic acid $C_{18}H_{32}O_2$	280	30:49	–	–	+	
Eicosyne $C_{20}H_{38}$	278	31:45	–	+++	++	–
Eicosene $C_{20}H_{40}$	280	32:18	–	+++		
Hexadecanoic acid methyl ester $C_{17}H_{34}O_2$	270	32:40	++	–	–	++
Hexadecanoic acid $C_{16}H_{32}O_2$	256	33:24	+	++	+++	++
9,12-Octadecadienoic acid methyl ester $C_{19}H_{34}O_2$	294	35:57	+	–	–	+
(Z)-9-Octadecenoic acid ethyl ester $C_{20}H_{38}O_2$	310	36:05	–	–	–	++
Isomer of 9-Octadecenoic acid methyl ester $C_{19}H_{36}O_2$	296	36:11	+++	–	–	+
(Z)-9-Octadecenoic acid $C_{18}H_{34}O_4$	282	31:19	–	++	–	–
Isomer of 9-Octadecenoic acid methyl ester $C_{19}H_{36}O_2$	296	37:21	–	–	+++	++

◆◆ Steroids observed after retention time 40 min were not identified. They are most abundant in the stem and root extracts.
Compound names (*indicates unknown compound and/or unknown molecular weights (MW), (molecular formula (MF), retention times (RT) and relative abundance (main component +++, moderately abundant component ++, minor component +, compound absent – in extract) are shown in each table. Gas-chromatography column: Chrompack WCOT fused silica (30 m × 0.25 mm) with a CP-Sil 8 CB Low Bleed MS film (thickness 0.25 µm). Carrier gas: Helium 0.7 ml/min. Temperature programme: 50 °C isothermal for 3 min; 5 °C/min from 50 °C to 250 °C (requires 40 min); 20 °C/min from 250 °C to 300 °C (requires 2.5 min); 300 °C isothermal for 14.5 min.

collection. Components which are unstable, volatile or readily decompose were not detected in the twigs that were kept open in the store-room for two years. Such a compound is benzaldhyde which is converted in the presence of oxygen to benzoic acid (www.jtbaker.com/msds/b0696.htm – 07-08-2002). Compounds abundant in old twigs were detected, often as derivatives, in the newer samples. Thus, storage as well as the environmental factors (Låg, 1996) might play a role in the difference between the compounds detected in the two samples. According to the inhabitants of the area there was no rain in the period between the two times when plants materials were collected.

This study is the first study to report the presence of oleic, linolic and stearic acids in miswak. Our results supported the previous findings (Lewis & Elvin-Lewis, 1977; Chhabra et al., 1991; Galletti et al., 1993; Samuelsson et al., 1993) that miswak contains the following compounds: myristic, lauric and palmitic acids; lignin and polysaccharide derivatives of phenols and furans; sterols.

Conclusions

A chemical examination of extracts of roots and stems of *S. persica* has shown the presence of ethyl esters of fatty and other organic acids commonly found in plants, in addition to similar amounts of the corresponding methyl esters. The seeds contained the widest variety of compounds. The procedure applied for successive extraction using chloroform and then ethanol proved helpful in so far as it proved easy to identify more of the compounds detected. The possible significance of such compounds for the efficacy of the extracts deserves further investigation. Future investigations will be carried out into this possibility in parallel with continuing work on the components of the miswak extracts. Storage of *S. persica* twigs for two years prior to extraction did not seem to affect results of the chemical analysis greatly.

Acknowledgements

The authors would like to thank Lene H Søvik for her appreciated help during the laboratory work. This investigation was funded by the School of Dentistry and the Department of Chemistry, University of Bergen, Norway.

References

AbdELRahman HF, Skaug N, Francis GW (2002): *In vitro* antimicrobial effects of crude miswak extracts on oral pathogens. *Saudi Dent J 14*: 26–32.

Abo Al Samh DA, Al-Bagieh NH (1996): A study of the antimicrobial activity of the miswak ethanolic extract *in vitro. Biomed Lett 53*: 225–238.

Al-Bagieh NH, Almas K (1997): *In vitro* antimicrobial effects of aqueous and alcohol extracts of miswak (chewing sticks). *Cairo Dent J 13*: 221–224.

Al-Mohaya MA, Darwazeh A, Al-Khudair W (2002): Oral fungal colonization and oral candidiasis in renal transplant patients: The relationship to Miswak use. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod 93*: 455–460.

Almas K (1999): The antimicrobial effects of extracts of *Azadirachta indica* (Neem) and *Salvadora persica* (Arak) chewing sticks. *Indian J Dent Res 10*: 23–26.

Almas K, Al-Bagieh N, Akpata ES (1997): *In vitro* antimicrobial effects of freshly cut and 1-month-old miswak extracts. *Biomed Lett 56*: 145–149.

Almas K, Al-Bagieh NH (1999): The antimicrobial effects of bark and pulp extracts of miswak, *Salvadora persica*. *Biomed Lett 60*: 71–75.

Anonymous (2000): The proceedings of the FDI's second world conference on oral promotion: Consensus statement on oral hygiene. *Int Dent J 50*: 139.

Chan DCN, Dogen AU, Dogen MM (1987): SEM, XRF and EMPA evaluation of Middle Eastern toothbrush "*Salvadora persica*". *J Electron Microsc Tech 7*: 145.

Chhabra SC, Mahunnah RLA, Mshiu EN (1991): Plants used in traditional medicine in Eastern Tanzania. V. Angiosperms (Passifloraceae to Sapindaceae). *J Ethnopharmacol 33:* 143–157.

Darout IA, Christy A, Skaug N, Ekeberg PK (2000): Identification and quantification of some potentially antimicrobial anionic components in *Salvadora persica* extracts. *Indian J Pharmacol 32*: 11–14.

ElAmin HM (1990): *Trees and Shrubs of the Sudan*. Reading, Exeter: Ithaca Press, pp. 285–287.

Galletti GC, Chiavari G, Kahlie YD (1993): Pyrolysis/gas chromatography/ion trap mass spectrometry of the "Tooth Brush Tree" (*Salvadora persica* L.). *Rap Com Mass Spectrometry 7*: 651–655.

Harborne JB, Turner BL (1984): *Plant Chemosystematics, Hidden Metabolites*. London, Academic Press, pp. 180–215.

Hardie J, Ahmed K (1995): The miswak as an aid in oral hygiene. *J Phillip Dent Assoc 47*: 33–38.

Kamel MS, Ohtani K, Assaf MH, Kasal R, Elshanawani MA, Yamasaki K, Ali AA, Tanaka O (1992): Lignan glycosides from stems of *Salvadora persica*. *Phytochemistry 31*: 2469–2471.

Lewis WH, Elvin-Lewis MPF (1977): *Medical Botany: Plants Affecting Man's Health*. New York, Wiley, Oral Hygiene, pp. 266–270.

Låg J (1996): Relationships between chemical composition of biological tissues and geomedical problems. Chemical data of plant, animal and human tissues as basis of geomedical investigations. Oslo, Published with grant from Astri og Biger Torsteds legat, pp. 27–29.

Ra'ed IA, Almas K (1999): Miswak (chewing stick): A cultural and scientific heritage. *Saudi Dent J 11*: 80–87.

Saeed A (1988): *Salvadora persica*, Linn. (siwak) – its position and heritage in Islamic dentistry. *Hamdard Med 31*: 75–91.

Samuelsson G, Farah MH, Claeson P, Hagos M, Thulin M, Hedberg O, Warfa AM, Hassan AO, Elmi AH, Abdurahman AD, Elmi AS, Abdi YA, Alin MH (1993): Inventory of plants used in traditional medicine in Somailia IV. Plants of the families Passifloraceae-Zygophylaceae. *J Ethnopharmacol 38*: 1–29.

Sanogo R, Monforte MT, Daquino A, Rossitto A, Di Mauro D, Galati EM (1999): Antiulcer activity of *Salvadora persica L.*: structural modifications. *Phytomedicine 6*: 363–366.

World Health Organisation (1987): *Prevention of Oral Diseases*. Geneva, WHO Offset Publication No. 103, pp. 60–61.

Wu CD, Darout IA, Skaug N (2001): Chewing sticks: Timeless natural toothbrushes for oral cleansing. *J Perodont Res 36*: 275–284.