PHARMACOLOGICAL, THERAPEUTIC AND PHYTOCHEMICAL ATTRIBUTES OF "*SALVADORA PERSICA*" PLANTS

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ABSTRACT

Toothbrushes and dentifrices are widely used in the modern era for cleaning teeth. For centuries, twigs from medicinal plants were used as natural toothbrushes. Arabs, Greeks and Romans used twigs and chewing sticks as oral hygiene aids since 3500 BC [1]. The twigs of plants were considered teeth cleaners in the age of Babylonians, some 7,000 years ago [1,2]. Ancient Egyptians, Israelites, Romans [3] and Arabian ancestors utilized these twigs to make their teeth white and shiny. More than 180 plant species have been used as teeth cleaning and chewing twigs in different parts of the world. The most popular and most extensively used of these have been Miswak twigs from the *Salvadora persica* tree [4]. Miswak contains many phytochemical constituents, making it a good oral hygiene aid.

Dental *caries* and *periodontal* diseases are caused by bacterial plaque. Dental plaque removal is very effective in treating gingivitis [5]. Mechanical and chemical methods are available for the maintenance of dental plaque removal and oral health. The use of *Miswak* for oral hygiene serves a dual function, i.e., mechanical plaque control by friction between plant fibers and tooth surfaces and chemical plaque control due to its phytochemical composition [2]. The Miswak chewing stick removes plaque from interproximal sites as well as more accessible sites. [6] As *subgingival microbiota* is similar irrespective to gender and ethnicity, [7] the benefits of Miswak provide a means of progressive dental hygiene.

In 1986 and 2000, the World Health Organization (WHO) endorsed the use of Miswak twigs in an international consensus statement on oral hygiene for regions where its use is customary, and concluded that further research was required to document its effects [8-12]. Several pursuant studies suggest it contains a number of medically beneficial properties, including being antiseptic, astringent, abrasive, detergent, enzyme inhibiting and fluoridative [2,13-16].

Fresh samples of different parts of the *S. persica* tree were collected in replicate from different geographical locations, viz., Yemen, Sudan, Egypt, Algeria, Saudi Arabia, Syria, India and Pakistan, and were chemically analyzed. Phytochemical data found approximate qualitative agreement with other investigators while quantitative results varied slightly from location to location and season to season. Samples from Yemen, Sudan and Egypt showed slightly higher yields on average compared to yields in the samples from other countries.

S. persica is a large, well-branched, evergreen shrub or a tree resembling *Salvadora oleoides* found in the Middle East, Africa and the arid regions of India and Pakistan. *S. persica* has multiple reports of potential medicinal characteristics. *S. persica* is identified to have *antiurolithiatic* [17], *antiplaque*, *analgesic*, *anticonvulsant*, *antibacterial*, *antimycotic*, *cytotoxic*, *antifertility*, *deobstruent*, *carminative*, *diuretic*,

astringent, *hypolipidemic*, *antiulcerative, antibilious* and *antirheumatic* characteristics. This research will identify for the first time the pesticidal characteristics of *S. persica*. In addition, various *phytochemical*, *therapeutic* and *pharmacological* attributes, along with quantification of the chemical constituents containing *periodontal* health benefits from different parts of *Salvadora persica* tree are discussed in this paper.

Keywords: *Antibacterial*, *hypolipidemic*, *Miswak*, *Salvadora persica*

1. INTRODUCTION

Salvadora persica Linn., commonly known as Arak [18], *Galenia asiatica*, *Siwak* or *Meswak* or *Miswak*, Peelu, Pīlu, *Salvadora indica*, or toothbrush tree or mustard tree, is a species of *Salvadora* [19,20]. It is widely distributed in the Middle East, Africa and the arid regions of Indo-Pak sub-continent and often on saline soils too. The use of *Miswak* predates the inception of Islam, which was adhered to by the ancient Arabs to get their teeth white and shiny [2,21]. Elvin Lewis has mentioned that *Miswak* root and bark have been traditionally used over 1000 years as a chewing-stick or natural toothbrush to strengthen the gums [22]. The end of a pencil-sized stick is shaped into a brush through biting or chewing on one end, which serves to separate its fibers frayed into a brush and release the healing herbal powers of the twig.

The beneficial effects of *Miswak* with respect to oral hygiene and dental health are partially due to its mechanical action and partly owing to its pharmacological action. Some investigators have identified various chemical constituents in different parts of *Miswak* tree, which are responsible for its pharmacological activities, e.g., Farooqui, *et al.* isolated *benzyl isothiocyanate* from the root of *S. persica* and claimed to have found *saponins* along with *tannins*, silica, a small amount of resin, *trimethylamine* and a fairly large amount *alkaloidal* constituents [23]. Ray, *et al.* isolated *β-sitosterol* [Fig. 6], *m-anisic acid*, and *salvadourea* (1,3-Bis-(3-methoxy-benzyl)-urea) [24]. Lewis and Elvin-Lewis reported high minerals content of 27.06% in the root [25].

1.1 Botanical & Ecological Descriptions

1.1.1 Taxonomy

Kingdom: *Plantae* ***Division:*** *Magnoliphyta* ***Class:*** *Magnoliopsida* ***Authority:*** *L.* ***Order:*** *Brassicales* ***Family:*** *Salvadoraceae* ***Genus:*** *Salvadora* ***Species:*** *Salvadora persica persica oleoides* ***Unranked:*** *Angiosperms; Eudicots; Rosids* ***Synonym(s):*** *Salvadora cyclophylla Chiov., Salvadora indica Wight, Salvadora wightiana Planch* [26].

1.1.2 Common Names: *Aarak, Arak, Arrak, Arraka, el Rak, Kabats, Shaow, Shau, Aiwak, Siwak, Sewak, Swak* (Arabic); *Darakhte Miswak (Persian); Mustard tree, Salt bush, Toothbrush tree* (English); *Arbre a cure-dents, Arbre a frotte-dents* (French); *Rregte mosterdboom* (Afrikaans); *Msuake, Mswaki, Musuake* (Swahili); *Ade, Adhei* (Somali); *Mefaka* (Ethopian), *Mswaki* (Tanzanian), *Aday, Yeharer-mefaqya* (Amharic); *Adai, Hadai* (Tigrigna); *Kayu Sugi (Malay); Misvak* (Turkish); *סלוודורה פרסית* (Hebrew); *Koyoji* (Japanese), *Khakhin Kickni* (Marathi); *Pelu, Peelu, Pīlu, Peelo, Miswak, Meswak,* (Urdu); *Jhak, Harjal, Datun* (Hindi); *Kharijal* (Gujrati); *Jhal* (Bengali); *Kalawa, Karkol, Perungoli, Ughaiputtai, Vivay,* (Tamil); *Ghunia* (Telgu); *Goni-Mara* (Kanada); *Kotungo* (Oriya) [27,28].

Its generic name was given by a famous botanist, Dr. Laurent Garcin, in 1749 in the honor of Juan Salvadory Bosca (1598-1681), an apothecary of Barcelona. The true specimen of this species came from

Persia (Iran), as its specific name '*persica*' indicates and the abbreviation *L.* is used to indicate Carl Linnaeus (1707-1778), a Swedish botanist, physician and zoologist, the father of modern taxonomy. The Botanic Gardens Conservation International has counted totally eight *S. persica* plants in conservation [29].

1.1.3 Vegetal Characteristics

S. persica is *Phanerophyte*, upright evergreen shrub or a small tree, reaching normally to a maximum height of about 3 m. The main trunk, which is seldom > 1 foot in diameter, is erect or trailing with profusely branched, wide crown of crooked, straggling and drooping branches [Fig. 2] and has soft whitish yellow wood. The young branches are green and the bark is soft, scabrous and cracked, whitish with pendulous extremities. But, the bark of old stems is rugose, drooping, glabrous, terete, finely striate, shining and almost white [Fig. 1].

The leaves are light to dark green, sharp-tipped, somewhat fleshy, glaucous, 3.8-6.3×2-3.2 cm in size, oblong-elliptic lanceolate or ovate, less commonly rounded, obtuse and often mucronate at the apex, which is broadly tapering to rounded, and sometimes with wart-like glandular dense dots. The base is broadly tapering and usually acute. The main nerves are in 5-6 pairs. The petioles are 1.3-2.2 cm long and glabrous. The leaves grow in opposite pairs [Fig. 4]. The tree sheds the leaves from late December to January. The leaves break with a fine crisp crackle when stepped over.

The flowers are pedicellate, greenish-white or greenish-yellow, very small, slender-branched with 5-12.5 cm long axillary and terminal panicles, numerous in the upper axils, 1.5-3 mm long pedicels with bracts underneath, ovate and very caducous. The calyx is 1.25 mm long, glabrous, cleft halfway down with rounded lobes. The corolla is very thin, 3 mm long, deeply cleft, persistent, with 2.5 mm long lobes, oblong, obtuse and much reflexed. The stamens are shorter than corolla, but exserted owing to reflexed corolla lobes. The drupe is 3 mm in diameter, globose or round and smooth, which becomes red when ripe [Fig. 4]. The flowering period is usually from January to April.

The fruit is small, pea-sized, spherical berry, fleshy, single seeded, 5-10 mm in diameter and pink to scarlet in color when mature [Fig. 3]. And the seeds are (1-4 mm) sub-globose, smooth, semi-transparent when mature and turn from white to pink or purple-red. The root bark is light brown (sand like color) and the inner surface has even lighter brown shade.

Fig.1: *S. persica* (Tooth-stick)

Fig. 2: *S. persica* Tree

Fig. 3: *S. persica* Fruits

Fig. 4: ***S. persica*** **Leaves**

Fig. 5: ***S. persica*** **Dry Fruits**

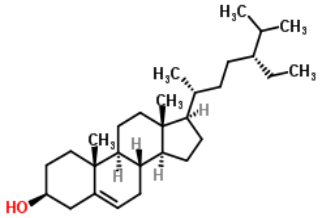

Fig. 6: β-sitosterol ($C_{29}H_{50}O$)

1.2 Distribution

1.2.1 Geographic Distribution

It is widely distributed in the Middle East and most of the African countries [30], like Algeria, Angola, Cameroon, Chad, Egypt, Eritrea, Ethiopia, Kenya, Libyan Arab Jamahiriya, Malawi, Mali, Mauritania, Mozambique, Niger, Nigeria, Republic of Sudan, Zambia, Zimbabwe, Senegal, Tanzania, Uganda, Somalia, South Africa, Kingdom of Saudi Arabia, The Hashemite Kingdom of Jordan, Syrian Arab Republic, Sultanate of Oman, Republic of Yemen, Islamic Republic of Iran, Israel, Republic of India, Islamic Republic of Pakistan and Sri Lanka [5,26,31,32].

1.2.2 Natural Habitat

S. persica is widespread, notably in thorn shrubs, desert floodplains, river and stream bank vegetation and grassy savannahs. It prefers areas where groundwater is readily available, e.g., by riverbanks, perimeters of waterholes, seasonally wet sites and along the drainage lines in the arid zones. It is also found in valleys dunes and termite mounds. *S. persica* is a *thermophilous* plant; hence able to tolerate a very dry environment with mean annual rainfall of less than 200 mm. It is highly salt tolerant and hence can also grow on coasts and inland saline soils [26]. In Pakistan, these sturdy trees are more closely associated with the graveyards.

1.2.3 Biophysical Limits

Altitude: 0-1800 m; **Rainfall:** 300-1000 mm; **Soil / Weather Type:** *S. persica* prefers clays, but also found on loam, black soils and sand. It is adapted to alkaline or very saline soils, usually clay-rich, and soils without salt. Apart from salt tolerance, it is drought resistant and well-adapted to arid conditions.

1.3 Botanical Management

1.3.1 Propagation & Seedling Management

S. persica readily germinates from seeds, which exhibit no dormancy but the fruit pulp contains germination inhibitors, which should be removed before sowing. The soaked and de-pulped seed usually germinates in 24-72 hours at 30-35°C, but under saline conditions the absorption of water depends upon the osmotic pressure of the medium and the cell sap. The seeds are raised in the nursery for up to 3 years prior to transplanting in the field [26].

1.3.2 Tree Management

S. persica is generally a slow-growing tree and grown in plantations or hedges. For high seed settings and seed oil content, harvesting is recommended 3 months after seed setting. It may be due to the

utilization of food reserve in the cotyledons for the development of fruit pulp. Coppicing is beneficial for the tree's usage as fuel. The branches are repeatedly pruned to produce short stems that are harvested for tooth-sticks [26].

1.3.3 Germplasm Management

The seeds of *S. persica* can be traditionally dried slowly under ambient conditions at 15-20% relative humidity (eRH) and stored with low moisture content. If the seeds are not properly dried and stored under optimal conditions, its storage life and viability is reduced. There are about 3400 seeds/kg [26].

Other useful literature referring to botanical, ecological and functional uses of *S. persica* are [33-51].

1.3.4 S. persica Twig Preservation

Some companies, such as Al-Khair, Al-Falah, Al-Areesh International, Sewak-us-Sunnah, Siwak As-Safa, Sewak Al-Khaleej, Sewak Al-Badr, Sewak Al-Harmain, Sewak Al-Muslim, Gowo Miswak Sticks, Miswak Club Stick, Baton De Miswak, Al-Jannatal Firdous Miswak, etc., have taken initiatives to process and preserve *S. persica* tooth-sticks in vacuum-packed sachets for increasing the twig's shelf life to a period of over six months without adding any preservative or *formaline*- soaking and use it for commercial purpose.

1.4 Traditional Uses of Various Parts of *S persica* Tree

Multiple functional uses of *S. persica* tree have been extensively reviewed in the literature [52-54]. Some traditional uses of different parts or products of the tree are as follows:

1.4.1 Food: Its fruit has a sweet, agreeable, aromatic, slightly pungent and peppery taste and can be eaten raw, cooked or dried and stored [Fig. 5]. When ripe, the fruit (with or without seeds) contains about 1.7-1.86% sugars. In some parts, fermented drink is reported to be made out of its fruits. The fresh leaves of *S. persica* are slightly bitter and aromatic with a mustard-like taste and cooked to be used as sauce and eaten with couscous or as green vegetable in the eastern tropical part of Africa. The tender shoots and leaves are eaten as salad too. Its seeds and seed oil are edible and edible salts are obtained from its ashes.

1.4.2 Fodder: The leaves and young shoots are browsed by all livestock but the cattle are usually not reared in the driest part of the *S. persica* distribution range. Hence, the leaves and young shoots are used as the fodder for the camels, sheep and goats. Due to high water content, between 15-36%, its leaves make a good forage. The high salt content in the leaves is believed to affect the taste of milk increase the lactation in cows.

1.4.3 Apiculture: *S. persica* is also reported as a good source of nectar.

1.4.4 Fuel: Sometimes the wood is used for firewood and charcoal but not used to cook the meat as it leaves a foul taste.

1.4.5 Timber: The wood being soft, white, easy to work, and above all safer to termite attack, is used for coffins and clubs.

1.4.6 Gum or Resin: The resin that drips from the tree is used in the production of varnishes.

1.4.7 Lipids: The seeds of *S. persica* have bitter taste and contain about 30-40% of a greenish-yellow non-edible oil that has > 50% of *lauric*, *myristic* and *palmitic* acids. The unrefined oil has unpleasant odor with high melting point but the low percentage of C_8 and C_{10} fatty acids in its oil is the most important aspect and of course of great economic significance. This oil is an alternative source of oil for soap and detergent industries.

1.4.8 Medicine: The roots and small branches of about 3-5 mm diameter of *S. persica* have been used as toothbrushes for oral hygiene for over thousands of years [3]. The chewing-stick from *S. persica* is an ancient traditional way to clean the teeth. The best *Siwak* is that taken from the subterranean roots of *S. persica*. It has a pleasant fragrance but pleasantly bitter taste. *S. persica* twig is still a popular chewing-stick throughout Arabian Peninsula and_the wider part of Muslim world [55] including India, Pakistan and African countries for its virtues quoted and extolled from the Prophet Muhammad (m.p.b.u.h.) [56]. Apart from strengthening the gums, it prevents tooth decay, eliminates toothaches and halts further decay that had already set in. It creates a fragrance in the mouth, eliminates bad odor, improves the sense of taste and causes the teeth to glow and shine [21].

Akhtar, *et al.,* have reported that other parts of the *S. persica* tree also have therapeutic values as corrective, *deobstruent*, liver tonic, *diuretic*, *analgesic*, *anthelmintic*, *astringent*, *lithontriptic*, *carminative*, *aphrodisiac*, and *stomachic* [21]. Al-Lafi, Homer and Almas, *et al.,* have demonstrated *in vitro* that the aqueous extracts of *S. persica* have growth inhibitory effects on several oral microorganisms [57-59]. Its natural antiseptics have a bactericidal action, killing harmful microorganisms in the mouth, the *tannic acid* it contains has astringent qualities, which protect the gums from disease, and its aromatic oils increase salivation. Because of its built-in antiseptics, the *S. persica* needs no cleaning.

Several biologically active chemical constituents identified in its bark and wood have been suggested to prevent dental *caries* [60] and the *antimicrobial* agents suppress the bacterial growth and formation of plaque. Apart from cleaning teeth, the tooth-stick made of its root relieves from toothache and gum diseases. Decoctions of *S. persica* leaves are used as a mouthwash and ground leaves for tooth and gum problems. The leaves are also used in traditional medicine for cough, asthma, scurvy, rheumatism, piles, de-obstruent, astringent to the bowels, tonic to the liver, diuretic, analgesic, anthelmintic, useful in ozoena and other nasal problems, scabies, leukoderma, lessening inflammation and strengthening of teeth. Its pungent leaves are considered as antidote to all sorts of poisons in Punjab, a northern state in India, and an external application in the treatment of rheumatism in Mumbai and Kokan belt on the southern west coast of India. The flowers are used as a stimulant and are mildly purgative.

The fruits of *S. persica* possess *deobstruent*, *carminative*, *diuretic*, *lithontriptic* and *stomachic* properties and used in biliousness and rheumatism. The stem bark is used as an ascarifuge and a remedy in gastric troubles. Herbal Practitioners (both Greek and *Ayurvedic*) have used the decoction of its root to treat gonorrhea, spleen sufferings, general stomachache, body pain, back pains, vesical catarrh and some chest diseases. Its roots are prepared as a salve and rubbed on the face for headaches. The paste of the root is also applied as a poultice or substitute to mustard plaster to heal boils and the latex from scratched bark is used for treating sores. The root bark is used as a vesicant and employed as an ingredient of snuff. A decoction of the bark is used as a tonic in amenorrhea, a stimulant in low fevers and an *emmenagogue*. The seeds of *S. persica* are used as purgative, diuretic and tonic and its oil is externally applied in case of rheumatism [61,62]. In Pakistani province of Sindh, it is believed that its fruit has curative effect on snakebites.

1.4.9 ***Shade & Shelter:*** *S. persica* is planted as shelterbelts and windbreaks to protect habitation of farms, gardens and orchards.

1.4.10 Reclamation & Anti-desertification: *S. persica* is planted for reclaiming sand dunes and saline soils. Dr. Rami Mohammed Diabi, an internist at the University of Kentucky Albert B. Chandler Hospital, Lexington, Kentucky, USA, reported that *S. persica* is contributing to fight against desertification, fixing the soil and affecting the environment and global climate [63,64].

1.4.11 Some Other Uses: Crusted leaves of *S. persica* with the leaves of *Pergularia tomentosa* were used earlier to remove the hair from tanned hides. Its roots contain a *urea* derivative, called *salvadourea.*

2. PHYTOCHEMICAL COMPOSITION & THEIR USEFUL IMPACTS

Different parts of the *S. persica* plant have shown various chemical components when analyzed by different methods [25,65-68]. The chemical and phytochemical analysis of *S. persica* root bark showed containing large amount of chlorine, c*arbohydrates* and/or a *tri-methylamine*, an *alkaloid*, which may effectively be *salvadorine*, and a resin [69]. Further investigations revealed it to contain chloride, sulfur, *terpenes*, vitamin C, *glycosides*, large amounts of fluoride and silica, small amounts of *tannins, saponins, flavonoids* and *sterols* [32,69,70]. El-Mostehy, *et al.*, also reported finding *Tri-methylamine*, an *alkaloid*, chlorides, high amounts of fluoride, Silica (SiO_2), Sulfur, vitamin C, and small amounts of *tannins*, *saponins*, *flavenoids* and *sterols* [71]. Manu Arora, *et al.*, isolated the flavonoids and flavonoids glycosides from *S. persica* [72].

Dr. William Carl, a Senior Cancer Dental Surgeon at Roswell Park Cancer Institute and Clinical Associate Professor of Fixed Prosthodontics at the UB School of Dental Medicine, reported that *S. persica* sticks not only serve as natural toothbrushes when used correctly, but contain oral health promoters, such as chlorides, fluoride, silica, vitamin C and *flavonoids* [16]. Some investigators listed the presence of *sinigrin*, *salicylic acid*, *calcium oxide* and *sodium bicarbonate* in the *S. persica* extracts. Attar also indicated that *S. persica* plant fibers contain *sodium bicarbonate* [73]. Akhtar and Ajmal found resin and large amounts of salts containing chlorine [70] and Chawla reported that *S. persica* contain a reasonable amount of fluoride [74].

When the extract of *S. persica* stem was subjected to more advance phytochemical investigations, it yielded *octacosanol*, *1-triacantanol*, *β-sitosterol*, and *β-sitosterol-3-O-β-D-glucopyranoside* [75]. During initial screening, Thin Layer Chromatography (TLC) revealed that the stem extract is a mixture of two compounds, which were later separated by Column Chromatography. One of the compound in this mixture in white needle shape was characterized having a melting point (m.p.) of 136-7ºC, *m/z* = 414 (mass) and molecular formula $C_{29}H_{50}O$ (C = 83.75%, H = 12.25%). It gave positive Salkowski, Liebermann, Burchard reaction, Noller reaction, Brieskron, Tschagajew and yellow color with *tetra-nitromethane* [76-78]. In Infrared (IR) spectrum, the peaks at V_{max} ^{KBr} 3500, 1450, 1470, and 1145 cm^{-1} identified this compound to be *β-sitosterol*. The other white crystalline compound was found having a m.p. of 265-68ºC, molecular formula $C_{35}H_{60}O^6$ (C = 72.9%, H = 14%) and *m/z* $[\alpha]_D^{29}$ -36.2 gave positive result for *saponin* and on hydrolysis yielded *β-sitosterol* and glucose, identified as *β-sitosterol-3-O-β-D-glucopyransoside*. The essential oil contained *α*- and *β-thujones*, *camphor*, *cineole*, *β-cymene*, *limonene*, *β-myrcene*, *borneol*, *linalool* and *bornyl acetate*, whereas the nonvolatile fraction contained *humulene*, *caryophyllene*, *β-santatol* and *farnesol* [79]. Farooqui and Srivastava reported that the chemical analysis of *S. persica* demonstrated the presence *β-sitosterol*, *m-anisic acid*, chlorides, *salvadourea*, *gypsum*, organic compounds (such as, *pyrrolidine*, *pyrrole*, and *piperidine derivatives*),

glycosides (such as, *salvadoside* and *salvadoraside*) and *flavonoids*, including *kaempferol*, *quercetin*, *rutin* and *quercetin glucoside* [23].

Khalil, has also isolated *Benzylamide*. The compounds isolated were identified as *butanediamide*, *N1, N4-bis(phenylmethyl)-2(S)-hydroxy-butanediamine*, *N-benzyl-2-phenylacetamide*, *N-benzyl-benzamide*, and *benzyl urea*. The compound *N-benzyl-2-phenylacetamide* has a significant inhibitory effect on human collagen-induced platelet aggregation and a moderate antibacterial characteristics against *Escherichia coli* [80]. Ezmirly, *et al.*, have isolated *glucotropaeolin* from *S. persica* [81]. Mohammed A., *et al.*, have found the root of *S. persica* containing a steam-distillable oil composed of 90% *benzyl isothiocyanate* and 10% *benzyl nitrate* [82]. Feras Alali, *et al.,* when subjected the volatile oil extracted from *S. persica L.* leaves to GC-MS analysis, could identify *benzyl nitrile*, *eugenol*, *thymol*, *isothymol*, *eucalyptol*, *isoterpinolene*, and *g-caryophyllene* [83].

However, the leaf galls contain heavy metals, which possess some growth promoting principles. Table 1 represents the metal contents in the Control and the Gall samples of *S. persica* plants from different countries of origin. The data obtained indicates generally higher contents of Iron (Fe), Copper (Cu), Zinc (Zn), Sulfur (S), Manganese (Mn), Cobalt (Co), Nickel (Ni), Cadmium (Cd), Molybdenum (Mo), Lead (Pb), Vanadium (V), Cerium (Ce), Titanium (Ti) and Mercury (Hg) in the galls when compared to their respective contents in the control. These metals are known to interact with biological systems and plant diseases, which result from the excess or the lack of the element(s) [84].

Table 1: Average Concentration of Metals in Control & Galls of *S. persica Plant* (ppm)

Sample Source / Metals	India/Pakistan		Egypt/Sudan		Algeria		Saudi Arabia/Syria		Yemen	
	Controls	Galls	Controls	Galls	Controls	Galls	Controls	Galls	Controls	Galls
Iron (Fe)	4.8	51.5	5.40	48.20	4.80	36.70	6.32	49.73	5.80	48.6
Copper (Cu)	5.7	7.3	7.5	8.7	5.9	6.8	7.3	8.2	8.6	9.5
Zinc (Zn)	4.6	4.8	4.7	4.6	4.8	4.9	5.2	5.1	6.3	6.2
Sulfur (S)	6.2	8.3	7.4	8.8	7.6	8.6	7.2	8.5	7.8	9.1
Manganese (Mn)	17.3	21.9	18.0	19.5	19.5	23.4	18.7	21.0	21.8	24.8
Cobalt (Co)	13.0	14.6	12.2	10.81	10.64	9.50	13.33	13.80	14.92	15.23
Nickel (Ni)	6.2	8.4	8.8	10.3	8.4	9.6	7.9	8.5	9.2	10.9
Cadmium (Cd)	0.18	0.20	0.18	0.19	0.20	0.21	0.28	0.30	0.26	0.25
Molybdenum (Mo)	0.25	0.50	0.26	0.35	0.18	0.22	0.31	0.53	0.42	0.62
Lead (Pb)	0.16	0.18	0.21	0.24	0.16	0.15	0.08	0.09	0.22	0.21
Vanadium (V)	0.005	0.14	0.003	0.006	0.003	0.004	0.063	0.052	0.092	0.097

	Cerium (Ce)	0.005	0.006	0.003	0.001	0.002	0.002	0.003	0.004	0.007	0.007
	Titanium (Ti)	00.00	0.005	00.00	00.00	00.00	0.000	0.042	0.040	0.060	0.063
	Mercury (Hg)	00.00	00.00	00.00	00.00	00.06	00.08	00.00	00.00	00.02	00.01

2.1 Seasonal Changes in the *Proteins* and *Amino Acid* Contents

Notable changes in *protein* contents and *amino acid* concentrations in all samples of *S. persica were observed during summer, winter and monsoon seasons*. Earlier Maggio, *et al.,* had also reported such changes after analyzing 12 samples from soil (0-15) and plant materials every month from three saline habitats of *S. persica* near Bhavnagar, India. The results obtained in the samples of *S. persica* plant for *Asparagine*, *Aspartic*, *Alanine*, *Arginine*, *Glycine*, *Glutalic Acid*, *Glutamina*, *Isoleucine*, *Leucin*, *Prolin* and *Valin* are presented in Tables 2; whereas, Table 3 represents the seasonal variation of the mineral contents (Meq g^{-1} d.wt.) in *S. persica* leaf and stem. Minerals determined were Sodium (Na^{+}), Potassium (K^{+}), Calcium (Ca^{+2}), Magnesium (Mg^{+2}), Chloride(Cl^{-}) and Ash [85-87]. The range of variations in average concentrations found in the samples from different countries of origin, viz., Yemen, Sudan, Egypt, Algeria, Saudi Arabia, Syria, India and Pakistan, are indicated by ± values.

Table 2: Seasonal Variations in Average *Proteins* & *Amino Acid* Contents in *S. persica Plant*

Proteins / Amino Acids	**Stems**			**Leaves**		
	Summer	**Monsoon**	**Winter**	**Summer**	**Monsoon**	**Winter**
Asparagine	370±105	336±81	268±94	279±92	184±46	165±35
Aspartic	445±72	619±88	356±76	432±76	310±48	504±172
Alanine	160±21	122±23	98±10	226±25	232±34	180±26
Arginine	95±34	59±26	133±44	136±62	76±33	129±44
Glycine	33±10	35±5	27±7	56±17	56±17	83±26
Glutalic Acid	68±12	56±14	61±15	94±24	93±11	111±28
Glutamina	67±13	55±18	65±12	98±23	90±12	110±27
Isoleucine	59±23	54±13	21±6	89±34	66±16	62±17
Leucin	78±24	63±11	23±8	110±46	79±15	76±26
Prolin	26±9	29±4	22±5	40±6	35±10	24±9
Valin	44±12	190±25	41±11	47±19	77±14	65±25

Table 3: Seasonal Variations of Average Mineral Ions in *S. persica Leaf & Stem* (Meq g^{-1} d.wt.)

Plant Parts / Seasons / Ions	**Stems**			**Leaves**		
	Summer	**Monsoon**	**Winter**	**Summer**	**Monsoon**	**Winter**
Na^{+}	1.16±0.16	0.71±0.35	0.72±0.03	2.66±0.70	1.50±0.35	1.87±0.19
K^{+}	0.49±0.06	0.39±0.07	0.39±0.04	0.23±0.06	0.31±0.05	0.20±0.02
Na/K	0.05	0.04	0.03	0.26±0.04	0.07±0.03	0.14±0.03
Ca^{+2}	1.58±0.13	0.16±0.14	0.45±0.16	2.64±0.11	2.31±0.04	2.38±0.05
Mg^{+2}	0.66±0.08	0.53±1.05	0.64±0.09	1.51±0.26	0.94±0.06	1.14±0.15
Cl^{-}	2.10±0.22	1.62±0.15	1.72±0.15	3.52±0.05	2.17±0.15	2.91±0.16

Ash	0.21±0.04	0.15±0.02	0.13±0.06	0.34±0.04	0.24±0.02	0.21±0.01

2.2 Analytical Techniques Applied

For the elemental analyses, Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) and Atomic Absorption Spectroscopy (AAS) techniques were used. For the characterization of organic composition, various techniques were applied, viz., Liquid Chromatography Organic Carbon Detection (LC–OCD), Pyro/Gas Chromatograph Mass Spectrometer (GC–MS), Specific UV Absorbance (SUVA), Fourier Transform Infrared (FT–IR) Spectroscopy and Solid State ^{13}C-Nuclear Magnetic Resonance (NMR) Spectroscopy.

2.3 Useful Impacts of Different Phytochemical Constituents

2.3.1 Fibers: Various explanations for the cleansing efficacy of the *S. persica* have been offered, e.g., the mechanical effects of its fibers, the release of beneficial chemicals or a combination of both [88].

2.3.2 Silica: Silica found in *S. persica* acts as a mild abrasive material to remove stains and deposits from the tooth surface giving the teeth whiteness [1].

2.3.3 Tannins: *Tannin* or *tannic acid* is a mixture of *gallic acid* esters and *glucose* with varying composition depending to the source. *Tannic acid* is an *astringent* that precipitates *albumin*. Its topical use is limited to the treatment of bedsores, minor ulcerations, etc. [89]. *Tannic acid* shows *anti-tumor* characteristics on animals [90]. Treating denture bases with *tannic acid* reduced *Candida* <3/Mv?/75 attachments to the surfaces [91]. *Tannins* also inhibit the action of *glucosyl transferase* thus reducing plaque and gingivitis [92]. It exerts an astringent effect on the mucous membrane, thus reducing the clinically detectable gingivitis [89].

2.3.4 Resins: The resins are amorphous end-products of metabolism which are physically usually hard, transparent or translucent and soften or melt when heated. Chemically, resins are complex mixtures of resin acids (*resinolic* acids), resin alcohols (*resinols*), resin *phenols* (*resinotannols*), esters, and chemically inert compounds [89,93]. Resin usually forms a coating over the tooth enamel, which protects against microbial actions/caries.

2.3.5 Alkaloids: The *alkaloids* are usually the derivatives of Nitrogen-ring compounds found in plants having strong physiological or toxic effects on animal body. These are colorless crystals, bitter in taste, soluble in alcohol and partially soluble in water. The *alkaloid* found in *S. persica* is *salvadorine*, which yields *trimethylamine* on *hydrolytical* cleavage [94] that exerts a *bacteriocidal* and stimulatory effects on the gingiva [28].

2.3.6 *Sulfur*: The sulfur compounds found in *S. persica* have a *bactericidal* activity [95].

2.3.7 Essential (Volatile) Oils: The essential *volatile oils* found in *S. persica* possess characteristic aroma, exert *carminative* and *antiseptic* actions [71] and its mild bitter taste stimulates and increases salivation, which is antiseptic [96].

2.3.8 Vitamin C: The vitamin C in *S. persica* helps in promoting healthy gingivae by healing and repairing tissues.

2.3.9 Sodium bicarbonate: The *Sodium bicarbonate* or baking soda ($NaHCO_3$) is an alkalinizing agent, which subsequently buffers acid. It has mild abrasive properties that is used as a dentifrice [97] in addition to its mild germicidal action [98].

2.3.10 Chlorides: High concentrations of chloride found in *S. persica* inhibit calculus formation and help in removing stains from the teeth [23,28,99,100].

2.3.11 Calcium: The calcium saturation of saliva inhibits demineralizalion of tooth enamel and promotes remineralization [92,99,100].

2.3.12 Fluoride: The effectiveness of a topical fluoride released in fresh *S. persica* saps depends on its ability to wet the tooth enamel and adequately reach caries susceptible sites such as pits, fissures and interproximal areas.

2.3.13 Organic Compounds: *Benzyl isothiocyanate* is reported to have a *broad-spectrum bacteriocidal* activity [12,101]. Al-Bagieh, *et al*., reported that *benzyl isothiocyanate* inhibits the growth and acid production of *streptococcus mutans* [12,57].

3. PHARMACOLOGICAL PRACTICES

Various practices of *S. persica* are cited in Greek and *Ayurvedic* systems but many of them are also substantiated by modern *pharmacology*, as reported by Hoor, *et al.*, that *S. persica* is a unique plant with *anti-inflammatory*, *analgesic*, *antibacterial*, *antifungal, anti-ulcer*, *anti-seizure*, *antioxidant*, *anti-platelet*, *diuretic* and *lipid-lowering* characteristics hidden within a single plant [102].

3.1 Healing of Full-Thickness Wound Characteristics

Wound is a breach formed in the normal continuum of the cellular and molecular structure of the body, thereby creating a disruption in the cellular, anatomic and as well as in their functional continuity. Wound healing or wound repair is an intricate process in which the skin or organ or tissue repairs itself after injury [103]. Mahran, *et al.*, studied the effect of phonopheresis and topical *S. persica* on wound healing in rats. 5% gel of *S. persica* aqueous extract was prepared in Carbopol 934P and applied. Results from repeated measures ANOVA test revealed a significant sequential reduction in Wound Surface Area throughout the treatment phases. Both the transdermal drug delivery, known as or *phonopheresis*, and the topical applications of *S. persica* extract gel exhibited positive effects in accelerating wound healing process in rats [104]. The *phonopheresis* method, which is introducing the gel using ultrasonic therapy, provide better results than the topical method The acceleration of wound-healing process may be attributed to the phytochemical constituents present in *S. persica*, either any individual property or the combined effect.

Getie, *et al.*, explained the healing mechanism as follows: The *flavonoids* are known to reduce lipid peroxidation, not only by preventing or slowing the onset of cell *necrosis*, but also by improving vascularity. Any drug that inhibits lipid peroxidation is believed to increase the strength of *collagen fibers* or the viability of *collagen fibril*, increase the circulation, preventthe cell damage and by promote the DNA synthesis [105]. Tsuchiya, *et al.*, mentioned that *flavonoids* promote the wound-healing process mainly due to their *astringent* and *antimicrobial* property, which seems to be responsible for wound contraction and increased rate of *epithelialization* [106]. It is also explained that the results achieved may be due to the presence of *benzyl isothiocyanate* component in the extract of *S. persica* in addition to the *flavonoids*.

3.2 Anti-hypolipidemic Characteristics

The stem decoction of *S. persica* shows distinctive *hypocholesterolemic* properties. Galati, *et al.*, studied the hypolipidemic characteristics of the extract of *S. persica* in rats [107].The effect of prolonged

administration of a *lyophilized* stem decoction of *S. persica* was evaluated in diet-induced *hypercholesterolemic* rodents. The preparation was administered for 15 and 30 days and the *Cholesterol*, High Density *Lipids* (HDL), Low Density *Lipids* (LDL) and *Triglycerides* (TG) levels were assayed. The *S. persica* decoction significantly lowered the *Cholesterol* and LDL *plasma* levels in rodents, and was found more active at 30 days of treatment. The systemic administration of *Triton* resulted in the rise of *plasma Cholesterol* and TG levels. The results thus obtained showed that *S. persica* decoction was inactive at 18 hours after treatment, whereas at 27 hours it was able to reduce *Cholesterol* and LDL *plasma* levels. In all these experiments, the HDL and TG levels remained unchanged [107].

3.3 Anti-diabetic Characteristics

The aqueous extract of *S. persica* at 500mg/kg dose level in comparison to other extracts was reported to have significant *hypoglycemic* and *hypolipedemic* effects and regenerated *pancreatic* beta cells in *streptozocin* treated *diabetic* rats [108].

3.4 Antiulcer Characteristics

The antiulcer activity of decoction of *S. persica* has been reported in rats. The Ulcer Index decreased significantly after the treatment with a *lyophilized* decoction of *S. persica* (500 mg/kg, os), once daily for 7 days, with respect to controls [109]. *S. persica* possesses significant protective action against *ethanol* and stress-induced *ulcers*. A study was designed to confirm the antiulcer activity of *S. persica* decoction using optical microscopy. The elements of *gastric mucosa* tended to be re-established normally when tested in treated rodents [110].

3.5 Anticonvulsant & Sedative Characteristics

The effect of *S. persica* was identified as an *anticonvulsant* using the extracts from the stem. On rodents, the stem extracts are reported to show potentiation of *Sodium pentobarbital* characteristics and also on generalized *tonic-clonic seizure* produced by *pentyl entertazol* (PTZ). The extracts of *S. persica* extended sleeping-time, decreased induction-time induced by *Sodium pentobarbital* and showed protection against PTZ-induced *convulsion* by increasing the *latency* period and diminishing the death rate [111].

3.6 Antifertility Characteristic

S. persica extract did not have much effect on female mouse fertility although it significantly decreased the relative weights of *ovary* and increased the weights of *uterine*. Exposure of male mice to *S. persica* extracts resulted in the reduction of pregnancies by 72% in untreated females impregnated by test males. The relative weights of *testes* and *preputial glands* were found significantly increased whereas the *seminal vesicles* significantly decreased in the test males. These results indicate that *S. persica* has adverse effects on male and female *reproduction systems* and *fertility* [112].

3.7 Antiplaque Characteristics

S. persica inhibits the formation of dental plaque chemically and the value of *Miswak* is due primarily to its mechanical cleaning action [113]. Lower plaque scores were reported following the proper use of *Miswak* as an oral hygiene aid comparing to those using conventional toothbrushes [114]. In a study, which compared the efficacy of *Miswak* with the use of toothbrush, revealed that the use of *Miswak* was associated with a significant reduction of dental plaque and gingivitis along with comparable or superior oral hygiene effect [115]. It was also reported that the habitual *Miswak* users had lower gingival bleeding [116,117].

3.8 Antibacterial Characteristics

Al-Bayati, *et al.*, reported that extracts of *S. persica* possesses several biological characteristics, including *antibacterial* [57,118]. Several *in vitro* studies have indicated that *S. persica* contains substances that possess dental plaque-inhibiting and *antimicrobial* properties against oral *microbes* [57,59,77,98,119-122]. Studies have indicated that *S. persica* contains substances that possess plaque inhibiting and *antibacterial* properties against several types of *cariogenic* (conducive to *caries*) bacteria and *periodonto pathogens*, which are frequently found in the oral cavity [78]. The growth of these bacteria and acid produced by them is inhibited by its acid inhibition properties [25,57,98,119]. Al-Lafi, *et al.*, reported that the use of *S. persica* inhibits the formation of dental plaque chemically and exert *antimicrobial* effect against many oral bacteria [57]. They tested its *antibacterial* characteristics against some oral aerobic and *anaerobic* bacteria and reported that the extract of these sticks had a drastic effect on the growth of *Staphylococcus aureus,* and a variable effect on other bacterial species [57]. Farooqui and Srivastava reported that the *Salvadorine*, an *alkaloid* present in *S. persica* may exert a bactericidal effect and stimulate the gingival [23].

In a clinical study, after professional instruction of the proper use of *Miswak* and toothbrush, *Miswak* was found to be more effective than use of tooth brush for reducing plaque and gingivitis in a sample of male Saudi Arabians [123,124]. Gazi, *et al.,* also reported that plaque and *gingivitis* were significantly reduced when *Miswak* was used 5 times a day compared with conventional toothbrush [117]. Almas showed that *S. persica* extracts had *antimicrobial* effects on *Streptococcus mutans* and *E. faecalis* [77]. In another study, pieces with identical size and weight of *S. persica* were tested against *Streptococcus mutans, Lactobacillus acidophilus, Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis* and *Haemophilus influenzae*. The results showed strong *antibacterial* effects against all *bacteria* tested due to the presence of a volatile active *antibacterial* compounds. Al-Otaibi M., *et al.,* also observed that the use of *Miswak* significantly reduced the amount of *A. actinomycetemcomitans* in the *subgingival plaque* indicating that the *S. persica* extracts might interfere with the growth and *leukotoxicity* of *A. actinomycetemcomitans* [125]. In another in vitro study, the derivatives of *S. persica* also demonstrated strong *antimicrobial* effects on the growth of *Streptococcus sp.* and *Staphylococcus aureus gram stain* [57]. Similarly, the *benzyl isothiocyanate* is considered the main *antibacterial* component in *S. persica* root that has strong bactericidal characteristic against oral *pathogens* involved in periodontal disease, such as the *Gram-negative periodontal pathogens A. actinomycetemcomitans* and *P. gingivalis* [126]. Elvin-Lewis, *et al*., and Almas suggested that this effect may be due to the interaction with bacteria, which prevents their attachment on the tooth surface [77,127]. Ezmirly, *et al.,* reported that the extracts of *S. persica* root yielded *β-sitosterol* together with elemental Sulfur (S_8, a monoclinic form) as a constituent that has shown *antibacterial* characteristics. They found Sulfur-containing oil in the ash of the roots as high as 4.73% [60].

But when Abd El-Rahman, *et al.*, subjected *Actinobacillus actinomycetemcomitans* (ATCC 43717), *Actinomyces naeslundaii* (40110/87) *Candida albicuns* (ATCC 90028), *Lactobacillus acidophilus* (CCUG 5917), *Porphyromonas gingivalis* (W50 Black), *Prevotella intermedia* (VPI 4197) and *Streptococcus mutans* (CCUG 11877) strains for *antimicrobial* testing of the crude extracts of *S. persica*, differed from previous findings [57,59,98,119,120] that *S. persica* extracts possess considerable *antimicrobial* characteristics. According to this study the *S. persica* crude extracts inhibited, reduced or enhanced the growth of the test *microorganisms*. Most of the extracts exerted their *antimicrobial* activity only at the highest concentrations used while *chlorhexidine* and tea-tree oil showed inhibition and growth

reduction, respectively, at much lower concentrations. *S. Mutans* was the most susceptible strain to all the extracts and *L. acidophilus* was the most resistant. Regarding inhibition, the most potent extract was the root-*ethanolic* extract and the weakest one was the stem-water extract. The different reactions of each strain to the various extracts indicated that each solvent extracted different chemical components of *S. persica*. The strength of the *antimicrobial* activity may also depend on the pH of the extracts since the lowest pH was shown by the root-*ethanolic* extract while the stem water extract demonstrated the highest pH. It appears that *S. persica* has a relatively low *antimicrobial* activity against the selected *oral pathogens* when compared with 0.2% aqueous *chlorhexidine* [12,128]. Another comparative study of *alcoholic* extract of *S. persica* to its aqueous extract with regards to *antibacterial* characteristics also indicated that the *alcoholic* extract is more effective [120]. Almas, *et al.*, found no difference in the *antibacterial* characteristics between a fresh vs. one-month old *S. persica* extracts [119].

In a study, published in Journal of Agriculture and Food Chemistry in 2007, it was found that mints with *S. persica* extracts were 20 times more effective in killing *bacteria* than ordinary mints [12]. It showed that in 30 min 60% of *bacteria* was killed by mints with *S. persica* extracts when compared to a meagre 3.4% by ordinary mints [129]. A study was carried out comparing *Miswak*, *Neem*, *Banyan* and *Mango* sticks, which are popular among oral hygiene aids in India, concluded that *Miswak* is more superior in *antimicrobial* activity among these chewing-sticks [130]. Strong *antibacterial* effect of *S. persica* against oral *pathogens* was proved when an in vitro study showed both *S. persica* pieces embedded in the agar plate and suspended above the agar plate at a distance of 3mm showed a zone of inhibition around the stick for *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Lactobacillus acidophilus* and *Haemophilus influenza*. It suggested the volatile *antibacterial* agents present in *S. persica* [131].

However, Feras Alali, *et al.,* evaluated the toxicity of aroma using brine shrimp lethality test which gave an LC 50 > 1000 ppm. Using Disc Diffusion Test, it was found that the extract of the leaves of *S. persica* has a considerable *antibacterial* effect on several different oral *aerobic bacteria* with comparable results to known *antibiotics*. The extract can be used effectively as a natural tool for teeth cleaning and as a natural *analgesic* for the disturbing toothache [83]. In a recent study, the strongest *antibacterial* characteristic of the aqueous *S. persica* extract was demonstrated against *E. faecalis* [132,133].

Further, the effects of the extracts of *S. persica* and *derum* were investigated on the proliferation of Balb/C 3T3 mouse *fibroblast* by measuring the *mitochondrial dehydrogenase* characteristics. Similarly, the effect on the viability of *carcinogenic* bacteria was also determined. The results indicated that *S. persica* and *derum* extracts have adverse effects on the growth of *cariogenic microorganisms* but *derum* was found more active than *S. persica*. The extract of *S. persica* showed *cell proliferation* by 156% and the *derum* extract by 255% [28].

3.9 Anti-carcinogen, Anti-genotoxic, Antineoplastic, Antifungal and Anti-herpes Characteristics

Amongst the organic compounds found in *S. persica*, the *benzyl isothiocyanate* is classified as one of the *chemo-preventive* agents that are thought to prevent *carcinogenic* and other *genotoxic* compounds from reaching or reacting with the target sites on the treated tissue [81,134]. Al-Dosari *et al.*, studied the effect of *benzyl isothiocyanate* on the *epithelial* changes induced by *trauma* and *dimethyl benzanthracin* in the *hamster tongue*. Their results indicate that *benzyl isothiocyanate* retarded the development of *neoplastic* changes induced by *trauma* or *trauma plus dimethyl benzanthracin* [12,128,135]. A recent study showed that the *ethanolic* extract of *S. persica* has good *antifungal* characteristic compared to *clotrimazole* [136]. Al-Bagieh, *et al*., also noted its anti-fungal characteristic [121]. Sarvesh, *et al.*, found that the leaves of *S. persica* have anti-fungal, carminative and antiseptic characteristics [137]. The *benzyl*

isothiocyanate isolated from *S. Persica* is also reported to have a *virucidal* characteristic against *herpes simplex virus-1* (HSV-1) replication at a concentration of 133.3 mg/ml [12,136,138,139].

3.10 Antibiotic, Anti-inflammatory, Hypoglycemic Nontoxic & Anti-oxidant Characteristics

The aqueous and *ethanolic* extracts of *S. persica* have been screened for broad range of pharmacological activities and the data indicates its *antibiotic*, *anti-inflammatory* and *hypoglycemic* characteristics. *S. persica* decoction possesses significant anti-inflammatory activity. The aqueous extract showed weak *anti-inflammatory* characteristics in a conventional *foot edema* test, whereas the *ethanolic* extract showed mild *hypoglycemic* characteristics in glucose-loaded mice at 400 mg/kg. Both extracts were found *nontoxic* at doses up to 1200 mg/kg [60]. In another study, the *ethyl acetate* extract of *S. persica* showed potent *anti-inflammatory* characteristic nearly that of *indomethacin* characteristic, which may be due to the presence of *flavonoids* in *ethyl acetate* extract [104]. *S. persica* has also revealed potent *antioxidant* capacity, which may be attributed to the *furan* derivatives and the presence of *antioxidant enzymes*, such as *peroxidase*, *catalase*, *polyphenoloxidase* [140].

3.11 Anti-mycotic Characteristics

Farooqi and Srivastava, and Edi, *et al.,* demonstrated the aqueous extracts of *S. persica* to have an in vitro inhibitory effect on the growth of *Candida albicans*, which may be attributed to its high sulfate contents [23]. Such inhibition lasts for up to 36 hours at concentrations of 15% and above [141]. The investigation carried out by Al-Bagieh, *et al.*, also suggest similar results [123].

3.12 Cytotoxicity, Anti-plasmodial, Anti-HIV-1-RT & Anti-hemoflagellate Characteristics

Mohammad, *et al.,* investigated the *cytotoxic* potential of *S. persica* on *gingival* and other *periodontal* structures using the *agar* overlay method. The results showed no *cytotoxic* effect by a freshly cut and used *S. persica*. However, the same *Siwak* used after 24 hours contained harmful components. This *cytotoxicity* became evident only after 24 hours because the *agar* overlay method depends on diffusion of medicament to the *agar*. Based on these findings, it is recommended to cut off the used portion of the *Siwak* after it was used for a day and prepare a fresh part for new use [92,98,142,143]. Ali, *et al.*, studied in vitro the *anti-plasmodial*, *anti-hemoflagellates*, *cytotoxicity* and *tyrosine kinase Inhibitory* effects of *S. persica* [144].

3.13 Analgesic Characteristic

Mansour, *et al.,* studied the *analgesic* effect of *S. persica* decoction when injected into mice and found that *S. persica* decoction was more effective against thermal *stimuli* than chemical *stimuli*. It also showed an *analgesic* property [119,120,145-147]**.** Physiologically, the response of thermal *stimuli* is via skin pain receptors, whereas chemical *stimulus* has its response via *visceral* receptors. It was found that *S. persica* responds to *peripheral* pain, not the *visceral*. Hence, it sets a relief in the oral pain if applied to the oral mucosa [148]. Experiments on mice have proved that *S. persica* has a moderate analgesic effect that is related to interaction with the peripheral opiate system [149]. It has been noted that patients using *S. persica* (*Miswak*) regularly had low incidence of toothache compared to toothbrush users [150] and Eid, *et al.*, claim that *S. persica* havings analgesic, astringent and anti-inflammatory properties, makes it an effective treatment for primary periodontal diseases [151]. Should the *analgesic* characteristic of *S. persica* be confirmed in human beings to relieve dental pains, like superficial pain due to dental hypersensitivity to thermal, tactile or to chemical stimuli, *S. persica* will be of great practical value.

3.14 Calcium & Chloride Releasing Characteristic

Gazi, *et al.,* investigated the immediate and medium-term effects of *S. persica* on the composition of mixed saliva and reported that *S. persica* significantly increased the release of calcium by 22-folds and chloride by 6-folds while decreased phosphate and pH significantly. The saturation of saliva with calcium inhibits demineralization and promotes re-mineralization of tooth enamel and high concentration of chloride inhibits calculus formation [99,100].

3.15 Anti-addiction (Curative & Preventive) Characteristics

Dr. Rami Mohammed Diabi, an internist at the University of Kentucky Albert B. Chandler Hospital, Lexington, USA, researching the effects of *S. persica* on health, reported its anti-addiction effects on smokers, both curative and preventive sides, in his latest publications, "*Miswak Medicine Theory*", "*Sewak Puncture Medicine*", "*Beyond Sewak: World of Science and Research*" [64,152].

3.16 Anti-Halitosis

S. persica (*Miswak*) is also used to clean the tongue. It helps in fighting *halitosis* and effectively removes the white coat that develops on the dorsum of the tongue. It is usually done by brush end of *Miswak* [74].

3.17 Anti-Carious Characteristics

Several epidemiological studies revealed that *S. persica* had strong anti-carious effects. In a dental health survey conducted in Sudan, a lower caries prevalence was reported among *Miswak* users than toothbrush users [153]. In subsequent studies, similar lower caries incidences were found among schoolchildren using *Miswak* [154-156]. Dental loss was very low in the adults where *Miswak* was widely used [25].

3.18 Smear Layer Removal

Soaking the healthy and *periodontally* diseased root dentine in *S. persica* extract resulted in partial removal of smear layer, and occlusion of dentinal tubules was observed in dentine specimens brushed with *S. persica* solution [76].

3.19 Pesticidal Characteristic

The effect of aqueous extracts from the leaves, shoots and roots of *S. persica* was tested at laboratory scale to assess their *pesticidal* characteristics on food grains stocked for 3 months. The preliminary results obtained were very encouraging but this subject requires a thorough investigation under a well-designed research project. The experimental methodology adopted was to spray (0.1-1.0% w/w) extracts obtained from the leaves, shoots and roots of *S. persica* over 5 pre-weighed test samples of wheat, barley, millet, rice, corn, three type of pulses and the flours of wheat and rice each. Each set of treated food grain was separately exposed to the counted number of live insects, viz., beetles, moths and mites. Moderate humidity was maintained and temperature was controlled between 21-23 ^{o}C. The activity (mobility) of these insects were noted and the number of live pets counted every 24 hours. Most of these insects started exhibiting sluggish movement beyond 3-5 days and started dying after a duration of 7-10 days. After 15 days of exposure, not a single pest was seen alive. The dead pests were separated from food grains and flours. No powder of the grains or pulses or cereals was found in the test petry dishes containing solid food grains. Further, microscopic study revealed that the food grains were

absolutely intact with no holes or pitting or damages. The grain and flour samples were weighed again. The final weights were found exactly the same as the initial weights.

The test was repeated by administering 0.1-1.0% w/w seed oil of *S. persica* on 5 pre-weighed test samples of wheat, barley, millet, rice, corn, three type of pulses and the flours of wheat and rice, and exposing to the counted number of live beetles, moths and mites. The results were almost the same, except that the pests in this case showed sluggishness from the second day onwards and started dying just after fifth day of exposure. Some of these characteristics are summarized in Table-4.

Table 4: Different Parts of *S. persica Plant* & their Common Uses

Parts of the Plant	Characteristics Reported
Leaves	Antibacterial, *Antifertility*, Anti-pests
Stem	*Beta-sitosterol* elucidated, *Hypolipidemic*, *Antiulcer*, *Anticonvulsant*, Sedative, Anti-pests
Leaves & Stem	*Hypoglycemic*, *Hypolipidemic*, *Anti-pests*

4. COMMERCIALLY EXPLOITED THERPEUTIC APPLICATIONS

Due to the amazing qualities of tooth-stick, the extract of *S. persica* has been transformed into effective toothpastes, toothpowders, mouthwashes and chewing gum preparations. These products are now commercially available in the market.

4.1 Toothpastes

In recent times *S. persica* has been successfully used in commercial toothpastes by a number of overseas manufacturers to provide their oral health benefits in the most user-friendly forms. *S. persica* paste or its extract is used as an effective ingredient in the toothpaste for cleaning teeth [2,12], removal of plaque, naturally disinfecting the mouth, stop bleeding and refreshing the breath. It is claimed that the plant fibers, used in toothpaste, provide mild abrasive system while in contact with moisture, swell and become soft, and consequently do not cause wear on the surface of the tooth. The resins present in the plant may place a protective coating on the tooth enamel, which helps to prevent the development of sensitive teeth and tooth decay. The extract used in the toothpaste also helps to provide antimicrobial compounds that are useful to eliminate harmful plaque bacteria. It also helps in healing and repairing tissues and promotes remineralization to build tooth enamel.

Elvin Lewis claimed to have detected limited antimicrobial spectrum in the aqueous extracts for oral organisms [157]. However, suggested the possibility that the enzymatic action of saliva on the *isothiocyanates* of this species could release unstable products (*hydrogen peroxide* - H_2O_2) that exert antimicrobial characteristics, which is produced only in the oral environment, and helps to prevent deposits build-up (tartar and plaque formation). Other factors in selection might be its high chloride content removing tartar and other stains from the teeth, silica acting as mild abrasive, resin forming a coat over the enamel to prevent tooth decay, and vitamin C promoting healthy gingivae [158].

Research studies conducted by a Swiss Pharmaceutical Company and later confirmed by King Saud University, Riyadh (Saudi Arabia) and Indiana University, Bloomington (USA), indicated that *S. persica* extract contains antibacterial substances, which destroy the harmful germs in the mouth causing gum infections and tooth decay, and thus making it a 'total' care ingredient in the toothpastes [60].

Farooqui, *et al.,* have claimed that toothpaste containing extracts of the plant *S. persica*, preserve the gums and the teeth in perfect health and give the latter a dazzling whiteness [23,159,160]. Although Dr. J. Mittleman, D.D.S., the Former President, International Academy of Preventive Medicine, USA, has also endorsed toothpaste products of *S. persica*, but the users of *S. persica* twig don't find the toothpaste a

true alternate practice of using it in its original form. A scientific study comparing the use of *S. persica* twig with plastic toothbrushes concluded that the results were in clearly favor of the users of natural *S. persica* twig [123]. Darout, *et al.,* conducted a study among 213 males, aged 20-65 years, to evaluate the periodontal status of *S. persica* (*Miswak*) and toothbrush users and reported that the periodontal status of *S. persica* users in a Sudanese population was better than that of toothbrush users, suggesting that the efficiency of *S. persica* use for oral hygiene is comparable or slightly better than toothbrush [100]. Batwa, *et al.,* carried out a study at the Institute of Odontology, Karolinska University Hospital at Karolinska Institutet, Sweden. Photogrammetric techniques giving measurements in an interval scale were used to obtain the measurements of the plaque area [161-163]. The *S. persica* was found as effective as a conventional toothbrush in reducing plaque in all of cleaning periods, i.e., after 30, 60 and 120 seconds. The clinical part also confirmed the experimental findings were in agreement with earlier reports that chewing-sticks was equally effective compared to toothbrushes in children and adults [164,165]. The results obtained are illustrated in Table-5. Some other investigators have also found that chewing-sticks were effective in reducing plaque and gingival inflammation.

Solaiman A. Amro, (Asst. Prof., Periodontology & Oral Medicine, Al-Azhar University), *et al.*, assessed all subjects using Simplified Oral Hygiene Index (OHI-S), Gingival Index, Pocket depth and Gingival Recession Measurement to nearest mm and concluded that apart from gingival recession that might be encountered in *S. persica* users as a result of improper technique, the *S. persica* exhibited a significantly higher improvement in gingival and periodontal conditions compared to tooth brush [14]. When properly used, *S. persica* had been reported to be as effective as tooth brushing [100,116,117,123,157]. However, some other investigators disagreed and reported that there were more plaque formation and gingival bleeding in individuals who used chewing-sticks with comparison to toothbrush users [143,166]. A study reported that about 22% of the Saudi schoolchildren with gingival recession used *Miswak* [167], but this group had minimal calculus deposits which may be attributed to the use of *Miswak*. It was further reported that *Miswak* users had significantly more sites of gingival recession than did the toothbrush users. In addition, the severity of the recession was significantly more pronounced in the *Miswak* users than that in the toothbrush users [168], which might be due to poor techniques.

Table 5: Comparative Mean Plaque Level After Brushing with *S. persica* & Toothbrush (Brushing Time & Maxillary Anterior & Posterior Regions) [100]

Location	Brushing Time (Sec)	*Salvadora persica*		Toothbrush		t	P
		Mean %	95% Cl	Mean %	95% Cl		
Maxillary Anterior Region	0	53.4	40.9, 65.8	53.9	41., 66.7	-0.1	0.95
	30	22.5	13.0, 32.0	24.3	15.0, 34.4	-0.3	0.73
	60	13.1	7.4, 18.7	14.5	8.2, 20.7	-0.4	0.72
	120	6.9	4.2, 9.6	6.6	3.3, 9.8	0.2	0.87
Maxillary Posterior Region	0	55.5	42.4, 68.3	64.6	53.8, 75.4	-1.1	0.25
	30	20.7	15.2, 26.3	25.0	16.5, 33.5	-0.8	0.37
	60	12.2	7.9, 16.5	13.4	8.8, 18.0	-0.4	0.67
	120	6.5	4.2, 8.8	8.6	4.7, 12.5	-0.9	0.32

Some toothpastes produced from *S. persica* plant extract commercially available in the Middle East, South Asia, Southeast Asia, Europe and North America are Sarkan Toothpaste (UK), Quali-*Miswak* Toothpaste (Switzerland), Misvak Özlü (Turkey), Dentacare *Miswak* Plus (Saudi Arabia), Epident Toothpaste (Egypt), Siwak-F Toothpaste (Indonesia), Miswak Herbal Dental Gel (Australia), Jaris Miswak Toothpaste (Singapore), Isha Ayurveda Misvak (China), Fluroswak *Miswak* (Pakistan), *Miswak* Hamdard

Peelu Toothaste (India), *Meswak* Dabur Toothpaste (formerly owned by Balsara Hygiene, India), etc. [169,170].

4.2 Mouthwashes

S. persica extract is being used as an ingredient in the mouthwashes [2,12]. Mustafa, *et al.*, found a reduction in plaque formation by *S. persica* based mouthwash [171]. Several other studies indicate that *S. persica* extract is somewhat comparable to other mouthwashes or oral disinfectants and anti-plaque agents, like *triclosan* ($C_{12}H_7Cl_3O_2$) and *chlorhexidine gluconate* ($C_{34}H_{54}Cl_2N_{10}O_{14}$), if used at a very high concentration [13,169]. At physiologic pH, *chlorhexidine* and *triclosan* dissociate and release positively charged cations. The bactericidal effect is a result of binding of these cationic molecules to the negatively charged bacterial *enoyl-acyl* carrier protein reductae (ENR) enzyme, which is encoded by the gene FabI. This binding increases the enzyme's affinity for *nicotinamide adenine dinucleotide* (NAD^+) and appears *bacteriostatic* targeting bacteria by formation of a stable complex (ENR-NAD^+- *chlorhexidine* / *triclosan*), which at low concentrations primarily inhibits fatty acid synthesis that is necessary for building and reproducing cell membranes; but at high concentrations acts as biocides with multiple cytoplasmic and membrane disruptions causing the death of bacterial cells [172-174]. Since humans do not have ENR enzyme are not affected by this mode of action.

Some of the nonalcoholic mouth-rinse preparations commercially available in market are Listerine Miswak Mouthwash, Siwak-F Mouthwash (Indonesia), Jaris Miswak Mouthwash (Singapore), Dabur Miswak Mint Fresh/Germ Kill Mouthwash (India), Himalaya Miswak Pomegranate Mouthwash (India), etc. [123,131].

4.3 Endodontic Irrigation Solution

Due to the *antimicrobial* characteristics, *S. persica* extract has been reported to be utilized as an ingredient in endodontic irrigation solution [2,12]. Abo Al-Samh, *et al.,* evaluated, *in vitro*, the effect of different concentrations of *S. persica* extract on L929 cell-line in tissue culture and compared the results with *sodium hypochlorite* (NaOCl) and found a concentration-dependent morphological change in L929 cell-line when exposed to both *S. persica* extract and NaOCl, the former showing inhibition effects at much higher concentrations than NaOCl; and suspected the recovery of the cells after a 4-h exposure period to different *S. persica* extract concentrations [98,125,175-177].

5. SUPPLEMENTARY THERPUTIC APPLICATIONS

Apart from cleaning both the teeth and the tongue and treating toothache, *S. persica* has various other therapeutic applications also, which require further clinical investigations. These are its functional aspects of chewing as a jaw exerciser following *traumatic* injuries to the jaw and *temporomandibular* joint, a reflex induction of copious saliva or *Sialogogue*, which is beneficial to the oral hygiene, general health and development of *dentition* during eruption of the wisdom teeth [28], improving appetite and regulating *peristaltic* movements of the *gastro-intestinal* tract [2,12,178].

6. REFERENCES

1. Almas K. and Al-Lafi T., (1995), The Natural Toothbrush, World Health Forum, 16:206-210.
2. Al-Sadhan Ra'ed and Khalid Almas, (1999), *Miswak* (Chewing-stick): A Cultural & Scientific Heritage, Saudi Dental Jour., 11(2):80-88.
3. Gerrit Bos, (1993), The Miswak, An Aspect of Dental Care in Islam, Medical History, 37:68-79.
4. Almas K., (2001), The Antimicrobial Effects of Seven Different Types of Asian Chewing-sticks, Odontostomatol Trop., 24:17-20.

5. Batwa, Mohammed, Jan Bergström, Sarah Batwa and Meshari F. Al-Otaibi, (2006), Significance of Chewing-sticks (*Miswak*) in Oral Hygiene from a Pharmacological View-Point, Saudi Dental Jour., 18(3):125-133.
6. Danielsen B., Baelum V., Manji F. and Fejerskov O., (1989), Chewing-sticks, Toothpaste & Plaque Removal, Acta Odontol Scand, 47:121-125.
7. Schenkein H.A., Burmeister J.A., Koertge T.E., Brooks C.N., Best A.M., Moore L.V. and Moore W.E., (1993), The Influence of Race & Gender on Periodontal Microflora, Jour. Periodontol., 64:292-296.
8. Undersøkelse av en Aktuell Eldgammel Munnrengjøringsmetode (Norwegian), http://www.uib.no/info/dr_grad/2003/darout.htm (28 January 2020)
9. World Health Organization, (1987), Prevention of Diseases, WHO, Geneva.
10. Consensus Statement on Oral Hygiene, (2000), The Proceeding of the FDI's Second World Conference on Oral Promotion, Int. Dent. Jour., 50:139.
11. Halawany HS, (2012), A Review on *Miswak* (*Salvadora Persica*) & its Effect on Various Aspects of Oral Health, The Saudi Dental Jour., 24:63-69.
12. Kshirsagar JTK and Jareen AJ, (2017), The Miracle Twig - *Miswak*, Int. Jour. of App. Dental Sci., 3(2): 66-70.
13. Almas, Khalid, (2002), The Effect of *Salvadora persica* Extract (*Miswak*) & *Chlorahexidine Gluconate* on Human Dentin: A SEM Study, Jour. of Contemporary Dental Practice, 3(3):27-35.
14. Amro S.O., Hatem E. A. and Batwa M., (2007), Oral Hygiene & Periodontal Status Associated with the Use of *Miswak* or Toothbrush Among Saudi Adult Population, Cairo Dental Jour., 23(2):159-166.
15. Araya Yoseph (2008), Contribution of Trees for Oral Hygiene in East Africa, Ethnobotanical Leaflets, 11:38-44.
16. Spina Mary, (1994), Toothbrushes – The *Miswak* Tree (TXT), University at Buffalo Reporter, 25(26).
17. Geetha K., Manavalan R. and Venkappayya D., (2010), Control of Urinary Risk Factors of Stone Formation by *Salvadora persica* in Experimental *Hyperoxaluria*, Exp. Clin. Pharmacol., 32(9):623-629.
18. Andrews F.W., (1956), The Flowering Plants of the Anglo-Egyptian Sudan, Khartoum: Arbroath, Scotland: Sudan Government, T. Buncle & Co., 286-288.
19. *Salvadora persica*, World Agroforestry Center.
20. *Salvadora persica*, Food & Agriculture Organization of the United Nations.
21. Akhtar J., Siddique K.M., Bi S. and Mujeeb M., (2011), A Review on Phytochemical & Pharmacological Investigations of *Miswak* (*Salvadora persica Linn*), Jour. Pharm. Bioallied Sci.
22. Elvin Lewis M., (1979), Empirical Rationale for Teeth Cleaning Plant Selection, Medical Anthropology, 3:431-454.
23. Farooqui MI & Srivastava JG, (1968), The Toothbrush Tree (*Salvadora persica*), Quart. Jour. Crude Drug Res., 8:1297-99.
24. Ray A.B, Lai Chand and Dutta S.C., (1975), Chemistry & Industry, 15:517.
25. Lewis W.H. & Elvin-Lewis M.P.F., (1977), Oral Hygiene, Medical Botany, John Wiley & Sons, N. York, 226-270.
26. AgroForestryTree Database: http://www.worldagroforestrycentre.org/Sea/Products/AFDbases/AF/asp/SpeciesInfo.asp?SpID=1477 (28 January 2020)
27. Ronse De Craene L. and Wanntorp L., (2009), Floral Development & Anatomy of Salvadoraceae, Ann. Bot., 104:913-923.
28. Almas K., (1993), *Miswak* (Chewing-stick) & its Oral Health, Postgraduate Dentist, 3:214-218.
29. Botanic Gardens Conservation International, (2009), Plant Search Database.
30. El-Amin H.M., (1990), Trees & Shrubs of the Sudan, In: A/Bari E., Ed. England: Ithaca Press, 285-287.
31. Al-Shammary SF., (2008), Effect of Saline Irrigation on Growth Characteristics & Mineral Composition of Two Local Halophytes Under Saudi Environmental Conditions, Pak Jour. Biol. Sci., 11:216-221.
32. Ahmed Salah, Soaad Esmaeil Essawy El-Gengaihi, Mohamed El-Sayed Ibrahim and Ewald Schnug, (2008), Preliminary Phytochemical & Propagation Trial with *Salvadora persica* L., Agric. & Forest. Res., 1/2(58):135-138.
33. Phulwaria M., Ram K., Gahlot P. and Shekhawat N.S., (2011), Micropropagation of *Salvadora persica* – A Tree of Arid Horticulture & Forestry, Jour. New Forests, Nov., Springer Science & Business Media B.V., 42:317-327.
34. http://en.wikipedia.org/wiki/Salvadora_persica (28 January 2020)
35. Khatak M., Khatak S., Siddqui AA., Vasudeva N., Aggarwal A., and Aggarwal P., (2010), *Salvadora persica*, Pharmacogn Reviews, July-Dec., 4(8):209-214.
36. Anon, (1986), The Useful Plants of India, Publications & Information Directorate, CSIR, New Delhi, India.
37. Beentje HJ., (1994), Kenya Trees, Shrubs & Lianas, National Museums of Kenya.
38. Bein E., (1996), Useful Trees & Shrubs in Eritrea, Regional Soil Conservation Unit (RSCU), Nairobi, Kenya.
39. Bekele-Tesemma A., Birnie A. and Tengnas B., (1993), Useful Trees & Shrubs for Ethiopia, Regional Soil Conservation Unit (RSCU), Swedish International Development Authority (SIDA).

40. Booth FEM. and Wickens GE., (1988), Non-timber Uses of Selected Arid Zone Trees & Shrubs in Africa, FAO Conservation Guide, No. 19, Rome.
41. Coates-Palgrave K., (1988), Trees of Southern Africa, C.S. Struik Publishers Cape Town.
42. Eggeling, (1940), Indigenous Trees of Uganda, Govt. of Uganda.
43. Hines DA. and Eckman K., (1993), Indigenous Multipurpose Trees for Tanzania: Uses & Economic Benefits to the People, Cultural Survival Canada & Development Services Foundation of Tanzania.
44. Hong T.D., Linington S. and Ellis R.H., (1996), Seed Storage Behavior: A Compendium, Handbooks for Genebanks: No. 4, IPGRI.
45. ICRAF, (1992), A Selection of Useful Trees & Shrubs for Kenya: Notes on their Identification, Propagation & Management for Use by Farming & Pastoral Communities, ICRAF.
46. Kokwaro JO., (1976), Medicinal Plants of East Africa, East African Literature Bureau.
47. Leeuwenberg AJM., (1987), Medicinal & Poisonous Plants of the Tropics, Pudoc. Wageningen.
48. Makwana MT., Patolia JS. and Iyengar ERR., (1988), Salvadora Plant Species Suitable for Saline Coastal Wasteland, Transactions of Indian Society of Desert Technology, 2:121-131.
49. Mbuya LP., *et al.,* (1994), Useful Trees & Shrubs for Tanzania: Identification, Propagation & Management for Agricultural & Pastoral Communities, Regional Soil Conservation Unit (RSCU), Swedish International Dev. Authority (SIDA).
50. Sahni KC., (1968), Important Trees of the Northern Sudan, United Nations & FAO.
51. Vogt K., (1995), A Field Guide to the Identification, Propagation & Uses of Common Trees & Shrubs of Dryland Sudan, SOS Sahel International, UK.
52. Watt J.M., (1962), Medicinal & Poisonous Plants of Southern & Eastern Africa, Livingstone, London, p. 926.
53. Albrecht J., (1993), Tree Seed Hand Book of Kenya, GTZ Forestry Seed Center Muguga, Nairobi, Kenya.
54. Von Maydell HJ., (1986) Trees & Shrubs of the Sahel - Their Characteristics & Uses, GTZ 6MBH, Eschborn.
55. National Institute of Industrial Research, (2003), Herbs Cultivation & Their Utilization, Asia Pacific Business Press, Delhi, Chapter: 2, ISBN 978-81-7833-064-8.
56. Islam Kotob, Muslims & Science, Islamic Books, p. 30.
57. Al-Lafi T. and Ababneh H., (1995), The Effect of the Extract of the *Miswak (*Chewing-stick) Used in Jordan & the Middle East on Oral Bacteria, Int. Dent. Jour., 45:218-222.
58. Homer K.A., Manji F. and Beighton D., (1992), Inhibition of Peptidase & Glycosidase Activities of Porphyromonas Gingivalis, Bacteroides Intermedius & Treponema Denticola by Plant Extracts, Jour. Clin. Periodontol, 19:305-310.
59. Almas K. and Al-Bagieh N.H., (1999), The Antimicrobial Effects of Bark & Pulp extract of *Miswak, Salvadora persica,* Biomedical Letters, 60:71-75.
60. Ezmirly S. T., Chang J.C. and Wilson S. R., (1979), Saudi Arabian Medicinal Plants – *Salvadora persica*, Jour. Planta Medica (Med. Plant Research), vol. 35(2), p. 191-192.
61. Atassi F., (2002), Oral Home Care & the Reasons for Seeking Dental Care by Individuals on Renal Dialysis, Jour. Contemp. Dent. Pract., 3:31-41.
62. Bukar A., Danfillo IS., Adeleke OA. and Ogunbodede EO., (2004), Traditional Oral Health Practices Among Kanuri Women of Borno State, Nigeria, Odontostomatol Trop., 27:25-31.
63. http://web.africa.ufl.edu/asq/v4/v4i3a3.htm (28 January 2020)
64. https://en.wikipedia.org/wiki/Miswak?oldid=648772212 (28 January 2020)
65. Darout I.A., Christy A., Skaug N. and Egeberg P.K., (2000), Identification & Quantification of Some Potentially Antimicrobial Anionic Components in *Salvadora persica* Extracts, Indian Jour. Pharmacol., 32:11-14.
66. Galletti G.C., Chiavari G. and Kahie Y.D., (1993), Pyrolysis/Gas-Chromatography/Ion-trap Mass Spectrometry of the 'Tooth Brush Tree' (*Salvadora persica L.*), Rap Com Mass Spectrometry,7: 651-655.
67. Kamel M.S., Ohtani K., Assaf M.H., Kasai R., El-Shanawani M.A. and Yamasaki K., (1992), *Lignan Glycosides* from Stems of *Salvadora persica* Tree, Phytochemistry, 31:2469-2471.
68. Chan D.C.N., Dogen A.U. and Dogen M.M., (1987), SEM, XRF & EMPA Evaluation of Middle Eastern Toothbrush '*Salvadora persica*', Jour. Electron. Microsc. Tech., 7:145.
69. Elvin-Lewis M. and Lewis W. H., (1977), Oral Hygiene, Medical Botany, John Wiley, Interscience, N.York, p. 515.
70. Akhtar, M.S. and Ajmal M., (1981), Significance of Chewing-sticks (*Miswak*s) in Oral Hygiene from a Pharmacological View-point, J. Pakistan Medical Asso., 31(4):89-95.
71. El Mostehy M.R., Al-Jassem A.A. and Al-Yassin I.A., (1983), *Miswak* as an oral health device, Preliminary Chemical & Clinical Evaluation, Hamdard, 26:41-50.

72. Arora M., Siddiqui A.A. and Paliwal S., (2013), Separation of Flavonoids from Alcoholic Extract of *Salvadora persica* by HPLC, International Jour. of Pharmacy & Pharmaceutical Sciences, 5(Suppl. 4):207-210.
73. Attar Z.A., (1979), The Miswak, Nature's Toothbrush, Bull. History of Dentistry, 27:39-40.
74. Chawla H.S., (1983), A New Natural Source for Topical Fluoride, Jour. Indian Dent. Assoc., 55:419-422.
75. Jain M. and Saxena VK., (1984), Chemical Constituents of the Stem of *Salvadora persica,* Acta Ciencia Indica, 10:127.
76. Almas K., (2001), The Effects of Extracts of Chewing-sticks (*Salvadora persica*) on Healthy and Periodontally Involved Human Dentine: A SEM Study, Indian Jour. Dent. Res., 12:127-132.
77. Almas K., (1999), The Antimicrobial Effects of Extracts of *Azadirachta indica* (Neem) & *Salvadora persica* (Arak) Chewing-sticks, Indian Jour. Dent. Res., 10(1):23-26.
78. Al-Otaibi M., (2004), The *Miswak* (Chewing-stick) & Oral Health, Studies on Oral Hygiene Practices of Urban Saudi Arabians, Swed. Dent. Jour. Suppl., 167:2-75.
79. Hyson JM., (2003), History of the Toothbrush, Jour. Hist. Dent., 51:73-80.
80. Khalil AT., (2006), Benzylamides from *Salvadora persica,* Arch. Pharm. Res., 29:952-956.
81. Ezmirly ST. and El-Nasr MS., (1981), Isolation of *glucotropaeolin* from *Salvadora persica*, Jour. Chem. Soc. Pak., 3:9-12.
82. Mohammad A. and Turner J.E., (1983), In vitro Evaluation of Saudi Arabian Toothbrush Tree *(Salvadora persica),* Odontostomatol Trop., 3:145-148.
83. Feras Alali and Taha Al-Lafi, (2003), GC-MS Analysis & Bioactivity Testing of the Volatile Oil from the Leaves of the Toothbrush Tree *Salvadora persica* L., Natural Product Res.: Formerly Natural Product Letters, vol. 17(3), p. 189-194.
84. Raj KP. and Aggarwal YK., (1979), Heavy Metal Contents of the Leaf-gall, Sci. Cult., 45:35.
85. Maggio A., Reddy MP. and Joly RJ., (2000), Leaf Gas Exchange & Solute Accumulation in the Halophyte *Salvadora persica* Grown at Moderate Salinity, Environ. Exp. Bot., 44:31-38.
86. Quinlan R., Robson G. and Pack AR., (1994), A Study Comparing the Efficacy of a Toothpaste Containing Extract of *Salvadora persica* With a Standard Fluoride Toothpaste, J.N.Z. Soc. Periodontol, 77:7-14.
87. Joshi AJ., Krishan MK. and Mali BS., (1993), Seasonal Changes in Proteins, Amino Acids & Minerals in *Salvadora persica* With Reference to Saline Habitats, Indian Jour. Plant Physiol., 17:202-4.
88. Hardie J. and Ahmed K., (1995), The Miswak as an Aid in Oral Hygiene, Jour. Phillip Dent. Assoc., 47:33-38.
89. Khoory T., (1983), The Use of Chewing-sticks in Preventive Oral Hygiene, Clinical Preventive Dentistry, 5:11-14.
90. Tyler V.E., Bradly L.R. and Robebers J.E., (1988), Pharma-cognosy, IXth Ed. Lea & Febiger, 80-106.
91. Gali H.U., Perchellet E.M. and Perchellet J.P., (1991), Inhibition of Turner Promoter Induced Ornithine Decarboxylase Activity by Tannic Acid & Other Polyphenols in Mouse Epidermis in vivo, Cancer-Res. 51:2820-2825.
92. Kubota K., Tanaka T., Murata Y. and Hirasawa M., (1988), Effect of Tannic Acid on Adherence of Candida to Denture Base, Jour. Dent. Res., 67:183.
93. Gazi M.l., Davies T.J., Al-Bagieh N. and Cox S.W., (1992), The Immediate & Medium-term Effects of Meswak on the Composition of Mixed Saliva, Jour. Clin. Periodontol, 19:111-113.
94. Dorland W.A. Newman, (1988), Dorland's Illustrated Medical Dictionary, 27ed., Philadelphia, W.B. Saunders Co.
95. Grant J., (1990), Miswak-Toothbrushes that Grow on Trees, Todays – FDA, 2: 60.
96. Dorner W.G., (1981), Active Substances from African & Asian Natural Toothbrushes, Chemische Rundschau, 34:19-23.
97. George E.T. and William C.E., (1985), Pharmacognosy, 12th Ed. Bailliere Tindall, 95.
98. Abo Al-Samh D. and Al-Bagieh N.A., (1996), Study of Antibacterial Activity of the *Miswak* Extract In-vitro, Biomed. Lett., 53:225-238.
99. Amin TT. and Al-Abad BM., (2008), Oral Hygiene Practices, Dental Knowledge, Dietary Habits & their Relation to *Caries* on Male Primary School Children in Al-Hassa, Saudi Arabia, Int. Jour. Dent. Hyg., 6:361-370.
100. Darout IA., Albandar JM. and Skaug N., (2000), Periodontal Status of Adult Sudanese Habitual Users of *Miswak* Chewing-sticks or Toothbrushes, Acta Odontol Scand., 58:25-30.
101. Brown JM, Jacobs JW., (1979), An Investigation into Antibacterial Activity in Chewing-sticks Against Oral Streptococci, Odontostomatol Trop., 2:25-30.
102. Hoor T., Farooqui R., Shaikh J.M. and Karim N., (2014), *Salvadora persica*: Anti-inflammatory Activity in Rats, Professional Med. Jour., 21(1):70-74.
103. Nilani P., Pranavi A. and Duraisamy B., (2011), Formulation & Evaluation of Wound Healing Dermal Patch, Afr. Jour. Pharm. Pharmacol., 5(9):1252-1257.
104. Mahran G.H. and Aborehab M.A.S., (2014), Phonopheresis Versus Topical Salvadora Persica in Healing of Full-Thickness Wound: An Experimental Study, Inter. Jour. of Advanced Research, 2 (5):919-928

105. Getie M., Gebre MT, Reitz R. and Neubert RH, (2002), Evaluation of the Release Profiles of *Flavonoids* from Topical Formulations of Crude Extract of the Leaves of *Dodonea viscosa (Sapindaceae)*, Pharmazie, 57:320-322.
106. Tsuchiya H., Sato M,, Miyazaki T., Fujiwara S., Tanigaki S., Ohyama M., Tanaka T. and Iinuma M., (1996), Comparative Study on the Antibacterial Activity of Phytochemical *Flavanones* Against Methicillin Resistant Staphylococcus Aureus, Jour. Ethnopharmacol., 50:27-34.
107. Galati EM., Monforte MT., Forestieri AM., Miceli N., Bader A. and Trovato A., (1999), *Salvadora persica* Hypolipidemic Activity on Experimental Hypercholesterolemia in Rat, Phytomedicine, 6(3):181-185.
108. Trovato A., Galati EM, Rossitto A., Monforte MT, Aquino A. and Forestieri AM, (1998), Hypoglycemic Effect of *Salvodora Persica* in the Rat, Phytomedicine, 5:129-132.
109. Monforte MT, Miceli N., Mondello MR, Sanogo R., Rossitto A. and Galati EM, (2001), Antiulcer Activity of *Salvadora persica* on Experimental ASA Induced Ulcer in Rats: Ultrastructural Modifications, Pharma Biol., 39:289-292.
110. Sanogo R., Monforte MT., Daquino A., Rossitto A., Maur DD. and Galati EM., (1999), Antiulcer Activity of *Salvadora persica* Structural Modifications, Phytomedicine, 6:363-366.
111. Monforte MT., Trovato A., Rossitto A., Forestieri AM., Daquino A. and Miceli N., *et al.,* (2002), Anticonvulsant & Sedative Effects of *Salvadora persica* Stem Extracts, Phytother. Res., 16:395-397.
112. Darmani H., Al-Hiyasat AS., E1betieha AM. and Alkofahi A., (2003), The Effect of An Extract of *Salvadora persica* (Meswak Chewing-stick) on Fertility of Male and Female Mice, Phytomedicine, 10:63-65.
113. Akpata E. and Akinrimisi E., (1977), Antibacterial Activity of Extracts from Some African Chewing-sticks, Oral Surg. Oral Med. Oral Pathol., 44(5):717-722.
114. Gazi M., (1988), Photographic Plaque Assessment of the Antiplaque Properties of *Sanguinarine* & *Chlorhexidine*, Jour. Clin Periodontol., 15:106-109.
115. Danielsons B., Baelum V., Manji F. and Fejerskov O., (1989), Chewing-stick, Toothpaste & Plaque Removal, Acta Odontol Scand., 47:121-125.
116. Olsson B., (1978), Efficiency of Traditional Chewing-sticks in Oral Hygiene Programs Among Ethiopian Schoolchildren, Community Dent. Oral Epidemiol., 6:105-109.
117. Gazi M., Saini T., Ashri N. and Lambourne A., (1990), Miswak Chewing-stick Versus Conventional Toothbrush as An Oral Hygiene Aid, Clin. Prev. Dent., 12:19-23.
118. AL-Bayati F.A. and Sulaiman K.D., (2008), In Vitro Antimicrobial Activity of *Salvadora persica L.* Extracts Against Some Isolated Oral Pathogens in Iraq, Turk. Jour. Biol., 32:57-62.
119. Almas K., Al-Bagieh N. and Akpata ES., (1997), In-vitro Antibacterial Effect of Freshly Cut 1-Month-Old *Miswak* Extracts, Biomed. Lett., 56:145-149.
120. Al-Bagieh N. and Almas K., (1997), *In-vitro* Antibacterial Effects of Aqueous & Alcohol Extracts of *Miswak* (Chewing-sticks), Cairo Dent. Jour., 13:221-224.
121. Al-Bagieh N.H., Idowu A. and Salako O., (1994), Effect of Aqueous Extract of *Miswak* on the *in vitro* Growth of *Candida albicans*, Microbios, 80:107-113.
122. Abd El-Rahman H.F., Skaug N. and Francis G.W., (2002), *In vitro* Antimicrobial Effects of Crude *Miswak* Extracts on Oral Pathogens, Saudi Dental Jour., 14(1):26-32, Jan.-Apr.
123. Al-Otaibi M., Al-Harthy M., Gustafsson A. and Angmar B., (2003), Comparative Effect of Chewing-sticks &Tooth Brushing on Plaque Removal & Gingival Health, Oral Health Prev. Dent., 1(4):301-307.
124. Almas K., Albaker A. and Felembam N., (2000), Knowledge of Dental Health & Diseases Among Dental Patients, A Multicenter Study in Saudi Arabia, Indian Jour. Dent. Res. 11:145-155.
125. Al-Otaibi M., Al-Harthy M., Gustafsson A., Johansson A., Claesson R. and Angmar B., (2004), Sub-gingival Plaque Microbiota in Saudi Arabians After Use of *Miswak* Chewing-stick & Toothbrush, J. Clin. Periodontol, 31:1048-1053.
126. Abier S., Ellen M. and Muhammad A., (2011), Benzyl Isothiocyanate, A Major Component from the Roots of Salvadora Persica Is Highly Active Against Gram-Negative Bacteria, PLoS ONE, 6(8):e23045.
127. Elvin-Lewis M., (1980), Plants Used for Teeth Cleaning throughout the World, Jour. Prev. Dent., 6:61-70.
128. Wattenberg LW., (1977), Inhibition of Carcinogenic Effects of Polycyclic Hydrocarbons by Benzylisothio-cyanate & Related Compounds, Jour. Natl. Cancer Inst., 58:395-398.
129. Husain A. and Khan S., (2015), *Miswak*: The Miracle Twig, Arch. Med. Health Sci., 3:152-154.
130. Elangovan A., Muranga J. and Joseph E., (2012), Comparative Evaluation of the Antimicrobial Efficacy of Four Chewing-sticks Commonly Used in South India: An in vitro Study, Indian Jour. Dent. Res., 23:840.
131. Sofrata A.H., Claesson R.L., Lingstram P.K. and Gustafsson A.K., (2008), Strong Antibacterial Effect of *Miswak* Against Oral Microorganisms Associated with Periodontitis & *Caries*, Jour. Periodontol, 79(8):1474-1479.

132. Sarmad G. M., (2013), Comparative Study of in vitro Antibacterial Activity of *Miswak* Extracts & Different Toothpastes, American Jour. of Agricultural & Biological Sciences, 8(1):82-88.
133. Al-Bayati F. and Sulaiman K., (2008), In vitro Antimicrobial Activity of *Salvadora persica L.* Extracts Against Some Isolated Oral Pathogens in Iraq, Turk. Jour. Biol., 32:57-62.
134. Hecht S.S., (2000), Inhibition of Carcinogenesis by Isothiocyanates, Drug Metabolism Reviews, 32(3-4):395-411.
135. Al-Dosari A., Kafrawy A., Standish S., (1992), The Effect of Benzyl Isothiocyanate on Epithelial Changes Induced by Trauma and DMBA in the Hamster Tongue, Saud. Dent. Jour., 4(1):4-10.
136. Al-Bagieh N., (1992), *Anti-Herpes* Simplex Virus Type-I Activity of *Benzyl Isothiocyanate*, Biomed. Lett., 47, 67-70.
137. Paliwal Sarvesh., Chauhan R., Siddiqui A.A., Paliwal Shailendra and Sharma J., (2007), Evaluation of Antifungal Activity of *Salvadora Persica Linn*. Leaves, Natural Product Radiance, 6(5):372-374.
138. Mahmoud Y.M.T., (2008), Antiviral Effect of Ethanolic Extract of *Salvadora Persica* (*Siwak*) on Herpes Simplex Virus Infection, Al–Rafidain Dent Jour., 8(1):50-55.
139. Al-Bagieh NH and Weinberg ED, (1988), *Benzylisothiocyanate*: A Possible Agent for Controlling Dental Caries, Microbios, 39:143-151.
140. Saleh A.M. and Khan J.A., (2013), Antioxidant Capacity of Chewing-stick *Miswak Salvadora persica*, BMC Complementary & Alternative Medicine, 13:40.
141. Edi MA. and Selim HA., (1994), Retrospective Study on the Relationship Between *Miswak* Chewing-stick & Periodontal Health, Egyptian Dent. Jour., 40:589-592.
142. Baeshen HA., Kjellberg H., Lingstr AP. and Birkhed D., (2008), Uptake & Release of Fluoride from Fluoride-impregnated Chewing-sticks *In-vitro* & *In-vivo*, Caries Res., 42:363-368.
143. Norton MR & Addy M., (2000), Chewing-sticks Verses Tooth Brushes in West Africa, Clin. Prev. Dent., 11:11-13.
144. Ali H., Konig G.M. AND Khalid S.A., (2002), Evaluation of Selected Sudanese Medicinal Plants for Their in vitro Activity Against Hemoflagellates, Selected Bacteria, HIV-1-RT & Tyrosine Kinase Inhibitory & for Cytotoxicity, Jour. Ethnopharmacol, 83:219-228.
145. Mansour M., Al-Khateeb T.L. and Al -Mazraoo A.A., (1996), The Analgesic Effect of *Miswak*, SDJ, 8:87-91.
146. Darmine H., Nusayr T. and Al-Hiyasat A.S., (2006), The Effects of Extracts of *Salvadora persica* Also Examined on Proliferation Balb/C 3T3 of Fibroblast & Viability of Carcinogenic Bacteria, 4:62-66.
147. Almas K. and Al-Zeid Z., (2004), The Immediate Antimicrobial Effect of a Toothbrush & *Miswak* on Cariogenic Bacteria: A Clinical Study, Jour. Contemp. Dent. Pract., 5:105-114.
148. Sulaiman M., Al-Khateeb T. and Al-Mazraoo A., (1996), Analgesic Effects of *Miswak*, The Saudi Dent. Jour., 8:140-144.
149. Hayes AG and Tyers MB, (1983), Determination of Receptors that Mediate Opiate Side Effects in the Mouse, Br. Jour. Pharmacol., 79:731-736.
150. Wu CD, Darout IA and Skaug N., (2001), Chewing-sticks: Timeless Natural Toothbrushes for Oral Cleansing, Jour. Periodontal Res., 36:275-284.
151. Eid MA and Selim HA, (1994), A Retrospective Study on the Relationship between *Miswak* Chewing-stick & Periodontal Health, Egypt Dent. Jour., 40:589-592.
152. http://quitcliniconline.com/Activities/Lectures/UIA-13-7-2007.htm (28 January 2020)
153. Emslie R., (1966), A Dental Health Survey in the Republic of the Sudan, Br. Dent. Jour., 120(4):167-178.
154. Baghdady V. and Ghose L., (1979), Comparison of the Severity of Caries Attack in Permanent First Molars in Iraqi & Sudanese Schoolchildren, Community Dent. Oral Epidemiol., 7(6):346-348.
155. Sathananthan K., Vos T. and Bango G., (1996), Dental Caries, Fluoride Levels & Oral Hygiene Practices of Schoolchildren in Matebelel & South Zimbabwe, Community Dent. Oral Epidemiol., 24(1):21-24.
156. Younes S. and El-Angbawi M., (1982), Dental Caries Prevalence in Intermediate Saudi Schoolchildren in Riyadh, Community Dent. Oral Epidemiol., 10(2):74-76.
157. Elvin Lewis M., (1982), The Theraputic Potential of Plants used in Dental Folk Medicine, Revue Odontostomatologique Tropicale, 5:107-117.
158. Memory Elvin – Lewis, (1983), The Anthropology of Medicine - From Culture to Method: Part III, Empirical Analysis of Non-Western Medicine, Pub. Lola Romanuci-Ross, Daniel E. Moerman and Laurence R. Tancredi, M.D., Prager, USA, p. 201-220.
159. Portereres RJ., (1974), Jour. Agricult. Trop. Bot. Appl., Vol. 21, p. 1.
160. Evenari M. and Gutterman Y., (1973), Flora (Jena), Vol. 162, p. 118.
161. Quirynen M. and van Steenberghe D., (1989), Is Early Plaque Growth Rate Constant with Time? Jour. Clin. Periodontol., 16:278-283.

162. Bergström J., (1981), Photogrammetric Registration of Dental Plaque Accumulation in vivo, Acta. Odontol. Scand., 39:275-284.
163. Lang NP, Cumming BR, Löe H., (1973), Tooth-brushing Frequency as it Relates to Plaque Development & Gingival Health, Jour. Periodontol., 44:396-405.
164. Sarita P.T.N. and Tuominen R., (1992), Tooth Cleaning Methods & Their Effectiveness Among Adults in Rural Tanzania, Proc. Finn. Dent. Soc., 88:139-145.
165. Nörmark S. and Mosha H.J., (1989), Relationship Between Habits & Dental Health Among Tanzanian Children, Community Dent. Oral Epidemiol., 17:317-321.
166. Mengel R., Eigenbrodt M. and Schunemann T., (1996), Flores-de-Jacoby L., Periodontal Status of a Subject Sample of Yemen, Jour. Clin. Periodontol, 23:437-443.
167. Younes SA and El-Engebawi MF, (1983), Gingival Recession in Mandibular Central Incisor Region of Saudi Schoolchildren Aged 10-15 Years, Community Dent. Oral Epidemiol., 4:246-249.
168. Eid MA, Selim HA and Al-Shammery AR, (1991), The Relationship between Chewing-sticks (*Miswak*) & Periodontal Health, Relationship to Gingival Recession, Quint Int., 22:61-64.
169. Almas K., Skaug N. and Ahmad I., (2005), In-vitro Antimicrobial Comparison of *Miswak* Extract with Commercially Available Non-Alcohol Mouth Rinses, Int. Jour. Dent. Hyg., 3:18-24.
170. Tubaishat RS., Darby ML., Bauman DB. and Box CE., (2005), Use of *Miswak* Versus Toothbrushes: Oral Health Beliefs & Behaviors Among a Sample of Jordanian Adults, Int. Jour. Dent. Hyg., 3:126-136.
171. Mustafa M.H., Abd el-A. M.M. and Abo el-Fadl K.M., (1987), Reduced Plaque Formation by *Miswak*-based Mouthwash, Egypt Dent. Jour., 33:375-384.
172. Leikin, Jerrold B., Paloucek and Frank P., (2008), *Chlorhexidine Gluconate*, Poisoning & Toxicology Handbook, 4th ed., Informa, p. 83-84.
173. Russell AD., (2004), Whither Triclosan?, Jour. Antimicrob. Chemother. May, 53(5):693-695.
174. The WHO Guidelines on Hand Hygiene in Healthcare (Advanced Draft), (2006), WHO, p. 37 http://www.who.int/patientsafety/information_centre/Last_April_versionHH_Guidelines% (28 January 2020)
175. Khan M.K., Khan A.A., Hosein T., Mudassir A., Mirza K.M. and Anwar A.J., (2009), Comparison of the Plaque-Removing Efficacy of Toothpaste & Toothpowder, Int. Acad. Periodontol, 11:147-150.
176. Al-Otaibi M. and Angmar B., (2004), Oral Hygiene Habits & Oral Health Awareness Among Urban Saudi Arabians, Oral Health Prev. Dent., 2:389-396.
177. Abo Al-Samh D., and Al-Nazhan S., (1997), In vitro Study of the Cytotoxicity of the *Miswak* Ethanolic Extract, Saudi Dental Jour., 9:125-130.
178. Al-Khateeb TL, O'Mullane DM, Whelton H. and Sulaiman MI, (1991), Periodontal Treatment Needs Among Saudi Arabian Adults & their Relationship to the Use of the *Miswak*, Community Dent. Health, 8:323.