# Preliminary phytochemical and propagation trial with *Salvadora persica L.*

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## Abstract

Seeds of *Salvadora persica L.* ("toothbrush tree" or "siwak") were collected from wild plants grown at Gabal Elba a mountain in East South Egypt on the coast of the Red Sea. The seeds germinate after one month. Phytochemical screening revealed the occurrence of carbohydrates and/or glycosides, sterols, terpenes, flavonoids and alkaloids.

The alcohol extract possesses antimicrobial activity against *Proteus vulgaris*. The herb contained 0.16 % volatile oil from which heptadecene γ-carbonic acid is the major. In a protocol for *in vitro* propagation of *S.persica* L. MS medium supplemented with 0.5 of each NAA and BA gave the best results for proliferated shoots and MS supplemented with IBA for rooting.

*Keywords: Salvadora persica L., germination, antimicrobial, in vitro propagation.*

## Zusammenfassung

### Untersuchungen zur Phytochemie und Vermehrung des Zahnbürstenbaums (*Salvadora persica L.*)

Samen von *Salvadora persica L.* ("Zahnbürstenbaumes" oder "Siwak") wurden von Wildbeständen in der Gebirgsregion "Gabal Elba" an der Küste des Roten Meeres im Südosten Ägyptens gesammelt. Die Samen keimten nach einem Monat. Ein Screening der phytochemischen Zusammensetzung wies Kohlenhydrate, Glycoside, Sterole, Terpene, Flavonoide und Alkaloide nach. Der alkoholische Extrakt erwies sich als antiseptisch wirksam gegen das Fäulnisbakterium *Proteus vulgaris*. Die krautigen Pflanzenteile enthielten 0,16 % flüchtige Öle, deren Hauptbestandteil Heptadecen γ-Karbonsäure war. Die *in vitro* Vermehrung von *Salvadora persica L.*war bei Schösslingen am erfolgreichsten auf einem MS Medium mit Zusatz von jeweils 0,5 mg/l NAA und BA und bei Wurzeln auf einem MS Medium mit Zusatz von IBA.

*Schlüsselwörter: Salvadora persica L., Keimung, antimicrobiell, in vitro Vermehrung.*

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## 1 Introduction

The tooth brush tree *Salvadora persica L.* is a small tree or shrub with a crooked trunk, seldom more than one foot in diameter, bark scabrous and cracked, whitish with pendulous extremities. The root bark of the tree is light brown and the inner surface are white. Its odor is like cress and taste is warm and pungent. Its fibrous branches have been used as tooth brushes by many Islamic communities, and are called "siwak". It was recommended to be used before every prayer. The users of siwak have shown a remarkable lack of tooth decay when compared with other tooth sticks and no tooth cleaning at all (El-Batanony, 1999). Today siwak is also sold in western countries as a natural means for teeth maintenance. A 5 - 8 cm long piece of the root is chewed until frayed and resembling a small brush (Figure 1).

Source: Manufactum, 2009.

Figure 1:
Frayed stick of *Salvadora persica L.*

Chemically, the air dried stem bark of *S. persica* is treated with 80 % alcohol and then with ether and other solvents which showed that it is composed of alkaloid may be salvadurine, tannins, saponins, flavonoids and sterols (El-Sadhan and Almaz, 1999). It has been reported that *S. persica* L. contains substances that possess antibacterial properties, which encourage some tooth paste manufacturers to incorporate powdered stems and or root material of siwak in their products.

In Egypt there are few plants concentrated in Gabal Elba a mountain in East South Egypt on the coast of the Red Sea, at the border between Egypt and Sudan. As this plant is endangered, so this study aims to propagate and sustain propagation of this plants by ordinary cultivation or by using *in vitro* propagation. In addition, a preliminary phytochemical and antimicrobial investigation of the alcohol extract of the herb were performed. In India, it is important to local people because of its oil yielding, pharmaceutical, fuel, and fodder value and small but edible fruits (Ramoliya et al., 2004).

## 2 Materials and methods

Seeds of *Salvadora persica L.*were collected from plants grown in the region of Gabal Elba on December 2005 and were sown in peat moss pots on autumn 2006. The seeds germinated after one month and the seedlings were kept in a green house. Shoot tips were obtained for tissue culture propagation. Mature plants collected from the Sermetay vally in the same region were distilled with water to retrieve the volatile oil. The oil obtained was analysed by gas chromatography mass spectrometry (GC/MS) employing the conditions given in Tables 1 and 2.

Table 1:
Settings for standard gas chromatography

| | |
|---|---|
| Instrument | Hewlett Packard 5890 Series 11 Plus |
| Column | 50 meter Carbowax<br>Hewlett Packard Capillary, Int. diameter 0.32 mm<br>Film thickness 0.33 µm |
| Sample size | 0.3 µl. |
| Oven temperature | 60 to 200 °C |
| Program rate | 3 °C/min |
| Injection port temp | 150 °C |
| Detector (FID) | 250 °C |
| Carrier gas | Helium |
| Carrier gas flow rate | 1 ml/min |
| Split ratio | 1:100 |
| Attenuation | 3 |

Table 2:
Settings for gas chromatography mass spectrometry

| | |
|---|---|
| Column | Hewlett Packard Capillary Column<br>50 meter Carbowax<br>Int. diameter 0. 2 mm<br>Film thickness 0.33 µm |
| Sample size | 0.5 µl |
| Oven temperature | 40 to 200 °C |
| Injection temp | 150 °C |
| Detector (FID) | TIC |
| Carrier gas | Helium |
| Carrier gas flow rate | 1 ml/min |
| Split ratio | 1:100 |
| MS ionization voltage | 70 eV |
| Start, Stop masses | 35 - 400 |
| Electron multiplier voltage | 1800 eV |

Identification of the volatile oil constituents was performed by matching with lhe Welly library of the MS apparatus (data reported in Adams, 1995) and in the Eight-peak Index of mass spectra 1974.

Phytochemical screening for the *S. persica* L. plants has

been carried out according to the method of Harborne (1997). The antimicrobial test for alcohol extract was adopted using the method of Abou Zeid and Schatu (1969).

## 3 Tissue-culture conditions

The shoot tips of *S. persica* L. were surface sterilized with 70 % ethanol for 1min. followed by 20 % commercial chlorox (contained 5.25 sodium hypochlorite) for 20 minutes. After three successive rinses in sterile distilled water the explants (about 0.25 cm in length) were placed in glass tubes containing 20 ml of MS (Murashige and Skoog, 1962; USDA, 2007) basal medium supplemented with 30 g/l sucrose, 100 mg/l myo-inositol and solidified with 7 g/l agar.

Four concentrations of the cytokinin BA (0.25, 0.5, 1, 2 mg/l) in combination with 0.5 mg/l NAA as auxin were added to the media prior to autoclaving (121 °C and a pressure of 1.2 kg/cm$^2$ for 20 min) and the pH was adjusted to 5.8 (using 1 M NaOH or HCL).

After 4 weeks the produced shoots were excised from proliferating shoot cultures and placed on MS medium supplemented with 0.5 mg/l each of NAA ($\alpha$-naphthaleneacetic acid) and BA (6-benzyladenine) as the best medium in starting stag. For rooting shoots were transferred (after 30 days of cultivation) to MS medium supplemented with different concentrations of IBA (indolebutyric acid; 0.0, 1, 2 and 3 mg/l). All cultures were maintained in a growth chamber at 25 ± 2 °C under a 16-h photoperiod (irradiance of about 40 µmol$^{-2}$ s$^{-1}$ provided by cool white fluorescent lamps).

## 4 Results and discussion

### *4.1 Seed germination*

Seeds of *Salvadora persica L.*were collected from plants wildly grown at Gabal Elba. The seeds were sown in peat moss pots 20 cm in diameter in the experimental garden of the National Research Center. The seeds germinated after one month. Each pot contained from 1 - 4 seeds which after successful germination, were thinned to only one plant after emergence of the second leaf, then all pots were transferred to the green house to complete growth.

The phytochemical screening revealed that the herbaceous parts of *Salvadora persica L.* contained carbohydrates, glycosides, sterols, terpenes, flavonoids, tannins, alkaloids and but were deprived of saponins, coumarins and anthraquinons.

The antimicrobial activity of alcoholic extracts of herbaceous parts of *S. persica* L. revealed a higher inhibition zone to Proteus valgaris amounted to 12.00 mm, in addition to the acidity of the sap of herb that reached 6.53, which confirms *S. persica* L. contains substances with antibacterial properties.

In this context it was reported that *S. persica* L.contained high amounts of fluorides and silica which had an important role in oral hygiene. Another investigation proved that siwak and other tree twigs could act as an effective tool in removal of soft oral deposits.

The herbaceous parts of siwak plants were hydrodistilled using a Clevenger apparatus and the amount of volatile oil retrieved was 0.16 %. The oil obtained was dried over anhydrous sodium sulphate and then injected to GLC with the conditions described in Tables 3 and 4. The result obtained revealed the presence of different monoterpene compounds, hydrocarbons and oxygenated derivates beside some short chain fatty acids.

The different constituents are compiled in Table 5 which indicate that the major constituent of the oil is heptadecene 8- carbonic acid, which amounted to 39.24 % followed by indole- (18.2 %) and hexadecanoic acid (13.4 %).

### *4.2 In vitro propagation of Salvadora persica L.*

A protocol is established to regenerate *Salvadora persica* L. using shoot tip explants. These shoot tips were surface sterilized with 70 % ethanol for 1min, followed by 20 % commercial chlorox (5.25 sodium hypochlorite) and the procedure was completed as described in material and methods.

Table 3:
Phytochemical screening of herbaceous parts of *Salvadora persica L.*

| Carbohydrates and/or glycosides | Sterols & terpenes | Flavonoids | Tannins | Alkaloids | Saponins | Coumarins | Anthraquinone |
|---|---|---|---|---|---|---|---|
| + | + | + | + | + | - | - | - |

Table 4:
Inhibition zone of alcoholic extracts of *Salvadora persica L.* to some organisms

| pH | B.subtilis | E.coli | Chromobacter sp. | Pseudomonas fluroscens | Lactobasillis breveis | Proteus vulgaris | Staphilococcus aureus | Salmonella typhi | Candida albicans | Aspragillus niger |
|---|---|---|---|---|---|---|---|---|---|---|
| 6.53 | - | - | - | - | ± | 12.00 | - | - | - | - |

Table 5:
Qualitative analysis of volatile oil from Salvadora persica

| Peak | Compounds | Rt. (min) | Area % |
|---|---|---|---|
| 4 | D-Limonene | 21.574 | 0.77 |
| 6 | Linalool | 22.560 | 0.57 |
| 7 | Benzaldehyde | 23.00 | 0.29 |
| 8 | Linalyl acetate | 23.70 | 0.63 |
| 10 | E-2-decanal | 25.89 | 0.17 |
| 11 | 2-Undecanone, 6-10-dimethyl | 26.71 | 0.24 |
| 12 | Delta.(7)-Methanone-2 | 27.37 | 0.34 |
| 13 | 2-Cyclohexen-1-one, 3-methyl-6-(1-methylethanyl) | 28.64 | 1.07 |
| 15 | β-Damascenone | 30.62 | 0.21 |
| 16 | 5,9-Undecadien-z-one, 6-10-dimethy | 30.97 | 0.54 |
| 17 | α-Ionone | 31.35 | 0.24 |
| 19 | Indole | 33.38 | 18.20 |
| 22 | Hedycaryol | 36.15 | 0.53 |
| 23 | 2-Pentadecanone, 6-10,14-trimethyl | 36.79 | 3.10 |
| 25 | Nonanoic acid | 37.53 | 0.36 |
| 30 | (-)-, Delta-selinene | 40.22 | 0.08 |
| 31 | β-Eudesmol | 40.57 | 0.35 |
| 32 | Tricosane | 40.77 | 0.24 |
| 34 | Dedecanoic acid | 47.72 | 0.41 |
| 36 | 2-Hexadecen-1-ol,3,7,11, 15-tetramethyl | 54.23 | 0.53 |
| 37 | Octadecanoic acid | 55.76 | 8.29 |
| 39 | Nonadecane | 59.26 | 0.14 |
| 41 | Tetra dedecanoic acid | 59.79 | 0.68 |
| 42 | 9-Octadecanoic acid | 62.05 | 0.21 |
| 43 - 58 | Heptadecene-8-carbonic acid | 62.09 - 63.99 | 39.14 |
| 54 - 78 | 9,12-Octa dedecenoic acid | 76.70 - 78.29 | 3.50 |
| 79 - 80 | Hexadecanoic acid | 80.24 | 13.40 |

Table 6 represents the effect of different concentrations of auxins as NAA and kinetin as BA on proliferation of shoots.

From the previous table, it was observed that MS medium supplemented with 0.5 of each of NAA and BA gave the best result for number of proliferated shoots. For rooting of shoots, it were excised and transferred to MS medium supplemented with different concentration of IBA of 0.0, 1, 2 and 3 mg/L. All cultures were maintained in a growth room at 25 ± 2 °C under a 16 h photoperiod.

After one month shoots were rooted in a MS medium supplemented with 3mg/L IBA. Table 7 shows that the MS medium supplemented with 3mg/L IBA followed by MS media containing 2.0 mg/L BA provided the optimum conditions for rooting.

Rooted shoots were transferred to pots containing peat moss and sand in a ratio of 1:1. The plantlets were covered with plastic bags and incubated in the growth chamber for one month. After one month these plants were transferred to the green house to complete their growth until flowering. In conclusion to this investigation, the endangered *Salvadora persica L.* plant can be propagated by seeds collected from wild grown plants if available. The second choice to propagate this plant is by tissue culture which simply adopted, however the plant needs time for ordinary or *in vitro* propagation.

Table 6:
Effect of different concentrations of NAA and BA on proliferation of *Salvadora persica L.* shoots

| Growth regulators concentration (mg/l) | No. of shoots | Shoot length (cm) | Time of shoot initiation (days) |
|---|---|---|---|
| 0.5 (NAA) + 0.25 (BA) | 5.0 ± 0.1 | 2.0 ± 0.09 | 10 |
| 0.5 (NAA) + 0.50 (BA) | 20 ± 0.2 | 7.0 ± 0.3 | 8 |
| 0.5 (NAA) + 1.0 (BA) | 15 ± 0.3 | 4.0 ± 0.1 | 7 |
| 0.5 (NAA) + 2.0 (BA) | 18 ± 0.2 | 3.0 ± 0.2 | 7 |

Table 7:
Effect of different concentrations of IBA on rooting of proliferated shoots of *Salvadora persica L.*

| IBA concentration (mg/l) | No. of roots | Root length (cm) | Time of roots initiation (days) |
|---|---|---|---|
| 0.0 | 3.0 ± 0.1 | 2.0 ± 0.09 | 20 |
| 1.0 | -- | -- | -- |
| 2.0 | 1.0 ± 0.3 | 4.0 ± 0.1 | 25 |
| 3.0 | 4 ± 0.2 | 6.0 ± 0.2 | 15 |

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