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Research Article

IDENTIFICATION OF ETHNOMEDICINAL COMPOUNDS AND ANTIMICROBIAL STUDIES OF *SALVADORA PERSICA* L. (SALVADORACEAE)

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ABSTRACT

Objective: *Salvadora persica* L. is a dense foliaceous evergreen shrub or small tree with diversified medicinal properties. The objective of this work was to do a comparative study on phytochemical composition between different plant parts of *S. persica* collected from the southern region of India.

Methods: The phytochemical analysis of ethyl acetate fraction of ethanolic extracts from leaves, tender stems and tree bark of *S. persica* was done by gas chromatography-mass spectrometry/mass spectrometry (GC-MS/MS). Also, the anti-bacterial and anti-fungal activity of extracts was analyzed *in vitro* by Disc-diffusion method.

Results: GC-MS/MS analysis of *S. persica* showed 29 phytocompounds. Among them, except for eugenol, caryophyllene, benzyl isothiocyanate, oleic acid, and fatty acid, the remaining 24 phytocompounds were newly reported in the present study. For the first time, a maximum amount of benzyl isothiocyanate (73.5%) was identified from tree bark extract of *S. persica* and this extract showed higher *in vitro* antimicrobial activity against gram-positive, gram-negative bacteria and fungi than leaves and tender stems.

Conclusion: The study demonstrated that benzyl isothiocyanate could be the major antimicrobial component in *S. persica*.

Keywords: *Salvadora persica*, Toothbrush tree, Gas chromatography-mass spectrometry/mass spectrometry, Benzyl isothiocyanate, Antimicrobial activity.

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INTRODUCTION

Bioactive plant compounds have served as templates for several synthetic drugs, and precursors used in the production of semi-synthetic drugs [1-4]. *Salvadora persica* of Salvadoraceae, popularly called Miswak, Arak, Rak and Toothbrush tree in the Arab and other tropical countries is an evergreen shrub or small sized tree with a life span of 25 years. In a floristic survey, *S. persica* has been recorded as one of the 61 ethnomedicinal plant species from the Aravalli hills of Mewar region of Rajasthan, India [5], though its distribution has been recorded elsewhere in India. The commercial *S. persica* sticks display antimicrobial action against both gram-positive (*Streptococcus mutans* and *Streptococcus gordonii*) and gram-negative (*Porphyromonas gingivalis*) oral bacteria [6]. Chewing sticks of *S. persica* have been used for centuries for tooth cleaning, and are recommended by the World Health Organization, in areas where their use is customary. A number of scientific studies have demonstrated that *S. persica* possesses anti-bacterial, anti-fungal, anti-viral, anti-cariogenic, anti-plaque, anti-inflammatory, hypoglycemic, hypolipidemic, anti-osteoporosis, anti-oxidant, anti-ulcer, anti-convulsant, sedative and analgesic effects [7-11]. *S. persica* extracts exhibited antibacterial activities against 10 multidrug-resistant bacterial clinical isolates other than oral pathogens *in vitro* [12]. A detailed survey was conducted in different parts of Saudi Arabia about the folklore uses, knowledge of local people and traditional healers to obtain information about this popular plant. Based on the results obtained, it was concluded that *S. persica* is a versatile medicinal plant used to treat enormous human and livestock ailments [13,14].

Analytical methods such as gas chromatography-mass spectrometry (GC-MS) and liquid chromatography with tandem MS are now used for analysis of plant extracts to identify its phytochemicals [15-17]. The composition of the essential oil from the roots of *S. persica* collected in Jordan and analyzed by GC and GC-MS showed seventeen compounds, the main constituents being benzyl isothiocyanate (70%), limonene (9.4%) and γ -pinene (8.7%) [18]. In another study, GC-MS analysis of the volatile oil extract from leaves of *S. persica* was identified with following compounds: Benzyl nitrile, eugenol, thymol, isothymol, eucalyptol, isoterpinolene, and gamma-caryophyllene [19]. However, no study had compared between different parts of *S. persica* for its phytochemical constitution analytically by GC-MS.

The present investigation has adopted GC-MS to do a comparative study to identify and analyze phytocompounds in ethanolic extracts from different plant parts of *S. persica* (leaves, tender stems, and tree bark) collected from the southern region of India, elucidating their molecular formula, molecular weight and other data as revealed by GC-MS spectrum. Subsequently, the anti-bacterial and anti-fungal activity of *S. persica* extracts was studied *in vitro* by Disc-diffusion method.

METHODS

General

Solvents such as ethanol and ethyl acetate were used for the extraction of active compounds from plant materials which were purified before as mentioned in Harborne [20]. Ethyl alcohol was purified by drying with anhydrous potassium carbonate. It was filtered and distilled at 78°C. Ethyl acetate was shaken with anhydrous potassium carbonate, filtered and pure ethyl acetate was distilled at 77°C [21,22].

Plant material and extractions

S. persica was collected from the dry deciduous forest of the Pulivallam reserved forest areas of Tiruchirapalli district, Tamil Nadu. The specimens were identified by Dr. S. John Britto at the Rapinat Herbarium,

Tiruchirapalli (RHT) and the voucher specimens were deposited at the RHT.

Fresh leaves, tender stems, and tree bark of *S. persica* were dried for 7 days in a drafty place, protected from light. The dried parts were ground separately by an electric mill to the desired particle size (typically <1 mm) [23]. First finely ground leaf powder (250 g) had been taken in Soxhlet apparatus [20]. Solvent (800 ml) had been taken in the 1000 ml round bottom flask. The extraction was carried out for 8 h. The same procedure was repeated thrice (3×250 g of leaf powder). The whole extract was collected, filtered and concentrated under reduced pressure. The dried extract was treated with 100 ml of ethyl acetate. Ethyl acetate fraction was filtered and filtrate named as Sample A. The whole procedure was repeated for tender stems and tree bark separately. Extracts from tender stems and tree bark were named as Sample B and Sample C respectively.

Microorganisms

The test microorganisms of Gram-positive bacteria: *Staphylococcus aureus*, *Streptococcus faecalis*, *Bacillus subtilis,* Gram-negative bacteria: *Pseudomonas aeruginosa, Eschericha coli, Klebsiella aerogenes,* and Fungi: *Candida albicans, Aspergillus niger, Penicillium* sp., were obtained from National Chemical Laboratory, Pune and maintained by periodical subculturing on nutrient agar and Sabouraud dextrose medium for bacteria and fungi respectively.

Phytochemical screening by GC-MS

GC-MS spectra were recorded using a Finnigan Matt GCQ Mass Spectrometer. Analysis of the plant extracts were performed at the Food Analysis Laboratory, Indian Institute of Crop Processing Technology, Thanjavur - 613 007, Tamil Nadu, India. These three samples A, B and C were separately analyzed in Finnigan Matt GCQ Mass Spectrometer.

GC-MS program

Column: Elite-1 (100% Dimethyl polysiloxane), 30 m×0.25 mm ID×1 µmdf, Equipment: GC Clarus 500 PerkinElmer, Carrier gas: Helium 1 ml/min, Detector: Mass detector-Turbo mass gold-Perkin Elmer, Software-Turbomass 5-1. Sample injected: 1 µl was injected with a Hamilton syringe to the GC-MS manually. Split: 10:1. Oven temperature program: 110°C - 2 min hold up to 280°C at the rate of 5 deg/min - 9 min hold, Injector temperature: 250°C, Total GC time: 45 min.

MS program

Library used: NIST Ver.2.1, Inlet line temperature: 200°C, Source temperature: 200°C

Electron energy: 70 ev, Mass scan: (m/z) 45–450, MS Time: 46 min.

Antimicrobial analysis

In vitro antimicrobial activity of the plant extracts (sample A, B, and C) were separately analyzed by Disc-diffusion method [24]. Circular discs of 6 mm diameter were prepared from Whatman No. 1 filter paper and sterilized in an autoclave. Each paper disc was impregnated with plant extracts for overnight and placed in nutrient agar plates seeded with the test bacterium and Sabouraud dextrose plates seeded with the test fungi. The test bacterial plates were incubated at 37°C for 24 h and the test fungi plates were incubated at 25°C for 24 h. After 24 h the zone of inhibition around each disc was measured and recorded. Each extract was tested four times to ensure the reliability of the result. The effect produced by the sample was compared with the effect produced by the positive control (reference standard: Ciprofloxacin 5 µg/disc for bacteria and clotrimazole 10 µg/disc for fungi).

RESULTS

Extensive studies on phytochemical investigation on *S. persica* reported amino acids and organic acids [25], heavy metals [26], inorganic constituents [27,28], glycosides [29], alkaloids [30], seed fat [31], fatty acids [32], phenols and forensic substances [28], essential oils [18,33], and benzyl amides [34]. However, the use of GC-MS is rather a few. In the present study, the phytocompounds present in *S. persica* plant extracts were investigated by GC-MS, the detailed elucidation of their retention time, molecular formula, molecular weight, and peak area in percentage as revealed by GC-MS spectrum was separately shown in following Tables 1-3. The results showed that except eugenol, caryophyllene, benzyl isothiocyanate and oleic acid, many other phytocompounds from leaves (Fig. 1), tender stems and bark (Fig. 2) extracts were reported for the first time.

The *in vitro* antimicrobial activity as determined by the zone of inhibition [35] of *S. persica* leaves, tender stems, and bark extracts on some bacterial and fungal strains were shown in Table 4.

DISCUSSION

The comparative study of phytochemical reports from leaves, tender stems and tree bark of *S. persica* showed that n-hexadecanoic acid was present in higher concentration in leaves (51.6%) than tender stems (20.94%) and tree bark (8.88%) extracts (Tables 1-3). The concentration of oleic acid was higher in tender stems (34.85%) than tree bark (16.09%) extract (Tables 2 and 3). Similarly, the tetradecanoic acid concentration was higher in tender stems (33.84%) than tree bark (2.06%) extract (Tables 2 and 3). The reported presence of eugenol and caryophyllene in *S. persica* leaves [19] and caryophyllene in *S. persica* stems extract [29,36] were also identified in *S. persica* tender stems extract for the first time as shown in Table 2.

Many of the earlier papers showed the antimicrobial activity of *S. persica* [37-40]. The acid production of *C. albicans* was inhibited by benzyl

Table 1: GC-MS revealed the phytocompounds present in ethyl acetate fraction of *S. persica* leaves extract

S.No.	RT	Name of the Compound	Molecular formula	Molecular weight (g/mole)	Peak Area %
1	8.02	N, N"-di-benzyoyloxy- Heptanediamide	$C_{21}H_{22}N_2O_6$	398	9.19
2	9.84	3-Oxo-4- phenylbutyronitrile	$C_{10}H_9NO$	159	1.27
3	16.67	2 (4H) Benzofuranone, 5,6,7, 7a-tetrahydro-4,4,7a- trimethyl-(R)	$C_{11}H_{16}O_2$	180	0.39
4	17.38	Dodecanoic acid	$C_{12}H_{24}O_2$	200	1.64
5	17.78	Diethyl phthalate	$C_{12}H_{14}O_4$	222	0.34
6	19.28	4-hydroxy-3,5-dimethoxy Benzaldehyde	$C_9H_{10}O_4$	182	0.35
7	21.67	5-Isopropyl-6-methyl-hepta-3,5-dien-2-ol	$C_{11}H_{20}O$	168	1.57
8	21.77	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	2.06
9	22.26	Dihydronopol	$C_{11}H_{20}O$	168	0.31
10	23.50	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	3.44
11	25.86	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	51.68
12	28.68	Phytol	$C_{20}H_{40}O$	296	3.24
13	29.10	Oleic Acid	$C_{18}H_{34}O_2$	282	16.09
14	29.50	Octadecanoic acid	$C_{18}H_{36}O_2$	284	2.87
15	35.64	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	5.57

S. persica: *Salvadora persica*, GC-MS: Gas chromatography-mass spectrometry, RT: Retention time

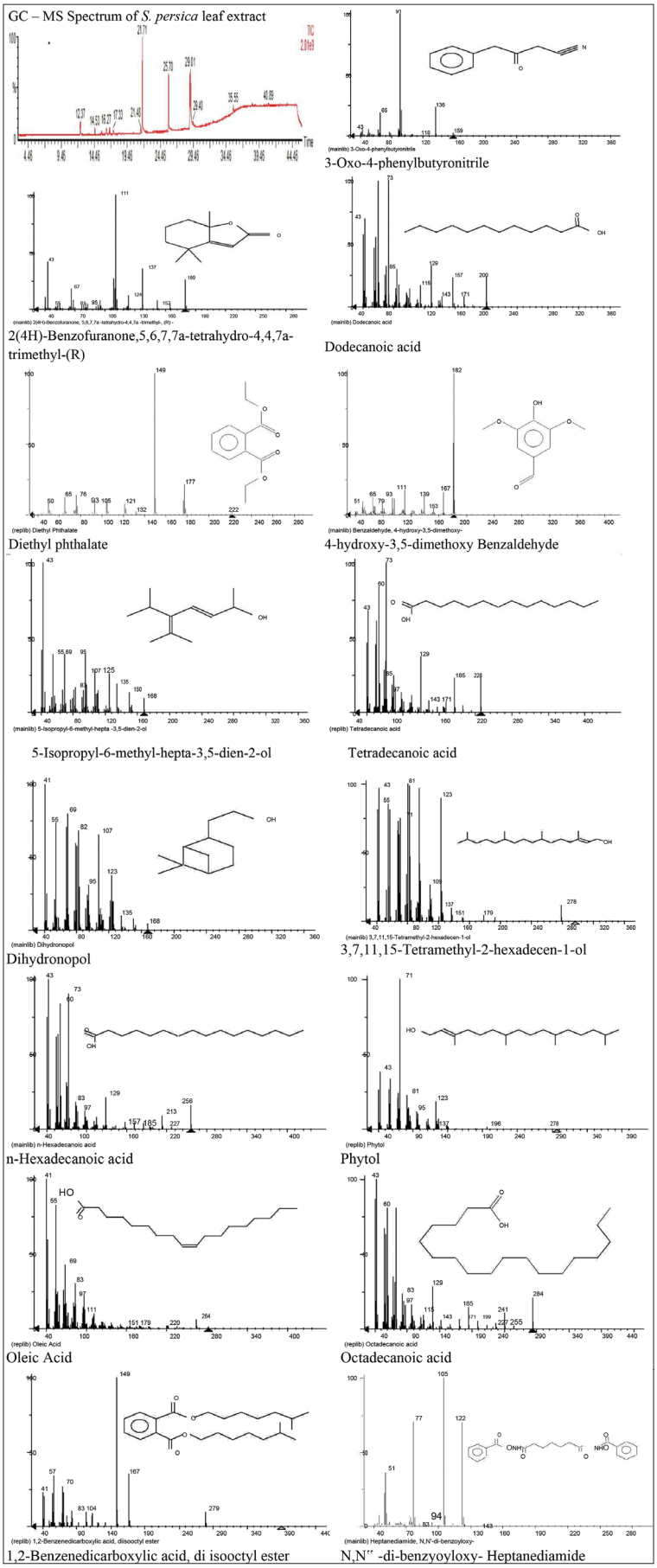


Fig. 1: Mass spectrum and structure of phytocompounds present in *S. persica* leaves extract

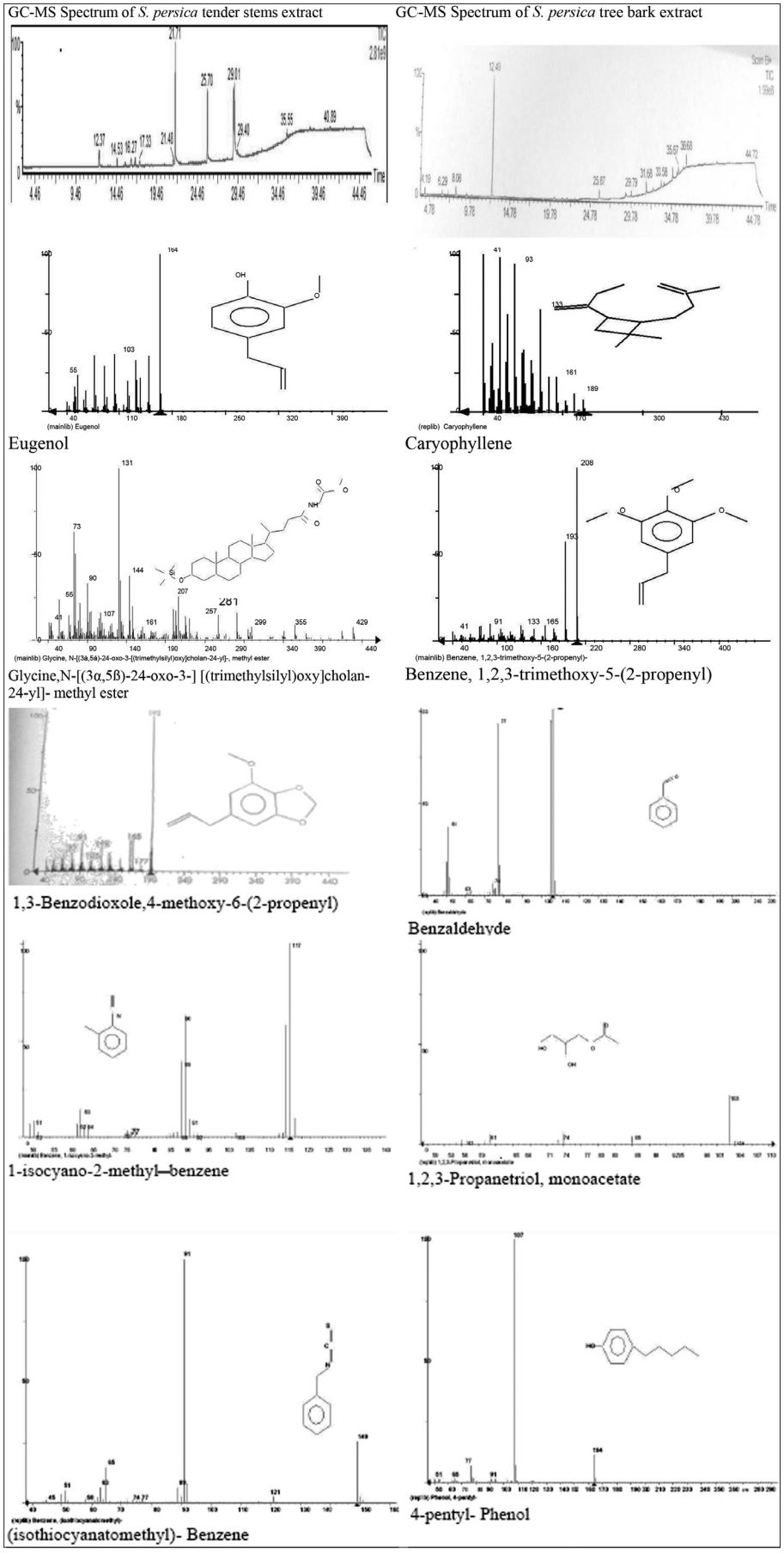


Fig. 2: Mass spectrum and structure of phytocompounds present in *S. persica* tender stems and tree bark extracts

Table 2: GC-MS revealed the phytocompounds present in ethyl acetate fraction of *S. persica* tender stems extract

S.No.	RT	Name of the Compound	Molecular formula	Molecular weight (g/mole)	Peak area %
1	12.37	Eugenol	$C_{10}H_{12}O_2$	164	4.26
2	14.53	Caryophyllene	$C_{15}H_{24}$	204	1.43
3	15.54	Glycine, N-[(3α,5ß)-24-oxo-3-[(trimethylsilyl) oxy] cholan-24-yl]- , methyl ester	$C_{30}H_{53}NO_4Si$	519	0.77
4	16.27	4-methoxy-6-(2-propenyl)- 1,3- Benzodioxole	$C_{11}H_{12}O_3$	192	2.48
5	16.80	1,2,3-trimethoxy-5-(2- propenyl)- Benzene	$C_{12}H_{16}O_3$	208	1.44
6	21.71	Tetradeconic acid	$C_{14}H_28O_2$	228	33.84
7	25.70	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	20.94
8	29.01	Oleic acid	$C_{18}H_{34}O_2$	282	34.85

S. persica: *Salvadora persica*, GC-MS: Gas chromatography-mass spectrometry, RT: Retention time

Table 3: GC-MS revealed the phytocompounds present in ethyl acetate fraction of *S. persic*a tree bark extract

S.No.	RT	Name of the compound	Molecular formula	Molecular weight (g/mole)	Peak area %
1	6.30	1,2,3-Propanetriol, monoacetate	$C_5H_{10}O_4$	134	3.15
2	7.08	1-isocyano-2-methyl- Benzene	C_8H_7N	117	2.18
3	12.50	Benzene, isothiocyanatomethyl Or (Benzyl isothiocyanate)	C_8H_7NS	149	73.53
4	14.19	Benzenamine, 4-butyl	$C_{10}H_{15}N$	149	0.65
5	20.28	4-pentyl- Phenol	$C_{11}H_{16}O$	164	1.69
6	25.86	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	8.88

*S. persic*a: *Salvadora persica*, GC-MS: Gas chromatography-mass spectrometry, RT: Retention time

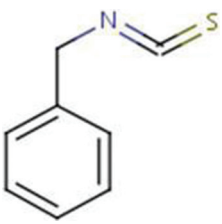
Table 4: *In vitro* antimicrobial activities of *S. persica* - leaves, tender stems and bark extracts

S. No.	Name of the microorganism	Zone of inhibition (mm)			Standard
		Sample A *S. persica* leaves extract	Sample B *S. persica* tender stems extract	Sample C *S. persica* bark extract	
1	*Staphylococcus aureus* (NCIM 2079)	12	15	14	25
2	*Bacillus subtilis* (NCIM 2063)	11	10	18	25
3	*Staphylococcus faecalis* (NCIM 2080)	14	12	17	25
4	*Escherichia coli* (NCIM 2065)	0	14	25	25
5	*Pseudomonas aeruginosa* (NCIM 2036)	16	21	24	28
6	*Klebsiella aerogens* (NCIM 2098)	20	10	20	30
7	*Aspergillus niger* (NCIM 1005)	0	10	30	18
8	*Penicillum* sp. (NCIM 3106)	8	15	15	16
9	*Candida albicans* (NCIM 3102)	12	14	32	15

Standard: Ciprofloxacin – 5 µg/disc for bacteria, Clotrimazole – 10 µg/disc for fungi. *S. persica*: *Salvadora persica*

isothiocyanate solutions in 10, 50 and 100 µg/ml concentrations [37] and this volatile oil benzyl isothiocyanate were extracted from roots of *S. persica* by Sofrata *et al.* [41]. Also, Bader *et al.* [18] identified a high concentration of benzyl isothiocyanate (70%) from *S. persica* root extracts. Together benzyl isothiocyanate found in the root of *S. persica* showed higher antimicrobial activity against gram-negative bacteria [41]. A recent study evidenced the antimicrobial component benzyl isothiocyanate in the oral cavity of humans chewing miswak sticks [42]. In the present study, this benzyl isothiocyanate was found higher in *S. persica* tree bark extract compared to leaves and tender stem extracts (Table 3). The concentration of benzyl isothiocyanate in tree bark was 73.5% which could be mainly responsible for the higher antimicrobial property of *S. persica* as shown in Table 4.

Structure of Benzyl isothiocyanate



The overall results of this investigation in *S. persica* indicated that the extracts from leaves, tender stems, and tree bark hold a potential source of plant drugs. They appear most promising not only as a blueprint for antimicrobial drugs but also have several valuable phytochemicals for other ailments.

CONCLUSION

The presence of several medicinal phytocompounds justifies the traditional ethnopharamacological uses of the plant, *S. persica*. The quantum of phytochemical data added more knowledge to the existing data about *S. persica*. Investigation in the future can take these data as starting points of drug developments. High-throughput screening along with combinational biosynthesis will have to be devised for better use of *S. persica* natural products. Also, automated real-time polymerase chain reaction process will enable the proper quality control and genetic identification of a rich variety of *Salvadora* species for its manifold medicinal uses.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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