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Studies on antibacterial potential and GC-MS analysis of *Salvadora persica*

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Abstract
Salvadora persica is commonly known as khara jaal or miswak tree. It belongs to family salvadoraceae. It is a small tree or large shrub which is used as chewing sticks against oral pathogens. This plant is therapeutically used as anticonvulsant, antibacterial, antiplaque, analgesic, antifungal and antimycotic. The present paper investigates the antibacterial activity of plant against various Gram positive and Gram-negative bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, *E. coli*, *Agrobacterium tumefaciens*, *Pseudomonas putida*, *Pseudomonas syringae* and *Enterobacter aerogenes*. The highest activity was present against *Enterobacter aerogenes* and *Staphylococcus aureus*. The GCMS analysis revealed the presence of 47 bioactive compounds such as 2-Amino-1,3-propanediol, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, Benzoic acid and Heptadecanal etc. which were responsible for the antimicrobial, antioxidant and antitumor activity of the plant.

Keywords: *Salvadora persica*; antibacterial; gcms analysis; oral pathogens

Introduction

Herbal drugs have been used since 5000 years in India [1]. People are more dependent on herbal medicines than synthetic drugs due to the natural origin and lesser known side effects of naturally derived drugs. It is also reported that naturally derived drugs are more efficacious and improves long term fitness and also cure various diseases in humans [2]. India is one of the richest floristic regions of the world and is well known for its ancient heritage regarding medicinal plants and plant drugs. The Indian system of medicine has identified 1,500 medicinal plants, of which 500 species are mostly used in preparation of drugs. In all cultures medicinal plants are utilized as medicative resource. In developing and developed countries the quality, safety and effectiveness of herbal plants and plant derived drugs became an important issue. The acceptance of herbal plants is increasing progressively and they are currently in great demand[3]. Plants are used as antimicrobial agents due to presence of antimicrobial and therapeutic properties[4]. *Salvadora persica* is commonly known as miswak[5] . In Urdu it is known as peelu[6]. It is an upright evergreen small tree of 3 meters [7,5]. It is member of family salvadoraceae[5]. *S. persica* chewsticks are used as toothbrush to maintain oral hygiene [8,5,9]. *Salvadora persica* has antibacterial and plaque inhibiting properties against several bacteria present in oral cavity [7]. The organic toothbrush obtained by miswak is more efficacious than the regular brush for removal of the plaque [5]. There is growing demand for safe, efficient and cost effective alternative preventative strategies as people worldwide show an interest in using natural products for oral hygiene [9,10]. Abdellatif *et al.* (2024) [11] have studied the efficacy of natural toothbrush prepared from miswak for the removal of plaque and gingivitis. This herbal plant is used as a conventional substitute for toothpaste and toothbrushes throughout the diverse regions of the world [11]. The chewing sticks of miswak are popular in Arab culture. These sticks are obtained from the *Salvadora persica* (Arak) tree in Saudi Arabia and different regions of the Middle East. The *S. persica* plant extract is used as mouthwash and as chewing gum due to its antimicrobial and antiplaque activity[12]. *Salvadora persica* is active against several oral cavity cariogenic bacteria and also possesses antifungal properties [13]. Its antibacterial potential inhibits the growth of oral anaerobic and aerobic bacteria [14]. The antibacterial activity of miswak plant is mainly due to the presence of benzyl isothiocyanate, it is active against all gram positive bacteria and periodontal microbes [7]. This plant has various bioactive compounds such as salvadorine, linoleic acid, saponins, cardiac glycosides, stearic acid etc. [15]. Salvadorin alkaloid and salvadorena are responsible for antibacterial [16,15], antifungal, cytotoxic and stimulatory activity [5,16]. The leaves are small, thick and round in shape with aroma. The flowers and fruits are small in size. The fruits can be eaten fresh or dried and they look like berries [16,7,5]. The small sticks of this plant are used as toothpicks [17]. *Salvadora persica* exhibits anti-inflammatory, antimicrobial, antioxidant and antiviral effects which makes it useful against various oral problems in developing and developed countries [18]. The present study is an attempt to exhibit the antimicrobial potential of different plant parts of *S. persica*.

Materials and Methods

Fresh plant parts of *Salvadora persica* were collected from residential gardens and farm houses, at different localities, in Jodhpur. Their identity was confirmed from the literature available in Department of Botany, J.N.V., University, Jodhpur. The whole plant of *Salvadora persica* was thoroughly washed and then dried under shade at 28± 20°C for about 10 days. The dried plant samples were ground well into a fine powder in a mixer grinder and sieved to give particle size of 50–150mm. The powder was stored in air sealed polythene bags at room temperature before extraction. 25g of dried powder was packed in a Whatman filter paper no.1 and was extracted in a Soxhlet apparatus using 100ml of solvent. Solvents used for extraction were Petroleum ether, Chloroform, Ethanol and Aqueous; and the extracts were dried. The dried extracts were stored in a refrigerator at 4°C. Finally, concentration of 5 mg per disc was loaded on each disc.

Antimicrobial Susceptibility Test

All the plant part extracts were screened against *E. coli, Enterobacter aerogenes, Bacillus subtilis, Pseudomonas putida, Pseudomonas syringae, Staphylococcus aureus* and *Agrobacterium tumefaciens* pathogenic bacterial strain. The disc diffusion method was used to test the antimicrobial activity of the plant extracts [19]. 20ml of sterilized nutrient agar medium for pathogens were poured into each sterile petri dish. The plates were allowed to solidify and 0.1% inoculum suspension was swabbed uniformly. The entire agar surface of each plate was inoculated with this swab, first in the horizontal direction and then in a vertical direction, which ensures the even distribution of organism over the agar surface. The filter paper discs (5mm in diameter) loaded with 5 mg/disc of dry extract were placed on the surface of the bacteria seeded agar plates and the compound was allowed to diffuse for 5 minutes and then the plates were incubated at 37°C for 24h. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimetre. These studies were performed in triplicate.

Phytochemical screening

Plant extracts were subjected to standard phytochemical analyses to find out the presence of various bioactive compounds present in the plants. The fresh plant of *Salvadora persica* was collected and washed thoroughly to remove debris and soil particles from the plant parts. The plant is allowed to kept at room temperature for 10 -15 days. After complete drying of plant, it is grinded into fine powder and were stored into air tight container or zipper bag. 25 gm. of grounded plant material was soaked in 250 ml. of methanol (99.99%) and distilled water for 48-72 hours. The plant samples were filtered using muslin cloth. The solvents which are methanol and distilled water were evaporated until semi solid form is obtained using water bath. The semi solid extract was weighed and stored into container. The preliminary phytochemical analysis was performed on aqueous and methanolic extract using the standard protocols [20,21,22].

GC-MS analysis

Extract preparation for GC-MS analysis: 10 gm. of dried sample was treated with 100 ml. of HPLC grade methanol and kept in dark for 48 hours with occasional stirring. Then the extract was filtered using Whatman filter paper no. 1 and centrifuged at 2500 rpm for 15 min. The supernatant was evaporated using water bath at 40°C temperature which results the crude syrupy extract. For GCMS analysis this syrupy extract was redissolved in methanol to make stock solution. 1 µl of stock solution was taken for further analysis.

Identification of chemical compounds: For GCMS analysis Shimadzu AP 2010 Plus with Thermal Desorption System TD20 was used. Helium gas was used as carrier gas with a constant flow rate of 16.3 ml. per min. and column flow rate of 1.21 ml. per min. The column oven temperature was kept at 60°C for 2 min., then gradually increased to 280°C at 26 min. The injection temperature was 260°C and the injection volume was 1 µl. The injection mode was normal. The total running time of GCMS was 50 min. and the samples were run at a range of 50/650 m/z and a mass spectrum graph was obtained. The identification of compounds was based on NIST and Willey libraries and also on the basis of their retention indices.

Results and discussion

In the present study, phytochemical profile of the plant *Salvadora persica* was performed using GCMS analysis. The antimicrobial potential was tested against 7 microorganisms using different solvents with disc diffusion method. The highest activity was observed in dry alcoholic leaf extract against *Enterobacter aerogenes*; and the dry and petroleum ether fruit extract has also shown potent antibacterial activity against *Staphylococcus aureus*. The dry chloroform leaf extract showed more activity against *Staphylococcus* aureus. Similarly Bissa and Bohra (2022) [23] screened the antibacterial potential of fruit peel of lemon against some pathogenic bacteria [23]. Singh *et al* (2021) [24] studied the antibacterial potential of some ethno medicinally important plants [24]. Among all aqueous dry stem extracts highest activity was observed against *Bacillus* subtilis. Similarly Bagheri *et al* (2020) [25] studied the antibacterial activities and phytochemical analysis of *M. oleifera* [25]. There is no activity of dry aqueous fruit extract against *Staphylococcus aureus* and the dry aqueous stem and leaf extract against *Pseudomonas putida*. In case if dried fruit extracts significant activity of petroleum ether extract was observed against *A. tumefaciens* as well as alcoholic extract against *E. aerogenes*. Khan and Ahmad (2012) [26] showed that ethanolic and methanolic bark extracts of *F. benghalensis* exhibit significant antibacterial activity against several Gram-positive and Gram-negative bacteria [26]. Hence from the above studies, it is concluded that the *Salvadora persica* is effectively more active against *Enterobacter aerogenes* and *Staphylococcus aureus.* 47 compounds were identified by the GCMS analysis among which some are responsible for the antimicrobial, anticancer, antioxidant and other medicinal properties. Such as 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- is responsible for anti-inflammatory, antimicrobial, antiproliferative, automatic nerve activity and antioxidant activity[27]; Heptadecanal has antioxidant, antimicrobial[28], induce apoptosis and antitumor activity[29]; STIGMAST-5-EN-3-OL, (3.BETA.) modifies the lipid profile of the membrane, reduces cell's cholesterol level[30] and also possesses antidiabetic properties[31].

Conclusion

Medicinal plants have various bioactive compounds which are having antimicrobial properties with no side effects. So the researchers are currently working to fill the gap of standard medicines by finding the herbal medicines. Effective treatment of a disease entails the development of new pharmaceuticals or some potential source of novel drugs. Commonly used medicinal plants of our community could be an excellent source of drugs to fight off this problem. There is still need to find out the natural compounds from various medicinally important plants for improvement of medicinal system and also the cost effectiveness.

Table 1: Antibacterial activity of dry extracts of *Salvadora persica*

Plant part	Plant extract	Zone of inhibition (mm)						
		A. tumefaciens	E. coli	B. subtilis	P. putida	P. syringae	S. aureus	E. aerogenes
Leaves	Aqueous	-	-	3.67±0.58	-	-	-	3±0.57
	Alcoholic	4±1.73	4.67±0.57	4.67±1.15	5.67±1.15	5.33±1.53	5.33±3.05	12±2.64
	Chloroform	4.67±0.58	6.33±0.58	3.66±0.57	7±0.58	9.33±0.58	10.33±1.15	9.33±3.21
	Petroleum ether	7±1	7.67±1.15	3.33±0.57	9.67±0.58	6.67±0.58	9.33±0.57	8.67±1.15
Stem	Aqueous	3±1	3.33±0.58	5.33±1.53	-	-	4.67±0.58	2.67±0.58
	Alcoholic	7.33±1.53	5.33±1.15	5.33±0.57	4.66±0.58	6.66±2.08	7.33±0.57	5.33±0.58
	Chloroform	6.33±2.08	8±1.00	6±1.73	7.33±1.53	5.66±0.58	6.66±0.57	5.67±1.15
	Petroleum ether	5.66±0.58	7.67±0.58	7.66±1.15	4±2.00	6±1.00	7.33±0.57	7.33±1.15
Fruit	Aqueous	3.67±0.58	1.33±0.58	2±1	-	2.67±1.15	-	-
	Alcoholic	8.67±1.53	5±1.73	5.33±1.15	5±1.73	3±1	7.33±2.08	11±1.73
	Chloroform	8.33±1.15	4±1.53	7.33±1.15	8±2	7±1.73	12±2.64	6.67±1.53
	Petroleum ether	11.33±0.58	6±1.00	9.67±2.65	10.33±0.58	9.67±1.53	10±1.73	6.33±1.53

Table 2: Phytochemicals present in *Salvadora persica* fruit extract

S.No.	Compounds	Methanolic extract
1.	Terpenoids	+
2.	Flavonoids	+
3.	Saponins	+
4.	Lignins	+
5.	Tannins	+
6.	Alkaloids	+
7.	Stearic acid	+

Table 3: GC-MS profile of methanolic fruit extract of *Salvadora persica*

Peak #	R. Time	Area	Area%	Name
1	4.447	10190031	1.57	butanoic acid, 2-ethyl-3-oxo-, methyl ester
2	5.087	3959541	0.61	piperidine, 1-methyl-
3	5.621	4896948	0.75	2-furancarboxaldehyde, 5-methyl-
4	5.879	3056206	0.47	2,4-dihydroxy-2,5-dimethyl-3(2h)-furan-3-one
5	6.210	232833	0.04	2-amino-1,3-propanediol
6	7.037	2431420	0.37	3-heptene, 2,6-dimethyl-
7	7.270	1511875	0.23	1,4-dimethylpiperazine
8	7.524	2686092	0.41	2,5-dimethylfuran-3,4(2h,5h)-dione
9	7.661	7458998	1.15	2,5-dimethyl-3-furanthiol
10	7.802	316994	0.05	pentanoic acid, 4-oxo-
11	8.358	765573	0.12	4-octanone, 2-methyl-
12	8.516	21273375	3.27	benzyl nitrile
13	8.894	58313254	8.98	4h-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
14	9.061	1932659	0.30	1h-imidazo[1,2-b] pyrazole, 2,3-dihydro-
15	9.306	694067	0.11	benzoic acid
16	9.470	2240871	0.34	2-methyl-2h-pyran-3,4,5(6h)-trione
17	9.827	2942724	0.45	5-formyl-2-furfurylmethanoate
18	9.931	564558	0.09	benzofuran, 2,3-dihydro-
19	10.679	356977637	54.95	5-hydroxymethylfurfural
20	10.844	6566603	1.01	hexanoic acid, 3-hydroxy-, methyl ester
21	10.992	833878	0.13	5-acetoxymethyl-2-furaldehyde
22	11.094	499558	0.08	2-methoxy-4-vinylphenol
23	11.632	6512093	1.00	2,4,4-trimethyl-3-nitroso-1,3-oxazolidine #
24	11.809	2307702	0.36	benzene, (isothiocyanatomethyl)-
25	12.677	5934776	0.91	furazan-3-ol, 4-amino-
26	13.014	2068279	0.32	isopropylphosphonic acid, dicylcopentyl ester
27	13.651	12214974	1.88	benzeneacetonitrile, 3-hydroxy-
28	14.557	11687338	1.80	. beta. -d-glucopyranose, 1,6-anhydro-
29	16.138	36535124	5.62	2,7-dioxa-tricyclo [4.4.0.0 3,8] decane-4,5-diol
30	17.291	190885	0.03	2,6,10-trimethyl,14-ethylene-14-pentadecne
31	18.206	640744	0.10	hexadecanoic acid, methyl ester
32	18.710	12669511	1.95	n-hexadecanoic acid
33	19.073	32153117	4.95	2-furancarboxaldehyde, 5,5'-[oxybis(methyl)
34	19.667	839394	0.13	13-hexyloxacyclotridec-10-en-2-one
35	19.851	238608	0.04	9,12-octadecadienoic acid, methyl ester
36	19.909	574051	0.09	hexadecadienoic acid, methyl ester
37	20.409	12100082	1.86	erythro-(cis) (1,4),(cis)(1',4')-4,4'-dihydroxybicyclooctyl
38	21.941	10453013	1.61	heptadecanal

39	23.495	965163	0.15	hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
40	23.613	614650	0.09	1,2-benzenedicarboxylic acid
41	24.185	1016531	0.16	2-furaldehyde azine
42	24.744	297893	0.05	glycidyl oleate
43	25.084	275916	0.04	1h-imidazole-4-carboxamide, 5-amino-
44	25.899	499376	0.08	pyrazole-4-carboxaldehyde, 1-methyl-
45	26.760	2639470	0.41	9-octadecenoic acid, 12-hydroxy-
46	27.102	3524867	0.54	13-hexyl-oxa-cyclotridec-10-en-2-one
47	34.932	2351366	0.36	stigmast-5-en-3-ol, (3. beta.)-
		649650618	100.00	

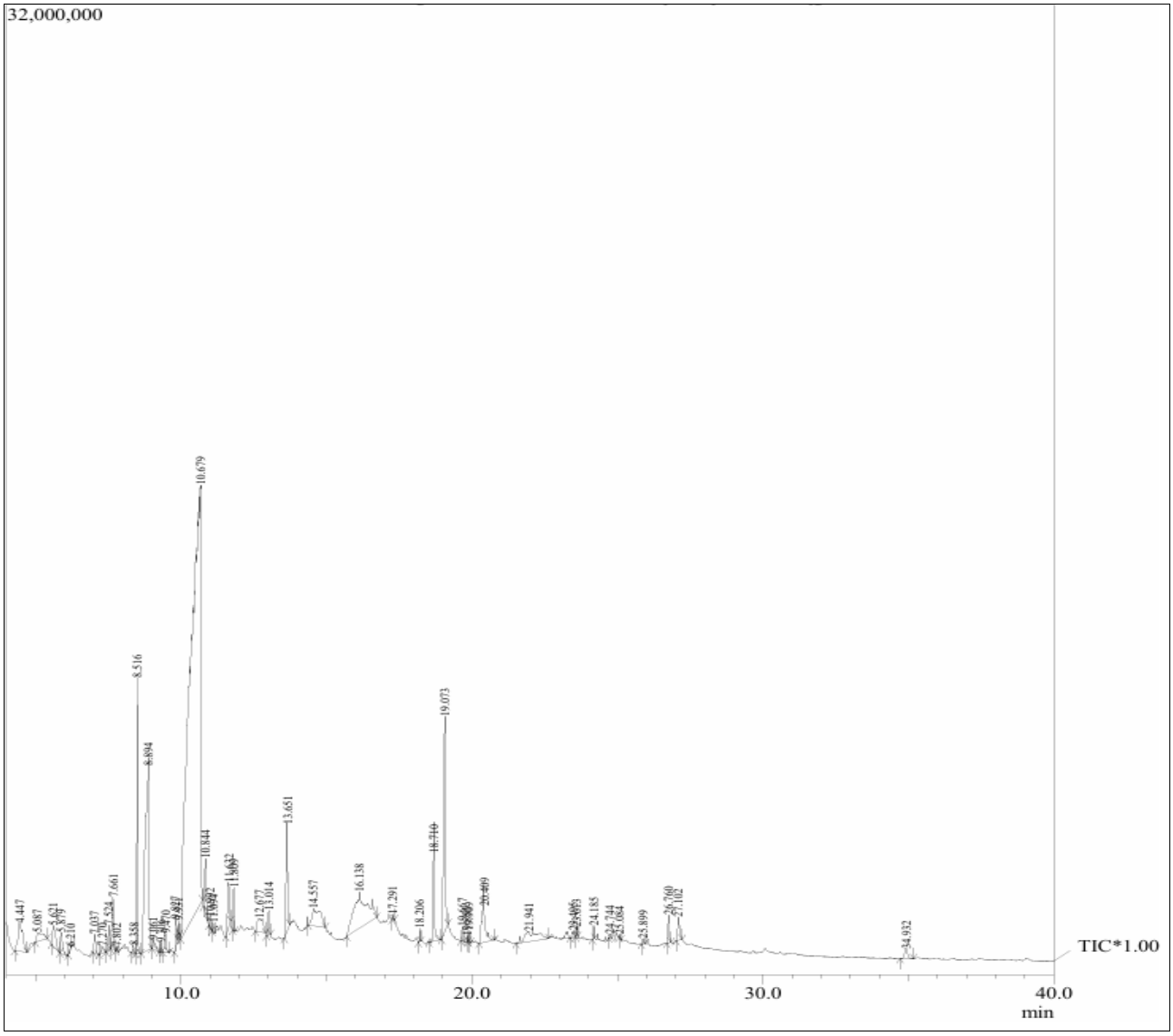

Fig. (A) showing Chromatogram of methanolic fruit extract of *Salvadora persica*

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