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Phytochemical analysis and antibacterial potential of *Salvadora persica* L.

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ABSTRACT

Salvadora persica L. is an important genus of family Salvadoraceae and is known worldwide as a toothbrush tree. Phytochemicals recorded in *S. persica* help to resolve tooth decay related issues and improves oral health. Preliminary phtytochemical analysis of Petroleum ether, chloroform, ethyl acetate, acetone, methanol, and aqueous extracts of *S. persica* leaves tested positive for carbohydrates, protein and amino acid, fats, saponins, sterols, terpenoids, alkaloids, phenols, tannins, flavonoids, anthocyanins, anthraquinones and cardiac glycosides. GC-MS analysis of methanolic extract of leaves recorded eight compounds. *S. persica* leaves methanolic extract revealed strong antibacterial action against *E. coli* proposing its candidature for future antibacterial plant.
Keywords: antibacterial. GC-MS, Phytochemical diversity, *S. persica*.

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INTRODUCTION

Salvadora persica L. is an evergreen small tree found in dry and arid regions of India belonging to family Salvadoraceae. In Persian language this plant received a name as "Darakht-e-miswak" meaning a tooth brush tree, as twigs of this plant were used as natural tooth brush. This plant is extensively used by Muslim community throughout the world to take care of oral health [1]. In India, *S. persica* is known by various trivial names like, Bengali (jhal); Hindi (jhak, piludi); Tamil (vivay, perungoli, kalawa); and English (toothbrush tree) [2]. About ten *Salvadora* species were documented from Africa and Asiatic regions [3]. Use of *S. persica* as tooth brush strenthene gum strengthening, prevents tooth decay, alleviate toothaches and stop the progression of existing decay. It freshens breath, removes awful odors, enhances the taste sense, and makes teeth appear brighter and shinier [4]. Considering these features, World Health Organization has endorsed the use of *S. persica* chewing sticks for cleaning teeth. Matured berries of *S. persica* were consumed fresh pertaining to peppery flavour and are act as a flatus-relieving and help to trigger appetite [5]. Despite worldwide recognition of this plant, very scanty studies were recorded towards its phytochemical analysis and determination of antibacterial potential. Present study aims to analyse phytochemical diversity using preliminary phytochemical analysis and GC-MS techniques. Plant extract was also investigated for their inhibitory action against selected microbes for estimation of antibacterial potential.

MATERIAL AND METHODS

Plant material

Leaves of *S. persica* were obtained from Daryapur region of District Amravati, Maharashtra, India. Leaves were brought to laboratory and shade dried. Dried leaves turn in to powder and kept in air tight bottle until further use.

Extract Preparation

Phytochemical extraction was performed using Soxhlet extraction method. Six distinct solvents viz. Petroleum ether, chloroform, ethyl acetate, acetone, methanol, and water were opted for extraction process. Subsequently, each solvent underwent 6 hour circulation through the plant material, persisting until the solvent within the upper chamber achieved colorlessness, a standard process duration averaging approximately 24 hours [6] and the resulted extracts were stored within a refrigerated environment at 4^0C until further use.

Preliminary Phytochemical Tests

The preliminary phytochemical analysis of *S. persica* was performed [7]. It included tests for carbohydrates, amino acids, lipids, saponins, sterols, alkaloids, flavonoids, terpenoids, phenol, tannine, anthocyanin, anthraquinones and cardiac glycoside.

GCMS Analysis

To gain a deeper understanding of the chemical composition of the plant extracts, Gas Chromatography-Mass Spectrometry (GCMS) analysis was conducted [8]. This comprehensive analysis was performed at IIT Powai, Bombay.

Antibacterial activity

Antibacterial activity of *S. persica* leaves methanol extract was tested according to Gupta et al.[9] using disc diffusion method against gram-ve *E. coli*. In order to compare antibacterial potential of *S. persica*, methanolic extracts of *P. glabrum* and *C. oppositifolia* were also tested along with standard antibiotic Ofloxacin. Sterile forceps was used to place phytochemical saturated discs on *E. coli* culture medium. Plates were allowed to incubate at 37 ^{0}C for 24 h and after incubation period the zone of inhibition was determined.

OBSERVATION AND RESULTS

Preliminary Phytochemical Analysis

Phytochemical analysis of *Salavadora persica* leaves was done using various solvent extracts and it revealed the presence of carbohydrates, protein and amino acid, fats, saponins, sterols, terpenoids, alkaloids, phenols, tannins, flavonoids, anthocyanins, anthraquinones and cardiac glycosides (Table 1). *S. persica* leaves methanol and aqueous extracts was rich in carbohydrates whereas other extract found negative. Except petroleum ether and chloroform extract all other were tested positive for protein and amino acids. Sudan test was the only test reporting fats in all extracts except petroleum ether. Acetone, methanol and water extract of *S. persica* leaves was rich in saponins and flavonoids from. Methanol extract of *S. persica* leaves showed rich in alkaloids. Majority of extract confirmed occurrence of phenols and tannins. Methanol and water extracts were rich in anthraquinones and cardiac glycosides, these two extracts along with choloroform and acetone reported presence of anthocyanins. Methanol followed by aqueous extract was the two best solvents reporting presence of almost all tested phytocompounds from *S. persica* leaves.

Table 1: Phytochemical analysis of *S. persica* leaves extract.

Sr. No.	Chemical Constituents	Test	Petroleum ether	Chloroform	Ethyl acetate	Acetone	Methanol	Water
1	**Carbohydrates**	Molisch test	-	-	-	-	+	+
		Fehlings test	-	-	-	-	+	+
		Benedicts test	-	-	-	-	+	+
2	**Protein and amino acid**	Millons test	-	-	+	-	+	+
		Biuret test	-	-	+	+	+	+
		Ninhydrin test	-	-	+	+	-	-
3	**Fats**	Stain test	-	-	+	-	-	-
		Saponification test	-	-	-	-	-	-
		Sudan test	-	+	+	+	+	+
		Test for glycerol	-	-	-	-	-	-
4	**Saponins**	Foam test	-	-	-	+	+	+
		Froth test	-	-	-	+	+	+
5	**Sterols**	Salkowaski test	-	+	-	-	-	-
		Liebermanburchard test	-	+	-	+	-	-
6	**Terpenoids**	Test for terpenoids	+	-	+	-	-	-
7	**Alkaloids**	Mayers test	-	-	-	-	+	-
		Hagers test	-	-	-	-	+	-
		Wagners test	-	-	-	-	+	-
		Dragendrffs test	-	-	-	-	+	-
8	**Phenols**	Ferric chloride test	+	-	-	+	+	+
		Liebermans test	+	-	-	-	-	-
9	**Tannins**	Lead Acetate test	-	-	-	-	+	+
		Ferric chloride test	+	+	+	+	+	+
10	**Flavonoids**	Lead acetate test	-	-	-	+	+	+
		Shinoda test	-	-	-	+	+	+
		Alkaline Reagent test	-	-	-	+	+	+
11	**Anthocyanin**	Dilute HCL acid test	-	+	-	+	+	+
12	**Anthraquinones**	Borntraggers test	-	-	-	-	+	+
13	**Cardiac glycosides**	Kellar killiani test	-	-	-	-	+	+

GC-MS analysis of *S. persica* leaves extract

Chromatogram GC-MS investigation of methanolic extract of *S. persica* leaves recorded 8 major peaks (Figure 1) and its analysis showing retention time, names of the compound, peak area, molecular weight (MW) and molecular formula and are shown in table 2.

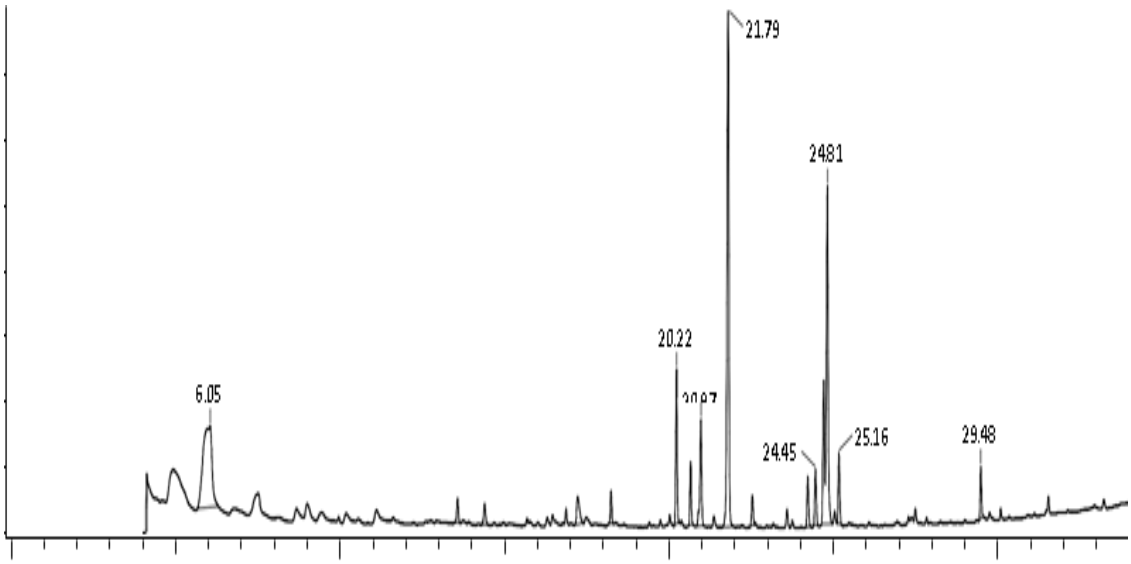

Figure 1: GC-MS chromotogram of methanolic extract of *S. persica* leaves.

Following phyto compounds were observed in the GC-MS analysis of methanolic extract of *S. persica* extract viz. Silane, dimethylphenyl-; 5-Methyl-2-pyrazinylmethanol; 4-n-Dodecylresorcinol; Hexadecanoic acid, methyl ester; 1-Piperazinecarboxamide, N,n-diethyl-4-methyl-; Benzenamine, 2-(1-methylethyl)-; Heptadecanoic acid, 16-methyl-, methyl ester; and 1,3-Octanediol (Fig. 2).

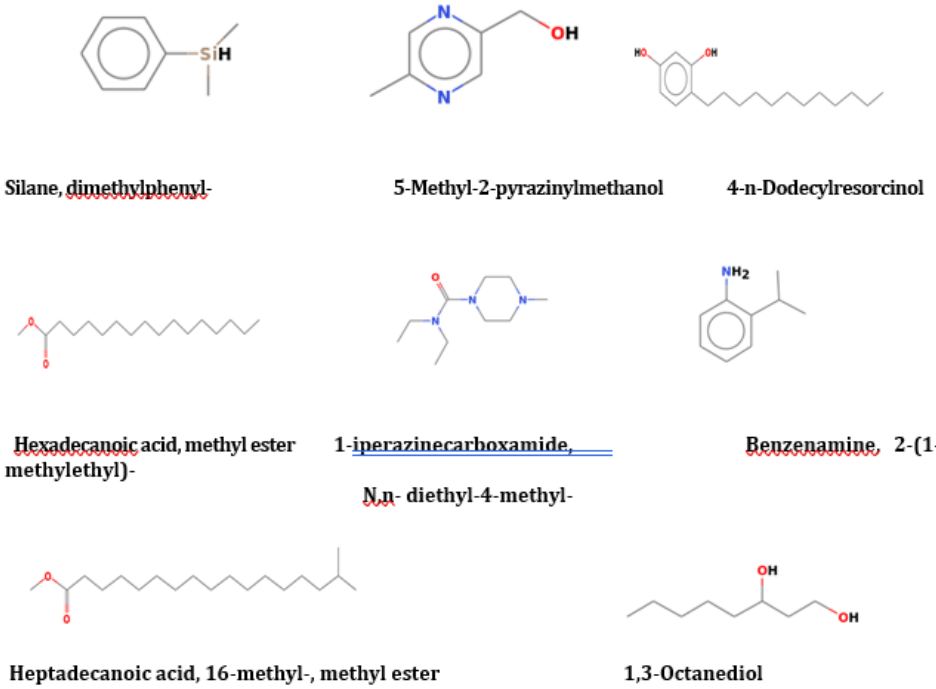

Figure 2: Structures of compounds detected from methanolic extract of *S. persica* leaves.

Table 2: Bioactive compounds identified from methanol extract of *S. persica* leaves.

Sr No.	R.T	Compounds	% Peak area	Molecular weight	Formula
1	6.05	Silane, dimethylphenyl-	6.87	136.07	$C_8H_{12}Si$
2	20.22	5-Methyl-2-pyrazinylmethanol	25.06	124.14	$C_6H_8N_2O$
3	20.97	4-n-Dodecylresorcinol	3.43	278.33	$C_{18}H_{30}O_2$
4	21.79	Hexadecanoic acid, methyl ester	31.32	270.29	$C_{17}H_{34}O_2$
5	24.45	1-Piperazinecarboxamide, N,n-diethyl-4-methyl-	0.61	199.20	$C_{10}H_{21}N_3O$
6	24.81	Benzenamine, 2-(1-methylethyl)-	8.59	135.14	$C_9H_{13}N$
7	25.16	Heptadecanoic acid, 16-methyl-, methyl ester	2.57	298.32	$C_{19}H_{38}O_2$
8	29.48	1,3-Octanediol	21.49	146.15	$C_8H_{18}O_2$

Antimicrobial activity of *S. percica* extract

Methanolic extract of *S. persica* leaves and other two plants were evaluated against gram negative bacterial strain *E. coli* for determination of antibacterial potential (Table 3). *S. persica* extract revealed strongest activity against *E. coli* with 20 mm zone of inhibition which was at par with standard antibiotic Ofloxacin (Figure 3), whereas methanolic extract of *P. glabrumi* showed approximate similar activity with 18 mm zone of inhibition. Surprisingly *C. oppositifolia* failed to show any activity against *E. coli*.

Table 3: Antibacterial activity of methanolic extract of *S. persica* and other two plants against *E. coli*.

Sr. No.	Tested plant extract	Antibacterial sensitivity test against bacteria after 24 hrs at 37^{0}C temperature (Zone of inhibition in mm)
		Gram –ve bacteria *E. coli*
1	*S. persica*	20
2	*P. glabrum*	18
3	*C. oppositifolia*	--
4	Control	--
5	Reference (Ofloxacin)	20

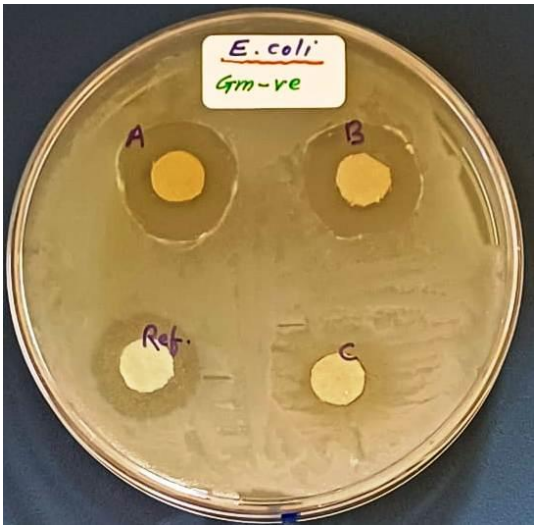

Figure 3: Antibacterial activity of methanolic extract of *S. persica* and other two plants against *E. coli* showing zone of inhibition. A- *S. persica*; B- *P. glabrum*; C- C. *oppositifolia*; Ref- Ofloxacin.

DISCUSSION

In phytochemical analysis methanolic extract was tested positive in various tests for carbohydrates, protein and amino acid, fats, saponins, sterols, terpenoids, alkaloids, phenols, tannins, flavonoids, anthocyanins, anthraquinones and cardiac glycosides. Alkaloids, flavonoids, glycosides, steroids, tannins, terpenoids and saponins were identified from stem and bark of *S. persica* revealing all parts of *S. persica* are rich in diverse phytochemicals signifying its medicinal potential [10]. Kumar and Sharma [11] also revealed that methanol, ethanol and aqueous extract of *S. persica* also had same phytochemical composition. Preliminary phytochemical analysis of *S. persica* herbaceous parts reported occurance of carbohydrates, glycosides, sterols, terpenes, flavonoids, tannins, alkaloids but tested negative for saponins, coumarins, and anthraquinons which was partially contradicted to our observations, it might be due to use of seed germinated plant material in their study [12]. Methanolic extract of *S. persica* roots bark also reported findings similar to present investigations [13]. Chromatogram GC-MS investigation of methanolic extract of *S. persica* recorded 8 major peaks. Hameed et al [14] also performed similar study showing 21 compounds. In both studies Hexadecenoic acid was the common compound proving its commonness. GC-MS investigation performed using petroleum ether extract of *S. persica* sticks and reported 32 compounds including Hexadecenoic acid [15]. *S. persica* leaves extract ethyl acetate fraction recorded 15 compounds which is in accordance with present study [16]. Methanolic extract of *S. persica* reported strong inhibitory action against *E. coli*, in accordance to our observations [10] this study also reported superiority of methanolic extract over other tested extract against various bacteria as well as fungi. Methanol extract of *S. persica* also recorded strongest inhibitory action against *E. coli* [11]; however the zone of inhibition was quite small compared to present investigation. In other studies [12, 15] nil action against *E. coli* using alcoholic extract and ethyl acetate fraction of *S. persica* leaves extract. In conclusion, *S. persica* is an important medicinal plant having huge phytochemical diversity and proven inhibitory potential against microbes. In coming future, *S. persica* need to be evaluated further to get more insights regarding its role in controlling other ailment.

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