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Antimicrobial potential and bioactive constituents from aerial parts of *Vitis setosa* Wall.

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Plants still represent untapped sources of novel compounds with potential therapeutic effects. Hence, the present investigation was carried out to study the *in vitro* evaluation of antimicrobial potential and to assess the bioactive constituents of the aerial parts of *Vitis setosa* Wall. The powdered aerial material was extracted with different solvents and examined for antimicrobial activity against *Escherichia coli, Salmonella typhi, Bacillus subtilis, Streptococcus pyogenes, Candida albicans, Fusarium solani* and *Trichophyton rubrum* using well diffusion method. An ethanol extract of *V. setosa* showed significant antimicrobial activity against all the tested pathogens. The fresh aerial parts contained eight primary metabolites (chlorophylls, carotenoids, total soluble sugars, total soluble proteins, total free amino acids, total phenol, hydroxyl phenols and lipids) and alkaloids, tannins, saponins, triterpenoids and phenolic compounds. Gas chromatography-mass spectrometry (GC-MS) results also showed 26 bioactive compounds including n-hexadecanoic acid, 9,12,15-octadecatrienoic acid (z,z,z)- and τ-tocopherol. In conclusion, the aerial parts of *V. setosa* are a promising source of antimicrobial bioactive compounds.

Key words: Bioactive constituents, *Vitis setosa*, GC-MS analysis, ethanolic extract, antimicrobial activity, folk medicine.

INTRODUCTION

In the plant kingdom, there are thousands of plants, known and unknown, that yield medicines or drugs useful to man. These plants are the gold mines to treat the diseases of men and animals, and serve as cure in the natural way (Jain, 1979). Medicinal herbs constitute the cornerstone of traditional practice world-wide and they have been used for centuries as remedies for human diseases because they contain chemical components of

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therapeutic value (Christina and Muthumani, 2013) and the plants are known to possess various secondary metabolites which show inhibitory effect against the various human diseases (Bhardwaj and Laura, 2009; Gobalakrishnan et al., 2013). These herbs are relatively cheap, easily available and their use depends on ancestral experience (Jamilu et al., 2008). Plants still represent a large untapped source of novel compounds that might serve as leads for the development of novel drugs (Cowan, 1999) and they are an important source of new biochemical substances with potential therapeutic effects (Nadaf et al., 2012), inhibiting the growth of microbes by interfering with their specific physiological characters or metabolic functions (Arekemase et al., 2011).

The first step towards this goal is the biological and phytochemical screening of plant extracts from traditional preparations used in the popular medicines (Alonso-Paz et al., 1995). Drug resistance of human pathogenic bacteria has phytochemical substances which have been commonly and widely reported in literature (Sarac and Ugur, 2007). Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, many scientists have focused their attention on the extracts and biologically active compounds isolated from medicinal plant species for herbal medicines (Essawi and Srour, 2000). Now-a-days, there is a growing interest in the antimicrobial screening of extracts and essential oils from plants to discover new antimicrobial agents. Keeping this in view, the present study was undertaken to investigate the natural bioactive constituent analysis and antimicrobial potential of the aerial parts extract of *Vitis setosa* (Family: Vitaceae; Pulinaralai in Tamil), a medicinal vine herb that grows only in selective areas of the Indian subcontinent.

MATERIALS AND METHODS

Plant

Aerial parts of fresh *V. setosa* plant were collected during January to April, 2011 from various regions of the Pudukkottai district in Tamilnadu, India. Plant was identified using the facility of Rapinat Herbarium, St. Joseph's College, Tiruchirappalli and the identified voucher specimen was deposited in the Research and PG Department of Botany, H.H The Rajah's college, Pudukkottai, Tamilnadu, India. Plants were thoroughly washed with tap water and aerial parts were separated and kept in between the filter papers in a dark room at room temperature to get rid of moisture until further analysis.

Preparation of extract

Dried aerial part materials were powdered with Waring blender, at room temperature and 2 g of the sample powder was soaked in 20 ml of different solvents (ethanol, ethyl acetate, chloroform, hexane, benzene and water) overnight. Later, the samples were filtered under vacuum using Whatman No.1 filter paper and stored in airtight screw-capped bottles at 5°C for further analysis.

Preparation of inoculum

Seven clinical pathogenic organisms were obtained from the Microbial Clinical Laboratory, KMC Hospital, Tiruchirappalli. Out of the seven, four strains were bacteria (*Escherichia coli, Salmonella typhi, Bacillus subtilis* and *Streptococcus pyogenes*) and three strains were fungi (*Candida albicans, Fusarium solani* and *Trichophyton rubrum*). Stock culture was maintained at 5°C on slopes of nutrient agar for bacteria and potato dextrose agar (PDA) for fungi. Under the sterile conditions, active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to the test tubes of respective media for respective tested organisms and incubated without agitation for 24 h at 37 ± 2°C for antibacterial activity and at 25 ± 2°C for 48 h for antifungal activity. Muller-Hinton broth (for bacteria) and PD broth (for fungi) were prepared for streaking and fresh slant cultures were prepared and stored in refrigerator at 5°C for future requirements.

In-vitro antimicrobial tests

Spectrum of antibacterial activity was studied by using the techniques described by Bauer et al. (1966). Gentamycin sensitivity disc (30 mg; Hi-Media) was used as a positive control and respective solvents were taken as negative controls. At the end of incubation, inhibition zones formed around the discs were measured with transparent ruler in millimeter. These studies were performed in triplicate.

Biochemical screening

Biochemical tests: Chlorophylls *a*, chlorophylls *b*, total chlorophylls, total sugars and total proteins (Sadasivam and Manickam, 2005), carotenoids (Goodwin and Britton, 1988), total free amino acids (Troll and Cannan, 1953), total phenols and hydroxyl phenols (Swain and Hillis, 1959) and lipids (Jayaraman, 1981) were quantitatively revealed of the fresh aerial part of *V. setosa*. Secondary metabolites were tested by the standard methods of Harborne (1973) and Odebiyi and Sofowora (1978).

Preparation of extraction in GC-MS Analysis

Aerial parts of *V. setosa* were shade dried. 20 g of the powdered materials was soaked in 95% ethanol for 12 h. The extracts were then filtered through Whatman filter paper No. 41 along with 2 g sodium sulfate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulfate was wetted with 95% ethanol. The filtrate was then concentrated by bubbling nitrogen gas into the solution. The extract contained both polar and non-polar phytocomponents of the plant material used and these extracts (2 µl of injection sample) were employed for GC/MS analysis (Merlin et al., 2009).

GC-MS analysis

GC-MS analysis was performed in the Indian Institute of Crop Processing Technology (IICPT), Thanjavur, India. Prepared alcoholic samples were analysed in a Perkin Elmer GC Clarus 500 MS

Table 1. Antimicrobial activity of *V. setosa* extracts in different solvents.

Tested organisms	Inhibition zone of diameter (mm)[a].						
Bacteria	**A**	**B**	**C**	**ET**	**EA**	**H**	**Gentamycin (+) disc**
E. coli	-	-	-	15.0±0.3	-	-	23.5
S. typhi	-	-	-	11.3±0.5	-	-	28.3
B. subtitles	-	-	-	10.3±0.2	12±0.3	-	25.8
S. pyogenes	-	-	-	10.6±0.4	-	-	31.7
Fungi							
C. albicans	13.7±0.5	-	12.1±0.2	11.9±0.4	12.5±0.2	-	35.6
F. solani	-	-	-	12.3±0.5	13.5±0.3	-	28
T. rubrum	-	-	-	10.3±0.3	14.3±0.6	-	26.2

Aqueous (A), Benzene (B), Chloroform (C), Ethanol (ET), Ethyl acetate (EA), Hexane (H), No activity (-).[a] - Values are mean ± standard deviation of three determination.

system for different components present in the extract, under the following conditions: column – dimethyl polysiloxane DB-1 fused silica capillary column (30 m × 0.25 mm × 0.1 µm of film thickness); carrier gas - helium (1 ml/min); injector temperature 250°C; detector temperature 200°C; column temperature 35 to 180°C at 4°C/min; then 180 to 250°C at 10°C/min; MS electron impact 70 $_e$V. Identification of the constituents was achieved with the aid of the respective Kovarts Indices and comparison of the mass spectra with those in the library (NIST Ver.2.1).

RESULTS

Present study investigated the antimicrobial activity of aerial parts of *V. setosa* crude extract against four bacteria (*E. coli, B. subtilis, S. typhi, S. pyogenes*) and three fungi (*C. albicans, F. solani* and *T. rubrum*), and the results are presented in Table 1. Antimicrobial activity of ethanolic extract of *V. setosa* aerial parts showed significant antimicrobial activity against all the tested pathogens. Ethanolic extract had maximum (15.0 mm) inhibition against *E. coli*. Whereas, the ethyl acetate extract exhibited moderate significant antimicrobial activity and maximum (14.3 mm) inhibition against *T. rubrum*.

Results of the biochemical screening extract of fresh aerial parts of *V. setosa* were presented: Chlorophyll *a* (1.868 mg g^{-1}), chlorophyll *b* (1.350 mg g^{-1}), total chlorophyll (2.300 mg g^{-1}), carotenoids (0.448 mg g^{-1}), total soluble sugars (47.50 mg g^{-1}), total soluble proteins (51.30 mg g^{-1}), total free amino acids (5.78 mg g^{-1}), total phenol (12.27 mg g^{-1}), hydroxyl phenols (6.870 mg g^{-1}) and lipids (20 mg g^{-1}). Further, alkaloids, tannins, saponins, triterpenoids and phenolic compounds were also present in the *V. setosa*.

Gas chromatography and mass spectroscopy analyses were carried out on the alcoholic extract of aerial parts of *V. setosa* and various bioactive compounds were identified. Active principles with their retention time (RT), molecular formula (MF), molecular weight (MW), concentration (%) and nature of the compounds are presented in Table 2. In the present investigation, a variety of compounds has been detected: Pentanoic acid; 2-acetyl-4-methyl- ethyl ester, 1-Butanamine;2-methyl-N- (2-methylbutylidene), benzeneacetaldehyde, propan,1,2,3-triethoxy-, 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl, β-D-glucopyranose;4-O-β–D-galactopyranosesyl, dodecanoic acid; methyl ester, 2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trymethyl-(R)-[Syn:Dihydroactinidiolide], dodecanoic acid, phosphonofluridic acid; (1-methylethyl)- cyclohexyl ester, tetradecanoic acid, 2-pentadecanone,6,10,14-trimethyl-[Syn:Hexahy drofarnesyl acetone], pentadecanoic acid, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, n-hexadecanoic acid, hexadecanoic acid ethyl ester, phytol, 9,12-octadecadienoic acid (z,z)-, 9,12,15-octadecatrienoic acid (z,z,z)-, Octadecanoic acid, 4,8,12,16-Tetramethylheptadecan-4-olide, 1,2-Benzenedicarboxylic acid; diisooctyl ester, Squalene, Cholesta-4,6-dien-3-ol,(3β), τ-Tocopherol and 9,10-Seco Cholesta-5,7,10(19)-trine-3,25,26- triol,(3β ,5Z,7E). GC-MS spectrogram showing the peak identities of the compounds is depicted in Figure 1.

DISCUSSION

In recent years, various scientists have accelerated research on the drugs and dietary supplements from the plants (Cowan, 1999) and used them as herbal medicines for the treatment of infectious diseases (Madureira et al., 2012), since no literature is currently available to substantiate antimicrobial prospective of the *V. setosa* aerial part. Therefore the present study was made on

Table 2. Compounds identified from ethanol extract of *V. setosa.*

RT	Compound	MF	MW	Peak area (%)	Compound Nature
3.57	Pentanoic acid,2-acetyl-4-methyl-,ethyl ester	$C_{10}H_{18}O_3$	186	1.38	Halogen- acids
4.13	1-Butanamine,2-methyl-N-(2-methylbutylidene)	$C_{10}H_{21}N$	155	1.55	Butyl alcohol
4.24	Benzeneacetaldehyde	C_8H_8O	120	5.05	Aromatic hydrocarbons
4.48	Propan,1,2,3-triethoxy-	$C_9H_{20}O_3$	176	1.24	Ether compound
5.79	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl	$C_6H_8O_4$	144	5.05	Flavonoid fraction
7.85	β-D-Glucopyranose,4-O-β –D-galactopyranosesyl- [syn:-Lactose]	$C_{12}H_{22}O_{11}$	342	4.21	Sugar compound
10.34	Dodecanoic acid, methyl ester	$C_{13}H_{26}O_2$	214	0.34	Lauric acid ester
10.75	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro- 4,4,7a- trymethyl-(R)-[Syn:Dihydroactinidiolide]	$C_{11}H_{26}O_2$	180	1.32	Heterocyclic Compounds
10.97	Dodecanoic acid	$C_{12}H_{24}O_2$	200	1.21	Lauric acid
11.15	Phosphonofluridic acid,(1-methylethyl)-,cyclohexyl ester	$C_{18}H_{18}FO_2P$	208	1.52	Phosphorus Compounds
13.39	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	2.12	Myristic acid
14.4	2-Pentadecanone,6,10,14-trimethyl- [Syn: Hexahy drofarnesyl acetone]	$C_{18}H_{36}O$	268	5.35	Diterpenoids
14.75	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	0.59	Lauric acid
15.04	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	0.55	Terpene alcohol
16.16	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	19.68	Falmitic acid
16.46	Hexadecanoic acid ethyl ester	$C_{18}H_{36}O_2$	248	2.19	Fatty acid
18.41	Phytol	$C_{20}H_{40}O$	296	4.98	Diterpene
18.73	9,12-Octadecadienoic acid(z,z)-	$C_{18}H_{32}O_2$	280	1.96	Linoleic (poly unsaturated fatty acid)
18.84	9,12,15-octadecatrienoic acid,(z,z,z)-	$C_{18}H_{30}O_2$	278	7.25	Linoleic acid
19.15	Octadecanoic acid	$C_{18}H_{36}O_2$	284	3.75	Myristic acid
21.95	4,8,12,16-Tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$	324	2.36	Carbohydrates
24.68	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	2.92	Plasticizer compound
28.92	Squalene	$C_{30}H_{50}$	410	2.6	Triterpenoids
33.01	Cholesta-4,6-dien-3-ol,(3β)-	$C_{27}H_{44}O$	384	3.63	Steroids
33.78	τ-Tocopherol	$C_{28}H_{48}O_2$	416	5.53	Vitamin E groups
35.29	9,10-Secocholesta-5,7,10(19)-trine-3,25,26-triol,(3β ,5Z,7E)-	$C_{27}H_{44}O_3$	416	11.67	Steroids

on antimicrobial study of *V. setosa* aerial parts extract to provide scientific evidence for its use as a folk medicine. Results revealed that the plant has a potential antimicrobial activity against all the tested pathogens. Ethanolic extract showed effective antibacterial activity and it has better solubility compared to other solvents. It is worth mentioning here that the ethanol formulations are relatively safe for human consumption as com-pared with other organic solvents (Wendakoon et al., 2012).

The preliminary biochemical tests are significant and helpful in finding chemical constituents in the plant materials that might lead to the source of pharmacologically active compounds (Iqbal, 2012). Chanda and Kaneria (2011) reported that the bioactive compounds are normally accumulated as secondary metabolites in all plant cells but their concentration could vary in different plant

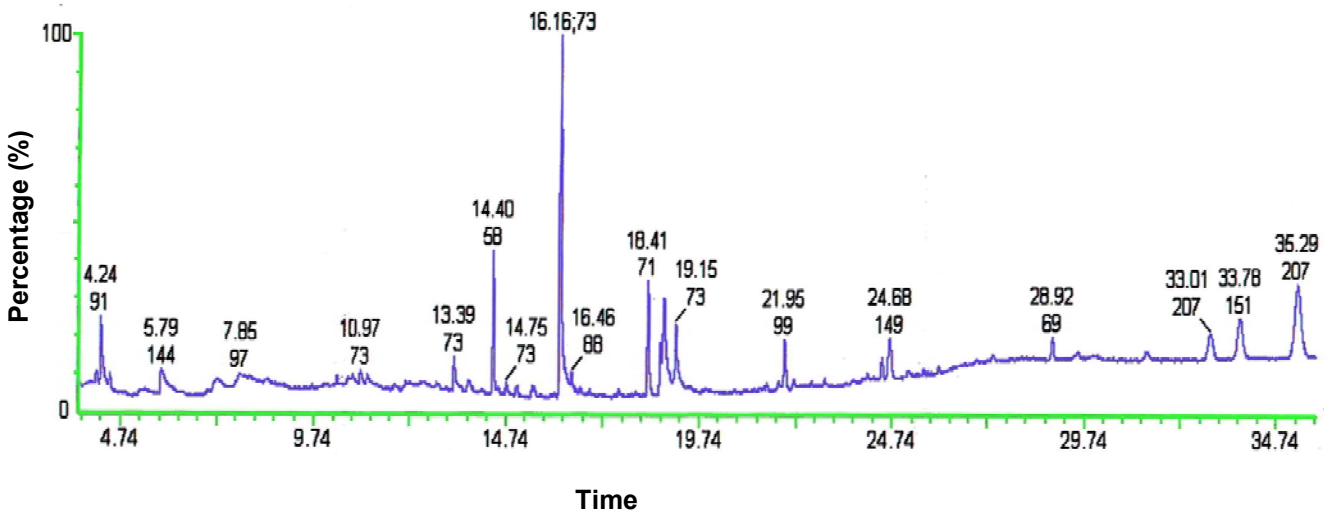

Figure 1. GC-MS spectrogramof ethonolic extract of the *V. setosa.*

parts. In this regard, aerial part is one of the highest accumulatory plant parts and its compounds are generally preferred for therapeutic purpose (Pires De Abreu et al., 2003).

Generally, alkaloids, saponins, tannins, flavonoids and phenolic compounds are important antimicrobials (Yadav and Agarwala, 2011). This lends support to the present study which revealed that those phytochemicals were identified in *V. setosa*. Similarly, previous studies have revealed that plant phytochemicals obtained from *V. setosa* leaf could act as antimicrobial drugs (Misra, 2009; Hemayet et al., 2012). Action mechanism of such compounds has not been unequivocally established, but they may interfere with peptidoglycon bacterial cell wall synthesis in the effected organisms (Rasooli and Mirmostafa, 2002) and in many other ways such as inhibiting protein synthesis, interfering with nucleic acid synthesis, breaking the peptide bonds, acting as chelating agents, inhibiting the metabolic pathway, lysis of cells and preventing the utilization of available nutrients by the microorganisms.

Knowledge of chemical constituents of plants is desirable because such information will be important for synthesis of chemical substances (Yadav and Agarwala, 2011). It could be qualified for application in pharmaceutical industry (Iqbal, 2012). Therefore, present study revealed that in the aerial part extract of *V. setosa* were identified 26 different compounds by GC-MS. Especially, n-Hexadecanoic acid, 9,10-Seco Cholesta-5,7,10(19)-trine-3,25,26-triol,(3β,5Z,7E, 9,12,15-octade-catrienoic acid (z,z,z) and τ-Tocopherol contributed more percentage than the other compounds. Compounds except pentanoic acid; 2-acetyl-4-methyl-ethyl ester, 1-butanamine-2-methyl-N-(2-methylbutylidene)-benzene-acetaldehyde, propan,1,2,3-triethoxy, 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trymethyl-(R)-phosphonofluridic acid;(1-methylethyl)- cyclohexyl ester, 2-Pentadecanone,6,10,14-trimethyl and Cholesta-4,6-dien-3-ol,(3β) were identified.

n-Hexadecanoic acid was extracted from the *V. setosa* aerial part (19.69 %), which is higher than that of the other extracted compounds. Harada et al. (2002) reported that this compound acts as an anticancer drug and Graikou et al. (2011) observed antioxidant and antimicrobial property for this compound. Similarly, Praveenkumar et al. (2010) reported that n-Hexadecanoic acid act as an antioxidant, hypocholesterolemic nematicide, pesticide, anti-androgenic flavor, hemolytic and 5- alpha reductase inhibitor.

9,12,15-Octadecatrienoic acid-(z,z,z) belonging to linoleic acid group was extracted from *V. setosa* (7.25%) and this compound acts as antiinflammatory, hypocholesterolemic cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic antieczemic, antiacne, 5-Alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary, insectifuge (Praveenkumar et al., 2010) and also as an antimicrobial agent (Senthilkumar and Kamaraj, 2010). τ-Tocopherol was obtained from *V. setosa* (5.53%) and it belongs to vitamin E which has reactivity with radicals and shows some antioxidant effect (Yoshida et al., 2007). It also has important implications in the anti-inflammatory effects and can control neutrophil oxidative burst (Varga et al., 2008). Further, vitamin E is associated with a decreased risk of heart diseases and certain cancers (Dickinson, 2002;

Jaialal and Devaraj, 2003).

In conclusion, ethanolic extract of aerial parts of *V. setosa* possess significant antimicrobial activity and this potential may be due to the presence of bioactive compounds like alkaloids, tannins, saponins, triterpenoids and phenolic compounds. Hence, the present study was justified on its use in the traditional folk medicine. GC-MS analysis also identified a variety of natural bioactive compounds including n-hexadecanoic acid, 9,12,15-octadecatrienoic acid (z,z,z)- and τ-tocopherol. However, a further detailed study on *V. setosa* is necessary for the development of novel drugs in the arena of antioxidant, anti-inflammatory, anticancer, antiandrogenic, antiarthritic and anticoronary activity.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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