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PHYTOCHEMICAL SCREENING AND GC-MS ANALYSIS OF n-HEXANE/ETHYL ACETATE LEAF EXTRACT OF *VENTILAGO DENTICULATA WILLD*

KONGARA SHOBHA RANI

Lecturer in Chemistry, VSR & NVR College, Tenali, Guntur Dist. AP, India

ABSTRACT

In the present research work the phytochemical analysis on leafs of *Ventilago denticulata Willd* was carried out. The leaves indicated the presence of Steroids, resins, steroids, tannins, glycosides, reducing sugar, carbohydrates, saponins, terpenoids, acidic compounds, phenols, alkaloids, flavonoids. In the GC-MS analysis of the *Ventilago denticulata Willd* extract result shows the presence of 14 compounds in the leaf extract by comparing their retention time and by interpretation of their mass spectra. The extracts of *Ventilago denticulata Willd* leaves have a broad spectrum of anti-bacterial activity and support the traditional use of these plants as medicines. This study also helped to identify the formula and structure of biomolecules which can be used as drugs.

Key words: *Ventilago denticulata Willd*, phytochemical screening, anti-bacterial activity, gas chromatography- mass spectrometry.

INTRODUCTION

India is known for its wealth of medicinal plants which are found in its diverse climatic and physiographic conditions. Medicinal plants are widely used for the management of different disease conditions and to play a beneficial role in human health[1,2]. Plant derived drugs have a market of about 20 billion annually in the United State alone. It is also estimated that only 5-15 % of potential useful plants have so for been systematically explored for useful chemical[3]. Plants are found to be sources of many chemical compounds, most of which account for their various uses by man. The most important of these compounds are alkaloids, terpenoid, steroid, phenolic compound, glycosides and tannin[4]. Traditional folk treatment from wild plants has always guided researchers to search for novel medications to develop healthy life for humans and animals[5]. In addition, some medicinal plants are still obscured within the plant which needs to be scientifically evaluated. The phytochemical research based on ethanopharmacological information is considered an effective[3]. The chemical substances used by plants for approach in the discovery of new agents from higher plants defense system and serve as the bioactive principle for various drugs in modern chemotherapy[6] .The GCMS is composed of two major building blocks; the gas chromatograph and the Mass spectrometer. The gas chromatograph utilizes a capillary column which depends on the columns dimension as well as the phase properties. The GCMS has been widely heralded as a "gold standard'' for forensic substance identification because it is used to perform a specific test.

Ventilago is a genus of plants in the family Rhamnaceae (Figure 1). It includes about 35 species found in the tropics southern India. *Ventilago denticulata Willd* is a Climbing shrub, stems sometimes twining. Leaves alternate, pinnately veined. *V. denticulata Willd* commonly called the Red Creeper is an extensively branched, woody climber with hanging branches. The stem and root bark of this plant is a source of a red dye 'ventilagin', which is used for coloring cotton, wool and tasar. Stem bark when powdered and mixed with

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sesame oil, can be externally applied to treat skin diseases and sprains. Root bark is used for atonic dyspepsia, mild fever and debility. Sap is used for the treatment of deafness. The ethanolic extract of plant also shows antiinflammatory activity.[7] The plant is rich in many pharmaceutical active ingredients. The stem bark contains friedelin and several anthraquinones. The root contains anthraquinones, ventinones A and B. Major constituents of the root bark are emodin, its glucoside and corresponding analogues, ventiloquinones. The fruit, leaves and stem give lupeol, beta-sitosterol and its glucoside.[8] The ethanolic extract from Rhang Dang leaves exhibited a strong antioxidant activity and prevented hemolysis. It showed the highest amount of phenolics (91.03 ± 12.43 mg of gallic acid equivalents/g extract) and flavonoid compound (69.76 ± 10.84 mg of catechin equivalents/g). Interestingly, this extract was more cytotoxic to HepG2 cells than PBMC[9]. Considering the medicinal importance of *Ventilago denticulata Willd,* an attempt has been made to investigate the phytochemical and anti-microbial activities of n-hexane/ ethyl acetate extract (60:40) from leaves of *V. denticulate.* Furthermore, the phytochemical constituents were identified from leaf extract of this plant by using gas chromatography-mass spectrometry (GC-MS) analysis.

Botanical Classifications
Tracheophyta
Magnoliopsida
Rosales
Rhamnaceae
Ventilago
Ventilago denticulate

Figure 1: *Ventilago denticulata Willd*

Experimental

MATERIALS AND METHODS

***Chemicals*:** All Chemicals used in the entire study were AR grade obtained from SD fine, Merck chemicals, India, Pvt Ltd.,

***Plant Material*:** Fresh roots of *Ventilago denticulata Willd* were collected in the month of September 2015 from Nallamala forest area and shrub is botanically identified by the department of Botany, Acharya Nagarjuna University, and Guntur, India. Leafs were collected in bulk (3 kgs) and completely dried for one month in sunlight to eliminate surface moisture. Then dry leaf mass packed into envelops and kept in oven at 55^{o}C temperature for further dryness. Dried mass later grinded separately in a mortar obtained fine powder and sieved; which was then kept in plastic bags for further use.

Preparation of plant extract: The dry leaf material of *Ventilago denticulata* passed through sieve (100□). The coarse powdered drug (200grams) was extracted in Soxhlet apparatus for 48 h with n-hexane: ethyl acetate (60:40 ratio) (60-75°C, 2L) extract obtained was concentrated under reduced pressure in rotatory evaporator below 60°C temperature to get semisolid sticky residue (20gm)

***GC-MS Analysis*:** GC-MS analysis of each extract sample was performed using a Perkin-Elmer GC Clarus 500 system and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with Elite-I, fused silica

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capillary column (30mm x 0.25mm 1D x 1 µMdf, composed of 100% Dimethyl poly siloxane). For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.99%) was used as the carrier gas at constant flow rate 1mL/min and an injection volume of 2µl was employed (split ratio of 10:1); Injector temperature 250°C; Ion-source temperature 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5seconds and fragments from 45 to 450 Da. Total GC running time was 72 minutes. The relative % amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a Turbomass. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained (Table 2 & Figure 2).

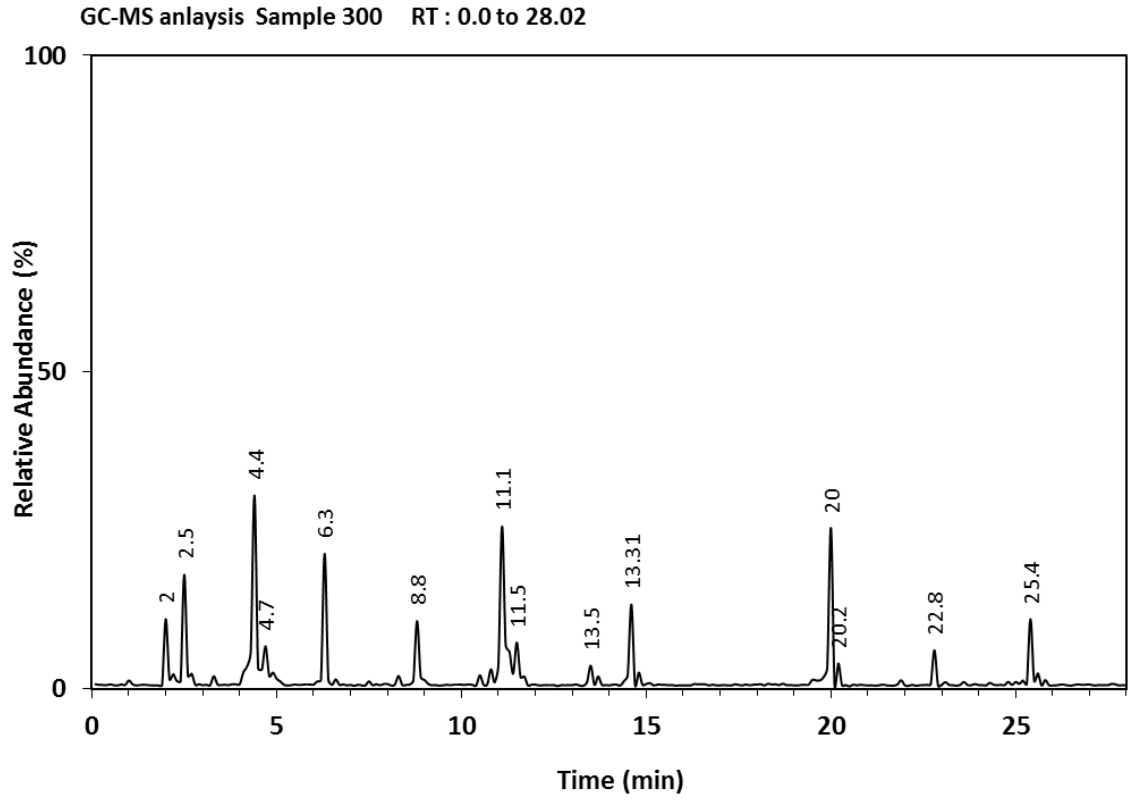

Figure 2: GC-MS spectra of n-hexane/ethyl acetate extract

Preliminary phytochemical analysis

Anti-Bacterial Activity by Disc Diffusion Method[11]

Preparation of Inoculum: *Escherichia coli, Staphylococcus aureus* strains were used. 50ml of nutrient broth was prepared in 100ml conical flask. It was sterilized & then inoculated with inoculum with the help of sterile loop in laminar air flow from preserved slants. They were then kept in incubator at 37°Cfor 24 hours for organism to grow.

Preparation of Media: 200ml of nutrient agar media (NAM) was prepared and the pH was maintained at 7.0 to 7.2.

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Pour Plate Method: 1ml of prepared inoculum was poured in sterile Petri dish & then 15 ml of NAM was poured in it & allowed to solidify.

Disc Diffusion Method: After solidification the disc of whatman 42 filter paper imbibed with 20 µl plant extracts were carefully placed with the help of forceps at the centre of the Petri dish and then kept in incubator for 24hrs.

Measurement of Zones: With the help of antibiotic zone scale the zone of inhibition (ZOI) were measured

Phytochemical Qualitative Analysis

Phytochemical screening of leaf extract has been analysed, which revealed at the presence of constituents which are known to exhibit medicinal active compounds as well as physiological activities. Analysis of the plant extract gives a positive test in the qualitative analysis for alkaloids, Terpenoids, carbohydrates, saponin, tannin, glycosides, steroidal glycosides; phenolic compounds etc., identified by standard tests[10] and the functional groups are shown in the table.1

Results and Discussion

The phytochemical constituent of *Ventilago denticulata* was qualitatively analyzed and the results are presented in Table 1. In the GC-MS analysis the mass spectra of identified compounds from n-hexane/ethyl acetate (60:40) leaf extract of *V. denticulata* were matched with those found in the NIST spectral database are given in Table 2 along with the medicinal properties (based on existed literature) of the analysed phytochemical constituents and the chromatographic peak are represented in Figure 1. The identification of compound based on comparison of their mass spectra with those of NIST[12]. This phytochemical screening aids as an initial step for future determination of its activity like antioxidant, anticancer, antiinflammatory, antimutagenic etc.

The GC/MS analysis showed that at least 14 compounds were present in leaf extract of *Ventilago denticulate* corresponding mass spectra resembles with the identified copounds are given in Figure 3 (i-xiv). The fragmentation pattern of the major compound is (16.27%) L-(+) ascorbic acid retention time is 4.4 and peak area percentage is 16.27. The next highest found compound is (14.74%) Methyl hexadecanoate or Palmitic acid methyl ester retention time is 11.1 with 14.7 peak area percent. These compounds have good pharmacological activity viz., Antioxidant, Immunomodulator, Antiandrogenic, Flavor, Hemolytic, Antioxidant, anti-bacterial, Hypocholestrolemic Nematicide, Pesticide, 5-Alpha reductase Inhibitor. The compound Squalene with peak area percentage 4.57 and RT 25.4 has shown to improve human immunity. Phytol was also detected 10.7% relative amount with 20.00 retention time; this compound is known to possess an antimicrobial, antioxidant activity.

Table 1: Display the presence/ absence of different phytochemicals in the leaf extract of *Ventilago denticulata*

Phytoconstituents	**Test**	n-Hexane/ Ethyl Acetate
Alkaloids	Wagner's test	+ +
Amino acids	Ninhydrin Test	--
Carbohydrates	Molish test	++
Cardiac glycosides	Keller-Killani test	--
Flavonoids	Shinoda's test	++

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Phenolics	phenol test	++
Polysterols	Salkowski's Test	++
Proteins	Biuret test	--
Saponins	Frothing test	++
Steroids	Libermann-Buchard's test	++
Tannins	Ferric chloride test	--
Terpenes	Salkwaski's test	++

Table 2: Components detected in n-hexane extract of *Ventilago denticulata* leaf extract

S.No	RT	% of area	Compound name	M.F	MW	Nature of Compound	Medicinal Importance derived from Literature#
1	2	12	1,2-benzenedicarboxylic acid	C8H6O4	166.03	Aromatic dicarboxylic acid	Used as Softeners, Used in preparation of perfumes and cosmetics, Used as plasticized vinyl seats on furniture and in cars, and clothing including jackets, raincoats and boots. Used in textiles, as dyestuffs, cosmetics and glass making
2	2.5	9.67	Carbamult	C12H17NO2	207.13	Aromatic acetamide	Pesticide,INSECTICIDE
3	4.4	16.27	L-(+)ascorbic acid; Vitamin C	C6H8O6	176.03	Saturated carboxylic acid	Vitamin C, Antioxidant, Immunomodulator
4	4.7	2.14	Carvacrol	C10H14O	150.21	Terpenoid	Nematocide
5	6.3	10.2	n-Hexadecanoic acid			a saturated fatty acid that is the major fat in meat and dairy products	Antibacterial and antifungal activity
6	8.8	8.54	9,12-Octadecadienoic acid (Z,Z)-	C18H32O2	280.44	Ethyl Ester/Fatty acid	Anti-inflammatory, acne reductive, antioxidant

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7	11.1	14.74	Methyl hexadecanoate or Palmitic acid methyl ester	C17H34O2	270.45	a saturated fatty acid that is the major fat in meat and dairy products	Lubricant, Antiandrogenic, Flavor, Hemolytic, Antioxidant, Hypocholestrolemic Nematicide, Pesticide, 5-Alpha reductase inhibitor
8	11.5	1.57	Dronabinol	C21H28O4	344.44	Steroid	Analgesic, antiinflammatory
9	13.5	1.02	Acetylecysteine	C5H9NO3S	163.19	Acetyl group	used to treat paracetamol (acetaminophen) overdose and to loosen thick mucus such as in cystic fibrosis or chronic obstructive pulmonary disease
10	13.31	7.24	Pentadecanoic acid	C15H30O2	243	the saturated fatty acid with a 20-carbon chain	it is as a minor constituent of peanut oil (1.1%-1.7%) and corn oil (3%)
11	20	10.07	Phytol			acyclic diterpene alcohol	antimicrobial, antioxidant Cancer-Preventive
12	20.2	0.94	□-terpinene	C10H16	136.2	Mono terpenoid	Food additives, Flavoring Agents,alpha-Terpinene is found in allspice
13	22.8	1.09	9,12,15-Octadecatrienoic Acid, (Z,Z,Z)	C20H34O2	306	Ethyl Ester	Hypocholesterolemic, Nematicide Antiarthritic, Antihistaminic Anticoronary, Insectifuge, Antieczemic

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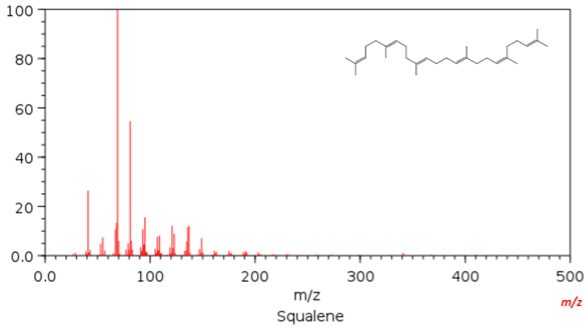
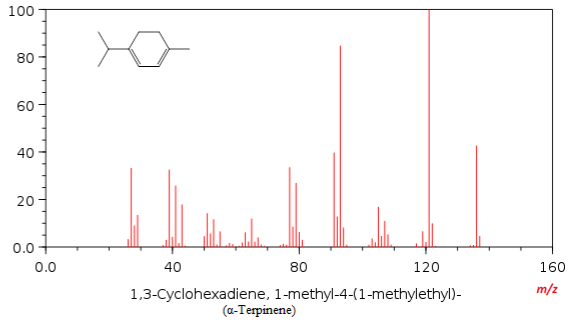
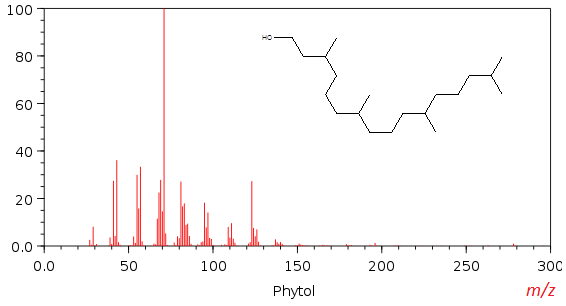
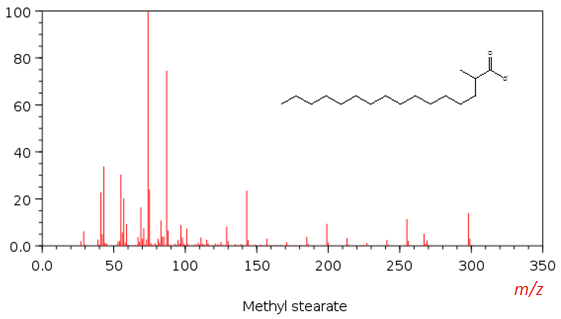
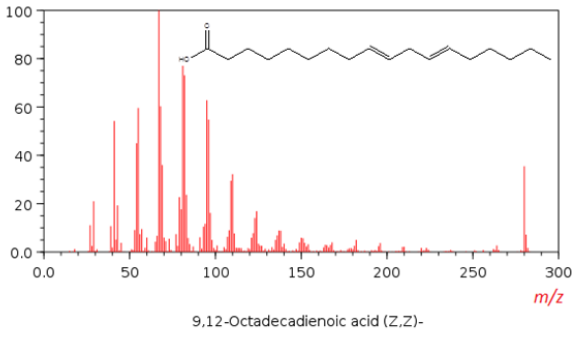
14	25.4	4.57	Squalene	C30H50	410.7	dehydrotriterpenic hydrocarbon	Antibacterial, Antioxidant, Antitumor, Cancer-Preventive, Chemopreventive, Immunostimulant and Lipoxygenase-Inhibitor
#Source: Dr. Duke's phytochemical and ethnobotanical databases [Online database].							

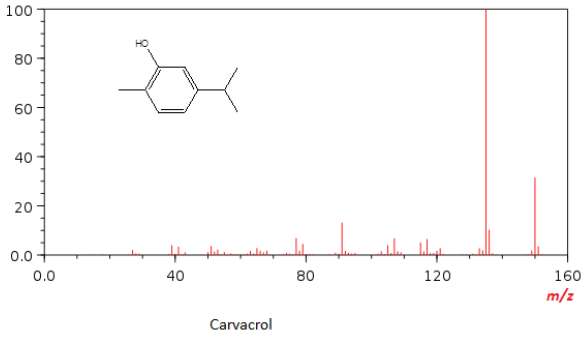












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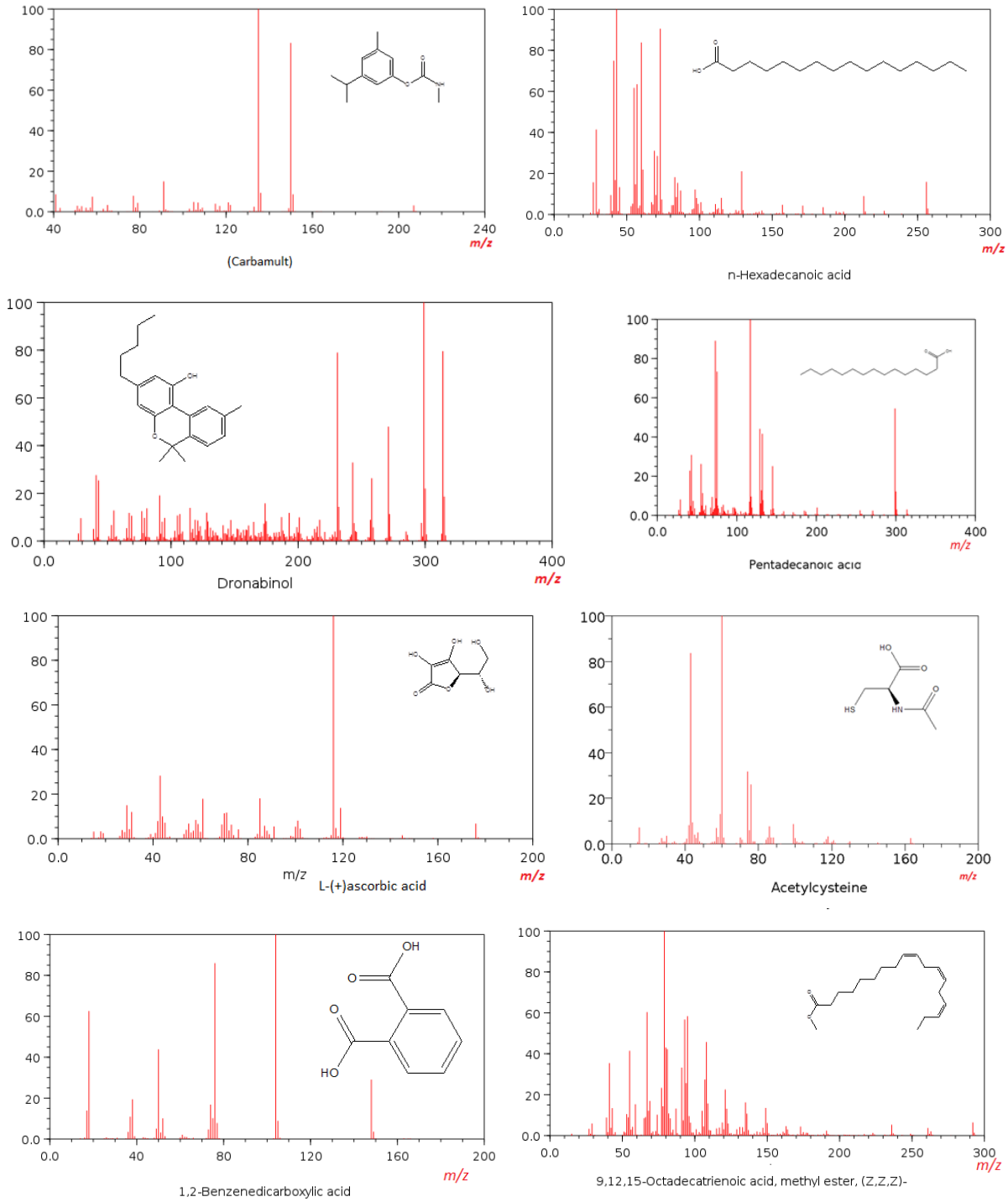

Figure 3: Mass spectra of identified compounds from leaf extract of *Ventilago denticulate (i to xiv)*

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Anti-bacterial Activity

The inhibitory action of identified compounds had been historically recognized and applied as a useful therapeutic agent for preventing wound infections. The antibacterial activities of extracted materials were investigated against two different types of bacterial strains like *Escherichia coli and Staphylococcus aureus.* (Figure 4) showed excellent antibacterial activity against tested bacterial strains at the volumes of 50 µL/well and 100 µL/well. The zone of inhibition (in mm) ranges identified for *Escherichia coli* (9.24 ± 0.22 & 14.25 ± 0.71) and for *Staphylococcus aureus* (7.08 ± 0.54 & 13.20± 0.22). The diameters of the inhibition zones for the all tested pathogens are listed in Table 3. Thus, our results show that root n-hexane/ethyl acetate extract sample has potential bacterial activity against *S. typhi* and *S.aureus*.

Table 3: Zone of Inhibition of selected microbial cultures

Bacteria	Zone of inhibition (mm) 100 µL/well	Zone of inhibition (mm) 50µL/well
Escherichia coli	14.25 ± 0.71	9.24 ± 0.22
Staphylococcus aureus	13.20± 0.22	7.08 ± 0.54

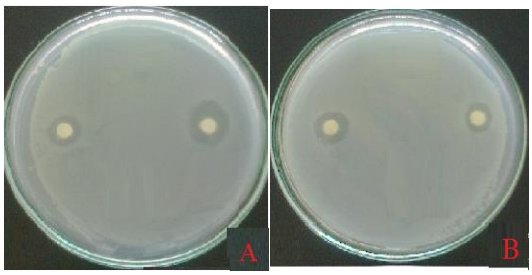

Figure 4: Showing antibacterial activity of n-hexane and ethyl acetate leaf extract of *Ventilago denticulata* against *Escherichia coli (A)* and *Staphylococcus aureus* (B) microbes at the volumes of 50 µL/well and 100 µL/well

Conclusion

In conclusion, the present study has shown that leaf extract of whole plant of *Ventilago denticulata* has significant anti-bacterial activity, but further studies are required to identify & isolate the actual phytoconstituents present in this plant which are responsible for other medicinal activities. The presence of phytochemicals such as total phenolics, alkaloids, phenols etc., (Table 2) in *Ventilago denticulata* provides some scientific evidence for the biological activities and also accounts for the multipharmacological use of this plant in traditional medicine. The results of this study offer a base of using *Ventilago denticulata* as herbal alternative for the synthesis of antimicrobial agents. It would be worthwhile to further isolate the compounds and determine their specific activity and also to understand the synergistic effect of compounds for therapeutic roles.

References

1. Krishna CS, Chandra R and Khaleel KM: Phytochemical screening and GC-MS studies of *Syzygium dhaneshiana* leaf and bark extracts. Int J Pharm Sci Res 2017; 8(5): 2277-81.doi: 10.13040/IJPSR.0975-8232.8(5).2277-81.
2. Raaman N: Phytochemical Techniques. New India Publishing Agency, New Delhi, India. 2006
3. K. Kalimuthu et al, Int. J. Pharm. Phytopharmacol. Res. 2014; 4 (3): 179-181
4. A. Zahir Hussain and S. Kumaresan J. Chem. Pharm. Res., 2014, 6(9):43-47

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Vol.4.Issue.2.2017 (April-June) ISSN:2455-0221(P), 2394-2606(O)

5. Rahman Gul, Syed Umer Jan, Syed Faridullah, Samiullah Sherani, and Nusrat Jahan, "Preliminary Phytochemical Screening, Quantitative Analysis of Alkaloids, and Antioxidant Activity of Crude Plant Extracts from Ephedra intermedia Indigenous to Balochistan," The Scientific World Journal, vol. 2017, Article ID 5873648, 7 pages, 2017. doi:10.1155/2017/5873648
6. Cragg GM, Newman DJ. Biodiversity: A continuing source of novel drug leads. Pure. Appl. Chem., 2005, 77 (1): 7 – 24
7. Akram MT,Dhenge RM, Itankar PR.Antiinflammatory activity of ethanolic extract of Ventilago denticulate. Indian J.Nat.Prod. 2009; 25(2):22.
8. Khare GP. Indian Medicinal Plants: An Illustrated Dictionary.Springer Private Ltd. New Delhi India; 2007. p.697.
9. Pongjanta A, Pangjit K, Srichairatanakool S., Antioxidant Activity and Cytotoxic Effect of Ventilago denticulata Willd Leaves Extracts.J Med Assoc Thai. 2016 Jan;99 Suppl 1:S51-7.
10. G. E. Trease and W. C. Evans, "Phenols and phenolic glycosides," in Textbook of Pharmacognosy, vol. 12, pp. 343–383, Balliese, Tindall and Co Publishers, London, UK, 1989.
11. Ncube NS, Afolayan AJ, Okoh AI. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. African Journal of Biotechnology 2008; 7 (12): 1797-1806
12. Dool HVD, Kratz PD. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. Journal Chromatography. 1963. 11: 463-471.