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Isolation and Identification of Bio Active Photochemical Compounds from *Ventilago denticulata* Stem using GC-MS

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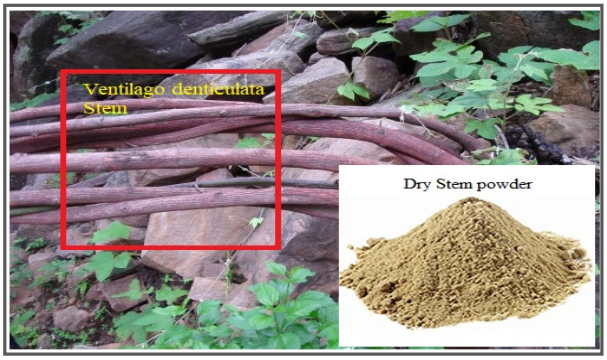
ABSTRACT

The present study explore the primary phytochemical study using gas chromatography-mass spectroscopy (GC-MS) and in vitro antimicrobial study (against gram positive bacteria and gram negative bacteria) was performed on n-hexane(50%)+Benzene (25%)+25% ethanol stem extract of Ventilago denticulata. Preliminary phytochemical screening revealed that plant contains 17bio active compounds with different concentrations. Qualitative analysis of the plant parts the presence of various components of therapeutic importance including tannins, saponins, phenolic compounds, glycosides, flavonoids etc., The present study provides information about the availability of some bio active phytoconstituents, which can be useful to provide dietary elements and it may also help in developing new drug formulations. There was a need to evaluate the extracts of the plant in order to provide scientific proof for its application and to explore the possibility of treating various diseases and disorders. Literature review indicates that very less work has been done on this plant and there is a wide scope for investigation.

Graphical Abstract



Ventilago denticulata stem [Family: Rhamnaceae]

Keywords: Isolation, Bio active photochemicals, *Ventilago denticulata,* Stem extract GC-MS.

INTRODUCTION

Plant secondary metabolites have been referred to as phytochemical compounds that are naturally occurring and have potential disease inhibiting capabilities. Phytochemicals are excellent sources of many bioactive compounds, such as volatile oils, steroids, alkaloids and natural antioxidants, i.e., flavonoids and other phenolic compounds, with beneficial effects on human health [1]. Herbal medicine has been practiced worldwide and it is recognized by WHO as essential building blocks for primary health care. WHO has estimated that up to 80 % of people still rely on traditional remedies, which are 21,000 plants around the world, among them 2500 species are in India, out of these 150 species are commercially used [2]. Hence standardization of medicinal plants and natural products will provide useful information with regards to its correct identity and will help to differentiate from other closely related species as well as from other commercially available crude drugs.

The development of microbial resistance towards antibiotics has highlighted the importance of the search for new potential effective plants and plant constituents against pathogenic microorganisms [3]. Antimicrobial screening of plant extracts and the phytochemical represent a starting point for antimicrobial drug discovery [4]. *V. denticulata* (Rhang Dang) belonging to the family Rhamnaceae (Figure 1). It is considered as an important medicinal plant by the traditional people of tribal people in India. Various parts of the plants used for treatment of many diseases. The plant is rich in many pharmaceutical active ingredients. The stem bark contains Friedel in and several anthraquinones that can be applied to treat skin diseases and sprains. The root contains anthraquinones, ventinones A and B, used for a tonic dyspepsia, mild fever and debility. The leaves give lupeol, betasitosterol and its glucoside [5]. The ethanolic extract of plant also shoes anti-inflammation and anti-microbial activity [6]. V. *denticulata* leaves are often used as tea products. Frequently drunk, it can help to diuretic cure, reduce cholesterol and blood sugar, serve as a relaxant, strengthen health, arthritis, reduce blood pressure and diet. The present study reports on the phytochemical analysis and antimicrobial activities of various extracts from *Ventilago denticulata* stem using GC MS analysis.

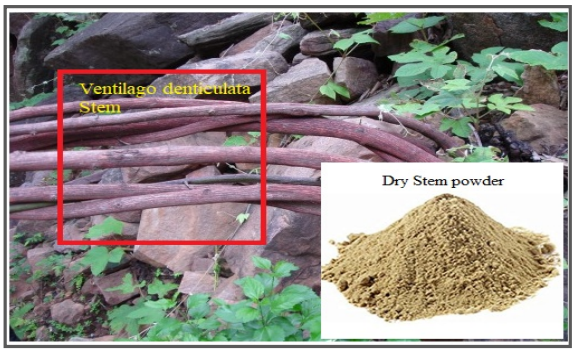


Figure 1. *Ventilago denticulata* stem [Family: Rhamnaceae]

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: Collection and identification of plant material *Ventilago denticulata* were collected in and around forest of Kondapalli, Krishna District, Andhra Pradesh, India, The authenticity of the plant was confirmed in Botanical Survey of India and Department of Botany Acharya Nagarjuna University, Guntur. The *V. denticulata* stem were collected in bulk, cut in small pieces and dried for one month. The shade dried samples were powdered separately using an electrical grinder. The powder was stored in screw cap bottles until further analysis.

Preparation of plant extract: The dry *V. denticulata* stem powder passed through sieve (100□microns). The coarse powdered drug (250 g) was extracted in Soxhlet apparatus for 48 h with n-hexane+ benzene+ethanol (50:25:25 ratio) (50-65°C, 2L) extract obtained was concentrated under reduced pressure in rotatory evaporator below 60°C temperature to get semisolid sticky residue (20g).

Qualitative analysis of phytochemical constituents: The phytochemicals screening of *V. denticulata* stem extract was carried out to determine the presence of the following compounds Terpenoids, carbohydrates, saponin, tannin, glycosides, steroidal glycosides; phenolic compounds etc., identified by standard tests [**8**] and the functional groups are shown in the table 1.

Table 1. Display the presence/absence of different phytochemicals in the stem extract of *V. denticulate*

Phytoconstituents	Test	Stem Extract
Alkaloids	Wagner's test	++
Amino acids	Ninhydrin Test	--
Carbohydrates	Molish test	++
Cardiac glycosides	Keller-Killani test	++
Flavonoids	Shinoda's test	++
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	++

GC-MS Analysis: GC-MS analysis of stem 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 capillary column (30 mm x 0.25 mm 1D x 1 mm, 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 1 mL min^{-1} and an injection volume of 2 µL 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 94 min. 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 65,000 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 and Figure 2).

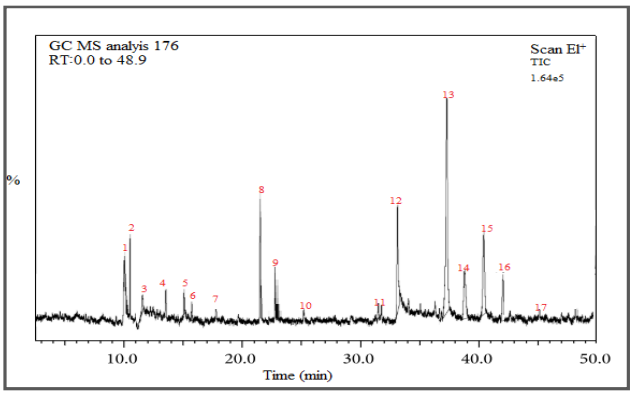


Figure 2. GC-MS spectra of 50% n-hexane+25% benxene+ 25% ethanol extract preliminary phytochemical analysis.

Table 2. Components detected in n-hexane/benzene/ethanol extract of *Ventilago denticulata* stem powder

S.No.	RT	% of area	Compound name	M.F	MW	Nature of Compound	Medicinal Importance derived from Literature#
1.	10.24	6.3	1,2-benzenedicarboxylic acid	$C_8H_6O_4$	166.03	Aromatic dicarboxylic acid	Used as Softeners, 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	11.02	6.9	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4	a saturated fatty acid that is the major fat in meat and dairy products	Antibacterial and antifungal activity
3	11.5	1.24	Methyl hexadecanoate or Palmitic acid methyl ester	$C_{17}H_{34}O_2$	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
4	14.24	1.36	Phytol	$C_{20}H_{40}O$	296.5	acyclic diterpene alcohol	antimicrobial, antioxidant Cancer-Preventive
5	15.1	1.61	α-terpinene	$C_{10}H_{16}$	136.2	Mono terpenoid	Food additives, FlavoringAgents,alpha-Terpinene is found in allspice
6.	16.02	1.02	Squalene	C30H50	410.7	dehydrotriterpenic hydrocarbon	Antibacterial, Antioxidant, Antitumor, Cancer-Preventive, Chemopreventive, Immunostimulant and Lipoxygenase-Inhibitor
7.	18.67	0.34	Carvacrol	$C_{10}H_{14}O$	150.21	Terpenoid	Nematocide
8.	21.64	17.3	Ledol				Antimicrobial, anti-inflammatory, antineoplastic
9.	23.64	5.1	Lauric acid, 3, 4-dichlorophenyl ester	$C_{10}H_{10}Cl_2O_2$	232	Fatty acid	Fatty acid; refatting agents for cosmetic formulations
10.	25.3	0.37	Phytofluene	$C_{40}H_{62}$	542	carotenoid	naturally in tomatoes and other vegetables.
11.	32.6	0.24	9,12,15-Octadecatrienoic Acid, (Z,Z,Z)	$C_{20}H_{34}O_2$	306	Ethyl Ester	Hypocholesterolemic, NematicideAntiarthritic, Antihistaminic Anticoronary, Insectifuge, Antieczemic
12.	33.9	10.26	Heptadecanolide	$C_{17}H_{32}O_2$	268	Fatty acid	Fatty acid; flavouring agent, as perfume
13.	37.8	35.61	Glycidyl palmitate	$C_{19}H_{36}O_3$	312	Fatty acid	Fatty acid; preparation of lysophosphatidic acids which inhibit apoptosis
14.	39.7	3.64	Eugenol	$C_{10}H_{12}O_2$	164	phenylpropene	Eugenol is used in perfumes, flavorings, and essential oils.
15.	40.3	5.61	Caryophyllene	C15H24	204	sesquiterpene	constituent of many essential oils
16	42.1	3.27	Cyclopentadecanone, 2-hydroxy-	$C_{15}H_{28}O_2$	240	Fatty acid ester	Volatile organic compound; component of paint, varnishes and glue
17.	44.9	0.2	Carazolol	$C_{18}H_{22}N_2O_2$	298	beta-adrenergic receptors	Pale yellow crystalline powder; act as beta adrenoceptor antagonist to prevent stress, to alleviate stress

#Source: Dr. Duke's phytochemical and ethnobotanical databases [Online database]

Anti-Bacterial Activity by Disc Diffusion Method

Preparation of Inoculum: *Escherichia coli*, and *Staphylococcus aureus* strains were used. 50 mL of nutrient broth was prepared in 100 mL conical flask. It was sterilized and 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^{o}C for 24 h for organism to grow [9].

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

Pour Plate Method: 1mL of prepared inoculum was poured in sterile Petri dish & then 15 mL of NAM was poured in it and 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 24 h.

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

RESULTS AND DISCUSSION

Earlier studies [9-10] on *V. denticulata* have been studied of active ingredient pharmacological and activities, including anti-inflammation, the anti-oxidative and antiproliferative activity of leaves and bark only but antimicrobial activity of stem remains unknown. The results showed the presence of some of the assessed phytochemical constituents in varying amounts in *V. denticulata* stem extract. Qualitative analysis results shown in table 1, and identified that alkaloids, carbohydrates, cardiac glycosides, flavonoids phenolics, polysterols, saponins, steroid and terpenes test positive reactions (table 1 and table 2) which is in line with many other studies conducted Worldwide[11]. Cardiac glycoside has been used in treatment of congestive heart failure due to its direct action which increases the force of myocardial contraction. Plant phenolics and flavonoids are considered as potent free radical scavengers. The moderate concentration of total phenolics and flavonoids in *Ventilago denticulata* stem indicated a notable antioxidant activity. Many studies strongly suggest that amount of polyphenol content should be considered as an important feature of herbal drugs. Some of its pharmacological effects like antioxidant, antiinflammatory, anticancer and diuretic activity can be attributed to the presence of these valuable many constituents [12].

The stem extract of *V. denticulata* revealed several peaks (17) which represents different compounds (17 compounds) as shown in the total ion chromatogram by Gas Chromatography-Mass Spectrometry analysis (Tables 1, 2). The peaks in the chromatogram were integrated and were compared with the database of spectrum of known components stored in the Gas Chromatography-Mass Spectrometry library. GC+MS Spectrometry analysis of the extract of *V. denticulata* stem revealed the presence of different fatty acids, fatty acids methyl esters and some volatile organic compounds. Glycidyl palmitate is a fatty acid with the molecular formula $C_{19}H_{36}O_3$ is essential in the preparation of lysophosphatidic acids which inhibit apoptosis. Heptadecanoyl is a fatty acid with the molecular formula $C_{17}H_{32}O_2$ is a component of flavoring agent and perfumes [13].

APPLICATION

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.* Table 3 showed excellent antibacterial activity against tested bacterial strains at the volumes of 25 µL well^{-1} and 50 µL well^{-1}. The zone of inhibition (in mm) ranges identified for *Escherichia coli* (6.24 ± 0.22 and 7.93 ± 0.71) and for

Staphylococcus aureus (6.08± 0.54 and 8.02± 0.22). The diameters of the inhibition zones for the all tested pathogens are listed in Table3. 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) 50 µL well^{-1}	Zone of inhibition (mm) 25µL well^{-1}
Escherichia coli	7.93 ± 0.71	6.24 ± 0.22
Staphylococcus aureus	8.02± 0.22	6.08 ± 0.54

CONCLUSION

The present study confirms that fractions of *Ventilago denticulata* stem extract having significant antibacterial activity along with valuable phytochemicals. The phytochemical analysis showed that the *Ventilago denticulata* stem extract contains a mixture of phytochemicals as reducing sugars, cardiac glycoside, phenolic compounds, flavonoids, and alkaloids. The broad spectrum of antimicrobial activity may help to discover new chemical classes of antibiotic substances that could serve as selective agents for infectious diseases, chemotherapy and control.

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