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Chemical composition and antimicrobial Activity of essential oil of leaves of *Vitex negundo* Linn. (Verbenaceae)

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Abstract: *Vitex negundo* Linn. (Verbenaceae) known as "Nergundi"; is used in folk medicine as a cure for arthritis and vermifuge. Essential oil present in the leaves is used as bathing oil and for sloughing wounds and ulcers. Literature survey revealed that an essential oil may have antibacterial activities responsible for bactericidal effect. The present study was carried out to analyze the chemical composition of essential oil by GC/MS and to investigate the antimicrobial properties of the essential oil of leaves of *Vitex negundo*. Essential oil was extracted from air dried leaves of plant by steam distillation using Clevenger apparatus to yield 1.6 % v/w essential oil, then it was dried over anhydrous sodium sulphate, and analysed by Gas chromatography/Mass spectroscopy (Shimadzu QP 2000 instrument with Ublon HR-1 GC column). The essential oil from leaves of *Vitex negundo* was tested against pathogenic microorganisms; *S. aureus, E. coli, K. pneumoniae, B. subtilis, M. luteus* and *Candida albicans*. The oil tested exhibited good antimicrobial activity against all the clinical isolates when compared with standard.

GC-MS analysis of essential oil of dried leaves of *Vitex negundo* identified ten (10) compounds of which three (3) compounds were characterized as sesquiterpenes (47.14%) present in high amount; α-Copaene (25.26%), a sesquiterpene was the predominant constituent present in oil. Other volatile constituents were five monoterpenes (45.50%) and two fatty acids (7.36%).

Keywords: Antimicrobial activity, Essential oil, Verbenaceae, *Vitex negundo.*

Introduction

Antibiotics provide the main basis for the therapy of microbial (bacterial and fungal) infections. Since the discovery of these antibiotics and their uses as chemotherapeutic agents there was a belief in the medical fraternity that this would lead to the eventual eradication of infectious diseases. However, overuse of antibiotics has become the major factor for the emergence and dissemination of multi-drug resistant strains of several groups of microorganisms.[1] The worldwide emergence of *E. coli*, *K. pneumoniae, Haemophilus* and many other β-lactamase producers has become a major therapeutic problem. Multi-drug

resistant strains of *E. coli*, *K. pneumoniae* are widely distributed in hospitals and are increasingly being isolated from community acquired infections.[2] *Candida albicans*, also a nosocomial pathogen, has been reported to account for 50-70% cases of invasive candidiasis.[3] Alarmingly, the incidence of nosocomial candidemia has risen sharply in the last decade.[4] All this has resulted in severe consequences including increased cost of medicines and mortality of patients.

Plants have traditionally been used in folk medicine as well as to extend the shelf life of foods, showing inhibition against bacteria, fungi and yeasts.[5] Most of their properties are due to essential oils and extracts produced by their secondary metabolism.[6] Essential oils and extracts from several plant species are able to control microorganism related to skin, dental caries and food spoilage, including Gram-negative and Gram positive bacteria.[7, 8, 9]

Aromatic plants and species have great importance for food, cosmetics and pharmaceutical industries. Their uses have been taken place since ancient times, and despite many of them were substituted by synthetic ones, the demand for natural products is increasing.[10] Leaves from *O. vulgare* L., *O. applii* L., *O. basilicum* L., *O. gratissimum* L., *M. spicata* L., *M. piperata* L. var. citrate have been used as spices and teas after drying, while the essential oil is utilized in cosmetics and pharmaceuticals. The essential oil contents in different species is influenced by genetic material, culture conditions and environment, and finally, by crop and post- crop processing.[11, 12]

Material and Methods

Plant material

The leaves of *Vitex negundo* were collected from Ganeshpur village, Saharanpur (UP -Uttarakhand border) and identified by Dr. Anjula Pandey, Taxonomist, National Bureau of Plant Genetic Resources (NBPGR), Pusa campus, New Delhi. A voucher specimen **(HS-19710)** is preserved in the herbarium section of taxonomic department of NBPGR, New Delhi.

Isolation of volatile oil

The fresh leaves (1.5 kg) were subjected to hydrodistillation by using a Clevenger-type apparatus for 3 hours according to the method recommended in the British Pharmacopoeia, 1998. The yield of volatile oil obtained was 1.6 % v/w. The collected volatile oil was dried with anhydrous sodium sulphate and stored at 4-6^{0}C in the dark.

GC analysis

The oil from the leaves of *vitex negundo* was analyzed using a varion 3300 GC gas chromatograph equipped with a flame ionization detector (FID) and a DB1 fused silica column (30 m x 0.25 mm id; film thickness 0.25µm). Injector temperature and detector temperature were 250°C and 300°C respectively. Carrier gas was nitrogen at a linear flow rate of 1.5 ml/min; injector volume for all samples was 0.1µl.

GC-MS analysis

GC-MS analysis was carried out on a Shimadzu QP-2000 instrument at 70 eV and 250^{0}C. GC column Ublon HR-1 fused silica capillary 0.25 mm X 50 m with film thickness 0.25 µm. The initial temperature was 100^{0}C for 6 min and then heated at a rate of 10^{0}C/min to 250^{0}C. Carrier gas Helium, flow rate 2 ml/min, detector used was FID.

Identification of essential oil

The volatile components were identified by comparing their retention indices of GC chromatograph with those of literature. Further identification was done by GC-MS. The fragmentation patterns of mass spectra were compared with those of the spectrometer database using NBS 54 AL and Wiley L – built libraries and also with those reported in the literature. Many constituents were identified by comparing their retention indices with those of authentic standards available in author's laboratory.

Microorganism

The microorganisms employed in the current study were procured from Institute of Microbial Technology, Chandigarh (India) which includes clinical isolates of *S. aureus, E. coli, K. pneumoniae, B. subtilis, M. luteus* and *Candida albicans.*

Media

Nutrient broth, Nutrient agar, Malt extract broth and Sabouraud dextrose agar, all product of Hi-media Laboratories, Mumbai (India) were used in this study.

Antimicrobial agent

Ciprofloxacin (10 mg/mL), Chloramphenicol (20 mg/mL)

Agar well diffusion bioassay

The antimicrobial activity of the essential oil was determined by using the agar well diffusion technique.[13] Nutrient agar plates were each seeded with 0.5 ml of an overnight culture of each bacterial, while the sabouraud dextrose agar plates were each similarly seeded with each fungal strain.

The 24 hrs broth culture of each bacterium and three days inoculated fungus culture were used to seed sterile molten nutrient agar and sabouraud dextrose agar at 45^{0}C respectively, allowed to set and well made by sterile cork borer and 100 µl (0.1 ml) solution of essential oil added in to in each well . Then

bacterial plates were incubated at 37^0C for 24 hrs and fungal plates were incubated at 25^0C for 2 days after which diameter of zones of inhibition were measured. Each well was filled with essential oil, Ciprofloxacin for bacteria along with control of each and Chloramphenicol in case of *Candida albicans.*

Result and Discussion

In this study, the volatile constituents of essential oil were identified. The method of identification and percentage composition of each constituent are mentioned.

The yield of essential oil obtained from fresh leaves of *Vitex negundo* was 1.6% (v/w). The constituents are arranged in order of GC elution on Ublon HR-1 column. Analysis of the isolate by GC and GC-MS resulted in the identification of ten components comprising 100% of the total volatile constituents. Kovate retention indices of the components are also included and confirmed the general elution sequence. [Table 1]

The volatile oil was characterized by a high amount of sesquiterpenes (47.14 %), monoterpenes (45.50%) and others (fatty acids) (7.36%). [Table 2] α-copaene was the predominant characterized constituent (25.26 %) of the isolate. Among sesquiterpenic components comprising 47.14% of total isolates, all were sesquiterpenic hydrocarbons and characterized as α-copaene (25.26%), β-elemene (19.16%) and t-caryophyllene (2.72%), respectively. Among five monoterpenic components comprising 45.50 % of total volatiles, four were hydrocarbons and one monoterpenic alcohol [Table 2]. The alcoholic monoterpene was linalool (3.69%) which is a well known and reported important aroma component and monoterpenic hydrocarbons were camphene (21.10%), α-thujene (9.86%), α-pinene (7.74%) and sebinene (3.11%), respectively. Two identified fatty acids were stearic acid (5.84 %) and behenic acid (n-docosanoic acid) (1.52 %). [Table 1]

The results obtained in antimicrobial assay are shown in table 3. The oil showed remarkable antimicrobial activity against all the microorganisms, except *E. coli*. The clinical isolates of *S. aureus, K. pneumoniae, B. subtilis, M. luteus* and *Candida albicans* showed maximum activity at a concentration of 1.0 v/v (24 mm, 12 mm,12 mm,18 mm and 13 mm of zone of inhibition respectively) and showed comparable antimicrobial activity with standard antibiotics. Minimum activity was shown by *E. coli* (18mm).

The current work has shown that the essential oil from the leaves of *Vitex negundo* is a potential source of antimicrobial agents and its activity against various clinical isolates may be sufficient to perform further studies for identification of active principles.

Table No. 1: Chemical composition of volatile oil of leaves of *Vitex negundo*

S.No.	Components	Rt	MS	IK	%	IT
1.	α - Thujene	6.433	11.96	922	9.86	AB
2.	α - Pinene	9.553	13.30	923	7.74	AB
3.	Camphene	19.263	15.46	939	21.10	AB
4.	Sabinene	13.991	16.36	960	3.11	AB
5.	Linalool	20.471	20.63	1084	3.69	AB
6.	α - Copaene	7.923	29.43	1364	25.26	AB
7.	β - Elemene	4.863	29.90	1382	19.16	AB
8.	t-Caryophyllene	7.663	30.23	1403	2.72	AB
9.	Stearic acid	21.170	46.96	1930	5.84	AB
10.	Behenic acid	11.018	68.33	1990	1.52	AB

Rt = Retention time on GC
MS = Retention time on Ublon HR-1
IK = retention index
IT = Identification techniques
A = MS
B = GC

Table No. 2: Percentage of various constituents of volatile oil of leaves of *Vitex negundo*

S.No.	Name of the compound	Number	Percentage
1	Total compound	10	100
2	Total identified compound	10	100
3	Total unidentified compound	NIL	-
4	Total monoterpenes	5	45.50
5	Total monoterpenic hydrocarbon	4	41.80
6	Total monoterpenic alcohol	1	3.69
7	Total sesquiterpenes	3	47.14
8	Total sesquiterpenic hydrocarbons	3	47.14
9	Others	2	7.36

Table No. 3: Antimicrobial activities of essential oil of leaves of *Vitex negundo*

S.No.	Organism	[a]Source of clinical isolates	Comparable antimicrobial agent	[b]Zone of inhibition		
				0.1(v/v)	0.5 (v/v)	1.0 (v/v)
1	*S. aureus*	MTCC-96	Ciprofloxacin (++++)	++	++++	++++
2	*E. coli*	MTCC-739	Ciprofloxacin (+++++)	++	++	+++
3	*K.. pneumoniae*	MTCC-109	Ciprofloxacin (++)	+	+	++
4	*B. subtilis*	MTCC-736	Ciprofloxacin (++)	_	+	++
5	*M. luteus*	MTCC-106	Ciprofloxacin (++++)	++	+++	+++
6	*Candida albicans*	MTCC-3017	Chloramphenicol (++)	+	++	++

[a]**Source of clinical isolates:** Institute of Microbial Technology, Chandigarh (India)

[b]**Zone of inhibition:** -, no zone; ++, (11-14mm); +++, (15-18mm) and ++++, (19-26 mm); +++++, (28mm and above)

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