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Phytochemical Analysis and Antimicrobial Activity of Leaves of *Vitex negundo* L.

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A B S T R A C T

Ethno- medicine in developing countries uses a wide variety of natural products in the treatment of some common infections. *Vitex negundo* L is a common plant in Indian Medicine belongs to family verbenaceae, is a woody, aromatic and medicinal Shrub. In the present study was carried out with an objective to investigate the phytochemical analysis, antimicrobial activity of the aqueous extract of the leaves of *Vitex negundo* Preliminary phytochemical analysis was done classical method and presence of most of the phytochemicals except steroids and terpenoids. Antimicrobial activity was carried out by using well diffusion method, and clear antimicrobial activity was observed against *Salmonella typhi.* GC-MS determination of methanol extract of the Vitex negundo leaves to find seven phytochemical constitutes have been identified by comparing the chromatogram , peak value of unknown compound with entries in NIST database, seven phytochemical compounds like [Acrylic acid (5- cyclo propylidenepentyl) ester], [Caryophyllene], [3-methyl -2(2-Oxopropyl) Furan], [5-Clorovaleric acid, 2-Formyl- 4,6-Dichlorophenyl ester], [Cyclododecanone, 2-(6-Chloro -1-Oxohexyl)], [Z,Z-6,28-Heptatriactontadien-2-one] and [5-(7A-Isopropenyl -4, 5-Dimethyl – Octahydroinden -4 YL) -3 –methyl pent -2-E]. The presence of various bioactive compounds confirms the application of *Vitex negundo* for various treatment.

***Key words*:** *Vitex negundo*, Phytochemicals, Antimicrobial, Flavonoid, Terpenoid, GC-MS

Plants are considered not only as dietary supplement to living organisms but also conventionally used for treating many health problems and the medicinal value of many plants still remains unexplored. Investigations of plants are carried out to find new drugs or templates for the development of new remedial agents [1]. The word Vitex take from the Latin word ‘vieo’ means to tie or bind, as its stems and twigs have the flexible nature. The Sanskrit word ‘Nirgundi’ for *V. negundo* means ‘that which protects the body from diseases’ [2]. Plants contain primary metabolites as well as secondary metabolites. The different phytocompound present in plants like anthraglycosides, arbutin, bitter drugs, flavonoids, alkaloids, saponins, coumarins, phenol carboxylic acids, terpenes and valepotriates. These phytoconstituents conferm specific characteristics and properties to plants. Therefore, this type of analysis would help in determining various biological activities of plants. Natural products, either as actual compounds or as homogenize plant extracts, provide unlimited opportunities for new drug [3]. *Vitex negundo* Linn, belonging to family Verbenaceae, commonly known as five leaved chaste trees [4]. These family comprising of about 75 genera and nearly 2500 species. *V. negundo Linn* [5]. Some common names are in Hindi -Nirgundi and in Sanskrit as Sindhuvara. It commonly bears tri- or Pentafoliate leaves on quadrangular branches, which give rise to bluish-purple colored flowers in branched tomentose [6]. Flowers occur throughout the year [7]. *V. negundo* has been used to several disorder like inflammation, eye disease, toothache, ulcers, fever, asthma, headache, digestion problems, sinuses, bronchitis, antibacterial, antipyretic, antihistaminic, analgesic, insecticidal, antidote for snake bite, etc. The leaves of these plants used mosquito repellent effects as well as ant. The plant also used to have anticancer, rheumatoid arthritis healing and hepatoprotective potentials. [8]. Whole plant is used, the leaves and the barks are the most important in the field of medicinal. The decoction of leaves is considered as tonic, vermifuge and is given along with long pepper in catarrhal fever [9]. The present study was carried out to analyze the phytochemical components and antimicrobial

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activities of the crude aqueous extracts of *V. negundo*. Also, study GC-MS analysis of the methanolic extract of *V. negundo* leaves to identify the presence of bioactive components.

MATERIALS AND METHODS

Collection of plant materials

Fresh leaves of *Vitex negundo* were collected from rice field and pond of Nalanipam village situated in the Dhemaji district of Assam. These plant samples were washed with tap water in order to remove the dust. Collected plant material were air dried followed by thermostatic oven drying at a considerably low temperature not exceeding 30°C for 24 hours. The plant material become well dried for grinding. After grinding the plant material were transferred into air tight containers with proper labeling for future use. 50 grams of powder extracted from leaves of *Ipomoea aquatica* Forssk. was taken and extracted with adequate amount of ethanol (4:1) using Soxhlet apparatus. The liquid part is stored at 4°C in separate container.

Chemicals and reagents

Distilled water, methanol, di-ethyl ether hexane, sodium phosphate buffer, DNS, starch, DPPH, ethanol, MH agar, sodium acetate, sodium hydroxide, hydrogen peroxide, 95% ethanol, 0.1% lead acetate, hydro chloric acid, sulphuric acid, sodium carbonate, chloroform.

Glass wares and plastic wares

Beaker, conical flask, test tube, measuring cylinder, pipette, Petri dish, test tube stands, plastic tray, 2 ml syringe for filtration, glass vial etc.

Equipment

Some of the equipment's utilized for the study include Electronic Balance (ANAMED), spectro-photometer, pipette, Soxhlet apparatus, micro pipettes, centrifuge, Hot plate, water bath, Laminar air flow, -70°C Temperature Freezer, Rotary evaporator and GC-MS Instrument (Perkin Elmer, USA) etc.

Water extract

The water extract was prepared using classical method, where 5gm of plant mass were weighted using an electronic balance and was crushed in 100ml of sterile water. Then the mixture was boiled at 50-60°C for 30 minutes on water bath and it was filtered through Whatman No.1 filter paper. Then the filtrate was centrifuged at 2500 rpm for 15 minutes. The extract was then collected, labeled and stored in sterile bottles at 4°C for further different experimental use.

Ethanol extract

Grinded samples (5gm) were mixed with 100 ml of 95% ethanol and kept in water bath at 70°C for 2 hours. The extracted samples were centrifuged and the supernatant was transferred into 50 ml volumetric flask and adjust the volume to 50 ml with 95% ethanol. The sample were stored at -4°C.

Qualitative analysis of phytochemicals

Chemical tests were carried out by using aqueous as well as ethanol extract to identify various phytochemicals, using standard methods [19-22]. The extracts were subjected to qualitative analysis for presence of chemicals constituents by performing various chemical tests such as Terpenoids (Salkowski test), Flavonoids (Alkaline reagent test, Sulfuric acid test, Lead acetate test), Tannins (Lead acetate test), Glycosides (killer kiliani test), Saponin (Forthing test) and coumarins (Sodium chloride test).

Test for the antimicrobial activity

For antimicrobial test *Escherichia coli and Staphylococcus aureus* were inoculate in already prepared 20 ml of sterilized Muller Hinton agar plates and incubate the plates at 37°C for 24 hours. A heat sterilized 10 mm cork borer was then used to make wells in the already inoculated medium and the plant extracts to be tested against each test organism were placed.

Gas chromatography –Mass spectrometry analysis

The phytochemical composition of was analyzed by GC–MS system (Perkin Elmer, USA) make GCMS instrument, Model: Clarus 680 GC & amp; Clarus 600C MS comprising a liquid auto-sampler). For GCMS analysis, 0.1 ml of each concentrated extracts (4mg/ml) was diluted to 1ml with solvent (Methanol) and transferred to standard GCMS sample. The Software used in the system was TurboMass Ver. 5.4.2. The capillary column used was 'Elite-5MS' having dimensions—length-60 m, ID-0.25 mm, and film thickness-0.25 µm, and the stationary phase is 5% diphenyl 95% dimethylpolysilox-ane. Helium (99.99%) was used as carrier gas (i.e., mobile phase) at a flow rate of 1 ml/min. An injection volume of 2 µl was employed in split less mode. Injector and ion-source temperatures were 280°C and 180°C, respectively. The oven temperature was programmed at 60°C (for 1 min), with an increasing rate of 7°C/min to 200°C (hold for 3 min) then again increased at rate of 10°C/min to 300°C (hold for 5 min). The total run time was of 60 min. The solvent delay was kept for 8 min. MS Protocol Mass Spectra was taken in Electron Impact positive (EI+) mode at 70 eV. A solvent delay of 8 min was there for MS scan. Mass range i.e., m/z range is 50–600 amu.

Identification of peaks

Interpretation of the peaks that appeared in the GC Chromatogram were done by library search of the mass spectrum of the corresponding peaks using the database software of National Institute Standard and Technology-2008 (NIST-2008).

Qualitative analysis of phytochemicals

Chemical tests were carried out by using aqueous as well as ethanol extract to identify various phytochemicals, using standard methods [10-13]. The extracts were subjected to qualitative analysis for presence of chemicals constituents by performing various chemical tests such as Terpenoids (Salkowski test), Flavonoids (Alkaline reagent test, Sulfuric acid test, Lead acetate test), Tannins (Lead acetate test), Glycosides (killer kiliani test) and coumarins (Sodium chloride test).

RESULTS AND DISCUSSION

Phytochemical analysis

Preliminary qualitative phytochemicals screening of the aqueous extract of *Vitex negundo* leaves confirmed the presence of Tannin, Flavanoide, Glycosides, Coumarins while the phytochemical terpenoids and Steroids are absent. Result of phytochemical analysis are listed in in (Table 1).

Table 1 Result of phytochemical analysis of *Vitex negundo*

Plants	Aqueous extract	Steroids	Terpenoids	Flavonoids			Tannins	Glycosides	Coumarins
		Salkowski test	Salkowski test	Alkaline reagent test	H_2So_4 test	Lead acetate test	Lead acetate test	Keller-Kiliani test	Sodium chloride test
Vitex negundo	Leaves	-	-	+	-	-	+	+	+

+ = present, - = absent

Table 2 Antimicrobial activity of *Vitex negundo*

Plants name	Aqueous extract	Bacterial pathogen	Diameter of zone of inhibition
Vitex negundo	leaves	*Salmonella typhi*	16mm

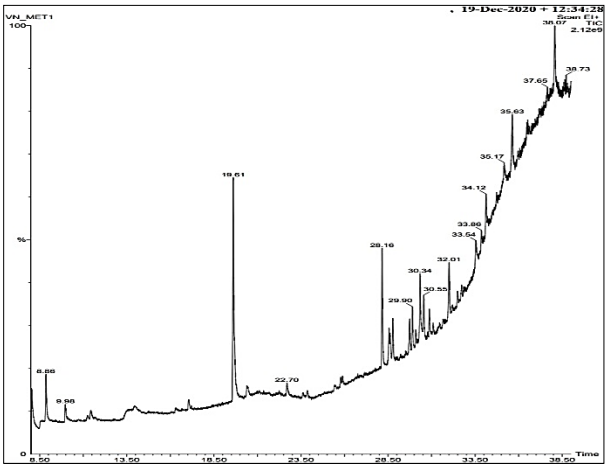

Fig 1 GC–MS chromatogram of methanolic extract of *Vitex negundo* [VN] Sample

Antimicrobial activity of Vitex negundo

Aqueous extract of leaves of *Vitex negundo* showed moderate antibacterial activity against Salmonella typhi. Results are tabulated in (Table 2).

GC-MS analysis of Vitex negundo leaf

GC-MS is one of the best techniques to identify the active phytochemical present in the plant extract. The chromatogram of the GC-MS analysis of the methanol extract of *Vitex negundo* leaf revealed the presence of number of compounds from the GC fractions and these compounds were identified with mass spectrometry attached to GC.

Present study GC–MS analysis of the methanolic extracts of the *Vitex negundo* detected 7 compounds like acrylic acid (5- cyclo propylidenepentyl) ester, Caryophyllene, 3-methyl -2(2-Oxopropyl) Furan, 5-Clorovaleric acid, 2-Formyl- 4,6-Dichlorophenyl ester, Cyclododecanone, 2-(6-Chloro -1-Oxohexyl), Z, Z-6,28-Heptatriactontadien-2-one and 5-(7A-Isopropenyl -4,5-Dimethyl – Octahydroinden -4 YL) -3 –methyl pent -2-E. The identified compounds their retention time [RT], molecular weight [MW], molecular formula [MF], peak area % are given in (Table 3).

Table 3 Phyto-compounds present in the methanolic extract of the *Vitex negundo* [VN] GC-MS peak report

RT	Name of the compounds	Molecular formula	Molecular weight	Peak area %
9.984	Acrylic acid (5- cyclo propylidenepentyl) ester	$C_{11}H_{16}O_2$	180	8.767
19.613	Caryophyllene	$C_{15}H_{24}$	204	6.008
22.699	3-methyl -2(2-Oxopropyl) Furan	$C_8H_{10}O_2$	138	0.385
28.772	5-Clorovaleric acid, 2-Formyl- 4,6-Dichlorophenyl ester	$C_{10}H_{19}ClO_2C_{13}H_{14}Cl_2O_3$	308	0.761
29.897	Cyclododecanone, 2-(6-Chloro -1-Oxohexyl)	$C_{18}H_{31}ClO_2$	314	0.753
30.873	Z,Z-6,28-Heptatriactontadien-2-one	$C_{37}H_{70}O$	530	0.52
28.161	5-(7A-Isopropenyl -4,5-Dimethyl –Octahydroinden -4 YL) -3 –methyl pent -2-E.	$C_{20}H_{39}O$	288	2.173

Among the identified phytochemicals acrylic acid (5-cyclo propylidenepentyl) ester is used in the production of hygienic medical products, detergents and wastewater treatment chemicals, Caryophyllene has potent anti-inflammatory antimicrobial, antibacterial, anti-oxidant properties. It is used to relieve anxiety and pain, reduce cholesterol, prevent osteoporosis, 3-methyl -2(2-Oxopropyl) Furan, 5-Clorovaleric acid, 2-Formyl- 4,6-Dichlorophenyl ester, Cyclododecanone, 2-(6-Chloro -1-Oxohexyl), Z, Z-6,28-Heptatriactontadien-2-one and 5-(7A-Isopropenyl -4,5-Dimethyl –Octahydroinden -4 YL) -3 –methyl pent -2-E. have no activity reported.

The various chemical constituents like flavonoids, flavone glycosides, volatile oil, triterpenes, tannins and lignin many others were identified and reported from *Vitex negundo* [14]. Also reported that the preliminary phytochemical analysis carried out on the crude ethanol extract indicated which indicated the presence of alkaloids, glycosides, lignin, flavonoids and saponins [15]. Previous study was also revealed the presence of alkaloids, carbohydrates, cardiac glycosides, flavonoids, glycosides, phenols, proteins, saponin and tannins in methanolic extract of *Vitex negundo* leaves [16]. While studying the Preliminary Phytochemical Screening and Antibacterial activity of *Vitex negundo*. They found that *V. negundo* contain various Phytochemical constituents like phenol, steroid, carbohydrates, quinines and flavonoids [17]. Our study showed that preliminary qualitative phytochemicals screening of the aqueous extract of *Vitex negundo* leaves confirmed the presence of Tannin, Flavanoide, Glycosides, Coumarins while the phytochemical terpenoids and Steroids are absent.

Previous studied antibacterial activity of leaves & bark of *Vitex negundo* against some gram +ve bacteria (*S. epidermidis, B. subtilis* and *S. aureus*) and Gram-ve bacteria (*E. coli, S. typhimurium, P. aeruginosa, V. cholera* and *V. alginolyteus*. All the extracts showed activity against *E. coli* and *S. aureus*. Ethanol and methanol extracts of leaf showed activity against gram +ve and gram -ve bacteria whereas petroleum ether and chloroform extracts of bark had better activity against gram +ve bacteria [18]. It is evident that

Methanolic extract has showed anti-bacterial activity and there were no zones observed in the other extracts like Ethanol, Acetone, Chloroform and Water Extracts. Gram negative bacteria *Pseudomonas aeruginosa, Salmonella Sps, Klebsiella pneumoniae, E. coli* are more susceptible than the gram-positive bacteria *Streptococcus pneumoniae, Staphylococcus aureus, Clostridium, Bacillus subtilis.* In Gram Positive bacteria maximum zone of inhibition 29 mm was observed for *Clostridium* whereas Ampicillin showed 24mm of zone of inhibition. The maximum zone of inhibition for Gram Negative bacteria 26 mm was found for *Pseudomonas aeruginosa while* streptomycin showed 20mm zone of inhibition respectively [19]. Our finding in aqueous extract, leaves of *Vitex negundo* showed moderate antibacterial activity against *Salmonella typhi* showed 16 mm of zone of inhibition.

In Vitex negundo GC-MS study also showed many Phytochemicals like Hexanoic acid, ethyl ester, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6- methyl-, Hexadecanoic acid, ethyl ester, Caryophyllene, Benzoic acid, 3-hydroxy-, Ledol, Aromadendrene oxide- (1), n-Hexadecaonoic acid, Phytol, 9,12,15- Octadecatrienoic acid, (Z, Z, Z)- and Vitamin E which contributes the activities like antimicrobial, antioxidant anticancer, Hypercholesterolemic, Antiulcerogenic and other activities [20]. Our present study GC–MS analysis of the methanolic extracts of the *Vitex negundo* detected seven compounds like Acrylic acid (5-cyclo propylidenepentyl) ester, Caryophyllene, 3-methyl -2(2-Oxopropyl) Furan, 5-Clorovaleric acid, 2-Formyl- 4,6-Dichlorophenyl ester, Cyclododecanone, 2-(6-Chloro -1-Oxohexyl), Z, Z-6,28-Heptatriactontadien-2-one and 5-(7A-Isopropenyl -4,5-Dimethyl – Octahydroinden -4 YL) -3 – methyl pent -2-E. It is used in the production of hygienic medical products, detergents and wastewater treatment chemicals., anti-inflammatory anti-microbial, antibacterial, anti-oxidant properties. It is used to relieve anxiety and pain, reduce cholesterol.

CONCLUSION

Medicinal plants, which are back bone of traditional medicine. The present study showed aqueous extract of *Vitex negundo* has phytochemical constituents, antimicrobial activities. GC-MS result also reveals the presence of seven major compounds in methanolic extract, which may be responsible for anti-inflammatory, anti- microbial, anti-bacterial, anti- oxidant properties. It might be recommended as a plant of phyto-pharmaceutical importance. However, isolation of individual phytochemical constituents and subjecting it to biological activity will definitely give fruitful results. Therefore, it could be concluded that *Vitex negundo* contain various bioactive compounds.

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