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RESEARCH ARTICLE **OPEN ACCESS**

PRELIMINARY PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF *VITEX NEGUNDO* (LINN.)

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ABSTRACT

'Nirgudi'plant *Vitex negundo* Linn. from family Verbenaceae is important medicinal plant in Indian Pharmacopoeia with variety of bioactive phytochemicals having importance in pharmacology and tribal medicines. It was found to contain many polyphenolic compounds, terpenoids, steroids, glycosides and alkaloids. Bioactivity guided preliminary phytochemical analysisof various extracts of leaves of *V. negundo* resulted in the qualitative analysis of ten metabolites.All the crude extracts from various solvents were evaluated for their antibacterial properties. They were found to have significant antibiotic activity against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherischia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Bacillus subtilis*. The significance of antibacterial activity was compared with standard antibiotic tetracycline.

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INTRODUCTION

Natural products are provided with numerous therapeutics effects and used as traditional medicines, since ancient times. Conventional plants are rich in various bioactive compounds that stimulate the immune system and protect against many diseases. Many of these compounds have passed clinical trials, and several others still remain unexplored (Gill and Kumar 2016).Natural products may be of plant or animal origin, although herbal bioactive products are derived from the plant origin, thus increasing the demand for systematic evaluation of novel bioactive compounds (Negi and Gill 2013). Plants are the rich sources of bioactive metabolites where every compound has antimicrobial properties. One such plant is *Vitex negundo* L. (Verbenaceae) commonly known as 'Nirgudi' in India. The genus *Vitex* has around 270 known species, ranging from shrubs to trees in the tropical, sub-tropical regions and temperate zones (Ganapaty and Vidyadhar 2005).

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The *Vitex* is used as a folk medicine in India, Bangladesh, China, Indonesia, Nepal, Pakistan, Philippines, and Sri Lanka (Vishwanathan and Basavaraju 2010, Gill *et al.* 2018). *Vitex negundo* is one such plant which is highly potential in the treatment of toothache, inflammation, eye disorders, leukoderma, spleen enlargement, skin-ulcers, auto immune disorders and sexually transmitted diseases. The extracts of this plant are used as tonics, vermifuge, lactogogue, antipyretic and anti-histaminic agents. The anti-oxidant potential is present in the leaves extracts with anthelminthic, dysmenorrhoeal, anti-hyperglycaemic, antifilarial and antibacterial activity (Kurapatti et al. 2017).Leaves are mostly used for the treatment of eye diseases, inflammation, leukoderma, toothache, spleen enlargement, skin-ulcers, gonorrhoea, rheumatoid arthritis, and bronchitis. Along with these, leaves are used as a vermifuge, lactagogue, tonics, antibacterial, antipyretic and antihistaminic agents (Gill *et al.* 2018). Previous phytochemical studies on *V. negundo* have afforded several types of compounds, such as volatile oils, lignans, flavonoids, iridoids, terpenes (triterpenes, diterpenes, sesquiterpenes), and steroids (Gautam*et al.* 2008). Even though, it constitutes a rare medicinal shrub with high biological value, very little survey research work has been done on this species in our country.

Taking into account of this ethnobotanical importance, the chemical constituents of the leaves of *V. negundo* have been investigated. The present study analyses the preliminary phytochemical analysis and evaluation of antibacterial activity of the *Vitex negundo* Linn.

Botanical Description of Plant *Vitex negundo* L.: *Vitex negundo* L. is a bushy shrub or small tree about 10-20 ft tall. Bark thin, grayish brown or grayish white, blaze yellow, branchletsterete or obtusely quadrangular, slightly pubescent, nodes annulate, internodes 3-9 cm long. Leaves palmately compound with 3-5 foliolate, rarely more, leaflets lanceolate-elliptic or ovate-lanceolate, middle leaflets 5-14 x 2-4 cm across, petiolules 1-3.5 cm long, lateral leaflets 2.5-4 x 0.8-1 cm across, petiolules 0.2-0.5 cm long, base cuneate or acute, margin entire or serrate, apex acuminate, chartaceous, dark green sparsely pubescent above, paler greyish pubescent beneath, lateral veins 10-18 on either side of the midrib, subparallel, margin arcuate, impressed above and prominent beneath, pubescent on midrib beneath, reticulate veinlets, petiole stout, slender, canaliculated, about 2-10 cm long. Inflorescence terminal panicles, sometimes dichotomously or trichotomously branched, about 10-30 cm long, peduncles, slender, obtusely quadrangular, pubescent, about 3-8 cm long, bracts leaf like, lanceolate, caduceus. Flowers bisexual, many, fragrant, pedicels about 1-3 mm long, Calyx campanulate, 5 toothed, teeth acute, purplish stripes inside, pubescent outside, about 3 x 2 mm across, Corolla sub infundibular, 5 lobed, 2-lipped, blue, purple or lavender, upper lip 2-lobed, light blue, lobes ovate, apex truncate, lower lip 3 lobed, midlobeobovate, light blue, concave, apex truncate, about 1 mm long, lower lobe, dark purple with white tinge, villous near the base, apex acute about 4 x 5 mm across, Corolla tube narrow, ampliate towards the apex, densely villous at throat with whitish hairs, pubescent outside. Stamens 4, didynamous, exserted, filaments slender, filiform, about 4-5 mm long, exserted, anthers oblong, light brown with white tinge, 2-celled, Ovary bicarpellary, 4 lobed, obovoid, glabrous, about 1 mm long, style slender, about 8 mm long, stigma bilobed, subequal, subulate. Fruit drupaceous, obovoid or subglobose, about 3-5 mm in diameter, green, glabrous, black when ripe, fruiting calyx cupular, pubescent (Indian Biodiversity Portal 2021).

MATERIALS AND METHODS

Collection and Authentication of Plant Material: The random surveys for collection of plant material from the Dang Surgana Forest in Western Ghats were conducted during June - December 2020. Fresh leaves of *Vitex negundo* were collected and washed thoroughly. The leaves were shed dried at the room temperature. The leaves without any moisture were blended until it becomes a fine powder. The plant *V. negundo* L. was authenticated and identified correctly and the voucher specimen were deposited at Herbarium Section, Department of Botany, MGV's Arts, Science and Commerce College Surgana, District Nashik, MH, India.

Process of Extraction: 100 grams of *Vitex negundo* Linn.powder was used for extraction with distilled water, ethanol, petroleum ether andchloroform using a Soxhlet extraction apparatus at the boiling point of the solvent for 48-72 hours or until the extracted solvent become clear. Then extracts were filtered with the help of Whatman filter paper and solvent was evaporated from extract in rotary evaporator. Then extract was kept in refrigerator at 4°C for future studies.

Phytochemical Analysis: A stock concentration of 1 % (W/V) was prepared using the respective solvent in each case. These extracts along with positive and negative controls were tested for the presence of active phytochemicals. Preliminary phytochemical analysis was carried out to find the presence of the active chemical constituents in extracts such as amino acids, carbohydrates, alkaloids, steroids, Cardiac Glycosides, flavonoids, saponins, tannins, terpenoids andphenols.In general, tests for the presence of phytochemical compounds involved the addition of appropriate chemical reagent(s) to the extract in test tubes (Harborne 1973; Trease and Evans 1989; Sofowra 1993; Kokate 1994; Mishra *et al.* 2011, Anbalagan*et al.* 2017, Bagul *et al.* 2021).

Test for Amino Acids: 2 ml of solvent extract was mixed with 2ml ninhydrin reagent and kept in hot water bath for 20 minutes. Appearance of purple color indicated the presence of amino acids in the sample.

Test for Carbohydrates: 2 ml of methanolic extract was mixed with 2 drops of Molisch's reagent and shake well. Add 2 ml of concentrated sulphuric acid in the sides of the test tube. A reddish violet color ring appeared at the junction of the two layers immediately indicated the presence of carbohydrates in the sample.

Test for Alkaloids: 1 ml extract was mixed with 1% HCl and 6 drops of Mayer's reagent and Dragendorf's reagent. A turbidity or precipitation indicated the presence of alkaloids in the sample.

Test for Steroids: 2 ml of acetic anhydride was mixed with 0.5 ml solvent extract and further added with 2 ml concentrated sulfuric acid. The color change from violet to blue or green indicates the presence of steroids.

Test for Cardiac Glycosides: 5 ml of solvent extract was mixed with 2 ml of glacial acetic acid containing one drop of ferric chloride solution already. This solution is further under layered with 1ml conc. H_2SO_4. A brown ring on the interface indicated a deoxy sugar characteristic of cardenolides. A violet ring might appear below the brown ring whereas the acetic acid layer, a greenish ring might form just gradually throughout thin layer.

Test for Flavonoids: Aqueous extract was added with 5 ml ammonia solution and conc. H_2SO_4. A yellow coloration confirms the presence of flavonoids which disappears on standing long.

Test for Saponins: Take small amount of extract with 20 ml of distilled water. Agitate the mixture for 15 minutes in graduated cylinder. The formation of 1cm layer of foam indicated the presence of saponins.

Test for Tannins: Take 5 ml of extract with few drops of lead acetate. A yellow precipitate confirms the presence of tannins.

Test for Terpenoids: Take 2 ml solvent extract with 2 ml of chloroform and 3 ml of conc. H_2SO_4 to form a monolayer of reddish brown coloration of the interface revealed presence of terpenoids.

Test for Phenols: 2 ml of extract was mixed with 3 ml of ethanol and a pinch of $FeCl_3$ to form greenish yellow color showing presence of phenols.

Microorganisms and Antibacterial Activity

Microorganisms: The microorganisms employed in the current study were obtained from Institute of Microbial Technology, Chandigarh (India) which includes clinical isolates of *Staphylococcus aureus* (MTCC 1430), *Klebsiella pneumoniae* (MTCC 432), *Escherischia coli* (MTCC 254), *Pseudomonas aeruginosa*(MTCC 424), *Salmonella typhi*(MTCC 733) and *Bacillus subtilis* (MTCC 121).

Antibacterial Activity: The antibacterial activity of *Vitex negundo* was performed by Kirby-Baeur disc diffusion method. The bacterial strains of *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherischiacoli*, *Pseudomonas aeruginosa*, *Salmonella typhi*and *Bacillus subtilis*were inoculated on the sterile Mueller-Hinton agar (MHA) plates with spread plate method using sterile bent glass rod. The sterile discs dipped and soaked with 30µg concentration of plant extracts were gently placed on the inoculated medium with standardtetracycline disc with the same 30µg concentration. These plates were incubated at 37°C for 24 hours for the observation of the zone of inhibition. Later the zones of inhibitionwere measured and compared with the standard antibiotics zones (Hudzicki 2009).

RESULT AND DISCUSSION

Preliminary phytochemical analysis of *Vitex negundo* L.leaves extracts: Preliminary phytochemical analysis of *Vitex negundo* L. was carried out by the solvents Distilled Water, Ethanol, Petroleum ether and Chloroform. Major 10 phytochemicals were screened for phytochemical analysis viz. amino acids, carbohydrates, alkaloids, steroids, cardiac glycosides, flavonoids, saponins, tannins, terpenoids andphenols.

- In distilled water extract amino acids, carbohydrates, alkaloids, flavonoids, saponins and tannins were found to be present while steroids, cardiac glycosides, terpenoids and phenols were absent.
- In Ethanol extracts amino acids, alkaloids, steroids, cardiac glycosides, flavonoids, tannins, terpenoidsand phenols were found to be present while carbohydrates and saponins were absent.
- In Petroleum ether extracts steroids, flavonoids, tannins and terpenoidswere found to be present whileamino acids, carbohydrates, alkaloids, cardiac glycosides, saponins and phenols were absent.
- In Chloroform extracts amino acids, carbohydrates, alkaloids, steroids, cardiac glycosides, flavonoids and terpenoids were present and saponins, tannins and phenols were found absent.

The compound flavonoids was present in almost all the extracts while saponins and phenols were found to be present only in the distilled water and ethanol leaves extracts respectively and theresults were plotted in Table 1.

Antibacterial Activity: The antibacterial assay of leaves extract of *Vitex negundo* L. had been investigated against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherischia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Bacillus subtilis*. The sterile discs were prepared with the leaves extract in 30µg concentration and the discs were placed on preinoculated Muller-Hinton agar plates with standard antibiotic tetracycline disc. T

Table 1. Preliminary phytochemical analysis of *Vitex negundo* L. leaves extracts

Sr. No.	Variable	Distilled Water Extract	Ethanol Extract	Petroleum Ether Extract	Chloroform Extract
1.	Amino acids	+	+	-	+
2.	Carbohydrates	+	-	-	+
3.	Alkaloids	+	+	-	+
4.	Steroids	-	+	+	+
5.	Cardiac Glycosides	-	+	-	+
6.	Flavonoids	+	+	+	+
7.	Saponins	+	-	-	-
8.	Tannins	+	+	+	-
9.	Terpenoids	-	+	+	+
10.	Phenols	-	+	-	-

(+) = Presence, (-) = Absence

Table. 2 Antibacterial activity of various extracts of *Vitex negundo* L. leaves

		Concentration of extract and zone of inhibition (mm)								
Sr. No.	Name of Bacteria	DW Extract 30µg	DW Negative Control	Ethanol Extract 30µg	Ethanol Negative Control	Petroleum Ether Extract 30µg	Petroleum Ether Negative Control	Chloroform Extract 30µg	Chloroform Negative Control	Standard Antibiotic Tetracycline 30µg
	1.Staphylococcus aureus	13.5	ND	14.5	5.0	ND	ND	7.5	2.0	24
	2.*Klebsiella pneumoniaee*	12.5	ND	14.5	4.5	ND	ND	9.5	2.0	19
	3.Escherichia coli	11.5	ND	11.5	3.5	ND	ND	9.5	1.5	13.5
	4.Pseudomonas aeruginosa	9.5	ND	10.5	3.0	ND	ND	8.5	1.0	13
	5.*Salmonella typhi*	11.0	ND	16.5	4.5	ND	ND	9.0	2.5	21
	6.Bacillus subtilis	12.5	ND	17.5	5.5	ND	ND	10.0	2.0	27

ND= Not Detected,

The blank pure solvents were used as the negative control for respective extracts assay. After incubation at 37°C for 24 hours for the zones of inhibition were observed. The ethanol extracts of *V. negundo* leaves showed greater antibacterial activity, the aqueous extract showed moderate antibacterial activity and choloroform extracts showed lower antibacterial activity while petroleum ether extract did not show any noticeable antibacterial activity.The negative control of ethanol and chloroform showed the antibacterial activity while distilled water and petroleum ether did not show any antibacterial activity (See Table 2).

- In aqueous extract of leaves the highest zone of inhibition was observed against *Staphylococcus aureus*(13.5 mm) and lowest zone of inhibition was observed against *Pseudomonas aeruginosa*(9.5 mm).
- In ethanol extract of leaves the highest zone of inhibition was observed against *Bacillus subtilis* (17.5 mm) and lowest zone of inhibition was observed against *Pseudomonas aeruginosa* (10.5 mm).
- In chloroform extract of leaves the highest zone of inhibition was observed against *Bacillus subtilis* (10 mm) and lowest zone of inhibition was observed against *Staphylococcus aureus* (7.5 mm).

The results clearly showed that distilled water and ethanol extracts were highly specific in action against the growth of bacteria and all the results of antibacterial assay were depicted in Table 2.

Conclusion

In conclusion, the *Vitex negundo* L. shows the presence of bioactive secondary metabolites such as alkaloids, steroids, cardiac glycosides, flavonoids, saponins, tannins, terpenoids and phenols. The knowledge and mode of inhibition due to specific bioactive compounds which are present in plant extracts, may contribute to the successful application of such bioactive metabolites for treatment of infections caused by pathogenic bacteria. It can be concluded that distilled water, ethanol and chloroform extract of *Vitex negundo* leaves exhibited potential bactericidal properties. Present investigations together with previous studies provide support to the antibacterial properties of *Vitex negundo* leaves. Therefore, it can be used a good alternative of antibiotics as antibacterial agent towards the development of new therapeutic drugs. Further pharmacological and clinical studies are required to understand the mechanism and the actual efficiency of these plant extract in treating various bacterial diseases.

Conflict of Interests: The author/s declares that they have no conflict of interest.

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