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Screening of Phytochemical Constituents and Antibacterial Property of Various Extract of *Vitex negundo* (Linn.)

Dr. Prabhat Kumar Saxena [1*], Dr. Archana Shrivastava [1]

Dept. of Biotechnology, College of Life Science, CHRI, Gwalior, M.P., India)

ABSTRACT

Plant Vitex negundo Linn. from family Verbenaceae is important medicinal plant in Indian Pharmacopoeia with variety of bioactive phytochemicals having importance in pharmacology and ancient times. It was found to contains many polyphenolic compounds, glycosides, alkaloids, steroids, and terpenoids. Bioactivity preliminary phytochemical analysis of various extracts of leaves of V. negundo resulted in the qualitative analysis of some phytochemicals. All the crude extracts from various solvents were evaluated for their antibacterial activities. They were found to have significant antibiotic activity against Staphylococcus aureus, Escherischia coli, Pseudomonas aeruginosa and Salmonella typhi. The significance of antibacterial activity was compared with standard antibiotic tetracycline.

Keywords: *Vitex negundo Linn*; Leaves extracts; Phytochemical analysis; Antibacterial activity; Microorganisms

INTRODUCTION

Plants having great potential to produce new drugs, through which humans can take benefit for the good healthy life [1]. Several infectious diseases can be treated with the traditional remedies [2]. The drugs which are used in the last 20 years to cure different type of diseases from which more than 25% are directly drive from plants [3]. The medicinal plants have been characterized for their bioactive compounds, which have been separated and subjected for their detailed structural analysis in the area of phytochemistry [4]. Naturally occurring drugs are easily available, less expensive, safe and having very less side effects [5].

Vitex negundo which belongs to the Verbenaceae commonly known as Nirgudi in India [6]. It is having large and aromatic shrub with bluish purple flowers spared in the region of Himalaya. It is genus tree and *vitex* have near about 800 species [7]. *Vitex* species are traditionally used in Indian system of medicine [8].

Literature survey of *vitex negundo* revealed that various extracts of *vitex negundo* shows the cytotoxicity, antimicrobial [9], anti-inflammatory, analgesic, anti-bacterial, anti-fungal , anti-insect [10], anti-oxidant, anticancer activities. Reema Murthy et al [11-12] also reported the presence of phytoconstituents like *glycosides, alkaloids, steroids, and terpenoids*, Sahajaya et al [13-14] also reports the presence alkaloids, phenolic compounds, saponin of various extracts of *vitex negundo Linn*.
Many researchers have studied and report the different activities of *vitex negundo Linn.* from the different regions throughout the world. For the present study we have selected the leaves of *vitex negundo Linn*. to know it medicinal properties.

MATERIALS AND METHODS

Collection and Authentication of Plant Material

The random surveys for collection of plant material from the Botanical Garden, College of Life Science, CHRI, Gwalior (M.P.) were conducted during June-December 2017. 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, College of Life Science, CHRI, Gwalior (M.P.), India.

Process of Extraction

100 grams of *Vitex negundo Linn*. powder was used for extraction with aqueous water, ethanol and chloroform 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 carbohydrates, amino acids, Cardiac Glycosides, alkaloids, terpenoids, steroids, flavonoids, saponins, tannins, and phenols. In general, tests for the presence of phytochemical compounds involved the addition of appropriate chemical reagent(s) to the extract in test tubes [10-13].

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

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 1 ml 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 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 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 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 flavonoids: Aqueous water 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 aqueous water. Agitate the mixture for 15 minutes in graduated cylinder. The formation of 1 cm 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 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 IMTech, Chandigarh (India) which includes clinical isolates of : -

1. *Staphylococcus aureus (MTCC-737)*
2. *Escherischia coli (MTCC-254)*

3. *Pseudomonas aeruginosa (MTCC-741)*
4. *Salmonella typhi (MTCC-733).*

Antibacterial activity: The antibacterial activity of *Vitex negundo* was performed by Kirby-Baeur disc diffusion method. The bacterial strains of *Staphylococcus aureus, Escherischia coli, Pseudomonas aeruginosa, and Salmonella typhi* 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 30 µg concentration of plant extracts were gently placed on the inoculated medium with standard tetracycline disc with the same 30 µg concentration. These plates were incubated at 37°C for 24 hours for the observation of the zone of inhibition. Later the zones of inhibition were measured and compared with the standard antibiotics zones.

RESULTS AND DISCUSSION

Preliminary Phytochemical Analysis of *Vitex negundo* Linn Leaves Extracts

Preliminary phytochemical analysis of *Vitex negundo L.* was carried out by the solvents Aqueous water, Ethanol and Chloroform. Major phytoconstituents were screened for phytochemical analysis such as carbohydrates, amino acids, Cardiac Glycosides, alkaloids, terpenoids, steroids, flavonoids, saponins, tannins, and phenols [14].

- In aqueous water extract carbohydrates, amino acids, 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, terpenoids and phenols were found to be present while carbohydrates and saponins 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 were present in almost all the extracts while saponins and phenols were found to be present only in the aqueous water and ethanol leaves extracts respectively and the results were arranged in Table 1.

Table 1: Preliminary phytochemical analysis of *Vitex negundo* Linn leaves extracts

S/No.	Phytoconstituents	Aqueous water extract	Ethanol extract	Chloroform extract
1.	Carbohydrates	+	-	+
2.	Amino acids	+	+	+
3.	Cardiac Glycosides	-	+	+
4.	Alkaloids	+	+	+
5.	Terpenoids	-	+	+
6.	Steroids	-	+	+
7.	Flavonoids	+	+	+
8.	Saponins	+	-	-
9.	Tannins	+	+	-
10.	Phenols	-	+	-
(+)=Positive, (-)=Negative				

Antibacterial Activity

The antibacterial assay of leaves extract of *Vitex negundo L.* had been investigated against *Staphylococcus aureus, Escherischia coli, Pseudomonas aeruginosa* and *Salmonella typhi.* The sterile discs were prepared with the leaves extract in 30 µg concentration and the discs were placed on preinoculated Muller-Hinton agar plates with standard antibiotic tetracycline disc. 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 [12].

The ethanol extracts of *V. negundo* leaves showed greater antibacterial activity, the aqueous water extract showed moderate antibacterial activity and choloroform extracts showed lower antibacterial activity. The negative control of ethanol and chloroform showed the antibacterial activity while aqueous water did not show any antibacterial activity (Table 2).

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

S/No	Name of bacteria	Aqueous water extract 30 µg	Aqueous water negative control	Ethanol extract 30 µg	Ethanol negative control	Chloroform extract 30 µg	Chloroform negative control	Standard antibiotic tetracycline 30 µg
1	Staphylococcus aureus	12.5	NF	14.5	5	7.8	2	24
2	Escherichia coli	11.5	NF	11.5	3.5	10.2	1.5	13.5
3	Pseudomonas aeruginosa	9.2	NF	10.8	3	8.5	1	13
4	Salmonella typhi	11	NF	15.5	4.5	9	2.5	21

- In aqueous water extract of leaves the highest zone of inhibition was observed against *Staphylococcus aureus* (12.5 mm) and lowest zone of inhibition was observed against *Pseudomonas aeruginosa* (9.2 mm).
- In ethanol extract of leaves the highest zone of inhibition was observed against Salmonella typhi (15.5 mm) and lowest zone of inhibition was observed against *Pseudomonas aeruginosa* (10.8 mm).
- In chloroform extract of leaves the highest zone of inhibition was observed against Escherichia coli (10.2 mm) and lowest zone of inhibition was observed against *Staphylococcus aureus* (7.8 mm).

The results clearly showed that aqueous water and ethanol extracts were highly specific in action against the growth of bacteria and all the results of antibacterial assay were portrayed 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 aqueous water, ethanol and chloroform extract of *Vitex negundo* leaves exhibited potential bactericidal properties. Present investigations together with previous studies provide support to the antibacterial activities of *Vitex negundo* leaves. Therefore, it can be used as 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.

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