Research Article

Phytochemical Analysis and Antibacterial Activity of *Vitex negundo* Leaf Extracts against Clinically Isolated Bacterial Pathogens

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ABSTRACT

Infectious diseases, the leading cause of premature deaths, in the world are killing almost 50,000 people every day. An increase in antibiotic resistant bacteria is threatening world population with the recurrence of infectious diseases that were once thought to be under control at least in developed countries (WHO 1994). In the recent years incidence of multi-drug resistance in Gram positive (*Staphylococcus aureus, Streptococcus pneumoniaee*), Gram negative (*E. coli, Shigella, Pseudomonas aueroginosa*) and other bacteria like *Mycobacterium tuberculosis* has been reported from all over the world. These multi-drug resistant bacteria have also created additional problems in cancer and AIDS patients. According to a report of World Health Organization, more than 80% of world's populations depend on traditional medicine for their primary health care needs During the last few decades there has been an increasing interest in the study of medicinal plants and their traditional use in different parts of the world but documenting the indigenous knowledge through ethno botanical studies is important for the conservation and utilization of biological resources. There are considerable economic benefits in the development of indigenous medicines and in the use of medicinal plants for the treatment of various diseases. *In vitro* antibacterial activity of leaves of *Vitexnegundo Linn.* was examined against 5strains of clinically isolated bacteria *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi*. They were treated with Ethanol, Methanol, Petroleum Ether and Chloroform extracts. Methanol and Ethanol showed the maximum bacterial growth inhibition.

Keywords: Antibacterial activity, World Health Organization, Bacterial growth inhibition, Solvent extraction, Herbal extract.

INTRODUCTION

India is rich in natural medicinal wealth, which is the birth place of Ayurveda. It has contributed to its maximum to the world of medicine. Medicinal plants have played a vital role especially among the natives, tribes and remote lives where the speck of allopathy was invisible. It has been traditional for ages which have brought about a drastic change in the medical field, solving the hazardous challenges in the ill-fell world.

Even though the scientists are busy digging the ditch of vaccines and medicines, still there are unsolved questions in the field of Microbiology where as there are hidden secrets of medicinal plants in the forests of India. Plants are the rich sources where every compound has antimicrobial effects. *Vitex negundo* is one such plant which is highly potential in the treatment of toothache, inflammation[1], eye disorders[2], leucoderma, spleen enlargement[3], skin-ulcers[4], auto immune disorders and sexually transmitted diseases[5]. The extracts of this plant are used as tonics, vermifuge[6-7], lactogogue, antipyretic[3] and anti histaminic agents[8].

The anti-oxidant potential[9] is present in the leaves extracts, antihelmenthic[3-5], medication and anti pain activity[10], dymenorrheal[11], anti-hyperglycemic, antifilarial (Sahare, 2008), opposed plant activity and antibacterial[12].

The present studies analyse the phytochemical and evaluate the anti bacterial activity of the *Vitex negundo Linn.*

MATERIALS AND METHODS

Plant Collection

Fresh leaves of *Vitex negundo* were collected from Kolli hills, Namakkal District of Tamil Nadu and washed thoroughly.

Blending the Leaves

The leaves were air dried at the room temperature. After the leaves are completely dried without any moisture, they were blended until it becomes a fine powder.

Process of Extraction

100 gms of the leaf powder of *Vitexnegundo* has been taken for the extraction and treated with Methyle alcohol, Ethyle alcohol, Chloroform and Fossil oil Ether. Soxhlet extraction equipment has been used to boil the solvent for 48-72 hours till it completely dissolves. Later the extract has been filtered with the filter paper. The solvent gets evaporated after the processing in rotary evaporator and induces the semi-liquid or thick consistency. At 4°C, the extract was unbroken in white goods and used for the further studies.

Phytochemical Analysis of Plant Extracts

To identify the phytochemicals present in the plant, qualitative chemical tests have been performed using the plant extract. Preliminary phytochemical screening by Harbrone's method[13] has been done to trace out the chemical constituents like alkaloids, flavonoids, tannins, carbohydrates, phenolic compounds, terpinoids, glycosides, steroids, fixed oils and fats. The general processing involves the addition of chemical reagents in the test tubes with the extracts.

Test for Alkaloids

Mayer's Test

Alkaloids are nitrogenous compounds with physiological and pharmacological activity. White yellowish precipitate is produced by the alkaloid solution after the addition of few drops of Mayer's reagent. It varies in the precipitation from a neutral or slightly acidic solution. The alcoholic extract was dried and the residue was heated with 2% hydrochloric acid on the boiling water bath. After cooling, the mixture has been filtered and followed by the addition of the Mayer's reagent which further reveals the presence of alkaloids, by noticing the turbidity or the yellow precipitate.

Test for Flavonoids

Lead Acetate Test

The extracts were being treated with few drops of 10% lead acetate solution. The formation of yellow precipitate indicates the presence of flavonoids.

Test for Glycosides

Treating with Aqueous Sodium Hydroxide

The extracts were treated with 1ml of Sodium Hydroxide and 1 ml of water, when the yellow precipitate forms indicate the presence of glycosides.

Test for Steroids

Salkowski Test

Few drops of chloroform and sulphuric acid have been added to the extract which gives out bluish red to cherry colour in chloroform layer. The formation of green fluorescence acid layer indicates the presence of steroids.

Test of Terpenoids

2 ml of Chloroform and 3ml of concentrated Sulphuric acid has been added to 5ml of each extract which forms a monolayer of reddish brown coloration interface which is positive to terpenoids.

Test of Phenols

3 to 4 drops of 5% Ferric chloride solution has been added to the extracts which forms the bluish black colours precipitate, indicating the presence of phenols.

Detection of Carbohydrates

The filtrates formed after dissolving the extracts in 5ml distilled water were used to test the presence of carbohydrates.

Benedict's test

Filtrates were treated with Benedict's reagent and gently heated for 10 minutes on the water bath gives the Brick red precipitate which indicates the presence of reducing sugars.

Antibacterial Activity of *Vitex negundo*

The antibacterial activity of *Vitex negundo* was performed by disc diffusion technique by Kirby-Bauer's method[14]. The clinically isolated strains of *Staphylococcus aureus, Klebsiella pneumoniaee, E.coli, Pseudomonas aeruginosa and Salmonella typhi* were spread on the sterile Muller Hinton agar plates with sterile cotton swabs.

30µg concentration of plant extracts were coated on the sterile discs and were gently placed on the medium with normal tetracyclin disc with the same 30µg concentration. The plates were incubated at 37^0C for 24 hours for the observation of the zone of inhibition. Later the zone of inhibition was measured and compared with the antibacterial agents.

RESULT AND DISCUSSION

The solvents used for the phytochemical analysis of V*itex negundo* are Methanol, Ethanol, Petroleum ether and chloroform.

In Methanol extract of *Vitex negundo* contain all the components, CHO, Alkaloids, flavonoids, tannin, terpenoids, glycosides, phenolic compounds and steroids are present.

Whereas the Ethanol extract of *Vitex negundo* contain all the constituents except CHO. Alkaloids, flavonoids, tannin, terpenoids, glycosides, phenolic compounds and steroids are present.

In Chloroform extract of *Vitex negundo* contain CHO, Alkaloids, flavonoids, terpenoids, glycosides, and steroids are present. Tannin and phenolic compounds are absent. Petroleum ether extract of *Vitex negundo* contains Steroids, flavonoids, tannin and Sugars are present.

The predominant components present in all the extracts of *Vitex negundo* are flavonoids, Terpenoids and steroids.

Chitra *et al*., (2009)[15] reported that the Preliminary phytochemical analysis carried out on the crude ethanol extract indicated the presence of alkaloids, glycosides, lignin, flavonoids and saponins.

The various chemical constituents like flavonoids, flavone glycosides, volatile oil, triterpenes, tannins and lignin many others were identified in *Vitex negundo.*

Antibacterial Activity of *Vitex negundo*

The antibacterial effects of Plant extract of *Vitex negundo* had been investigated against isolated *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniaee, Salmonella typhi* and *Pseudomonas aeruginosa.* The disc was prepared with plant extract in 30µg concentration and the discs were placed on Muller-Hinton agar plates with standard antibiotic tetracycline disc.

The antibacterial activity of the standard antibiotic tetracycline disc at the 30µg concentration, the zone of inhibition (22mm), against *Staphylococcus aureus.* The antibacterial activity of the ethanol extract of *Vitex negundo*, at the 30µg concentration was high (15mm and 11mm) against *S.aureus* and *Klebsiella pneumoniae* respectively. Minimum activity was recorded (10mm, 10mm, and 8mm) against *Escherichia coli, Pseudomonas aeruginosa, and Salmonella typhi.*

The antibacterial activity of chloroform extracts of *Vitex negundo*, at 30µg concentration the inhibition zone was high (11mm) against *Pseudomonas aeruginosa* and *Salmonella typhi*. Whereas minimum activity (08mm) against *Staphylococcus aureus.*

The antibacterial activity of methanol extracts of *Vitex negundo*, at 30µg concentration the inhibition zone was high (10mm) against *S.aureus*. Whereas minimum activity (2mm) against *Pseudomonas aeruginosa.* In petroleum ether extracts of *Vitexnegundo*, not inhibit the growth of bacteria.

The results clearly showed that ethanol and chloroform extracts were specific in action against the growth of bacteria.

Arjit chaturvedi and T.N.Nag (2011)[16] reported that the, extracts of *V.negundo* seeds and leaves showed maximum activity against all the microorganisms tested but their bound flavonoid fractions were found to be inactive against *S .typhimurium* and *T.viride.*

The zone of inhibition in the disc diffusion method showed that the leaf extract of *Vitexnegundo,* have the antibacterial activity against test pathogens namely *Escherichia coli, Staphylococcus aureus*, *Pseudomonous sp, Proteus vulgaris* and *Salmonella typhi*.

Antibacterial activity of *Vitex negundo* extracts against isolated organisms

Organisms	Concentration of extract and zone of inhibition (mm)				
	Tetracycline (30µg)	Methanol extract (30µg)	Ethanol extract (30µg)	Chloroform extract (30µg)	Petroleum ether extract (30µg)
Staphylococcus aureus	22	10	15	08	-
Klebsiella pneumoniaee	-	-	11	10	-
E.coli	-	06	10	10	-
Pseudomonas aeruginosa	-	02	10	11	-
Salmonella typhi	-	-	08	11	-

CONCLUSION

As medicinal plants play a vital role in traditional medicine, *Vitex negundo* takes a major place in proving its herbal values to face the infective challenges. This plant is rich in potential sources which help in the drug development by the pharmacological agencies. The vast research on this plant proves much medicinal significance as an antibacterial source which can be an answer to various unsolved infections. The active chemical compounds, has no doubt, be the best cure for many human ailments.

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