



Phytochemical and Antibacterial Screening on Leaf Extracts of *Vitex negundo* Linn

KEYWORDS

Phytochemicals, Antibacterial activity, *Vitex negundo*, Fluorescence test.

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ABSTRACT *In drug discovery, plants secondary metabolites have been the most potent source of safer drugs. Vitex negundo is one of the ancient medicinal herbs used by various tribal peoples and it is also available most common throughout Indian subcontinent. The phytochemical and antimicrobial assay of the different solvent extracts such as petroleum ether, chloroform, ethyl acetate, methanol and water were screened to show the presence of phytochemicals like alkaloids, steroids, tannins, phenolic compounds and terpenoids. The antimicrobial screening by disc diffusion method on different test pathogen showed potent inhibitory against Bacillus cereus, B. subtilis and Pseudomonas alimanta with the zone of inhibition ranging from 7- 25mm. Hence, this study states the effective use of V. negundo as a traditional medicine for many bacterial borne diseases.*

INTRODUCTION

Herbs are nature's gift to mankind. Herbal medicines have been a part of human life through the past centuries. It is high time to make concrete efforts to promote and propagate their great heritage. Consumption of drug have caused us irreparable loss and declining of health of rural and tribal population. Large numbers of plants are constantly being screened for their possible pharmacological value as per Chopra (1956) and Gamble (1974). The elucidation of the biogenetic pathways for the formation of medicinally active secondary metabolites of plants has afforded a photochemical foundation was reported by Banerji (1969), Harborne (1973) and Mathew (1984). In recent times, there has been a global trend towards revival of interest in the indigenous system of medicine. There is an increasing awareness among people that herbal remedy is the safest method of treatment due to the reduction of undesirable side effects which most of the modern synthetic drugs do as previously quoted by Auzi (1994), Zaidan (2005) and Gond (1998). As a result of modern isolation and pharmacological testing procedures, new plant drugs usually find their way into medicine as purified substances rather than in the form of other galenical preparations, as agreed by Sharma (1998), Khan (1998), Davokar (1998) and Ghosh (1980).

The leaf extracts of vitex negundo solvented by ethanol, showed the spectrum of inhibition on *S. paratyphi*. Most of the bacterial pathogens (*S. paratyphi*, *K. pneumonia*, *V. cholera*, *Streptococcus mutans* and *E. coli*) were found to be susceptible in leaf extracts of the vitex negundo. Petroleum ether leaf extract of vitex negundo showed good activity against *S. paratyphi* and *Enterobacter* (Merlin Rose, 2011).

Plant produces many secondary metabolites and constitutes an important source of pesticides, microbicides and pharmaceutical drugs. Some of the India indigenous essential oils have been reported to possess high therapeutic value and remarkable antimicrobial activity (Girgune, 1980, De Wit, 1979., Ananthanarayanan, 1998 and Heisey, 1992). Plant material *Vitex negundo* L. belongs to the family Verbanaceae is large shrub growing throughout India, Ceylon, Afghanistan, Tropical Africa, Madagascar, china and Philippines. It is large shrub upto 3 meters. Leaves are 7-9 cm long, petiolate opposite, exstipulate whitish green with agreeable aromatic odour. It has pungent, bitter, acrid taste, heating astringent, cephalic, somachic, athalmitic, promotes the growth of hair

useful in diseases of the eye, consumption inflammation, leucoderma, enlargement of the spleen, bronchitis, asthma, biolousness, painful teething of children (Kirtikaran and Basu, 1987 and Khan 1998). The present investigation involves the analysis of phytochemical profile and the antimicrobial activity of the leaf extracts of *Vitex negundo* Linn against the different strains of human pathogenic bacteria.

MATERIALS AND METHOD

Plant material

The leaves of *Vitex negundo* were collected in the local Sivakasi from lands. The collected leaves were dried under shade and powdered using a mechanical grinder and extracted using different solvents by hot extraction methods.

Preparation of Extract

The extract of the powered leaf material was prepared with different solvents sequentially petroleum ether, chloroform, ethyl acetate, methanol and water using soxhlet's apparatus and it was concentrated to get a crude green paste. The extracts were collected and stored a 4°C for further analysis of phytochemical and antibacterial assay.

Culture

Bacterial cultures like *Bacillus cereus*, *Pseudomonas alimanta* and *Bacillus subtilis* were obtained from the department of Microbiology of ANJA College, Sivakasi and subcultured in nutrient agar slants and used of the current investigation.

Phytochemical Analysis in the leaf powder of *Vitex negundo* Linn.

Preliminary Test

1. Nature of the leaf powder - coarse
2. Colour - pale green
3. Odour - characteristic
4. Taste - bitter

Fluorescent analysis

The powder was treated separately with 1N NaOH, 1N HCl, 50% H₂SO₄, 50% HNO₃ and Ethanol. Then, examined under ordinary UV light and the colour changes were recorded.

Screening of Antimicrobial activity - Disc diffusion method

The disc diffusion test was performed by using the standard procedure with some modifications. About 50µl of the test microbial suspension was spread on the Muller Hinton Agar (MHA) by using sterile cotton swabs. Sterile discs (5mm di-

ameter) were loaded with 50µl of each extracts and kept dry at room temperature. The discs were placed on the seeded MHA plates. Standard antibiotics (ampicillin) were used as the positive control (10mcg/ disc). Then, the plates were incubated at 37° C for 24 hours and the diameter of the clear zone of growth inhibition was measured in mm scale. Triplicates were maintained.

Preliminary Phytochemical Test- Evans Catherine (1997)

Test for Steroids- To the test solution, add minimal amount of chloroform and then add 3- 4 drops of acetic anhydride and one drop of Conc. H₂SO₄. A colour change from purple to blue colour indicates the presence of steroids.

Test for Alkaloids - To the test solution, add 2N HCl Shake and decant the aqueous layer and to IT add few drops of Mayer's reagent. The formation of white precipitate indicates the presence of alkaloids.

Test for Phenolic Compounds - To the test solution; add few drops of neutral FeCl₃. The formation of intense blue or green colour indicates the presence of phenolics compounds.

Test for Saponins - To the test solution, add water and shake well. Formation of foamy leather indicates the presence of saponins.

Test for Tannins - To the test solution; add water and then lead acetate. The formation of white precipitate indicates the presence of tannins.

Test for Anthraquinones- To the test solution; add magnesium acetate solution. The formation of pink colour indicates the presence of anthraquinones.

RESULTS

Preliminary phytochemical analysis of the leaf powder of *Vitex negundo* Linn

The Phytochemical analysis of the powder revealed the presence of alkaloids, steroids, tannins, phenolic compounds and terpenoids. The anthraquinones and saponins were absent (Table 1).

Table 1- Phytochemical Analysis of *Vitex negundo* Leaf extracts.

S. No	Leaf Extracts	Phytochemical Analysis				
		Steroids	Alkaloids	PC	Tannins	S & AQ
1	Petroleum ether	+	-	+	+	+
2	Chloroform	+	-	+	+	-
3	Ethyl acetate	+	+	+	+	+
4	Methanol	+	+	+	-	+
5	Aqueous	-	+	-	-	-

Legend: PC- Phenolic compounds, S- Saponins, AQ- Anthraquinones

Antibacterial susceptibility of various extracts of *Vitex negundo* Linn.

The diameter of the inhibition zone was between and when ranges one to eight millimeter, the organism is moderately sensitive (MS) and when exceeds eight millimeter the organism it is considered to be sensitive (SS). Different solvent extracts were tested for their antibacterial activity against different bacterial strains viz., *B. cereus*, *B. subtilis* and *Pseudomonas alimanta* by disc diffusion method. Inhibitory zone formation was observed with all extracts at a considerable value. The ethyl acetate, petroleum ether and aqueous extract of the plant showed better zone of inhibition against the

growth of the specific bacterium *Bacillus cereus* Significant inhibitory zones were found with the extracts of petroleum ether, chloroform and methanol on growth of the bacterium *Bacillus subtilis*. The chloroform and methanol extract showed a favorable inhibitory effect on the growth of the bacterium *Pseudomonas alimanta*, where as the aqueous extract showed a very minute effect (Table 2).

Table 2- Antibacterial effect of *Vitex negundo* Leaf extracts.

S. No	Leaf Extracts	Antibacterial activity (Diameter of Zone of growth inhibition- mm)		
		<i>B. cereus</i>	<i>B. subtilis</i>	<i>P. alimanta</i>
1	Petroleum ether	9	20	15
2	Chloroform	10	23	25
3	Ethyl acetate	14	-	-
4	Methanol	12	19	20
5	Aqueous	7	-	14

Legend: 1- 8 mm are moderately sensitive (MS) and > 8mm are Sensitive (SS)

Fluorescent analysis

The powder and extracts were examined under ordinary light UV light. The colour of the powder is different in both cases. The leaf powder as such, powder with 1N HCl, petroleum ether extract and chloroform extract was Green under UV light. Brown colour observed in powder with 1N NaOH and powder with 50% H₂SO₄. Dark green was observed in powder with 50% HNO₃, ethyl acetate extract and ethanol extract. Yellowish green was observed in methanol extract and benzene (Table 3).

Table 3- Fluorescent analysis of leaf powder and their extracts of *Vitex negundo* Linn

S. No	Treatment Nature	Ordinary light	UV light
1	Powder as such	Pale Green	Green
2	Powder +1N NaOH	Reddish Brown	Brown
3	Powder+ 1N HCl	Ash Green	Green
4	Powder + 50% HNO ₃	Golden Yellow	Dark Green
5	Powder+50% H ₂ SO ₄	Dark Brown	Black Brown
6	Ethanol and Ethyl acetate extract	Orlic Green	Dark Green
7	Petroleum Ether Extract	Yellowish green	Green
8	Chloroform extract	Brownish green	green
9	Methanol extract	Dark green	Yellowish green
10	Benzene	Yellowish	Light green

DISCUSSION

Plant products are gaining prominence as bactericides. Oil prepared from the juice is applied for sinusitis sores. It was also found that the oil obtained from the leaves showed mosquito repellent activity. Flavonoid rich fraction of the seed exhibits anti-androgenic activity (Wolf, 1979, Sureshkumar, 1991., Brindha, 1981 and Chetty, 1990).

One of the successful strategies for the investigation of medicinal agents from higher plants includes the pharmacological screening of plant extracts followed by bio assay guides fractionation of active plant extract and leading the isolation of the pure constitution of the pure constituents (Krishnaswamy, 1980). Lakshmi (1999) reported that in *Heterostemma tanjorensis*, all the polyphenols, the free oxidized and po-

lymerized components appeared to have the antimicrobial agents.

The phytochemicals in the leaves of *Vitex negundo* Linn, revealed the presence of alkaloids, steroids, tannins, phenolic compound and triterpenoids. From the seeds of the genus *Vitex* 5, 7, 3-trihydroxy, 6, 8, 4'-tri methoxy flavones have been elucidated (Bhargava, 1989, Bhatt, 1993., Chatterjee, 1993 and Rao, 1998). Methanol and chloroform extracts are very much inhibitory to *B. subtilis* and *P. alimanta*. Petroleum ether extract is inhibitory only for *B. Subtilis*. Other than these, the petroleum ether extract and ethyl acetate also showed poor inhibition on *Bacillus cereus* and it is found to be resistant to the leaf extract of *Vitex negundo* L, than the other bacterial strains *Pseudomonas alimanta* and *B. subtilis* was said by Vaghasiya (2007). The study in that way opens new vista to augment the exploration of herbal medicine for the effective and efficient treatment of diseases.

CONCLUSION

It is very necessary to introduce new and biological-lysafe and active drug seco friendly in nature and effective as antimicrobial agents. Usually medicinal plants contain several phytochemical compounds, which are very much necessary to control the growth of the microorganisms. Uniyal (2006) reiterates a popular local quote of the Bhangalis in the Western Himalayan region of India which translates as "A man cannot die of disease in an area where *vitex negundo* is found". From the antibacterial activities of *Vitex negundo* Linn; higher effect was showed on *P. alimanta* and *B. subtilis* than on *B. cereus*. This gave an insight, into the phytochemistry of the test plant. The antibacterial activities of the leaf are found due to the presence of the Phytochemicals. Hence, it can be used as an antibacterial agent.

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