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), Da-vokar (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 inleaf 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, exocarp whitish green with agreeable aromatic odour. It has pungent, bitter, acrid taste, heating astringent, cephalic, somachic, athalmintic, 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 Si-vakasi 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 alimenta 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 ne-gundo 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. H2 SO4. 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 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 alimenta 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 alimenta, while 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) |
|-------|--------------|---------------------------------------------------------------|
| 1     | Petroleum ether | B. cereus | B. subtilis | P. alimenta |
| 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% H2 SO4. 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% HNO3 | Golden Yellow | Dark Green |
| 5     | Powder+50% H2 SO4 | 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 bacteriodes. 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., Brintha, 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 tanjorensense, 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. alimenta. 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 alimenta 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 biologically safe and active drugsoeco 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) iterates a popular local quote of the Bangalis 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. alimenta 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.