INTRODUCTION

Though the traditional Indian system of medicine has a long history of use, they lacked adequate scientific documentation, particularly in light modern scientific knowledge. Vitex negundo (verbenaceae) commonly known as Nirkundi or Nallanocci. It is an aromatic large shrub or small tree about 3m in height with quadrangular branches and almost found throughout India, ascending to 1500m in the outer shrub or small tree about 3m in height with quadrangular branches and almost found throughout India, ascending to 1500m in the outer deciduous forests. The essential oil of vitex negundo leaves showed significant antifungal activity against trichloroderma viride, fusarium sp., colectrotrichum and helminthosporium. An ointment made from the juice is applied as hair- tonic. It is constituent of the Ayurvedic preparations "Vishagbartha thaila". The extraction of the leaves showed anti-cancer activity against Ehrlich ascites tumor cells. The seed extracts of V. negundo interfere with male reproductive function without producing adverse toxicity in other vital organs. The ash of the plant is a source of potassium carbonate and is reported to be used as alkali in dyeing. Leaf extract of Azadirachta indica inhibited the growth, sporulation and germination of Alternaria alternata. Leaf extract of Cinnamomum Camphora, Vitex negundo were less effective. Significant activity against Escherichia coli, Klebsiella aerogenes, Proteus vulgaris and pseudomonas aerogenes was shown by the extract of vitex negundo. The pentacyclic triterpenoids betulinic and ursolic acids isolated from leaves of vitex negundo, showed a very mild antibacterial activity. The plant extract was effective against curvularia lunata and rhizopus nodules. Though vitex negundo also finds use as a food crop and source of timber. The present study is designed to explore the preliminary phytochemical and antimicrobial analysis of vitex negundo which is responsible for its pharmacological properties.

MATERIALS AND METHODS

Plant material

The leaves of Vitex negundo were collected at the Rapinat Herbarium and Center for molecular systematic, St. Joseph's College, Tiruchirappalli, Tamil Nadu, India. The leaves were dried, ground into powder and stored in polythene bags before use.

Extraction of plant material

Dried ground leaves of 50 grams were extracted in soxhlet sequentially in 300ml of petroleum ether dichloromethane and ethanol. The process was run for 48 hrs at 31°C until complete exhaustion of the material. At the end of 48 hrs, the extracts were distilled. After distillation those extracts were stored in a refrigerator for the analysis. The compound in the leaf extracts were separated using TLC technique. Then the compounds were identified using UV spectroscopy.

Preliminary phytochemical investigations

The major secondary metabolites like, alkaloids, flavonoids, saponins, phenols, terpenoids, anthraquinones, proteins and amino acids, carbohydrates and glycosides were assessed according to the standard procedure described by Harborne.

Antibacterial screening test

Disc diffusion method

The paper disc diffusion method was used to determine the antibacterial activity of the extracts prepared from the vitex negundo leaves using standard procedure. Its essential feature is the placing of filter paper discs with the antibiotics on the surfaces of agar immediately after inoculation with the organism tested. Undiluted over night broth cultures should never be used as an inoculum. Routine direct application of discs to plates seeded with clinical material is not recommended because of problems with inoculum control and mixed cultures.

Streak plate method

The streak plate method was used. In this method a sterilized loop or transfer needle is dipped into a streak plate method offers in the most practical method of obtaining discrete colonies and pure cultures. Suitable diluted suspension of organisms, which is then streaked on the surface of an already solidified agar plate with plant extract, to make a series of parallel non-overlapping streaks. The aim of this method is to check whether the organisms are growing in plant extract containing medium or not based on granite of particular microorganisms.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

Phytochemical screening of the extracts of vitex negundo revealed the presence of alkaloids, steroids, flavonoids, aminocids, phenols, quinones and starch (table I). These compounds have significant application against human pathogens, including those that cause enteric infections. 5-hydroxy-3, 6, 7, 3’, 4’-penta methoxy flavones and 3, 5-dihydroxy-6, 7, 3’, 4-tetra methoxy flavonol were isolated from vitex negundo leaves.
Growth ethanol extract. Throughout the experiment dichloromethane showed very poor susceptibility even at the higher concentrations to *Streptococcus mutans* given using their sensitivity pattern to the extract solvents by ethanol and dichloromethane.

Only to the...show wide spectrum of inhibition to all the solvent extracts except all the solvent extracts. Dichloromethane.

In fine, this paper establishes that *vitex negundo* leaf solvent reveals nil inhibitory activity to the entire bacteria. The inhibition zone serially increases with the increased concentration of the extract to all bacterial culture.

Table 2 shows the effect of antibacterial activity of *vitex negundo* on disc diffusion method.

In streak plate method the petroleum ether extracts, the growth of the bacterial pathogens like *salmonella paratyphi* and *Enterobacter* were found to be maximum in both 25% and 50% concentration and as the concentration increases the degree of susceptibility decreases and growth is absent in both 75% and 100% concentration. Table 3 shows the effect of antibacterial activity of *vitex negundo* by streak plate method.

Table 2: Effect of antibacterial activity of *vitex negundo* on disc diffusion method-leaf

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Pathogen used</th>
<th>Number of discs (D) zone of inhibition in mm</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>D1</td>
<td>D2</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td><em>Klebsiela pneumoniae</em></td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td><em>Vibrio cholerae</em></td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td><em>Streptococcus mutans</em></td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td><em>Salmonella paratyphi</em></td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td><em>Eschericia coli</em></td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td><em>Klebsiela pneumoniae</em></td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td><em>Vibrio cholerae</em></td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td><em>Streptococcus mutans</em></td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td><em>Salmonella paratyphi</em></td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td><em>Eschericia coli</em></td>
<td>8</td>
<td>7</td>
</tr>
</tbody>
</table>

Table 3: Effect of different concentrated petroleum ether leaf extract on test bacteria (Streak Plate method)

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Growth of two bacteria on different concentration leaf extract</th>
<th>Control</th>
<th>25%</th>
<th>50%</th>
<th>75%</th>
<th>100%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella paratyphi</em></td>
<td></td>
<td>++++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td><em>Enterobacter</em></td>
<td></td>
<td>++++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

+++++ = Excessive growth; +++ = Moderate growth; ++ = Less growth; + = Nil

CONCLUSION

It is very necessary to introduce new and biologically safe and active drugs eco-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. Unjial et al. 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". In fine, this paper establishes that *vitex negundo* leaf extracts have good activity against *Klebsiela pneumoniae*, *Eschericia coli*, *Salmonella paratyphi* and *Enterobacter*.

REFERENCES