ABSTRACT

The *Flueggea leucopyrus* is a species of *Euphorbiaceae* family. It is understood from the literature that less work has been done on this plant. This fact prompted us to undertake a study on this much less explored plant. The present work is based on phytochemical screening and antimicrobial studies of the hydro-alcoholic leaf extract of *Flueggea leucopyrus*. And thus the medicinal plant is found to possess significant phyto-constituents. The presence of such a variety of phytochemicals may be attributed to the medicinal characteristics of this plant *Flueggea leucopyrus*. It is a disinfectant and it is useful in vitiated conditions of burning sensations, seminal weakness and general debility.

KEYWORDS: *Flueggea leucopyrus*, phyto-chemical screening, antibacterial, antifungal.
associated with side effects and have an enormous therapeutic potential to heal many infectious diseases.\cite{4}

About 70 – 80% of world’s population depends on traditional medicinal plants. Plant derived drugs serve as most effective and less toxic medicines for many of the diseases.\cite{5} *Flueggea leucopyrus* Willd is one of the medicinally used bushy weed. It is also known as Secruniga leucopyrus. *Flueggea leucopyrus* belongs to the family of *Euphorbiaceae* and the genus of *Flueggea*. It is a thorny bush weed. The male plant has greenish yellow colored clusters like leaves. And the female plant has red colored leaves. The fruits of *Flueggea leucopyrus* are white colored berries.\cite{6} Commonly known as *Katupila*. It is found in tribal regions of Srilanka, Shaurastra, Southern parts of Asia, Australia and Malaysia.\cite{7} The *Flueggea leucopyrus* have the dimensions of 1-3 × 1-2 cm. The tribes of Srilankause, *Flueggea leucopyrus* as a disinfectant and it is useful in vitiated conditions of burning sensations, seminal weakness and general debility.\cite{8} The plant has been used in preparations in traditional medicine for the treatment of cough, hay asthma, bowel complaints, disinfections, laxatives, for diarrhea, gonorrhea, constipation and mental illness and kidney stones.\cite{9,10} A paste prepared by mixing leaves of the plant with tobacco leaves has been used to destroy worms in sores.\cite{11} It is also used as fish poison. The leaves were boiled and taken orally a twice a day for stomachache. Recently the plant has been attracted interest as complementary and alternative medicine for cancer, especially in Sri Lanka. The decoction prepared from leaves of *F.leucopyrus* (Willd.) has been used by patients suffering from malignancy.

**EXPERIMENTAL METHODS**

**Collection of plants**

The plant *Flueggea leucopyrus* is thorny bush weed which are found in “Pachamalai” near Trichy district located at 119°N, 78°21’E of Trichy. The plant has been collected during the summer season (April-June 2014). The plant is authenticated in the Rapinat herbarium, St.Joseph’s College (Autonomous) Trichy.

**Extraction**

The fresh plant leaves are washed well and dried in shade. The shade-dried plant material is ground and taken for solvent extraction. The plant sample is first defatted using petroleum ether solvent. The defatted sample is again extracted by water-ethanol solvent (1:4). The aqueous – ethanolic extract is concentrated by flash evaporator.
Antimicrobial studies
The extract is taken for the antimicrobial studies in order to know the activity of the extract against the bacteria and fungi species and the % of inhibition is calculated.

Procedure for Antimicrobial activity study

Test Bacterial and Fungal species
*E.* coli, *Proteus mirabilis,* *Salmonella typhi,* *Serratia marcescens,* *Klebsiella pneumoniae,* *Pseudomonas aeruginosa,* Vibrio cholerae, Staphylococcus aureus and Bacillus cereus and the fungal species such as *Candida albicans* and *Candida tropicalis* were collected from Department of Microbiology, KAP Visvanatham Medical College, Tiruchirappalli. The microbial cultures were maintained on slants consisting of Nutrient Agar and Potato dextrose agar. The bacterial and fungal cultures were pre cultured in nutrient and potato dextrose broth overnight for antimicrobial screening.

Maintenance of Bacterial and Fungal Cultures
The tested microbes (bacterial and fungal species) were maintained in Nutrient Agar Slants. The cultures were sub cultured and then the cultured strains were allowed to grow about two days and they were stored at 5°C for the future studies.

Method of Antimicrobial Screening
The bacterial screening of the aqueous and the other organic solvent extracts of different parts of the test plant was investigated through different methods. The assays consisted of both anti-bacterial and anti-fungal evaluation.

Principle
The Disc Diffusion method is used for the rapid determination of a drug or a particular substance on a specific bacterium or fungus. This method consists of impregnating small circular disc of standard filter paper with the given amount of a chosen concentration of substance. These discs are placed on plates of culture medium that has been seeded with the tested bacterial and fungal inoculums. After incubation the diameter of the clear zone inhibition surrounding the deposit of the substance is taken as a result.

Disc Diffusion Assay (Maruzella and Henry, 1958):

Preparation of Disc: The discs were prepared using WhatmannNo.1 filter paper. A disc of 6mm diameter is cut from Whatmann No.1 filter paper with the help of the punching
machine. The paper discs were autoclaved at 120°C, 150 lbs. About 10 discs were dipped in the plant extract for each.

![Fig.1 Paper discs in pure solvent](image1)

![Fig.2 Paper discs in plant extract](image2)

**Procedure**

**Assay for antibacterial testing**

Antibacterial activity of an aqueous ethanolic solvent extracts were assayed separately using disc diffusion method. Petri plates containing 10 ml of Muller Hinton Agar medium were inoculated with $10^8$CFU/ml of each test bacteria. Sterile filter paper discs (6 mm in diameter) were impregnated with 10μl of the 3mg/ml plant extracts (30μg/disc) placed on the surface of the medium. Negative controls were prepared using the same solvents employed to dissolve the plant extracts. A standard disc containing chloramphenicol antibiotic drug (30μg/disc) was used as a positive control and they were incubated for 24 h. The assessment of antibacterial activity was based on the measurement of diameter of inhibition zone formed around the disc.

![Steriled Petriplate With Agar (15ml)](image3)

![Fig.3 Inhibition zone formation](image4)
Calculation for % of Inhibition: The zone diameter values were averaged to get the mean diameter value. The zone of inhibition is measured and the diameter values were used to determine the % of inhibition.

\[
\text{% of Inhibition} = \frac{d_{av} - d_s}{d_S} \times 100
\]

Where, \(d_{av}\) - average zone diameter by the plant extract
\([d_{av} = (d_1 + d_2 + d_3) / 3]\)
\(d_s\) - zone diameter of the pure solvent
\(d_S\) - zone diameter of the standard

Phytochemical Screening

The extract is taken for phytochemical screening analysis to find out the presence of the phyto-constituents such as alkaloids, flavonoids, terpenoids, saponins, etc. It is confirmed by phytochemical screening.

Table -1 Experimental procedure for phytochemical screening (Harborne, 1973):

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Name Of The Test</th>
<th>Experimental Procedure</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Test for Carbohydrates: Molisch’s test</td>
<td>2-3ml of extract, add two drops of alpha naphthol solution in alcohol shake and add conc. H\textsubscript{2}SO\textsubscript{4} from sides of test tube.</td>
<td>Violet coloration occurs.</td>
</tr>
<tr>
<td>2.</td>
<td>Test for Reducing Sugar: Fehling’s test</td>
<td>Mix 1ml Fehling’s A and 1ml Fehling’s B solutions, boil for one minute. Add equal volume of test solution. Heat in boiling water bath for 5-10 min.</td>
<td>Brick red colored precipitate is obtained.</td>
</tr>
<tr>
<td>3.</td>
<td>Test for Hexose Sugars:</td>
<td>Heat 3ml. Selwinoff’s reagent and 1ml. test solution in bearing water bath for 1-2 min.</td>
<td>Red color solution is obtained.</td>
</tr>
<tr>
<td>a)</td>
<td>Selwinoff’s test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b)</td>
<td>Cobalt chloride test</td>
<td>Mix 3ml test solution with 2ml cobalt chloride. Boil and cool. Add few drops of NaOH solution.</td>
<td>Solution is greenish blue (glucose) in color.</td>
</tr>
<tr>
<td>4.</td>
<td>Test for Non-Reducing Polysaccharides (Starch): Iodine test</td>
<td>Mix 3ml test solution and few drops of dilute iodine solution.</td>
<td>Blue color solution is obtained. It disappears on boiling and reappears on cooling.</td>
</tr>
</tbody>
</table>
5. **Test for Proteins:**
   - **a) Biruet test**
     - To 3ml test solution add 4% NaOH and few drops of 1% CuSO₄ solution. Pink color is obtained.
   - **b) Million’s test**
     - Mix 3ml. test solution with 5ml million’s reagent. White precipitate, warm precipitate turns brick red or the precipitate dissolves given red colored solution.

6. **Test for Amino Acids:**
   - **Test for tyrosine**
     - Heat 3ml of test solution and add 3 drops million’s reagent. Red color solution is obtained.

7. **Test for Steroids:**
   - **Salkowski test**
     - To 2ml of extract add 2ml chloroform and 2ml conc. H₂SO₄. Shake well. Chloroform layer appears red and acid layer layer shows greenish yellow florescence.

8. **Test for Glycosides:**
   - **a) General test:**
     - Add 10ml of 50% HCl to the 2ml test solution. Heat on boiling water bath for 30min. Add 5ml of Fehling’s solution and boiled for 5min. Brick red precipitate is obtained.
   - **b) General test**
     - To test solution adds 1ml of water and NaOH. Yellow coloration occurs.

9. **Test for Cardiac Glycosides:**
   - **Legal’s test (test for cardenolides)**
     - To aqueous or alcoholic extract, add 1ml pyridine and 1ml. sodium nitroprusside. Pink to red color change occurs.

10. **Test for Anthraquinone Glycosides:**
    - **a) Borntrager’s test (for Anthraquinone glycosides)**
    - **b) Modified Borntrager’s test**
      - To 5ml 5% FeCl₃ and 5ml dil. HCl. Heat for h min in boiling water bath. Cool and add benzene or any organic solvent. Shake well. Separate organic layer. Add dilute ammonia. Ammoniacal layer shows pinkish red color.

11. **Test for Saponin Glycosides:**
    - **Foam test:**
      - Shake the drug extract or dry powder vigorously with water. Persistent foam is formed.

12. **Test for Cyanogenic Glycosides:**
    - **Grignard’s test or sodium picrate test**
      - Soak a filter paper strip first in 10% picric acid, then in 10% sodium carbonate, dry. In a conical flask place moistened powdered drug. Cork it, place the above filter paper strip in the slit in cork. The filter paper doesn’t turn brick red or maroon color.

13. **Test for Alkaloids:**
    - **a) Dragendorff’s test**
      - To 2-3ml test solution add few drops Dragendorff’s reagent. Orange brown precipitate.
    - **b) Wagner’s test**
      - 2-3ml test solution with few drops Wagner's reagent. Reddish brown precipitate.
    - **c) Mayer’s test**
      - 2-3ml test solution with few drops of White or pale
14. **Test for Tannins:**
   a) Lead acetate test
   Mayer’s reagent.
   3-5 ml test solution with few drops of 1% lead acetate.
   Red precipitate is obtained.

   b) Ferric chloride test
   To 2-3ml test solution add 2ml of FeCl₃.
   Blue precipitate is obtained.

15. **Test for Phenolic Compounds:**
   Ferric chloride test
   To 1-2 ml test solution adds 2ml of water and 10% aqueous ferric chloride solution.
   Green color solution is obtained.

16. **Test for Flavonoids:**
   Shinoda test
   To test solution, add 5ml of alcohol. Few drops of conc. HCl and magnesium turnings.
   Pink coloration occurs.

17. **Test for Terpenoids:**
   a) To test solutions add 2ml of chloroform and 1ml of conc. H₂SO₄.
   Reddish brown coloration occurs.

   b) To test solution add 1ml of 2,4-dinitrophenyl hydrazine in 2M HCl.
   Yellow orange coloration occurs.

18. **Test for Saponins:**
   Foam test
   To test solution add drop of sodium bicarbonate. Shake well.
   Honey comb like froth is obtained.

**RESULTS AND DISCUSSION**

The evaluation of the antibacterial activity of an aqueous ethanolic extracts of leaf of *Flueggea leucopyrus* against both Gram positive and Gram negative bacteria by using the disc diffusion method is given in Table- 2. The *in vitro* results were observed in terms of inhibition zone around each disc caused by diffusion of antibacterial properties from the plant extract impregnated disc into the surrounding medium. The diameter of inhibition zones were noted in the leaf extracts, the aqueous ethanolic extract showed greater antibacterial activity against the test bacteria such as, *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus mutant*, *Pseudomonas aeruginosa* and *Bacillus cereus*. The zone of inhibition was higher in the case of *Klebsiella pneumoniae* (1.55), *Escherichia coli* (1.35), *Streptococcus mutast* (1.1), *Pseudomonas aeruginosa* (1.1), and *Bacillus cereus* (0.95). Moderate inhibition was observed in *Salmonella typhi* (0.85) and *Serratia marcescens* (0.8) and low inhibition was noted in Proteus mirabilis (0.7) and *Vibrio cholerae* (0.55). Even at a low concentration of hydro-alcoholic extract of the plant Flueggea leucopyrus inhibits the activity of the bacterial species such as *Serratia marcescens*, *Salmonella typhi*, *Escherichia coli* and *Staphylococcus mutan*. The percentage of inhibition zone was depicted in Fig.4. In addition, the inhibition zones formed by standard antibiotic disc (chloramphenicol 30 mcg/disc) and those filter paper discs injected with 70% ethanol (negative controls) are also listed in Table – The antifungal activity of hydro alcoholic extract of F. leucopyrus showed complete absence of inhibition zones against *Candida albicans* and *Candida tropicalis* (Table-3 ).
The qualitative phytochemical screening of aqueous ethanolic extract of Flueggea leucopyrus revealed the presence of phyto constituents such as carbohydrates, reducing sugar, Hexose sugar, starch, proteins, amino acids, tyrosine and steroids etc. (Table -4). Thus the phytochemical screening of the hydro-alcoholic extract is found to contain important secondary metabolites.

Table - 2 Anti-bacterial activity of hydro alcoholic extract of *F.leucopyrus*

The results given below are for 0.0624 g of extract in 5ml concentration.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Bacterial species</th>
<th>Zone length for standard (cm)</th>
<th>Zone length for pure solvent (cm)</th>
<th>Zone length for extract (cm)</th>
<th>Bacterial Activity</th>
<th>% of Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Serratia marcescens</td>
<td>2.8</td>
<td>0.6</td>
<td>0.8</td>
<td>+</td>
<td>7</td>
</tr>
<tr>
<td>2.</td>
<td>Salmonella typhi</td>
<td>2.5</td>
<td>0.6</td>
<td>0.85</td>
<td>+</td>
<td>10</td>
</tr>
<tr>
<td>3.</td>
<td>Escherichia coli</td>
<td>2.5</td>
<td>0.5</td>
<td>1.35</td>
<td>+</td>
<td>34</td>
</tr>
<tr>
<td>4.</td>
<td>Proteus mirabilis</td>
<td>0.8</td>
<td>0.8</td>
<td>0.7</td>
<td>_</td>
<td>Nil</td>
</tr>
<tr>
<td>5.</td>
<td>Staphylococcus mutant</td>
<td>2.7</td>
<td>0.5</td>
<td>1.1</td>
<td>+</td>
<td>22.2</td>
</tr>
<tr>
<td>6.</td>
<td>Pseudomonas aeruginosa</td>
<td>0.9</td>
<td>0.7</td>
<td>1.1</td>
<td>_</td>
<td>Nil</td>
</tr>
<tr>
<td>7.</td>
<td>Vibrio cholerae</td>
<td>2.0</td>
<td>0.6</td>
<td>0.55</td>
<td>_</td>
<td>Nil</td>
</tr>
<tr>
<td>8.</td>
<td>Bacillus cereus</td>
<td>1.2</td>
<td>0.5</td>
<td>0.95</td>
<td>_</td>
<td>Nil</td>
</tr>
<tr>
<td>9.</td>
<td>Klebsiella pneumoniae</td>
<td>2.8</td>
<td>0.6</td>
<td>1.55</td>
<td>_</td>
<td>Nil</td>
</tr>
</tbody>
</table>

Table – 3 Antifungal activity of hydro alcoholic extract of *F.leucopyrus*:

The results given below are for 0.0624g of extract in 5ml concentration.

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Fungal Species</th>
<th>Zone length for standard (cm)</th>
<th>Zone length for pure solvent (cm)</th>
<th>Zone length for extract (cm)</th>
<th>Fungal Activity</th>
<th>% of Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Candida albicans</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>_</td>
<td>Nil</td>
</tr>
<tr>
<td>2.</td>
<td>Candida tropicalis</td>
<td>2.0</td>
<td>Nil</td>
<td>Nil</td>
<td>_</td>
<td>Nil</td>
</tr>
</tbody>
</table>

Fig. 4 Percentage of inhibition of *F.leucopyrus* against pathogenic bacteria
Table – 4 Qualitative phytochemical screening of Hydro alcoholic extract of 
*F. leucopyrus*

<table>
<thead>
<tr>
<th>S.No</th>
<th>Phyto-constituents</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Reducing Sugar</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Hexose Sugar</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Non-Reducing Polysaccharides (Starch)</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Proteins</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Amino Acids</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Tyrosine</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Cardiac Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>Anthraquinone Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>Saponin Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>Cyanogenic Glycosides</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>15</td>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>16</td>
<td>Phenolic Compounds</td>
<td>+</td>
</tr>
<tr>
<td>17</td>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>18</td>
<td>Terpenoids</td>
<td>+</td>
</tr>
<tr>
<td>19</td>
<td>Saponins</td>
<td>+</td>
</tr>
</tbody>
</table>

CONCLUSION

This chapter summarizes the findings of the present project phytochemical screening and anti-microbial studies of the extracts of *Flueggea leucopyrus*. Thus the medicinal plant is found to possess significant *Flueggea leucopyrus* constituents. The presence of such a variety of phytochemicals may be attributed to the medicinal characteristics of this plant *Flueggea leucopyrus*. It is disinfectant and it is useful in vitiated conditions of burning sensations, seminal weakness and general debility.

REFERENCES