PUBLICATION
ANTI-BACTERIAL AND ANTI FUNGAL ACTIVITY OF METHNOL AND ETHYL ACETATE EXTRACT OF FLUGGEA LEUCOPYRUS

Nagarathna P. K. M*, Gopal. M. Adviraao

*Department of Pharmacology, Karnataka college of pharmacy, Bangalore, 560 064, Karnataka, India.

Department of Biochemistry, Kuvempu university, Shivagangothri, Davanagere, 577001, Karnataka, India.

Fluggea leucopyrus (Euphorbiaceae) have been popularly used for many ailments like diuretic and disinfectant. In order to evaluate this information, anti bacterial and antifungal activity of methanol and ethyl acetate extract from leaves part was investigated by using cup plate method, the minimum inhibitory concentration of both the extracts were studied in this experiment. Methanol and ethyl acetate extract of fluggea lecopyrus exhibited antibacterial and antifungal activity against different pathogens.

KEYWORDS: Fluggea leucopyrus, Euphorbiaceae, methanol and ethylacetate, anti-, bacterial and antifungal activity, chloramphenicol, nystatin.

INTRODUCTION

The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants. Plants are known to produce a variety of compounds to protect themselves against a variety of their pathogens and therefore considered as potential source for different classes of antimicrobial substances (Alamagboul A. Z. et al., 1985; Alkofani A. S. et al., 1990; Graye R.J. et al., 1994; O.D.K. et al., 1993). Plants used in traditional medicine contain a wide range of substances that can be used to treat chronic as well as acute infectious diseases. The substances that can either inhibit the growth of microorganisms or kill them are considered as candidates for developing new drugs for treatment of various infectious diseases (Haila F.N. et al., 1999). The plants used in traditional system of medicine are well known as remedies for diseases in the rural areas of developing countries (Catalano S et al., 1998; Martinez M.J. et al., 1996; Sunder R.K. 1996; Taylor R.S.L. et al., 1996). Herbal medicines have been used in developing countries as an alternative to Allopathic medicines. Our extensive review of literature has revealed a variety of medicinal plants possessing anti-inflammatory activity which also exhibited good antimicrobial properties (Valcheva K.S. et al., 2005; Sarker S.D. et al., 2004; Sandler C. et al., 2005; Hauser B. et al., 2005; Vuorela S. et al., 2005). On the basis of these reports the plant extracts were subjected for antimicrobial activity against various bacterial and fungal strains. Fluggea leucopyrus Linn. (Euphorbiaceae) The plant is sweet, cooling, diuretic, aphrodisiac and tonic, and is useful in vitiated conditions of pitta, burning sensation, strangury, seminal weakness and generally debility. The leaves act as a disinfectant and its paste is used by tribes to extract any extraneous materials from body tissues without surgery. Leaves are eaten. The juice or paste of the leaves is used along with tobacco to destroy worms in sores. The berry is sweet and edible. Slender branches are used for masking wicker-baskets, and for thatching. Wood is pink, hard and close-grained. It is used as fuel (Gammie et al.,). Anti ulcer activity of fluggea microcarpa was reported by (Goel R.K., et al., 1997), anti microbial activity of this plant was reported by (Bakshu et al.). It was reported that plant was used as fish poison by (Williamman et al). It was reported that an unidentified alkaloid as well as free triterpenes, flavonoids, diterpenes, steroids or relative polycyclic substances are present in the plant. Stem bark contains tannin. (Wealth of india vol no 9 p.no 267.)

EXPERIMENTAL

PLANT MATERIAL:

Plant materials were collected from the Ananthanahalli village near Davanagere disit, Karnataka, India during may 2007; leaves
were dried at room temperature. Authenticated Voucher specimens were deposited in the Institutional museum, Kuvempu University.

**Preparation and characterization of the extract:** 100g powdered leaves parts were subjected to successive extraction in a soxhlet extractor in different solvents from non-polar to polar solvents such as ethyl acetate and methyl alcohol. The different extracts obtained were concentrated in a rotary shaker evaporator to dryness to get a constant weight. Then the methanol extract was subjected to column chromatography on a silica gel, column eluted with acetone, methanol, formic acid and water mixture the component obtained from this is subjected to TLC to isolate the flavonoid, the isolated flavonoid was confirmed by LCMS, IR, MASS and NMR. Then the isolated compound was labeled as leucopyrosinol. In this experiment the antibacterial and antifungal activity of leucopyrosinol and ethyl acetate crude were compared with the standard drug chloramphenicol and nystatin.

**CHEMICALS:** chloramphenicol, nystatin.

**Test Compounds:** Leucopyrosinol and crude extract of ethyl acetate of *fluggea leucojus*.

**Test organisms:** The microorganisms used for the experimental were procured from the department of microbiology, IIISC, Bangalore.

**Methods for evaluating antimicrobial activity:**
The term microbiological assay is a biological assay performed with micro-organisms like bacteria, yeast, fungi, etc. This involves the measurement of the relative potency or activity of compounds by determining the amount or test material required for producing stipulated effect on suitable organism under standard conditions.

The procedures employed in microbial assay were,

a). Cylinder plate method or cup plate method

b). Turbidimetric or tube assay method (two fold serial dilution method).

In the present study, antimicrobial screening was carried out by both cup plate and tube assay (two fold serial dilution) method.

In cup plate method, the antimicrobial substance diffuses from the cup through a solidified agar layer in a petridish or a plate to an extent so that the growth of added micro-organism is inhibited entirely in a circular area or zone around the cavity containing the solution of a known quantity of antimicrobial substance. The antimicrobial activity is expressed as the zone of inhibition in millimeters, which is measured with a zone reader.

The leucopyrosinol and crude extract of ethyl acetate, leaves of *fluggea leucojus* was screened for antimicrobial activity against a wide spectrum of micro-organisms and the activity was compared with appropriate reference standards (chloramphenicol for both gram-positive and gram-negative micro-organisms and nystatin for fungal strains). Micro-organisms were grown in nutrient agar medium. Dimethyl sulfoxide (DMSO) and distilled water were used as control and the drug vehicles for the plant extract and reference standards respectively.

**Evaluation of antibacterial and antifungal activity:**
Determination of zone of inhibition by cup plate method (Indian Pharmacopoeia, 1996):
The cylinder plate assay of drug potency is based on measurement of the diameter of zone of inhibition of microbial growth surrounding cylinders (cups), containing various dilutions of test compounds.

A sterile borer was used to prepare four cups of 6 mm diameter in the agar medium spread with the micro-organisms and 0.1 ml of inoculum was spread on the agar plate by spread plate technique. Accurately measured (0.05 ml) solution of each concentration and reference standards were added to the cups with a micropipette.

All the plates were kept in a refrigerator at 2 to 8°C for a period of 2 hours for effective diffusion of test compounds and standards. Later, they were incubated at 37°C for 24 hours. The presence of definite zone of inhibition of any size around the cup indicated antibacterial activity. The solvent control was run simultaneously to assess the activity of dimethyl sulfoxide and water which were used as Drug vehicles. The experiments were performed three times. The
Table 1: Antibacterial activity of leucopyrosinol and ethyl acetate extract of fluggea leucopyrus.

<table>
<thead>
<tr>
<th>Plant material</th>
<th>Dose (µg/cup)</th>
<th>zone of inhibition* (diameter in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>gram (+)ve S.p. B.c B.p S.a E.c E.a P.a S.m</td>
</tr>
<tr>
<td>Fluggea leucopyrus:</td>
<td></td>
<td>gram (-)ve S.p B.c B.p S.a E.c E.a P.a S.m</td>
</tr>
<tr>
<td>Ethyl acetate (crude)</td>
<td>100</td>
<td>12 9 9 - 11 9 - -</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>13 13 11 9 13 12 10 -</td>
</tr>
<tr>
<td>Leucopyrosinol</td>
<td>100</td>
<td>14 24 22 16 20 20 18 20</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>19 29 27 19 26 28 23 25</td>
</tr>
<tr>
<td>Standard:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>30</td>
<td>19 18 17 16 20 18 12 18</td>
</tr>
<tr>
<td>Vehicle:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMSO</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Water</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Values are the average of triplicate; Includes the cup diameter (6mm).

S.p=Streptococcus pneumoniae; B.c=Bacillus cereus; B.p=Bacillus pumilis; S.a=Staphylococcus aureus; E.c=Escherichia coli; E.a=Enterobacter aerogens; P.a=Pseudomonas aeruginosa; S.m=Streptomyces marienensis; =No activity

Table 2: Antifungal activity of leucopyrosinol and ethyl acetate extract of fluggea leucopyrus

<table>
<thead>
<tr>
<th>Plant material</th>
<th>dose (µg/cup)</th>
<th>Zone of inhibition (diameter in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A.r. R.s S.c</td>
</tr>
<tr>
<td>Fluggea leucopyrus:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethyl acetate (crude)</td>
<td>100</td>
<td>17 13 31</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>19 15 36</td>
</tr>
<tr>
<td>Leucopyrosinol</td>
<td>100</td>
<td>21 17 19</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>25 24 20</td>
</tr>
<tr>
<td>Standard:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nystatin</td>
<td>30</td>
<td>18 26 21</td>
</tr>
<tr>
<td>Vehicle:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMSO</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Water</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

A.r=Aspergillus Niger; R.s=Rhizopus stolonifer; S.c=Sacharomyces cerevisiae; =No activity

Values are the average of triplicate; Includes the cup diameter (6mm).

Table 3: Determination of Minimum Inhibitory Concentration (MIC)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Antimicrobial concentration (µg/ml)</th>
<th>Growth Control</th>
<th>Sterility Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1000</td>
<td>500</td>
<td>250</td>
</tr>
<tr>
<td>E. coli</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S. aureus</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A. niger</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C. albicans</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

(+) Growth; (-) No growth
diameter of the zone of inhibition was measured and recorded.

**Determination of minimum inhibitory concentration (MIC) in liquid medium** (Indian Pharmacopoeia, 1996; Micheal J, 1998):

The turbidimetric assay to detect the drug potency is based on inhibition of microbial growth as indicated by measurement of the turbidity (transmittance) of a suspension of suitable micro-organisms in a fluid medium to which have been added, graded amounts of the test compounds and known concentration of reference material.

**DISCUSSION**

All the extracts at a concentration of 100 µg and 150 µg per each cup exhibited antibacterial and antifungal activities against one or the other organisms in dose dependent manner. The plant extracts have exhibited considerable activity on the tested fungi. Leucopyrosinol had produced good antibacterial activity against gram +ve and gram –ve bacteria and fungal strains when compared to ethyl acetate. *The plant did not produce inhibition against Staphylococcus aureus, Pseudomonas aeruginosa and Streptomyces marienensis* at a dose of 100 µg/cup.

Table 3 shows that test tubes containing 31.25, 62.5 & 125 µg/ml respectively of antimicrobial drug exhibited a growth evident in the form of turbidity when compared against the sterile control (blank), both in the case of bacteria and fungi. Whereas test tubes containing 250, 500 & 1000 µg/ml respectively of antimicrobial drug did not show any growth. Hence, 250 µg/ml is the minimum inhibitory concentration of the antimicrobial drug both in the case of bacteria and fungi.

The above results clearly demonstrated that the extracts had significant and considerable anti-microbial activity against variety of pathogens.

**REFERENCE:**


Indian Pharmacopoeia Vol-II, the Controller of Publications, New Delhi, 1996.

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Research Article


ANTI-INFLAMMATORY ACTIVITY OF METHANOL AND ETHYL ACETATE EXTRACT OF FLUGGEA LEUCOPYRUS

Nagarathna P.K.M*, Gopal M. Advirao*

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ABSTRACT - Fluggea leucopyrus (Euphorbiaceae) have been popularly used for many ailments like diuretic and disinfectant. In order to evaluate this information, anti inflammatory activity of methanol and ethyl acetate extract (crude) from leaves part was investigated by using in vivo method. For anti inflammatory activity carrageenan-induced hind paw edema model, were employed. Methanol and ethyl acetate extract (crude) of Fluggea leucopyrus exhibited notable inhibition against carrageenan induced hind paw edema in rats.

KEYWORDS - Fluggea leucopyrus, Euphorbiaceae, methanol and ethyl acetate (crude), Carrageenan, Phenyl butazone.

INTRODUCTION

Inflammatory diseases are among the most common health problems treated with traditional remedies. Therefore, it is crucial to evaluate the potential of herbal remedies discovery of novel bio-active compound that might serve as leads for the development of potent drugs. The present investigation represents a preliminary screening in an on-going program on plants used in traditional medicine for the treatment of inflammatory disease.

A survey of the literature revealed that the anti-inflammatory activity of this plant have not been studied so far, except the anti-ulcer activity (R.K.Gopal et al.). The present paper was taken to search for anti-inflammatory activity of the leucopyrosinol and ethylacetate (crude) extract of leaves parts of Fluggea leucopyrus in order to validate their medicinal utilization. Inflammation, an evidence of many diseases, is major concern for physicians throughout the world. Prolonged use of anti-inflammatory agents has been associated with gastrointestinal irritation. In India many ayurvedic practitioners are using various indigenous plants for the treatment of different types of arthritis and inflammatory conditions.

EXPERIMENTAL

CHEMICALS

Hi-media, Mumbai: Carrageenan.
Ugo Basile, Italy: Plethysmometer.
Nestor Pharma, India: Phenyl butazone.

Test Compounds: Leucopyrosinol and crude extract of ethyl acetate of Fluggea leucopyrus.

PLANT MATERIAL:

Plant materials were collected from the Ananthanahalli village near Davanagere disit, Karnataka, India during May 2007; leaves were dried at room temperature. Authenticated Voucher specimens were deposited in the Institutional museum, Kuvempu University.

Preparation and characterization of the extract: 100g powdered leaves parts were subjected to successive extraction in a soxhlet extractor in different solvents from non-polar to polar solvents such as ethyl acetate and methyl alcohol. The different extracts...
obtained were concentrated in a rotary shaker evaporator to dryness to get a constant weight. Then the methanol extract was subjected to column chromatography on a silica gel, column eluted with acetone, methanol, formic acid and water mixture the component obtained from this is subjected to TLC to isolate the falvonoid, the isolated flavonoid was confirmed by LCMS, IR, MASS and NMR. Then the isolated compound was labeled as leucopyrosinol. In this experiment the anti inflammatory activity

DATA SHOWING THE ANTI-INFLAMMATORY ACTIVITY OF LEAF EXTRACTS OF FLUGGIA LEUCOYRUS LINN

<table>
<thead>
<tr>
<th>GROUP</th>
<th>TREATMENT</th>
<th>DOSE mg/kg</th>
<th>PAW ODEMA VOLUME AFTER</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Control</td>
<td>0.2 ml</td>
<td>0.45</td>
</tr>
<tr>
<td>B</td>
<td>Phenyl butazone</td>
<td>100</td>
<td>0.54 ± 0.01</td>
</tr>
<tr>
<td>C</td>
<td>Leucopyrinol</td>
<td>100</td>
<td>0.48 ± 0.001</td>
</tr>
<tr>
<td>D</td>
<td>Leucopyrinol</td>
<td>150</td>
<td>0.51 ± 0.003</td>
</tr>
<tr>
<td>E</td>
<td>Ethyl acetate</td>
<td>200</td>
<td>0.52 ± 0.001</td>
</tr>
<tr>
<td>F</td>
<td>Ethyl acetate</td>
<td>100</td>
<td>0.48 ± 0.007</td>
</tr>
<tr>
<td>G</td>
<td>Ethyl acetate</td>
<td>150</td>
<td>0.51 ± 0.004</td>
</tr>
<tr>
<td>H</td>
<td>Ethyl acetate</td>
<td>200</td>
<td>0.52 ± 0.007</td>
</tr>
</tbody>
</table>

Values are ± SEM, n=6, Significance at p < 0.001*, p < 0.01**, p < 0.05*. Graph showing the % inhibition of oedema.

of leucopyrinol and ethyl acetate crude were compared with the standard drug phenyl butazone.

PHARMACOLOGICAL PROCEDURE
Albino rats either sex weighing between 150-200 gms were purchased from the Sri Venkateshwara animal breeding laboratories, J. P. Nagar, Bangalore. The animals left for 2 days for acclimatization to animal room conditions were maintained on standard pellet diet and water ad libitum.

The food was withdrawn on the day before the experiment but allowed free access of water. A minimum of six animals were used in each group. Throughout the experiment, animals were processed according to the suggested ethical guidelines for the case of laboratory animals.

PREPARATION OF TEST SAMPLES FOR BIO-ASSAY
Test samples were given orally to test animals after suspending in a mixture of distilled water and 0.5% sodium carboxymethylcellulose (CMC). The control group of animals received the same experimental handling as those of the test group except the drug treatment was replaced with appropriate volumes of dosing vehicle. Phenyl butazone 100mg/kg was used as reference drug.

ANTI-INFLAMMATORY ACTIVITY
Anti-inflammatory activity was carried out as suggested by winter et al (1962) Albino rats of either sex weighing between 150–200 grams were selected. They were maintained
on standard pellet feed Gold Mohr's Lipton India limited, for seven days. Water was supplied ad libitum. Anti-inflammatory activity was carried out as suggested by winter et al (1962).

The rats were divided into eight groups of six animals each.

Group A: Received 0.2 ml of normal saline by oral route which served as normal control.

Group B: Received 100 mg/kg body weight of phenyl butazone by oral route which served as positive control.

Group C, D, E: Received leucopyrosinol, at a dose of 100, 150, 200 mg /kg which served as test.

Group F, G, H: Received Ethyl acetate (crude) at a dose of 100, 150, 200, mg/kg which served as test.

The test extracts and standard reference phenylbutazone were administered orally to rats 1 hour before the injection of 0.1ml of 1% carrageenan suspension in normal saline. A No 26 gauge needle was used to inject 0.1 ml of the carrageenan suspension in to the subplantar region of the left hind paw and right hind paw served as control. Immediately there after the volume of injected paw were measured by mercury displacement method using plethysmograph. It was found that the swelling reached a peak between 3 to 5 hours and was stable for several hours. There after for assessing anti-inflammatory activity the oedema volume at predetermined intervals was noted.

The difference in oedema volume between the treated and control were measured and mean volume of oedema was calculated. From the data obtained. The percentage reduction in oedema volume was calculated using the formula:

\[ \frac{V_t - V_c}{V_c} \times 100 \]

Where \( V_t \) = paw volume after time t

\( V_c \) = paw volume of control (Carrageenan treated)

5.8.5. Statistical Analysis:

The results were expressed as mean ± SEM. The significance was evaluated by student t test compared with control and p < 0.001 implied significance (Woodson 1989).

Significance at p < 0.05* p < 0.01** and p < 0.001***

ACUTE TOXICITY

Animals employed in the carrageenan induced paw edema experiment were observed during 24 hours and morbidity or mortality was recorded, if happens, for each group at the end of observation period.

STATISTICAL ANALYSIS OF DATA

Data observed from animal experiments were expressed as mean standard error (SEM). Statistical and the controls were evaluated by anova and student T-tests. p < 0.05 was considered to be significant from the control test.

RESULT: There was dose dependent significant reduction in carrageenan induced rat paw edema at different doses of leucopyrosinol, ethyl acetate crude and at 100mg/kg phenyl butazone over a period of 4 hour as shown in table 1. There was dose dependent significant reduction in carrageenan induced rat paw edema at different doses of leucopyrosinol, ethyl acetate and at 100mg/kg phenyl butazone over a period of 4 hour as shown in table 1.

Significant anti-inflammatory activity was noted with all the three doses of leucopyrosinol and ethyl acetate (crude) at different time intervals (i.e. 1 hour, 2 hour, 4 hour ) and significant percent reduction in oedema volume with all the 3 doses were noted as leucopyrosinol 100mg/kg (6.41, 46.61%, 50.25%) 150mg/kg (13.47, 54.88%, 55.64%), 200mg/kg (15%, 55.91%, 57.89%, 69.94%), and ethyl acetate (crude) 100mg/kg (6%.10.75%,

![Graph](image.png)
The leucopyrosinol and ethylacetate (crude) extract have contained flavonoids, the active principles of this may be responsible for anti-inflammatory activity. Flavonoids are reported with anti-inflammatory activity. The extract on phytochemical studies revealed the presence of the above principles. This confirms their anti-inflammatory activity in animal studies.

**DISSCUSSION**

Topical and systematic application of herbal plants is common practice for the treatment of inflammation, particularly, arthritis (anu-vata) which has been followed for many years in the practice of Indian system of medicine ayurveda. In the present study, the action of the leaves menthol extract was examined by local application on the paw hind. This phlogistic agent provides a skin inflammation model suitable for the evaluation of topical anti-inflammatory agents. The majority of its activities appear to involve or depend on arachidonic acid and metabolism and interaction with protein kinase C (Young and De Yong, 1989).

Carrageenan induced hind paw edema is the standard experimental model of acute inflammation. Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is known to be antigenic and is devoid of apparent systemic effects. Moreover the experimental model exhibits a high degree of reproducibility. Carrageenan induced edema is a biphasic response. The 1st phase is mediated through the release of histamine, serotonin & kinins, where as the 2nd phase is related to the release of prostaglandin & slow reacting substances with peak at 3h. The results of Carrageenan experiment showed maximum activity at 1st & 4th hour after the injection. This explains the inhibition of 1st phase of inflammation which is mediated by histamines & serotonin. However then experiments were carried out using specific blocking agents, it was shown that this blocking of inflammation was due to histamine and not serotonin. Also, inhibition of inflammation at 24 hour due to PGE1 was significantly to decrease by extract treatment. Thus, it was confirmed that action of extract is more potent against the histamine & PGE1 & not serotonin. Thus, in the light of above results, Ayurvedic use of fuguja leucopyrosinol menthol explained.

**REFERENCES:**


