



Original Article

In Vitro Anthelmintic Activity of *Flueggea leucopyrus* by Using Earth Worms

V Deepika^{*}, T Umapoorani¹, M Raja², V Baskar Ananda Raj³, S Dhanasekar⁴

¹ Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai, 625020.Tamilnadu,India.

² Department of Pharmaceutics, college of pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore, 641044.Tamilnadu, India.

³ Associate Professor, Department of Pharmacognosy, SSMcollege of pharmacy,Chinniapalayam, Erode,638212.Tamilnadu,India.

^{*} and ⁴ SSM college of pharmacy,Chinniapalayam, Erode,638212.Tamilnadu,India.

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Objective: To prescreen the in vitro anthelmintic activity of the aerial parts of the plant *Flueggea leucopyrus* Family Euphorbiaceae by using Earth worms. **Method:** In the present study to investigate the effect of aqueous extract of *Flueggea leucopyrus* was selected for phytochemical and anthelmintic activity. Anthelmintic activity was determined by Earth worms were observed for the motility after incubating at 37°C. **Result:** Preliminary phytochemical screening of aqueous extract of *Flueggea leucopyrus* (AEFL) showed the presence of alkaloids, flavonoids, glycosides, carbohydrate, steroids, amino acids and proteins. The anthelmintic activity of AEFL determined by using Earth worms were observed paralysis after the treatment of drug at various concentrations. **Conclusion:** *Flueggea leucopyrus* have been used in medicine due to various biological activities. This study indicates that the aqueous extract of *Flueggea leucopyrus* possesses potential anthelmintic activity. The presence of alkaloids in aqueous extraction of the aerial parts of the plants *Flueggea leucopyrus* appears to contribute to its activity. Further investigation requires to confirm this activity.

Keywords: *Flueggea leucopyrus*, Euphorbiaceae, Anthelmintic activity, Earth worm, Albendazole.

1. INTRODUCTION

Medicinal plants are the most important source of life saving drugs and have been widely used for the treatment of diseases in traditional way for several years. An interaction between ancient medicine and biotechnological tools is to be established towards newer drug development. The interface between cell biology, structural chemistry and in vitro assays

Corresponding author *

V Deepika

SSM college of pharmacy,
Chinniapalayam, Erode,638212.Tamilnadu,India.

E-Mail: umapooranimpharm@gmail.com

will be the best way available to obtain valuable leads. The value of plants lies in the potential access to extremely complex molecular structure that would be difficult to synthesize in the laboratory. In spite of an increasing awareness and expenditure of resources, the incidence of chronic diseases like cardiac, cancer, diabetes etc. has not declined and in fact is rising at an alarming rate¹. Herbal medicine is based on the premise that plants contain natural substances that can promote health and alleviate illness. Diabetic mellitus is a metabolic disorder characterized by disturbances in carbohydrate, protein, lipid metabolism and by complications like microvascular (retinopathy, neuropathy and nephropathy) and macrovascular (heart attack, stroke and peripheral vascular disease) complications. Currently available synthetic antidiabetic agents produce serious side effects like hypoglycemic coma and hepato renal disturbances².

Flueggea leucopyrus Wild. (Family: Euphorbiaceae) locally known as 'bushweed'. The plant is found in many parts of Sri Lanka particularly in dry zones as shrubs. It is an erect shrub 1.5-4m tall with branches cylindrical or obtusely angular when young, gray. The plant grows in south eastern Queensland, southern India and Sri Lanka. The stem and leaves of the plant exude a white substance. The leaves of *F. leucopyrus* have been used in the treatment of cancer, boils, external ulcers and sores in traditional medicine in Sri Lanka³. The plant has been used in preparations in traditional medicine for the treatment of cough, hay asthma, bowel complaints, disinfections, antioxidant, immunomodulatory activities, laxatives, for diarrhea, gonorrhoea, constipation and mental illness and kidney stones⁴. Various species of the genus *Flueggea* are used to treat many diseases including epilepsy, malaria, jaundice, intestinal worms, edema, heavy menstruation, sterility, poliomyelitis and aplastic anemia in many African and Asian countries. Previous chemical investigations have shown the presence of alkaloids, flavonoids, glycosides, carbohydrate, steroids, amino acids and proteins. Most of the therapeutic properties of this plant are attributed to alkaloids which has considerable attention due to their pharmacological effects⁵.

2. MATERIALS & METHODS

All chemicals and reagents used for this study were of analytical grade and procured from approved organization.

Collection and authentication of plant materials.

The selected plant material was authenticated by Dr.M.Palanisamy, Scientist 'D' -In- Charge, Botanical Survey of India, Southern Regional Center, TNAU, Campus, Coimbatore, and certified as *Flueggea leucopyrus*, Euphorbiaceae.

Preparation of Extraction

Raw materials for extraction were powdered separately. The powdered raw material was taken in 1 liter round bottom flask and extracted with hydro-alcohol (1:1) by maceration extraction method for 3 days^{6,7}.

Preliminary phytochemical screening

Preliminary phytochemical screening was carried out using appropriate solvent extract of the plant to identify the presence and absence of various phytoconstituents like alkaloids, carbohydrates, flavonoids, etc.,^{8,9}

Determination of total phenolic content

The total phenolic content in AEAL was determined spectrophotometrically by Folin-Ciocalteu method calibrating against gallic acid standards and expressing the results in gallic acid equivalent and defined as mg gallic acid /L.¹⁰

Determination of total flavonoid content

The flavonoid content of AEAL was estimated by aluminium chloride method. In this method, aluminium chloride complexes with flavonoids of C3-C5 hydroxyl group and to produce intense colour in acidic medium. The intensity of the colour is proportional to the amount of flavonoids and can be estimated as quercetin equivalent at wavelength of 415nm.¹¹

Anthelmintic Activity¹²

Earth worms each of average length of 6 cm was placed in petri dishes containing 2 ml of various drug concentrations 0.6mg/ml, 0.8mg/ml, 1 mg/ml, 10mg/ml and 20mg/ml of solutions. Albendazole solution (10mg/ml) was used as reference standard drug and distilled water as control. The worms were observed for the motility after incubating at 37°C. This was done after pouring the petri dishes content in the wash basin and allowing the worms to move freely. By tapping the end of each worm with the index finger and applying a bit of pressure, the worms that were alive would show motility and those dead were non motile. The motile worms were returned to the respective petri dishes containing drug solutions and the incubation process was carried out again. In the control, the worms were viable for at least twelve days, which is similar to the findings reported earlier. The time taken for paralysis, motility activity of any sort, death time of worms were observed and recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water.

3. RESULTS

- Preliminary phytochemical screening of appropriate extract of the plant showed the presence of alkaloids, flavonoids, glycosides, carbohydrate, steroids, amino acids and proteins.
- Total phenolic content was found to be ± 0.56 mg/ml.
- Total flavonoid content was found to be ± 0.06 mg/ml.
- Fifteen earthworms were collected and immediately transferred to petridishes containing water. Each petridishes were named as batch no 1,2,3,4,5,6 and 7 with various concentrations of albendazole and extracts. The results of anthelmintic activity of drugs and extracts, based on time of paralysis and time of death is shown in the table no -1 and figure no -1

- The effect of the drug Albendazole at concentration 10mg/ml had more time of death (16.42 min) compared to *F.leucopyrus* leaves extract 10mg/ml (6.01 min). The time taken for paralysis in *F.leucopyrus* leaves extract at concentration 10mg/ml was less (5.01 min) as compared to Albendazole (15.23 min).

Table 1: PROVIDE THE CAPTION FOR TABLE

S.NO	Albendazole	AEFL					
		0.6	0.8	1	10	20	
1.	Concentration (mg/ml)	10					
2.	Time taken for paralysis (min)	15.23	18.23	15.07	13.34	5.04	2.24
3.	Time taken for death (min)	16.42	19.34	16.02	14.23	6.03	3.02

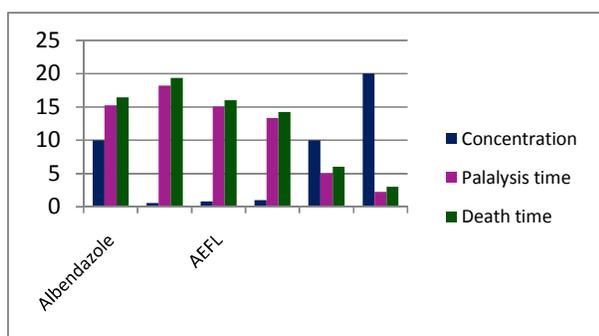


Fig 1: Anthelmintic activity of *Flueggealeucopyrus* using Earth worms

4. DISCUSSION

Antioxidant, cough, hay asthma, bowel complaints, disinfections, immunomodulatory activities, laxatives, for diarrhea, gonorrhoea, constipation, mental illness, kidney stones, epilepsy, malaria, jaundice, intestinal worms, edema, heavy menstruation, sterility, poliomyelitis and aplastic anemia.¹³⁻¹⁵ Preliminary phytochemical screening of appropriate extract of the plant showed the presence of alkaloids, flavonoids, isoflavones, simple phenolics, glucosinolates, cyanogenic glycosides, acids, aldehydes, saponins, triterpenes, glycosides, carbohydrate, steroids, amino acids and proteins.

The aqueous extract of *F.leucopyrus* showed the significant anthelmintic effect causing death of the earth worm at all the concentrations but the time of death was different in each case¹⁶⁻¹⁷. However, when observed the response of worms in case of paralysis, there was significant variation among the results produced by the different extracts at different concentrations like 25, 50, 100, and 200mg/ml. The aqueous extract showed more significant effect on paralyzing the worms, in terms of paralysis time, at every concentration compared to that of ethyl acetate, ethanol, chloroform, hexane extracts. However, further studies are underway to

isolate the lead molecules responsible for the activity and also to pinpoint on the mechanism of action of the same¹⁸⁻²⁰.

5. ACKNOWLEDGEMENT

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