

Phytochemical screening and Evaluation of Acute oral Toxicity of Methanolic Extracts of leaves and bark of *Flueggea leucopyrus* (WILLD.)

Authors:

Dr. S. Shoba^{1*}, J.Tamizhvani ², P.Sakthivel . ³

¹ Professor, Department of Pharmacology, Adhiparasakthi College of Pharmacy, Melmaruvathur, Tamil Nadu, India. Affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, Tamil Nadu, India.

² Postgraduate Scholar, Department of Pharmacology, Adhiparasakthi College of Pharmacy, Melmaruvathur, Tamil Nadu, India. Affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, Tamil Nadu, India

³ Assistant professor, Department of Pharmacology, Adhiparasakthi College of Pharmacy, Melmaruvathur, Tamil Nadu, India. Affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, Tamil Nadu, India

ABSTRACT

The present research work was undertaken to investigate the phytochemical constituents and acute oral toxicity of the methanolic extract of bark and leaves of *Flueggea leucopyrus*(WILLD.). Fresh bark and leaves were collected, shade-dried at room temperature, coarsely powdered, and extracted with methanol by continuous hot percolation method using Soxhlet apparatus. The extract was concentrated using an electrical water bath to obtain a semisolid crude extract. Preliminary phytochemical screening was carried out using standard procedures to identify the major phytoconstituents present in the extract. The acute oral toxicity of the test extract was determined in experimental animals and performed according to the Organisation for Economic Co-operation and Development (OECD) guideline (423) to determine the safe dose of the plant extract. The preliminary phytochemical evaluation of the methanolic extract of *Flueggea leucopyrus*(WILLD.) leaves and bark revealed the presence of alkaloids, flavonoids, tannins, saponins, phenolic compounds, glycosides, terpenoids, steroids, and carbohydrates. In the acute oral toxicity study, the methanolic extract of *Flueggea leucopyrus*(WILLD.) leaves and Bark did not cause any mortality or observable toxic symptoms upto to dose of 2000 mg/kg body weight respectively.

Keywords: *Flueggea leucopyrus*(WILLD.), leaves and Bark, Methanolic extract, Phytochemical screening, Acute oral toxicity studies.

INTRODUCTION

In the Ayurvedic system of medicine, the plant *Flueggea leucopyrus* (WILLD.) belonging to the family Euphorbiaceae and is commonly referred to as Katupila or Heen Katupila and is also associated with the name Sharapunkha. Other vernacular names include Slihashatru, Kantapunka, Kantalu, and Kalika. *Flueggea leucopyrus* (WILLD.) is a shrub widely distributed throughout the dry zones of Sri Lanka and has long been used in Leaves for the treatment of cancer⁽¹⁾. The species is also found in several regions of South India, where it grows naturally on

sandy coastal habitats and at elevations of upto 150 m above sea level. Owing to its ethnomedicinal significance, particularly its reported anticancer properties, *F. leucopyrus*(WILLD.) has attracted considerable scientific interest in recent years. In addition to its reported anticancer activity, *Flueggea leucopyrus*(WILLD.) (Katupila) has traditionally been employed in the management of various other ailments like sweet, cooling, diuretic, aphrodisiac. The plant is a small shrub that typically grows to a height of approximately 3–4 feet.⁽²⁾ It is characterized by round green leaves, small pale-green flowers resembling peppercorns, and tiny seeds similar in appearance to mustard seeds. Despite its common name, Katupila possesses only mildly developed thorns.⁽³⁾

The therapeutic potential of medicinal plants is largely attributed to their diverse phytochemical constituents, which are responsible for a wide range of biological activities. Understanding the relationship between phytoconstituents and their pharmacological effects is essential for the development of novel therapeutic agents targeting various diseases, including chronic disorders.^(4,5) Consequently, there is a growing need for systematic scientific investigations to validate the traditional uses of medicinal plants and to elucidate their mechanisms of action. Such studies will not only enhance our understanding of plant-based remedies but also promote their evidence-based utilization in modern healthcare system.⁽⁶⁾

Flueggea leucopyrus(WILLD.) has been widely recognized in traditional medicine for its diverse therapeutic applications. In Ayurvedic practice, the plant is described as possessing sweet, cooling, diuretic, aphrodisiac, and tonic properties, making it useful in the management of aggravated Pitta conditions, burning sensations, urinary disorders, seminal weakness, and general debility.⁽⁷⁾ Indigenous communities have traditionally used leaf paste as an antiseptic and disinfectant for cleansing infected or damaged tissues, thereby promoting healing without surgical intervention. Various parts of the plant have been employed in the treatment of numerous ailments, including kidney stones, mental disorders, diarrhoea, gonorrhoea, constipation, gastrointestinal complaints, cough, hay fever, and infectious diseases. The leaves are commonly applied for the management of fibroids and haemorrhoids (piles), while the roots are used to alleviate oedema and testicular enlargement.⁽⁸⁾ Traditional healers also utilize the whole plant in the treatment of cancers affecting the sole of the foot. Furthermore, *F. leucopyrus*(WILLD.) has been reported to be beneficial in conditions such as hepatomegaly, portal hypertension, and abdominal masses. The stem bark is traditionally used as a remedy for toothache.⁽⁹⁾



Fig. 1 -Leaves of *F. leucopyrus* (WILLD.)



Fig. 2 -Bark of *F. leucopyrus* (WILLD.)

MATERIALS & METHODS COLLECTION OF PLANT MATERIALS

Fresh leaves of *Flueggea leucopyrus*(WILLD.) were collected from a suitable geographical location. The plant material was authenticated at the Siddha Central Research Institute (CCRS), Chennai, under the Ministry of AYUSH, Government of India. The sample was identified as *Flueggea leucopyrus*(WILLD.) and assigned the authentication code O18032601E. The fresh leaves were collected between January to February 2026. The collected leaves were cleaned and shade dried at room temperature. The dried plant materials were coarsely powdered using a mechanical grinder and sieved through sieve No.10. The powdered material was further used for extraction process. The same procedure was carried out for the bark of *Flueggea leucopyrus*(WILLD.)

Preparation of Methanolic Extract of leaves and bark of *Flueggea leucopyrus* (willd.)

About 1 kg of powdered plant material of leaves and bark were separately first defatted with petroleum ether. Then about 150 g of the dried powdered material (leaves) was subjected to continuous hot extraction using methanol in a Soxhlet apparatus. The obtained methanolic filtrate of leaf extract was concentrated using an electrical water bath to obtain a semisolid residue. The obtained methanolic extract of *Flueggea leucopyrus* leaves (MFL) was subjected to preliminary phytochemical screening and Acute oral toxicity studies. The above same procedure was carried to obtain the methanolic extract of *Flueggea leucopyrus* bark (MFLB).

Preliminary Phytochemical screening⁽¹⁰⁾:

The preliminary Phytochemical test was conducted for MFL and MFLB to detect the presence of phytochemicals by following standard methods.

1. Detection of Alkaloids

a) Mayer's test:

The extract was treated with few drops of Mayer's reagent [Potassium mercuric iodide solution]. Formation of cream coloured precipitate indicates the presence of alkaloid.

b) Dragendroff's test:

The extract was treated with few drops of Dragendroff's reagent [Potassium bismuth iodide solution]. Formation of reddish-brown precipitate indicates the presence of alkaloids.

c) Wager's test:

The extract was treated with few drops of Wager's reagent [Solution of iodine in potassium iodide]. Formation of brown precipitate indicates the presence of alkaloids.

d) Hager's test:

The extract was treated with few drops of Hager's reagent [Saturated solution of Picric acid]. Formation of yellow colour precipitate indicates the presence of alkaloids.

2. Test for carbohydrates

a) Molisch's test:

The extract was treated with few drops of alcoholic alpha naphthol, then added few drops of concentrated sulfuric acid slowly through the sides of the test tube. Formation of purple to violet colour ring at the junction of test tube indicates the presence of carbohydrates.

b) Barfoed's test: The extract was treated with few drops of barfoed's reagent and heated for 2 minutes. Formation of red precipitate indicates the presence of carbohydrates.

c) Seliwanoff's test:

The extract was treated with 3ml of Seliwanoff's reagent and heated on water bath for 1 minute. Formation of rose red colour (ketoses) indicates the presence of carbohydrates.

3. Detection of reducing sugars

a) Benedict's test:

The extract was treated with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and boiled on water bath. Formation of reddish-brown precipitate indicates the presence of reducing sugar.

b) Fehling's test:

The extract was treated with Fehling's solution A and B, heated for few minutes. Formation of brick red precipitate indicates the presence of reducing sugar.

4. Detection of glycosides

a) Borntrager test:

The extract was treated with 5-10ml of dilute HCl, boiled on water bath for 10 mins and filtered. The filtrate was extracted with benzene or CCl₄ and added equal amount of ammonia solution to filtrate and shaken. Formation of pink or red colour in ammoniacal layer due to presence of anthraquinone moiety indicates the presence of glycosides.

b) Modified Borntrager test:

The extract was treated with 5ml of dilute HCl followed by 5ml ferric chloride. Boiled for 10mins and filtered. The filtrate was extracted with benzene or CCl₄ and added equal amount of ammonia solution to filtrate and shaken. Formation of pink or red colour in ammoniacal layer due to presence of anthraquinone moiety indicates the presence of glycosides.

5.Detection of proteins and amino acids**a) Biuret test:**

The extract was treated with 1ml of 10% sodium hydroxide, 1ml of 1% copper sulphate solution. Formation of violet colour indicates the presence of proteins and amino acids.

b) Ninhydrin test:

The extract was boiled with 0.2% solution of Ninhydrin (Indane 1,2,3 trione hydrate). Formation of violet colour indicates the presence of proteins and amino acids.

6.Detection of phenolic compounds

a) Iodine test: The extract was treated with few drops of diluted iodine solution. Formation of transient red colour indicates the presence of phenolic compounds.

b) Ferric chloride test:

The extract was treated with 1ml of water and boiled for few minutes then it is filtered. The filtrate was treated with 5% ferric chloride solution. Formation of bluish black colour indicates the presence of phenolic compound.

7.Detection of Tannins**a) Gelatine test:**

The extract was treated with 2ml of 1% gelatine and 10% of sodium chloride. Formation of white precipitate indicates the presence of Tannins.

b) 10% sodium hydroxide test:

The plant extract was treated with 4 ml of 10% of sodium hydroxide solution and shaken well. Formation of emulsion indicates the presence of tannins.

8.Detection of saponins**a) Foam test:**

The extract was treated with 10 ml of water and boiled for few minutes then it is filtered. The filtrate shaken well and noted for the stable foam

9.Detection of Phytosterols**a) Salkowski Test:**

The extract was treated with 2ml of chloroform, then added few drops of concentrated sulphuric acid, shaken and allowed to stand for some time. Formation of yellow colour ring at the junction which turns red after 2mins indicates the presence of phytosterols

b) Libermann Burchard Test: The extract was treated with 2ml of chloroform, small amount of acetic anhydride and 1ml of concentrated sulphuric acid. Change of colour from red to bluish green ring at the junction indicates the presence of phytosterols.

10.Detection of Quinones

a) Alcoholic KOH test:

To 1ml of plant extract, added few ml of alcoholic potassium hydroxide. Formation of red to blue colour indicates the presence of quinones.

b) Concentrated HCl test:

The extract was treated with few ml of concentrated HCl. Formation of green colour indicates the presence of quinones.

c) Concentrated Sulfuric acid:

The extract was treated with few ml of concentrated sulfuric acid. Formation of red colour indicates the presence of quinones.

11.Detection of fixed oils and fats

a) Stain test:

Little quantity of plant extract was pressed in between two filter paper. Appearance of oil stain indicates the presence of fixed oils and fats.

12.Detection of Flavonoids

a) Alkaline reagent Test:

The extract was treated with 10 ml of ammonium hydroxide. Formation of yellow fluorescence indicates the presence of flavonoids.

b) Ferric chloride test:

The extract was treated with few drops of 10% ferric chloride solution. Formation of Green precipitate indicates the presence of flavonoids.

c) Lead acetate test:

1 ml of the extract filtrate was taken in a test tube. 3–5 drops of 0.1% lead acetate solution were added, The formation of a gelatinous precipitate indicated the presence of tannins.

EXPERIMENTAL ANIMALS:

Healthy adult wistar Albino female rats weighing 180-200g, were selected for the experimental study. The animals were obtained from a registered CCSEA approved animals facility. Before the start of the experiment, the rats were acclimatized to the laboratory environment for 7 days. They were housed in clean

polypropylene cages under standard laboratory conditions, with a temperature of $25 \pm 2^\circ\text{C}$, relatively humidity of 45-55%, and a 12-hour light / 12-hour dark cycle. Standard pellet feed and safe drinking water were provided *ad libitum* throughout the study. All experimental procedures were conducted in accordance with CCSEA guidelines and were approved by the institutional animals ethics committee (IAEC) before the commencement of the research.

Acute oral Toxicity studies:

Procedure:

The acute oral toxic class method of the OECD 423 (2001) was followed to determine the maximum safe dose of the extract. For animal welfare reasons, the starting dose to be used is 300mg/kg bodyweight. Three Wistar Albino rats will be administered a single oral dose of 300 mg/kg of the extract overnight, fasted. On subsequent dosing, animals will be examined individually for the first 30 min, with particular focus for 4hrs, intermittently for 24hrs, and further assessed for 14 days.

Mortality rate observed in 2 or 3 animals considered as toxic dose. Mortality of one animal or no mortality observed; the same dose is repeated again for confirmation. Further higher dose will be decided based on the above results. Absence or presence of mortality of the animals dosed at one step will be determined in the next step (i.e.)

1. No further testing is needed
2. Dosing of three additional animals, with the same dose.
3. Dosing of three additional animals at the higher or the next lower dose.

Observation:

The following clinical observation were made and recorded

1. Pre-terminal deaths
2. Body weight
3. Cage side observation

The home cage activity, color and consistency of the feces, and behavior of the animal was observed once in two days.

4. Physical examination

Observations include skin and fur, eyes i.e. lacrimation, eye prominence, eye lid closure, corneal reflex, lighting reflex, and mucus membrane. Attention was directed for the observation of tremors, convulsion, salivation, lethargy, diarrhea, sleep and coma. Tail elevation, static limb position, head position, righting reflex, and pinna reflex were monitored.

RESULTS:

Preliminary phytochemical screening:

Table 1: Preliminary Phytochemical Screening of methanolic extract of *Flueggea leucopyrus* leaves (MFL) and methanolic extract of *Flueggea leucopyrus* bark (MFLB).

S.No	Name of the test to detect the Phytoconstituents	MFL	MFLB
1.	Detection of Alkaloids		
	a) Drangendorff's Test	Present	Present
	b) Hager's Test	Present	Present
	c) Mayer's Test	Present	Present
	d) Wagner Test	Present	Present
2.	Detection of Carbohydrates		
	a) Barfoed's Test	Present	Present
	b) Molisch's Test	Present	Present
	c) Seliwonoff's Test	Present	Present
3.	Detection of Reducing Sugar		
	a) Benedict, S Test	Present	Present
	b) Fehling's Test	Present	Present
4.	Detection of Glycosides		
	a) Borntrager's Test	Present	Present
	b) Modified Borntrager's Test	Present	Present
5.	Detection of Proteins and Amino Acids		
	a) Biuret Test	Absent	Absent
	b) Ninhydrin Test	Absent	Absent
6.	Detection of Flavonoids		
	a) Alkaline Reagent Test	Present	Present
	b) Ferric Chloride Test	Present	Present
	c) Lead Acetate Test	Present	Present
7.	Detection of Phenolic Compounds		
	a) Iodine Test	Present	Present
	b) Ferric Chloride Test	Present	Present

8.	Detection of Tannins		
	a) Gelatin Test	Absent	Absent
	b) NaOH Test	Absent	Absent
9.	Detection of Saponins		
	Foam Test	Present	Present
10.	Detection of Phytosterols		
	a) Salkowski's Test	Present	Present
	b) Libermann Burchard Test	Present	Present
11.	Detection of Quinones		
	a) Alcoholic KOH Test	Present	Present
	b) Con.Hcl Test	Present	Present
	c) Con. Sulfuric Acid	Present	Present
12.	Detection of Fixed Oils and Fats		
	Stain Test	Present	Present

Determination of Acute Oral Toxicity (LD₅₀)

Acute oral toxicity of the methanolic extract of *Flueggea leucopyrus*(WILLD.) leaves and bark was evaluated according to OECD Guideline 423 (2001). The Leaves and Bark extract was administered orally separately up to dose of 2000 mg/kg body weight respectively. No mortality or treatment-related signs of toxicity were observed during the 14-days observation period. Therefore, upto 2000 mg/kg body weight, the methanolic extract of *Flueggea leucopyrus* (WILLD.) leaves and bark are relatively safe for oral administration respectively.

DISCUSSION

The present study was carried out to evaluate the preliminary phytochemical constituents and acute oral toxicity of the methanolic extract of *Flueggea leucopyrus*(WILLD.) leaves and bark. Phytochemical screening revealed the presence of alkaloids, carbohydrates, reducing sugars, glycosides, phenolic compounds, saponins, phytosterols, quinones, fixed oils and fats, and flavonoids, whereas proteins and tannins were absent in leaves and bark of *Flueggea leucopyrus*. The presence of these secondary metabolites indicates that the plant is a rich source of biologically active compounds that may contribute to its medicinal properties.

Phenolic compounds and flavonoids are recognized for their antioxidant activity, while saponins and phytosterols have been reported to possess various pharmacological properties. Alkaloids, glycosides, quinones, and other phytoconstituents are also known to exhibit diverse biological activities. Therefore, the phytochemical profile of the methanolic extract suggests that *Flueggea leucopyrus*(WILLD.) may possess significant therapeutic potential.

Acute oral toxicity of the methanolic extract was evaluated according to OECD Guideline 423 (Acute Oral Toxic Class Method). The extract did not produce any morbidity or mortality when administered orally upto the limit dose of 2000 mg/kg body weight during the observation period of 14-days. Therefore, MFL and MFLB are relatively safe for oral administration. These findings support the use of the extract in further pharmacological and toxicological investigations

CONCLUSION

The findings of the present study suggest that the methanolic extract of leaves and bark of *Flueggea leucopyrus* (WILLD.) possesses alkaloids, carbohydrates, reducing sugars, glycosides, phenolic compounds, saponins, phytosterols, quinones, fixed oils and fats, and flavonoids, while proteins and amino acids and tannins were absent in leaves and bark. In acute oral toxicity studies, the methanolic extract of *Flueggea leucopyrus* (WILLD.) of leaves and bark are safe up to 2000mg/Kg. Based on the above data leaves and bark of *Flueggea leucopyrus* possess therapeutic potential which should be further screened for its individual therapeutic potential respectively.

REFERENCES

1. Bulugahapitiya VP, Munasinghe AA. Phytochemical profile, proximate composition and antioxidant properties *Flueggea leucopyrus* (Willd), a plant used as complementary and alternative medicine by cancer patients in Sri Lanka *Vingnanam J Sci*. 2020;15(1):19-26.
2. Raj AA, Vinnarasi J, Venkataraman r. Phytochemical and Pharmacognostical analysis of *Flueggea leucopyrus* (willd) *World J Pharm Res*. 2016;5(8):1227-1236.
3. Solangaarachchi SM, Perera BM. Floristic Composition and medicinally important plants in the under storey of the tropical day mixed evergreen forest at the Hurulu reserve of Sri Lanka. *Journal of National Science Council of Sri Lanka*. 1993; 21(2):209-226.
4. Silva ID, Soysa P, Wijayabandara J. Cytotoxic activity of the aqueous extract of *Flueggea leucopyrus* (katupila) leaves against Hep-2 cells. 3rd International Conference on Medicinal Plants and Herbal Products, 2011; Sri Lanka. p. 106-107.
5. Gopal TK, Sheela T, Chamundeeswari D, Umamaheswara Reddy C. Investigation of in-vitro antioxidant, anti-inflammatory and anti-arthritis activity of aerial parts of *Securinega leucopyrus* (Willd.) Muell procedures, *Indian Journal of Research in Pharmacy and Biotechnology*. 2013; 1(3):371-378.
6. Vajira P. Bulugahapitiya, Amila Bandara Munasinghe, Menik Hettihewa. Investigation of chemical composition of *Flueggea leucopyrus* (Willd.) *World Journal of Pharmacy and Pharmaceutical World J Pharm Sci*. 2014;3(8):79-94.
7. Afreed Muhammed N, Ranjith D, Vinayakraj M, Rahman M, Sivan V, Sanis J. Physical characteristics, extractive yield and qualitative phytochemical analysis of *Flueggea leucopyrus* Willd leaves. *J. Med. Plants*. 2018;6:175-9.

8. Soysa P, De Silva IS, Wijayabandara J. Evaluation of antioxidant and antiproliferative activity of *Flueggea leucopyrus Willd* (katupila) BMC Complement Altern Med. 2014; 14: 274.
9. Ravi V, Thalavai PA, Renuga R, Sivapriya O, Vijayakanth P. Antifungal activity of fruit extracts of *Flueggea leucopyrus*: IOSR J Pharm Biol Sci. 2019;14(1 Ser II):1-7.
10. Kokate CK, Purohit AP, Gokhale SB. *Pharmacognosy*. 57th ed. Pune: Nirali Prakashan; 2021. For phytochemical screening/investigation: P. 12.1–12.15.

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