

## INVESTIGATION OF PHYTOCHEMICALS, NUTRIENTS AND MAJOR ELEMENTS IN THE LEAVES AND THE BARK OF *FLUEGGEA LEUCOPYRUS* (WILLD.)

Vajira P. Bulugahapitiya<sup>1</sup>, M.M.A.B. Munasinghe\*<sup>1</sup>, Menik L.  
Hettihewa<sup>2</sup>

<sup>1</sup> Department of Chemistry, Faculty of Science, University of Ruhuna, Matara, Sri Lanka. \*amilamunasinghe1988@gmail.com

<sup>2</sup> Department of Pharmacology, Faculty of Medicine, University of Ruhuna, Matara, Sri Lanka.

**Key words:** *Flueggea leucopyrus* (Willd.), phytochemicals, major elements, proximate analysis

### Introduction

Plant-derived substances have become of great interest owing to their versatile applications and they have been used as medicine since time immemorial. Approximately one-third of the top selling drugs in the world are derived from natural products and their derivatives. The plant kingdom is a treasure house of potential drugs and in the recent years there has been an increasing awareness about the importance of medicinal plants in discovering potential compounds and potential applications. The bioactive substances in the medicinal plants are mainly secondary metabolites known as phytochemicals and provide important benefits for the plants themselves, specially providing defenses mechanisms for the plants against diseases and other environmental factors. These phytochemicals belong to the classes of alkaloids, terpenoids, tannins, steroids, glycosides, flavonoids, carbohydrates and phenolic compounds etc. (Yadav

and Agarwala, 2011). Knowledge of the chemical constituents of plants is important, not only for the discovery of therapeutic agents, but also because such information be of value in disclosing new resources of such chemical substances.

*Flueggea leucopyrus* (Willd.) commonly known as Katupila in Sinhala belongs to family Euphorbiaceae and grows in India, Burma and Sri Lanka. Various medicinal properties are attributed to the plant such as for cleaning wounds, as disinfections, laxatives, for diarrhea, gonorrhoea, constipation and mental illness etc. In recent past, *F.leucopyrus*(Willd.) has been received much attention as a complimentary and supplementary medicine for cancer, especially in Sri Lanka. The decoction prepared from leaves of *F.leucopyrus*(Willd.) has been used by patients suffering from malignancy. Some preliminary investigation on its anti-cancer properties has been

reported, and the compound Bergenin has been isolated (Wijayabandara *et al.*, 2012). There is no report on the complete study on phytochemicals, proximate and major elemental analyses of the leaf and bark of *F.leucopyrus* (Willd.). Therefore the objective of this study was to carry out the complete study on phytochemicals, proximate and major elemental analysis of leaves and bark of *F.leucopyrus*(Willd.).

#### **Materials and methods**

**Collection of plant material:** The fresh leaves and the barks of *F. leucopyrus* (Willd.) were collected from Vitharandeniya area in Tangalle. The plant material was taxonomically identified and authenticated with the help from Botany Department, University of Ruhuna. The leaves and bark were washed with running water followed by distilled water and air dried, grounded into coarse powder.

**Preparation of plant extracts:** 500 g of powdered bark and 500 g of powdered leaves of *F.leucopyrus*(willd) were extracted separately in 2000 mL of methanol by maceration technique at room temperature. The mixture was filtered and crude extract was obtained after evaporation of methanol under reduced pressure.

**Qualitative phytochemical analyses:** The methanolic crude was used for the screening of phytochemicals. The standard chemical screening methods eg: for alkaloids, for unsaturated sterols & terpenes, glycosides, carbohydrates and portions, for saponins and phenolics, for terpenoids (Yadav and Agarwala, 2011) and for

flavonoids & tannin were carried out to identify the classes of phytochemicals present in leaves and bark.

#### **Quantitative Analysis of Phytochemicals**

Following procedure described in the literature were used for isolation of major classes of phytochemicals such as alkaloids, saponins, tannins and phenolic compounds.

**Quantification of alkaloids:** Powdered plant material was moistened with water and mixed with sodium carbonate to make a paste. The paste was dried and repowdered. Free alkaloids were extracted into dichloromethane by maceration and it was agitated with dilute sulfuric acid and aqueous layer was separated. The extracted aqueous layers were combined and alkalined with ammonia. Free alkaloids (partially insoluble precipitate) were re-extracted into organic layer and alkaloids were obtained by evaporating the organic solvent under reduced pressure.

**Quantification of Saponins:** Plant material was defatted with n-hexane and extracted with methanol. The methanolic extract was concentrated under vacuum and suspended in deionized water. It was partitioned with n-butanol and saponin was precipitated with diethyl ether. **Quantification of Tannins:** Plant material was macerated in methanol for three days after filtration it was concentrated under vacuum. The formed solid was suspended in 90 % methanol and partitioned with hexane. The aqueous extract was obtained evaporating the methanol and concentrated again and dissolved in distilled water. The aqueous extract was extracted with

chloroform and washed with 1 % sodium chloride solution to extract tannins present in chloroform layer. The aqueous fraction was evaporated to dryness under reduced pressure and crude tannins were obtained.

**Quantification of Phenolic compounds:** Plant material was macerated in 70 % methanol for three days and aqueous crude fraction was obtained by evaporating methanol. It was defatted with petroleum ether (60-80 °C). Phenolic compounds were separated using acid base separation method.

**Quantification of Flavonoids:** Methanolic crude extracts of leaves and bark of *F.leucopyrus*(Willd.) were used for the flavonoid extraction. The extracts were washed with n-Hexane to remove less polar organic molecules and the crude was dissolved in distilled water. The aqueous solution was partitioned with ethyl acetate and n-butanol to extract flavonoids.

#### **Proximate and major elemental analysis**

Proximate and major elemental analysis of leaves and bark of *F. leucopyrus* (Willd.) were carried out. Fresh, healthy leaves and bark of *F.leucopyrus* (willd.) were washed thoroughly with deionized water to remove extraneous substances. The dried plant material was grounded and passed through 500 µm size mesh. The homogenized plant samples were

placed in paper containers and dried in an oven at 80 °C for 12 hours to stabilize the tissue and to stop enzymatic reactions. Dried, homogenized plant samples were heated at 550 °C in a muffle furnace for 8 hours to destruct organic matters (Dry ashing). The dry ash (organic matter destructed plant samples) was used for the quantitative determination of ash content, sodium (Na), potassium (K) and phosphorus (P) contents. The moisture content was determined by heating the pre-weighed plant material in an oven at 105 °C (preheated) for 2 hours and reweighing it into the nearest 0.001 g. Ash content by dry ashing method, lipid content described by (Folch *et al.*, 1957), crude fiber content by method described by (Sarkiyayi *et al.*, 2013), crude proteins and Nitrogen by Kjeldahl method, Sodium and Potassium content by Flame emission spectrophotometry and Phosphorus by Molybdenum blue method were determined (Brian *et al.*, 1989).

#### **Results**

Phytochemical analysis of methanolic extracts of leaves and bark of *F.leucopyrus*(Willd.) reveals that it contains alkaloids, phenolics, saponins, flavonoids, terpenoids, tannins, glycosides, unsaturated sterols and triterpenes, proteins and carbohydrates. The results for quantitative phytochemical analysis for leaves of *F.leucopyrus*(Willd.) is given below (Table 1);

Table 1: Percentages of phytochemicals present in leaves and bark of *F.leucopyrus* (Willd.)

Phytochemicals	Percentage (%)	
	Leaves	Bark
Alkaloids	0.13	0.02
Saponins	0.74	0.19
Tannins	3.84	4.10
Phenolics	1.66	0.90
Flavonoids	1.32	0.76

The proximate and major elemental analysis results of *F.leucopyrus*(Willd.) leaves and bark is given in Table 2.

Table 2: The proximate and major elemental analysis of *F.leucopyrus*(Willd.) leaves and bark

Component	Content g/100 g	
	Leaves	Bark
Moisture	10.20±0.01	11.82±0.61
Ash	7.06±0.44	6.03±0.29
Lipid	4.91±0.86	1.64±0.09
Fibre	8.44±0.83	14.56±0.48
Protein	21.20±0.30	12.87±0.08
Carbohydrates	48.73±0.44	53.07±0.85
N	3.39±0.05	2.06±0.01
Na	0.37±0.24	0.23±0.00
K	0.36±0.01	0.27±0.01
P	0.01±0.00	0.01±0.00

[The results are expressed as mean ± standard deviation (n=3)]

### Discussion

According to the above analyses, the plant *F. leucopyrus* contains large number of important phytochemicals such as alkaloids, phenolics, saponins, flavonoids, terpenoids, tannins, glycosides, unsaturated sterols and triterpenes, proteins and carbohydrates. The amount of alkaloids, saponins, flavonoids, tannins and polyphenols present in *F.leucopyrus* (Willd.) is considerably higher in the leaves than in the bark.

The results of the proximate analysis shows that the both bark and leaves are rich in moisture, fibre and carbohydrates, protein, lipid and ash. Lipids are present in comparatively lesser quantity in both leaves and bark. The analysis of major elements shows that the leaves contain higher quantities of Nitrogen, Sodium and Potassium than the bark and presence of phosphorus is negligible in both leaves and bark.

It is known that the alkaloids in the plants can be attributed to the medicinal value of analgesic antispasmodic and antibacterial properties and plant phenolics responsible for biological properties such as anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities (Yadav and Agarwala, 2011). Saponins have antitumor and anti-mutagenic activities and can lower the risk of human cancers, by preventing growth of cancer cells. Natural antioxidants mainly originate from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc. Therefore, the medicinal value of *F. leucopyrus*(Willd) can be justified with present of large number of phytochemicals and nutrients in the plant.

### Conclusions

According to this investigation *F.leucopyrus* (Willd.) contains large number of phytochemicals, such as alkaloids, tepenoids, glycosides, phenols, saponins, flavonoids and tannins. Therefore use of the plant as remedies for many diseases have scientific base. Tannins and phenolics are the major phytochemicals present in leaves and bark. Leaves of comprises higher quantities of Nitrogen, Sodium and Potassium than the bark. Further investigations for purification of the each fraction in order to isolate pure compounds in is in progress.

**Acknowledgement:** Financial assistance given under the Research Grant of Transforming University of Ruhuna to International Level (Grant No. Ru/DVC/Pro-136) is acknowledged.

### References

- Brian S. Furniss Antony J. Hannaford Peter W. G. Smith Austin R. Tatchell, 1989. *Text book of Practical organic chemistry*. Fifth edit ed. Longman Scientific & Technical.
- Folch, J., Lees, M. and Stanley, G.H.S., 1957. A simple method for the isolation and purification of total lipids from animal tissues. *The Journal of biological chemistry*, 226, pp.497-509.
- Sarkiyayi, S., Mohammed, M. and Yakubu, A., 2013. Comparative Analysis of Nutritional and Anti Nutritional Contents of Some Varieties of Mango ( *Mangifera indica* ) in Kaduna Metropolis-Nigeria. *Research Journal of Applied Sciences, Engineering and Technology*, 5(4), pp.387-391.
- Wijayabandara M.D.J, Mendis B.M.M.J, Iqbal Choudhary , Soysa S.S.S.B.D, A.M., 2012. *Characterization of biological activity and the major active constituent of Flueggea leucopyrus Willd*
- Yadav, R.N.S. and Agarwala, M., 2011. Phytochemical analysis of some medicinal plants. *Journal of Phytology*, 3(12), pp.10-14.