

Phytochemical profile, proximate composition and anti-oxidant properties *Flueggea leucopyrus* (Willd), a plant used as complementary and alternative medicine by cancer patients in Sri Lanka

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Abstract—*Flueggea leucopyrus* Willd (Phyllanthaceae), locally known as “katupila”, a plant has been known with vast applications in traditional medicine, and recently it has been widely used in the treatment of cancers in complementary and alternative medicine in Sri Lanka. Though chemical investigation of this plant has been carried out in other countries, no detailed study on phytochemical, proximate and anti-oxidant properties reported in Sri Lanka on this plant. In this study, methanolic extract of leaves of *F.leucopyrus* was prepared using maceration and tested for phytochemicals and proximate composition using standard procedures described in the literature. Phytochemical screening revealed the presence of alkaloids, terpenoids, unsaturated sterols, glycosides, saponins, phenolics, flavonoids and tannins. Gravimetric methods were used to quantify alkaloids and saponins, and they were found as $0.13\pm 0.00\%$ g and $0.74\pm 0.01\%$ g respectively. Colorimetric methods were used to quantify tannins, total phenolics and total flavonoids contents and results obtained were 1.15 ± 0.01 Tannic Acid Equivalent, 38.49 ± 0.30 mg/g Gallic Acid Equivalent and 11.48 ± 0.27 mg/g Quercetin equivalent respectively. Proximate composition was found to be 10.20 g/100 g, 7.06 g/100 g, 1.50 g/100 g, 8.44 g/100 g, 21.20g/100g and 48.73 for moisture, ash, lipids, fiber, protein and carbohydrates contents respectively. Main elements in the leaves were found as N: 3.39 ± 0.05 , Na: 0.37 ± 0.24 , K: 0.36 ± 0.01 and P: 0.01 ± 0.00 in g/100g. Anti-oxidant capacity was evaluated using DPPH and FRAP assays and plant showed great anti-oxidant capacity giving IC_{50} value of 402.58 ± 3.97 μ g/mL in DPPH assay and 148.65 ± 11.91 μ g/mL of FRAP value. As conclusion, this study gives the broad picture on phytochemical, proximate and major elements content, and anti-oxidant capacity of leaves proving the highly medicinal value of the plant.

Keywords – *Flueggea leucopyrus*, Phytochemicals, proximate compositions, anti-oxidant, DPPH assay, FRAP assay

I. INTRODUCTION

Plant-derived substances have become of great interest owing to their versatile applications in human wellbeing. Use of herbal medicine for the treatment of diseases is as old as mankind. According to the World Health Organization the use traditional medicine has proven to be efficacious and safe (WHO, 2002). In order to promote herbal medicines among the people and to identify its potential, scientific studies of medicinal plants should be more intensified, especially those have been used as folk medicines and as traditional medicine (Ali *et al.*, 2001; Nair *et al.*, 2005). Specific secondary metabolites namely phytochemicals present in the plants are known to be responsible for various pharmacological activities exerted by plants themselves. These phytochemicals have potential to be developed as herbal medicines or could serve as the precursors for modern medicine or can be used as crude drugs. Plant anti-oxidants namely phenolics and flavonoids play vital role in the plant itself as protective agents at unhealthy situations improving the immunity functions, and very importantly it is known that anti-oxidants can involve in free radical defense mechanism in human cells, resulting the reduction of excessive oxidative stress generated in the human cells under unfavorable conditions. There is a known correlation between excessive oxidative stress and non-communicable diseases (NCD) including cancers in humans (Young-Joon Surh, 2003).

Flueggea leucopyrus (Willd) synonyms; *Securinega leucopyrus*, (Willd), known as “bush weed”, has been known as multi-functional medicinal herb, grown in Sri Lanka, Southern parts of Asia, Australia and Malaysia, and has a long history of using in the treatments of vast array of pathological conditions in the traditional system of medicine (Gopal, 2013). Recently the plant has been attracted interest as complementary and alternative medicine for cancer, especially in Sri Lanka (Sozya *et al.*, 2014). The leaves of *F.leucopyrus* (Willd.) has been used by patients suffering from malignancy for boils and for external ulcers (Soysa *et al.*, 2014). The adequate research has been conducted on various pharmacological activities including anti-cancer activities, anti-microbial activities and wound healing activities etc. by various researchers of other countries including India, and phytochemical profile and anti-oxidant properties has been reported in few studies (Anuka *et al.*, 2015; Silva *et al.*, 2011). Though *F.leucopyrus* is a very popular medicinal herb in Sri Lanka, its phytochemical compositions, proximate composition and anti-oxidant properties have not been well documented. Therefore, this study was aimed on investigation of phytochemicals, proximate composition and antioxidant properties of methanolic extract of leaves of locally grown *F.leucopyrus* with the aim of developing the leaves as nutraceuticals to be used in reduction of the risk of NCDs.

II. MATERIAL AND METHODS

A. Collection of plant material

The fresh leaves of *F.leucopyrus* (Willd.) were collected from Vitharandeniya, Tangalle in Sri Lanka. The plant material were taxonomically identified and authenticated by the support from the taxonomist of Department of Botany, University of Ruhuna, Sri Lanka. The healthy leaves were washed, air dried and then grinded into coarse powder to be used in the extraction and analysis.

B. Preparation of Plant Extracts

Five hundred grams of powdered leaves of *F.leucopyrus* were extracted in 2000 mL of methanol using maceration for three days at room temperature. The resultant mixture was filtered and then evaporated under reduced pressure to obtain methanolic crude. The crude extract was stored in airtight bottle at 4°C to be used in the analysis.

C. Screening for Phytochemicals

Screening tests for the phytochemicals such as alkaloids, unsaturated sterols and triterpenes, phenols, flavonoids, tannins, triterpenes, glycosides and saponins were carried out by following the standard procedure described in the literature (Harborne *et al.*, 1998, Evans, 1989, Pandith Javid Iqbal, 2010, Yadav, 2012, Prohp, 2012).

D. Quantification of Phytochemicals

Quantification of alkaloids, saponins, tannins, polyphenols and flavonoids were done using the methods described below.

D.1. Determination of quantity of Alkaloids by STAAS-OTTO method

Powdered plant material (500 g of leaves) was moistened and mixed with sodium carbonate to make a paste and allowed for drying. Free alkaloids were extracted into dichloromethane. The dichloromethane extract was acidified by adding dilute acid and the aqueous layer was separated and made it alkaline adding ammonia, free alkaloids liberated was re-extracted into dichloromethane. The solvent was evaporated to obtain solid alkaloids (Kokate *et al.*, 2008).

D.2. Determination of quantity of Saponins

Crude methanolic extracts prepared using 400 g of dried leaves was defatted using petroleum ether and it was suspended in deionized water. The water suspension was extracted with n-butanol and saponin was precipitated by adding diethyl ether to the n-butanol fractions (Latif *et al.*, 2006).

D.3. Determination of Tannins

Tannin was extracted first and then quantified using colorimetric method (Morrison *et al.*, 1995). Extraction of Tannins: The methanolic extract of plant material (1.00 g) was

extracted into the mixture of 10 mL of petroleum ether (40-60°C) and 10 mL of acetone: water (7:3 v/v) at 30°C for 30 minutes. The solution was centrifuged and the supernatant was collected and filtered. After evaporation to dryness the crude solid was dissolved in 25.00 mL of methanol.

Quantification of Total tannin: Folin- Ciocalteu colorimetric method was used. Folin-Ciocalteu (10 % v/v) 500 µL, Na₂CO₃ (7.5 % v/w) (1 mL) and distilled water (7.50 mL) were mixed and the absorbance was measured at 725 nm after incubation of 30 minutes. Same procedure was repeated for the standard solutions (tannic acid) and plant extract at different concentrations. The total tannin content of leaves was determined using the calibration curve, $y = 0.0085x + 0.004$ (R²= 0.9992) as TAE.

D.4. Determination of Total Phenolic Content

Folin- Ciocalteu colorimetric method was used (Nurhanan *et al.*, 2012). A solution of Folin-Ciocalteu (10 % v/v) and Na₂CO₃ (7.5 % v/w,) the standard series of gallic acid and the stock solutions of crude methanolic extracts (100 µg/mL) in methanol were prepared. Folin-Ciocalteu reagent (2.5 mL) was added into the tests tube containing plant extract in methanol and gallic acid separately, mixed them and, 2.5 mL of 7.5 % Na₂CO₃ reagent was added to each test tube slowly followed by incubation of 2 hours. The absorbance was measured at 765 nm. The total phenolic content of each extract was determined according to the calibration curve, $y = 0.0135x + 0.029$ (R²= 0.9979) in GAE.

D.5. Determination of Total Flavonoid content

Aluminum chloride colorimetric method was used (Karuppusamy *et al.*, 2011). A solution series of quercetin and stock solutions of leaves extracts (100 µg/mL) in methanol, and Aluminum chloride solution (10 % distilled water) were prepared. The mixture contained 0.1 mL of 10 % aluminum chloride, 4.4mL of distilled water and 0.5 ml of plant extracts were added and mixed well. After incubation of 30 minutes, the absorbance was measured at 415 nm. The total flavonoid content of each extract was calculated according to the calibration curve $y = 0.0087x + 0.0015$ (R²= 0.9995) in QE.

E. Proximate analysis

Cleaned, air dried and powdered leaves was used for the testing of proximate composition (moisture, ash, lipid, protein, fiber) and for the major elemental analysis.

E.1. Determination of moisture content

Thermal drying method was used for the determination of moisture content ([AOAC, 1990). One hundred grams of powdered plant sample placed in crucible and in a pre-heated oven at 105°C for 3 hours. The sample was allowed to cool in a desiccator and then reweighed. The percentage moisture content was calculated by expressing the loss in weight on drying as below;

$$\text{Moisture content (\%)} = \frac{\text{Initial weight} - \text{Final weight (g)}}{\text{Initial weight (g)}} \times 100$$

E.2. Determination of ash content

A dried plant sample (0.300 g) was placed in crucible and placed it in a muffle furnace. The temperature of ashing was adjusted to 550 °C and maintained for 8 hours. White ash obtained was reweighed after cooling in a dissector (AOAC, 1990). The procedure was repeated until constant weight was obtained. The ash content was calculated as below;

$$\text{Ash content (\%)} = \frac{\text{Weight of ash (g)}}{\text{Sample weight (g)}} \times 100$$

E.3. Determination of lipids content

The quantity of 15.00 g of dried leaves was subjected to extraction using Soxhlet method with petroleum ether (40 - 60°C) and in the presence of anti-bumping granule (W1). Extraction was carried out for 4 hours and after evaporation of solvent under *vacuum*, the content in the flask was dried in an oven at 60 °C. After cooling the flask was reweighed (W2) (Nwinuka, 2005 *et al.*). The content of lipid was calculated as below;

$$\text{Percentage of lipids (\%)} = \frac{(W2 - W1) \text{ (g)}}{\text{Weight of the plant sample (g)}} \times 100$$

E.4. Determination of fiber content

A sample of 1.0 g of finely ground plant material was weighed into a 50 mL beaker and homogenized with petroleum ether for one hour. After adding 100 mL of 1.25 % H₂SO₄, the mixture was boiled under reflux for 30 minutes. After filtration and washing the residue with water, 100 mL of 1.25 % NaOH solution was added to the residue and the mixture was boiled under reflux for 30 minutes. After filtration of hot solution and washing the crude using water it was dried in an oven at 105 °C, cooled in a desiccator and weighed (M1). The weighed sample was incinerated in a muffle furnace at 300 °C for 30 minutes. It was cooled in a desiccator and reweighed (M2). Crude fibre content was calculated as below (Sarkiyayi *et al.*, 2013);

$$\text{Percentage of crude fibre(\%)} = \frac{(M1-M2)(g)}{\text{Sample weight (g)}} \times 100$$

E.5. Determination of protein content

A sample of 0.300 g of powdered leaves was digested adding conc. H₂SO₄ in digestion block of Kjeldahl setup (150-400°C). After completion of the digestion, the mixture was subjected to distillation by adding 50 mL of distilled water and 50 mL of 35 % NaOH. The distillate was collected into a 250 mL conical flask containing standard H₃BO₃ and it was titrated with standard 0.10 M HCl in the presence of methyl red indicator. The total nitrogen content and protein content was calculated as follows (Sarkiyayi *et al.*, 2013);

$$\text{Percentage of Nitrogen (\%)} = \frac{V \times C \times 1.401}{\text{Sample weight}}$$

Where; V- Volume of HCl; C- Concentration of HCl; 1.401 - milliequivalent weight of nitrogen

$$\text{Protein (\%)} = \text{Percentage of Nitrogen (\%)} \times 6.25$$

Where; 6.25 is the conversion factor

E.6. Determination of Carbohydrate content

The total carbohydrate content was determined by subtracting the sum of the percentage moisture, ash, crude protein and crude fibre from 100.

$$\text{Carbohydrates (\%)} = 100 - [\% \text{ Moisture} + \% \text{ Ash} + \% \text{ Fat} + \% \text{ Protein} + \% \text{ Fibre}]$$

F. Mineral analysis

Air dried plant samples prepared above was dried further in an oven at 80°C for 12 hours. About 0.2-1.0 g of prepared powdered plant material was placed in a porcelain crucible and it was placed in a muffle furnace and the temperature was raised up to 550°C and kept for 8 hours (Dry ashing). The clean, white colour ash obtained above was dissolved in 10-15 ml of conc.HCl and made the volume up to 25.00 ml with deionized water. These samples were used for the quantitative determination of sodium (Na), potassium (K) and phosphorus (P) (Gefry *et al.*, 1989).

F.1. Determination of Na and K

Standard stock solutions of NaCl and KCl (1.00 g/l) were prepared in deionized water and used for the measurements for calibration curves. The measurements were performed using Jenway Flame Photometer. The flame filter was selected as Na (589 nm). The calibration curve was plotted and Na content of plant material was calculated according to the calibration curve ($y = 0.0002x + 0.0044$, $R_2 = 0.9477$). Same procedure was followed to determine the K content using flame filter of 766 nm [calibration curve $y = 0.0119x + 0.0689$, $R_2 = 0.9889$].

F.2. Determination of P

Molybdenum blue method was used. The standard stock solution (50.00 mg/l) of PO₄³⁻ was prepared dissolving KH₂PO₄ in deionized water. Molybdenum blue solution was prepared by dissolving 4.800 g of ammonium Molybdate [(NH₄)₂Mo₇O₂₄.4H₂O] in 1.0 ml of 5 M H₂SO₄, and Hydrazinium sulfate solution was prepared by dissolving 4.800 g of hydraziniumsulphate in 1000.00 ml of deionized water. Previously prepared plant ash of 0.30 g was dissolved in 10 ml of conc.HCl and diluted to 25.00 ml. This solution was transferred into a 50.00 ml volumetric flask and molybdate solution (5 ml) and hydrazinium sulfate solution (2 ml) were added into it and diluted with water. After incubation the absorbance was measured at 830 nm. Using a calibration curve, P of the sample was determined (Jeffry *et al.*, 1989).

*G. Determination of anti-oxidant properties**G.1. DPPH free radical scavenging assay*

The free radical scavenging activity was determined according to the method described by Goyal *et al.* (2012) with slight modifications. Briefly, 3900 µL of freshly prepared 0.004 % DPPH solution was added into a test tube containing 100 µL of various concentrations (4-750 µg/mL) of plant extract in

methanol. Methanol was used as the control and ascorbic acid was used as the standard. The solutions were incubated in dark at room temperature for 30 minutes and the absorbance was measured at 517 nm. Percentage Inhibitions were calculated according to the absorbance values;

$$\text{Percentage Inhibition} = \left(A_0 - \frac{A}{A_0} \right) \times 100 \%$$

Where A_0 is absorbance of the control and A is absorbance of the sample. The IC_{50} values were calculated for each extract by using statistical software SPSS.

G.2. Ferric Reducing Power Antioxidant assay

Working FRAP reagent was prepared by mixing acetate buffer (300 mM, pH 3.6), 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) in 40 mM HCl and 20 mM $FeCl_3 \cdot 6H_2O$ solution in 10:1:1 ratio. *Assay:* Leaves extract of 100 $\mu\text{g/mL}$ (in methanol) was mixed with 3.0 mL of working FRAP reagent, incubated at 37 $^{\circ}\text{C}$ and absorbance was measured at 593 nm. The assay involved $FeSO_4 \cdot 7H_2O$ as the standard and FRAP value was determined using calibration curve (Shahwar *et al.*, 2012).

H. Statistical Analysis

Calculated mean values are expressed as (Mean \pm standard deviation). Standard deviation values were calculated using Excel 2010. Differences exist among the means were determined by ANOVA and pairwise multiple comparisons test; Duncan test were used.

III. RESULTS AND DISCUSSION

A. Qualitative and quantitative analysis of phytochemicals

The results of phytochemical screening showed that the methanolic extract of leaves of *F.leucopyrus* are rich with important specific secondary metabolites such as alkaloids, flavonoids, tannins, glycosides, terpenoids, steroids, phenolics and saponins. As *F.leucopyrus* has been known as multi-functional medicinal herb and has being used in traditional system of medicine for treating various pathological conditions including cough, hay asthma, bowel complaints, disinfections, laxatives, diarrhoea, gonorrhoea, constipation, mental illness, cancers, kidney stones and urinary troubles etc. (Soyza *et al.*, 2014; Ghodela *et al.*, 2013), such pharmacological activities exerted by leaves can be attributed to those specific classes of secondary metabolites present in.

It showed that quantities of alkaloids and saponins were 0.13 ± 0.00 % g and 0.74 ± 0.01 % g respectively (Table -1). Alkaloids have been known to contribute to pharmacological activities such as, analgesic antispasmodic, antibacterial properties antihypertensive effect, antiarrhythmic effect, antimalarial activity and anticancer actions (Yadav *et al.*, 2011; Roberts, 1998) etc. According to Prohp and Onoagbe (2012) saponins are known to show antitumor and anti-mutagenic activities and can lower the risk of human cancers via preventing growth of cancer cells. The non-sugar part of saponins has a direct antioxidant activity which may result in reduction of risk of cancer and heart diseases in humans.

Phytochemical class	Quantity
Alkaloids	0.13 ± 0.00 % g
Saponins	0.74 ± 0.01 % g
Tannins	1.15 ± 0.01 TAE
Phenolics	38.49 ± 0.30 mg/g GAE
Flavonoids	11.48 ± 0.27 mg/g QE

Data were expressed in mean values + SD in three replicate

Table 1: Quantification of phytochemicals present in leaves.

The quantities of tannins in Tannic Acid Equivalent (TAE) are given in the table -1, which was taken using the standard graph drawn with the equation of $y = 0.0085x + 0.004$, $R^2 = 0.9992$ for tannic acid standard (Figure -1) where y is absorbance at 725 nm. The tannin content was found to be 1.15 ± 0.01 TAE. Although tannins are considered as anti-nutrient, some tannins have shown medicinal properties such as anti-carcinogenic activity, reduction of mutagenic activity of number of mutagens, anti-microbial activity etc.

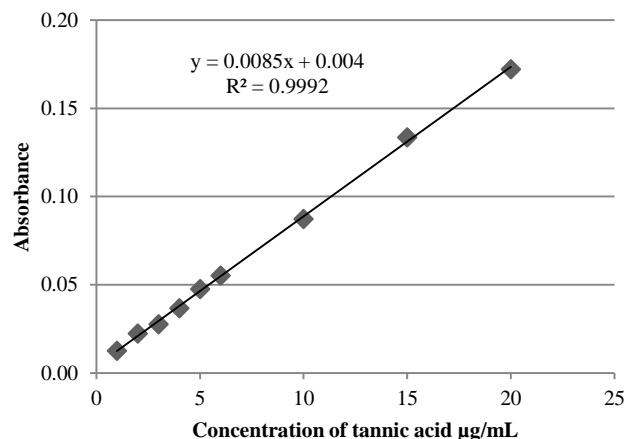


Figure 1: Standard curve of Tannic acid.

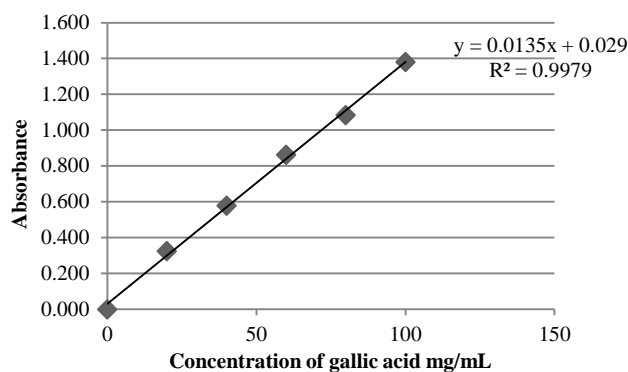


Figure 2: Standard curve of Gallic acid

The total phenolic content of the leaves was obtained using a calibration curve drawn for gallic acid (Figure-2) in which y as absorbance at 765 nm and x as concentration of gallic acid with the equation of $y = 0.0135x + 0.029$, $R^2 = 0.9979$. Total phenolic content of the leaves was found to be 38.49 ± 0.30 mg/g Gallic Acid Equivalent (GAE), which is relatively higher amount. As phenolics are best known as powerful natural anti-oxidants, many pharmacological activities exerted by leaves can be attributed to the higher content of phenolics compounds in the leaves. In previous study, Preethi Zoyza *et al* (2014) has found phenolic content of aqueous extract of leave as 22.15 w/w GAE. This less value can be rationalized as methanol is far better solvent for phenolics than water.

Using the standard curve drawn (Figure-3) where y as absorbance at 415 nm and x as concentration of Quercetin and with the equation of $y = 0.0087x - 0.0015$, $R^2 = 0.9995$, the total flavonoids content of the leaves was noted as 11.48 ± 0.27 mg/g in Quercetin Equivalent (QE) (Table1) which is significantly higher amount. Flavonoids are considered as primary antioxidants or free radical scavengers and shown the ability of acting against many non-communicable diseases including cancers (Vijay *et al.*, 2011; Hossain *et al.*, 2011).

B. Proximate analysis

As shown the results of proximate analysis in the table 2, the moisture content of leaves is about 10% which gives the possibility of leaves to formulate into different products as low moisture content is less favored for microbial growth and stability.

Ash value of any organic material represents its non-volatile inorganic components. In this study, the ash content of the leaves of *F.leucopyrus* showed as 7 % from dry weight, giving indication that leaves is rich enough with mineral. As lipid content is not higher (about 1 %), and protein content is relatively high (21.20 ± 0.30 g/100g) and carbohydrates content is 48.73 ± 0.44 g/100g, the leaves would be better for transforming into functional foods or nutraceuticals to be used in reducing the risk of NCDs, as easily available products. The elements such as nitrogen, sodium and potassium contents are 3.39 ± 0.05 , 0.37 ± 0.24 and 0.36 ± 0.01

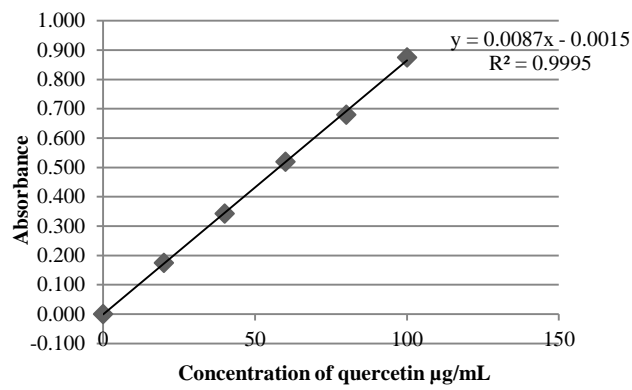


Figure 3: Standard curve of Quercetin

respectively and phosphorus content is very low (Table -2). Minerals are important for human health but knowing the amounts of minerals are important.

Proximate	Content g/100g
Moisture	10.20±0.01
Ash	7.06±0.44
Lipid	1.50±0.24
Fibre	8.44±0.83
Protein	21.20±0.30
Carbohydrates	48.73±0.44
Nitrogen	3.39±0.05
Sodium	0.37±0.24
Potassium	0.36±0.01
Phosphorous	0.01±0.00

Data were expressed in mean values + SD in three replicate

Table 2: Proximate composition of leaves

C. Anti-oxidant assays

C.1. DPPH free radical scavenging assay

This assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants. This is a direct and reliable method for determining radical scavenging action of plant extracts. Original DPPH solution is purple colour and it changed to yellow when plant secondary metabolites reduced it by donating electrons as hydrogen radical. The figure -4 showed the variation of percentage inhibition of DPPH free radical with the concentration of crude extracts of *F.leucopyrus* and ascorbic acid. According to that it exhibits concentration dependent DPPH free radical scavenging activity.

IC₅₀ is the concentration of plant extract where the response is reduced by half. The IC₅₀ values were calculated for the extract and the standard by probit analysis using SPSS. Accordingly, plant extract and ascorbic acid gave the IC₅₀ values of 402.58 ± 3.97 µg/mL and 92.66 ± 1.44 µg/mL

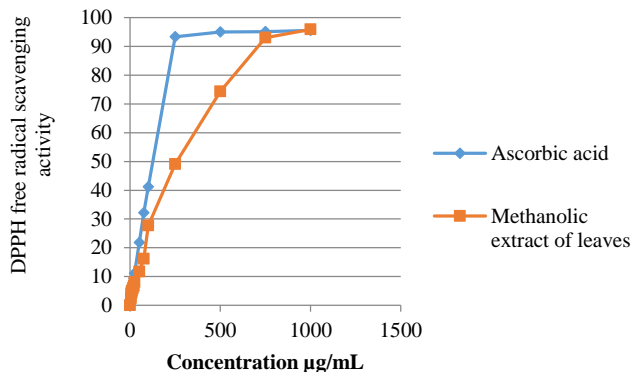


Figure 4: Concentration depend inhibition of DPPH

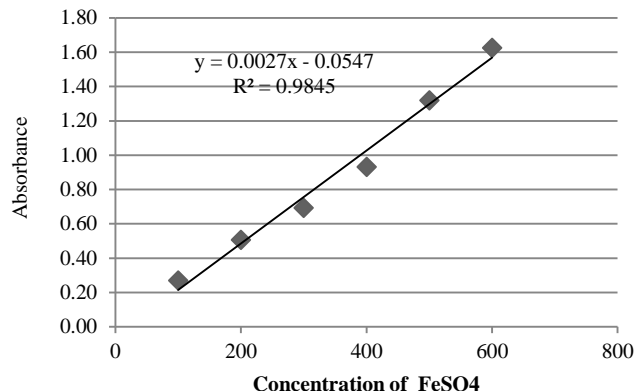


Figure 5: Standard curve of FeSO4

respectively, showing that moderate free radical scavenging activity of *F.leucopyrus*. Similar scavenging results were reported by Wanigasekera *et al.*, (2019) which was $353 \pm 15 \mu\text{g/mL}$ in contrast to that low value has been reported by Sozya *et al.*, (2014) which was $11.16 \pm 0.37 \mu\text{g/mL}$ for the same.

C.2. FRAP reducing assay

The FRAP assay treats the antioxidants contained in the samples as the reductants in a redox-linked colorimetric reaction and the value reflects the reducing power of the antioxidants (in which antioxidant reacts with Fe^{3+} TPTZ and produce a coloured Fe^{2+} TPTZ complex (Selvakumar *et al.*, (2011). The formed Fe^{2+} TPTZ complex was measured using standard curve prepared with $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ as given in Figure -5 in which y as the absorbance measured at 593 nm and x as the concentration of leave $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ with the equation of $y = 0.0027x - 0.0547$, $R^2 = 0.9845$.

The leaves showed FRAP value of $148.65 \pm 11.91 \mu\text{g/mL}$ whereas ascorbic acid, the standard showed as $364.70 \pm 2.14 \mu\text{g/mL}$. The results of FRAP assay further supports the moderate anti-oxidant capacity of the leaves. The concentration dependent reducing power of ferric tripyridyltriazine (Fe(III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe(II)-TPTZ) of leaves extracts of *F. leucopyrus* given in figure -6.

IV. CONCLUSION

The study revealed that leaves of *F.leucopyrus* is rich with important specific secondary metabolites and contains favorable proximate composition and major elements along with higher amount of total phenolics and flavonoids content with the moderate anti-oxidant capacity. The results support to validate the use of *F.leucopyrus* as multi-functional medicinal herb in traditional system of medicine and to prepare ready to use functional products and nutraceutical using leaves.

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Conflict of Interest: Authors declares no conflict of interest

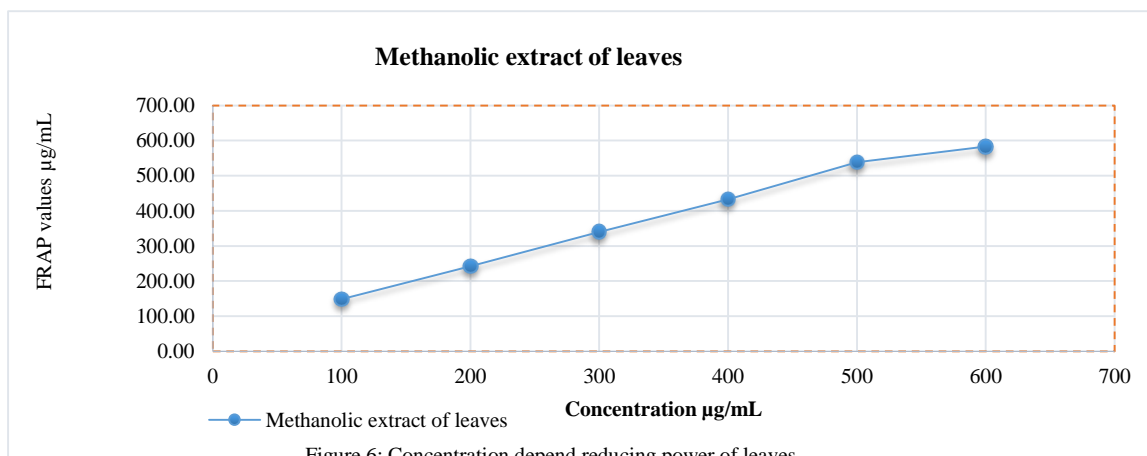


Figure 6: Concentration depend reducing power of leaves.

VI. REFERENCES

- Ali N, Julich W, Kusnick C, Lindequist U (2001). Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. *J. Ethnopharmacol* 74(2), 173–9.
- Anuka S, Mendis, Ira T, Sameera R, Samarakoon S, Kamani H.T (2015). Modulation of expression of heat shock proteins and apoptosis by *Flueggea leucopyrus* (Willd) decoction in three breast cancer phenotypes. *BMC Complementary and Alternative Medicine* 15, 404.
- AOAC: Official Methods of Analysis (1990). Association of Official Analytical Chemists, Inc. (Volume 1). 15th ed.
- Evans W.C. (1989). *Pharmacognosy*. 357.
- Ghodela Naresh Kumar, Vijay Kumar, Dudhamal Tukaram Sambhaji and Mukesh Kumar Naria (2016). A wonderful medicinal plant: *securinega leucopyrus* (willd) muell- a brief review. *International Journal of Science Inventions Today* 5, 472-484.
- Gopal T.K. (2013). Investigation of In-Vitro Anti-Oxidant, Anti-Inflammatory and Anti-Arthritic Activity of Aerial Parts of *Securinega leucopyrus* (Willd.) Muell, *Indian Journal of Research in Pharmacy and Biotechnology* 1, 371-378.
- Goyal, A.K., Middha, S.K. Sen, A (2012). Evaluation of the DPPH radical scavenging activity , total phenols and antioxidant activities in Indian wild *Bambusa vulgaris* “Vittata” methanolic leaf extract. *Journal of Natural Pharmaceuticals* 1, 42–45.
- Hossain, M.A., Shah, M.D., Gnanaraj, C. and Iqbal, M (2011). In vitro total phenolics , flavonoids contents and antioxidant activity of essential oil , various organic extracts from the leaves of tropical medicinal plant *Tetragonia* from Sabah. *Asian Pacific Journal of Tropical Medicine* 717–721.
- Jeffrey, G., Bassett, J., Mendham, J. and Denney, R (1989). *Vogel's Text book of Quantitative chemical analysis*.
- Harborne, A. (1998). *Phytochemical methods a guide to modern techniques of plant analysis*. Springer Science & Business Media, Springer Netherlands.
- Karuppusamy, S., Rajasekaran, K.. and Muthuraja, G (2011). Antioxidant activity of selected lesser known edible fruits from Western Ghats of India. *Indian Journal of Natural Products and Resources* 2(2), 174–178.
- Kokate CK, Purohit AP, Gokhale SB. (2008). *Pharmacognocny*.
- Latif S.D.S.Z, Gray A.I (2006). *Natural Products Isolation*. 2nd ed., The Humana Press Inc.
- Morrison I, Asiedu E, Stuchbury T, Powell A (1995). Determination of Lignin and Tannin Contents of Cowpea Seed Coats. *Annals of Botany* 76, 287–290.
- Nair R, Kalariya T, Chanda S (2005). Antibacterial Activity of Some Selected Indian Medicinal Flora. *Turkish Journal of Biology* 29, 41–7.
- Nurhanan A.R, Wan Rosli W (2012). Evaluation of Polyphenol Content and Antioxidant Activities of Some Selected Organic and Aqueous Extracts of Cornsilk (*Zea Mays Hairs*). *Journal of Medical and Bioengineering* 1, 48–51.
- Nwinuka N, Ibeh G, Ekeke G (2005). Proximate Composition And Levels of Some Toxicants In Four Commonly Consumed Spices. *J. Appl. Sci. Environ. Management* 9, 150–155
- Pandith Javid Iqbal. (2012). Phytochemical screening of certain plant species in Agra city. *Journal Drug Delivery Ther* 2, 135–138.
- Prohp T.P, Onoagbe I.O (2012). Determination of phytochemical composition of the stem bark of *Triplochiton scleroxylon* K . Schum.(*Struculiaceae*). *International Journal of Applied Biological Pharmacological Technology* 3, 68–75.
- Raman N, *Phytochemical Techniques* (2006). New India publishing agency, New Dilhi. 19-24.
- Roberts, MF. and Wink, M. eds. (1998). *Alkaloids Biochemistry, Ecology and Medicinal Applications*. In: *Alkaloids*. Plenum Press.
- Sarkiyayi S, Mohammed M, 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 Science Engineering Technology* 5, 387–391.
- Selvakumar K. Madhan R., Srinivasan G. and Baskar (2011). Antioxidant Assays in Pharmacological Research. *Asian Journal of Pharmacy and Technology* 1, 99-103.
- Shahwar, D., Raza, M.A., Bukhari, S. and Bukhari, G (2012). Ferric reducing antioxidant power of essential oils extracted from *Eucalyptus* and *Curcuma* species. *Asian Pacific Journal of Tropical Biomedicine journal* 2(3), 1633–1636.
- Silva I.D, Soysa P, Wijayabandara J. (2011). Cytotoxic activity of the aqueous extract of *Flueggea leucopyrus* (katupila)leaves against Hep-2 cells. 3rd International Conference on Medicinal Plants and Herbal Products.

Soysa, P., De Silva, I.S. and Wijayabandara, J (2014). Evaluation of antioxidant and antiproliferative activity of *Flueggea leucopyrus* Willd (katupila). *BMC complementary and alternative medicine* 14, 274.

Yadav R.N.S.2011. Agarwala M. Phytochemical analysis of some medicinal plants. *J. Phytology* 3, 10–4.

Young-Joon Surth, (2003).Cancer chemoprevention with dietary phytochemicals, *Nature Reviews Cancer*. 3, 768–780.

Vijay, T., Rajan, M.S.D., Sarumathy, K. and Sudha (2011). A.In vitro Cytotoxicity of *Grewia umbellifera*. *International Journal of Pharmacy & Life sciences*. 2,1293–1298

Wanigasekera, W.M.A, Joganathan, PA, Pethiyagoda, R Yatiwella, L.N and Attanayake, H.M.D.A.B (2019). Comparison of antioxidant activity, Phenolic and Flavonoid contents of selected medicinal plants in Sri Lanka. *Ceylon Journal of Science* 48, 155-162.

Wijayabandara M.D.J, Mendis B.M.M.J, Iqbal Choudhary , Soysa S.S.B.D (2012). Characterization of biological activity and the major active constituent of *Flueggea leucopyrus* Willd. 13th Congress of the International Society for Ethnopharmacology.

WHO Policy Perspective on medicine (2002); Traditional Medicine; Growing need and Potential: No 02, WHO, Geneva 2002.