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Physical characteristics, extractive yield and qualitative phytochemical analysis of *Flueggea leucopyrus* Willd leaves

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Abstract
In recent days, there is renewed interest towards the drugs of natural origin due to their nontoxic nature Therapeutic efficacy of medicinal plants depends upon the quality and quantity of chemical constituents. The misuse of herbal medicine or natural products starts with wrong identification. The most common error is the vernacular name given to two or more entirely different species. The problems can be solved by pharmacognostic identification of medicinal plants, which is essential to lay down standard specifications of medicinal plants. The present investigation attempts to study different facets of identification of *Flueggea leucopyrus* leaves including pharmacognostic parameters, physical parameters, Extractive yield and qualitative phytochemical analysis. The results suggests that all the parameters are were as per the prescribed guidelines, the extractive yield was highest with acetic acid and lowest with benzene and qualitative phytochemical analysis revealed the presence of maximum phytoconstituents in methanolic extract.

Keywords: Herbal medicine, pharmacognostic identification, flueggea leucopyrus leaves, physical parameters, extractive value, qualitative phytochemical analysis

Introduction
Medicinal plants being the genesis of most of the lifesaving drugs and are used in great extent for the treatment of multifarious infections traditionally for several years. According to the World Health Organization the use of traditional medicine has proven very effective and safe and estimated that about 85 – 90% of the world's population consumes traditional herbal medicines. Developing countries like Tanzania (60%), Rwanda (70%), India (70%), and Benin (80%) and developed countries Belgium (31%), USA (42%), France (49%) and Canada (70%) use herbal medicine for their primary health needs. *Flueggea leucopyrus* Willd. (Family: *Phyllanthaceae*) commonly known as 'bushweed'. Habitats in many parts of Sri Lanka, South eastern Queensland and southern parts of India. An armed large shrub with leaf characteristics like obtuse, alternate, cuneate, entire, elliptical and simple, fruits are of capsule like, white when ripe, fruiting season is from April- November and flowering season from February-May [1, 2]. The plant has multifaceted Ethnomedical significance, the leaves has been used in the treatment of boils, cancer, ulcers of skin and sores and various preparations in traditional medicine used for the treatment of bowel infection, in treatment of cough, hay asthma and used as antioxidant, immunomodulators, disinfectant and laxatives, further it has been claimed as therapeutic agent for mental illness and urolithiasis [3, 4] respectively. Aerial parts of the plants especially leaves are commonly used as an alternatives to commonly used antibiotics to destroy maggots in sores to treat myiasis and promote healing of wound and also in otitis media [5, 6]. Several lab studies reported the presence of major active constituents from *Flueggea leucopyrus* leaves is Bergenin and it has shown predominant antioxidant and immunomodulatory activity *in vitro* [7]. In Srilanka, it has been a common practice to use decoction of *F. leucopyrus* among cancer patients as dietary substituent in addition to anticancer treatments [8, 9]. Further, the paste prepared by mixing leaves of *F. leucopyrus* with tobacco leaves has been used to destroy worms in sores and also used as fish poison. The leaves were boiled and taken orally twice a day for stomachache. Recently the plant has been attracted interest as complementary and alternative medicine for cancer, especially in Sri Lanka. The decoction prepared from leaves of *F. leucopyrus* (Willd.) has been used by patients

suffering from malignancy [10, 3]. The present investigation was carried out to understand the pharmacognostic characteristics of *Flueggea leucopyrus* leaves.

Materials and methods

Collection of plant materials

The fresh leaves of *Flueggea leucopyrus* (Willd) were collected from Wayanad regions of Kerala. The plant materials are taxonomically identified and authenticated with the support from M S Swaminathan research foundation, Puthoorvayal, Wayanad, Kerala. The healthy leaves were washed with running water and then with distilled water, air dried and then ground in to coarse powder and stored in refrigerator till use.

Determination of swelling index

Introduce the specified quantity (1 gm) of the *F. leucopyrus* leaf powder, previously reduced to the required fineness and accurately weighed, into a 25-ml glass-stoppered measuring cylinder. The internal diameter of the cylinder should be about 16 mm, the length of the graduated portion about 125 mm, marked in 0.2 ml divisions from 0 to 25 ml in an upwards direction. Add 25 ml of water and shake the mixture thoroughly every 10 minutes for 1 hour. Allow to stand for 6 hours at room temperature. Measure the volume in ml occupied by the herbal material, including any sticky mucilage. Calculate the mean value of the individual determinations, related to 1 g of herbal material [11, 12].

Determination of foaming index

Reduce about 1 g of the herbal material to a coarse powder, weigh accurately and transfer to a 500-ml conical flask containing 100 ml of boiling water. Maintain at moderate boiling for 30 minutes. Cool and filter into a 100-ml volumetric flask and add sufficient water through the filter to dilute to volume. Pour the decoction into test-tubes and adjust the volume of the liquid with water to 10 ml. Stopper the tubes and shake them in a lengthwise motion for 15 seconds, two shakes per second. Allow to stand for 15 minutes and measure the height of the foam. The results are assessed as follows: If the height of the foam in tube is less than 1 cm, the foaming index is less than 100. If the height of the foam is more than 1 cm, the foaming index is over 1000. In this case repeat the determination using a new series of dilutions of the decoction in order to obtain a result [11].

Calculate the foaming index using the following formula:

$$\frac{1000}{a}$$

Where,

a = the volume in ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1 cm is observed.

Water solubility index (WSI) and Water absorption index (WAI)

Take 2.5 g of plant powder in a 50-mL centrifuge tube and add 30 mL of distilled water to it at 30 °C and stir intermittently for 30 min. Then centrifuge for 10 min at 5100 × g. Pour the supernatant carefully into a Petri dish and then allow both supernatant and pellet to dry overnight [13].

WSI = Amount of the solid in the dried supernatant / Weight of plant powder

WAI = Weight of dry solid / Weight of plant powder

Determination of Physical Characteristics of *Flueggea leucopyrus* leaves

Bulk density

It is the ratio of given mass of powder and its bulk volume. It is determined by transferring an accurately weighed amount of powder sample to the graduated cylinder with the aid of a funnel. The initial volume was noted. The ratio of weight of the volume it occupied was calculated [13]

Bulk density=W/V_0 g/ml

Where, W = mass of the powder, V_0 = untapped volume

Tapped density

It is measured by transferring a known quantity (2g) of powder into a graduated cylinder and tapping it for a specific number of times. The initial volume was noted. The graduated cylinder was tapped continuously for a period of 10-15 min. The density can be determined as the ratio of mass of the powder to the tapped volume [13]

Tapped volume= W/V_f g/ml

Where, W = mass of the powder, V_f = tapped volume.

Compressibility index

It is the propensity of the powder to be compressed. Based on the apparent bulk density and tapped density the percentage compressibility of the powder can be determined using the following formula [13].

Compressibility index = [(v0-vf)/v0] x 100,

Or

% Compressibility = [(tapped density – bulk density)] / tapped density] x 100

Hausner ratio

It indicates the flow properties of the powder. The ratio of tapped density to the bulk density of the powder is called Hausner ratio [13]

Hausner ratio= Tapped density/bulk density

Angle of repose

The internal angle between the surface of the pile of powder and the horizontal surface is known as the angle of repose. The powder is passed through funnel fixed to a burette at s height of 4 cm. A graph paper is placed below the funnel on the table. The height and the radius of the pile were measured [13].

Angle of repose of the powder was calculated using the formula

Angle of repose= tan-1(h/r)

Where, h=height of the pile, r = radius of the pile

Determination of pH range

The powder sample of *Flueggea leucopyrus* was weighed to about 5g and immersed in 100 ml of water in a beaker. The beaker was closed with aluminum foil and left behind for 24 hours in room temperature. Later the supernatant solution was decanted into another beaker and the pH of the formulation was determined using a calibrated pH meter [14]

Powder analysis of *Flueggea leucopyrus* leaves:

The pharmacognostic studies were performed as per the standard methods. The powder was analyzed for preliminary and micro chemical tests for identification of various cell types and presence of crystals in the leaves [15]

Determination of Extractive Yield of *Flueggea leucopyrus* leaf

The dry powder of plant material of *Flueggea leucopyrus* was

extracted with almost 20 solvents using maceration process. 2 gm of the coarsely powdered plant material was weighed in a weighing bottle and transferred into a dry 250 ml conical flask. Then the flask was filled with different solvents (30 ml) separately. The flasks were corked and kept aside for 24 hrs at room temperature, shaking frequently. The mixtures were filtered through Whatmann No. 1 filter paper into a 50 ml measuring cylinder. After the filtrate has obtained, it was then transferred into a weighed petry plates. The obtained extracts were concentrated to dryness by keeping filtrate for complete evaporation of solvent [16]

The extractive value in percentage was calculated by using following formula and recorded.

$$\text{Extractive value (\%)} = \frac{\text{Weight of dried extract}}{\text{Weight of plant material}} \text{ X } 100$$

Phytochemical screening of solvents extract of *Flueggea leucopyrus* leaf extract

The different phytochemical tests were performed for establishing the profile of plant extract for its phytochemical constituents with multifarious solvents [17]

Results

Table 1: Pharmacognostic analysis of F. leucopyrus leaf powder

Test	Results
Swelling index	0.8 cm
Foaming index	Less than 100
Water solubility index	14.8±0.8
Water absorption index	10.9±0.2
Bulk density (g/ml)	0.66
Tapped density (g/ml)	0.80
Hausner ratio	1.21
Compressibility index (%)	15.5
Angle of repose (^{0})	26.7
pH value	5.9

Table 2: Powder analysis of F. leucopyrus leaves – Preliminary test

Test	Observation	Inference
Colour	Green	Leaf drug
Odour	Specific	Aromatic drug
Taste	Acrid	Drug containing terpenoids

Table 3: Powder analysis of *F. leucopyrus* leaves – Microscopic observation

Reagent	Observation	Characteristics
Powder + Phloroglucinol + Conc. HCl	Pink colour	Lignified cells are present
Power + Ruthenium red	No pink colour	Mucilaginous cells are absent in epidermis
Powder + Sudan red III	Pink colour	Cuticle
Powder + Dilute iodine solution + Conc. Sulphuric acid	Blue colour	Hemicellulose present
Powder +Dilute HCl	insoluble	Calcium oxalate crystal are absent
Powder + Sulphuric acid.	Brown colour	Stone cell absent
Powder + Dil. Iodine Solution.	Blue	Endodermis with Starch

Table 4: Extractive values of *F. leucopyrus* leaves

Solvents	Extractive Yield (%)	Solvents	Extractive Yield (%)	Solvents	Extractive Yield (%)
Water	29.5	Aniline	28.9	Toluene	2.20
Hydro alcoholic	29.9	Ethyl acetate	2.1	Xylene	1.9
Acetic acid	37.6	Acetone	9.8	Cyclohexane	1.60
Methanol	18.5	CCL4	26.8	Petroleum ether	2.20
Ethanol	18.4	Dichloromethane	10.7	Hexane	2.10
Isopropanol	4	Chloroform	6.9	Diethylether	4.4
Acetonitrile	2.9	Benzene	1.5		

Table 5: Qualitative phytochemical analysis of *Flueggea leucopyrus* leaves with various solvents

Sl No.	Phytoconstituents		*F. leucopyrus* leaf extract																
			W	HA	AA	M	E	IP	AN	AN	EA	AC	CC	B	T	XL	CH	PE	H	Cl
1	Alkaloids	Dragendroff's test	P	P	P	P	P	P	P	A	P	A	P	P	P	P	P	P	P	P
		Wagner's test	P	P	P	P	P	P	P	P	A	A	P	P	P	P	P	P	P	P
		Mayer's test	P	A	A	P	P	A	P	A	A	A	P	A	A	A	P	A	A	P
		Hager's test	P	P	P	P	P	A	P	A	A	A	A	A	A	A	A	A	P	P
2	Flavonoids	Lead acetate test	A	A	A	P	P	P	P	A	A	A	A	A	A	A	A	P	A	P
		Sodium hydroxide reagent test	P	A	A	A	A	A	A	A	A	P	P	A	A	A	A	A	P	P
		Ammonium test	A	A	A	P	A	A	A	A	A	A	A	P	A	P	A	A	A	P
3	Glycosides	Bromine water test	P	A	A	A	A	A	P	A	A	P	A	A	A	A	A	A	A	P
		Borntrager's test	A	A	A	A	A	A	A	A	A	A	A	A	A	A	P	A	A	P
		Keller – Killiani test	P	A	A	A	A	A	A	A	A	A	P	P	A	A	P	P	A	A
4	Steroids	Salkowski test	P	P	P	P	P	P	A	A	A	P	A	A	A	A	P	P	A	P
		LB Test	P	P	P	P	P	P	A	A	A	A	A		A	A	P	A	A	A
5	Tannins	Lead acetate test	P	P	P	P	A	P	A	A	A	A	A	A	A	A	P	P	A	A
		Ferric chloride test	P	P	P	P	A	P	A	A	A	P	A	A	A	A	P	A	A	P
		Gelatin test	P	P	P	P	A	A	A	A	A	A	A	P	A	A	A	A	A	A
6	Phenolic compounds	Gelatin test	A	A	A	A	A	A	A	A	A	A	A	P	A	A	A	A	P	A
		Ferric chloride test	P	P	P	P	A	P	A	A	A	P	A	A	A	A	A	A	A	A
		Ellagic acid test	P	P	P	P	A	P	P	A	A	P	P	A	A	A	A	P	A	A
7	Terpenoids	Copper acetate	A	A	A	P	A	P	P	A	A	P	A	A	A	A	A	A	P	P
		Salkowski test	P	P	P	P	A	P	A	A	A	P	A	A	A	A	A	P	A	P

		LB Test	P	P	P	P	A		A	A	A	A	A	A	A	A	A	A	A	A
8	Saponins	Foam test	P	A	P	P	A	A	A	P	A	A	A	A	A	A	A	A	A	A
9	Carbohydrates	Fehling's test	A	P	A	P	A	P	A	A	A	A	A	A	A	A	A	A	A	P
		Benedicts test	A	A	A	P	A	P	A	A	A	A	A	P	A	A	A	A	A	P
10	Oils and Fats	Spot test	A	A	A	A	A	A	A	P	P	A	A	A	P	P	A	A	A	P
11	Proteins and amino acids	Biuret test	A	A	A	P	A	A	A	A	A	A	A	A	A	A	A	A	A	A
		Ninhydrin test	A	A	A	A	A		A	A	A	A	A	A	A	P	A	A	A	A
		Xanthoproteic	A	A	A	P	A	P	P	A	P	A	P	A	A	A	A	A	A	A

W- Water, HA – Hydro alcoholic, AA- Acetic acid, M- Methanol, E-Ethanol, IP-Isopropanol, AN-Acetonitrile, EA-Ethyl acetate, AC-Acetone, CC-CCL4, B-Benzene, T-Toluene, X- Xylene, CH-Cyclohexane, PE-Petroleum ether, H-Hexane, CL- Chloroform, A-Absent, P-Present.

Discussion

Many herbal materials are of specific therapeutic or pharmaceutical utility because of their swelling properties especially gums and those containing an appreciable amount of mucilage, pectin or hemicellulose. The swelling index is the volume in ml taken up by the swelling of 1 g of herbal material under specified conditions. Its determination is based on the addition of water or a swelling agent. The mixing of whole herbal materials with the swelling agent is easy to achieve, but cut or pulverized materials requires vigorous shaking at specified interval of time to ensure even distribution of materials in the swelling agents [18]. In the present study the swelling index is 0.8 cm. Many herbal materials contain saponins that can cause a persistent foam when an aqueous decoction is shaken. The foaming ability of an aqueous decoction of herbal materials and their extracts is measured in terms of a foaming index. If the height of foam in tubes is less than 1 cm, the foaming index is less than 100 (non-significant). If the height of the foam is more than 1 cm the foaming index is more than 1000 (significant). In the present study, the foaming index is less than 100, which is non-significant [18].

Water Solubility Index (WSI) is related to the quantity of soluble molecules which is associated with the conversion of starch during extrusion. At lower water solubility index, there is minor degradation of starch leads to less number of soluble molecules in the extrudates. At higher water solubility index, there is higher degradation of starch leading to more number of soluble molecules in the extruder, the difference in the water solubility index is associated with the difference in the extent of starch degradation [19, 20]. The Water absorption index (WAI) is an indicator of the plant powder to absorb water. Water is absorbed and bound to the starch molecules inducing change in the starch molecule structure. A decrease in WAI was attributed to the relative decrease in starch content caused reduced water absorption. The same trend of increased WAI with increased extrudates due to increase in starch and fibre content [21]. In the present study, the leaf powder of *Flueggea leucopyrus* showed a good WSI and WAI respectively.

The bulking properties of a powder are dependent upon the preparation, treatment and storage of the sample. The particles can be packed to have a range of bulk densities, which depends on both density of the powder particles and spatial arrangement of particles in the powder bed. The tapped density is an increased bulk density attained after mechanically tapping a container containing the powder sample [22].

The interparticulate interactions influencing the bulking properties of a powder are also the interactions that interfere with powder flow, a comparison of the bulk and tapped densities can give a measure of the relative importance of these interactions in a given powder. Such a comparison is often used as an index of the ability of the powder to flow, like Compressibility index or the Hausner ratio. The Compressibility index and Hausner ratio are measures of the propensity of a powder to be compressed. As such, they are measures of the powder ability to settle and they permit an assessment of the relative importance of interparticulate interactions. In a free flowing powder, such interactions are less significant, and the bulk and tapped densities will be closer in value. For poorer flowing materials, there are frequently greater interparticulate interactions, and a greater difference between the bulk and tapped densities will be observed [23].

When the pH value is low (acidic), the bacterial count was observed to be equally low, but at neutral or higher pH the level of contamination of the herbal preparations were observed to be higher. This suggests that a neutral or alkaline pH favored high contamination levels of the herbal preparations. This agrees with the observation that bacterial growth is optimal at more or less neutral pH, around pH 5–8.5 [24]. The presence study shows the leaf powder of *Flueggea leucopyrus* pH towards acidic side, means the chances of contamination of herb is distant.

Plants contain many active compounds such as alkaloids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols flavonoids etc, which are deposited in their specific parts such as leaves, flowers, bark, seeds, fruits, root, etc. The beneficial medicinal effects of plant materials typically result from the combination of these secondary products [24, 25]. Phytochemicals are known to possess antioxidant [26], antibacterial [27], antifungal [28], antidiabetic [29], anti-inflammatory [29], radio-protective activity [30] etc and due to these properties they are largely used for medicinal purpose. In these study, the extractive values of leaf powder were assessed with multifarious solvents viz. water, hydro alcoholic, acetic acid, methanol, isopropanol, acetonitrile, toluene, xylene etc. among the various solvents tested acetic acid extract showed highest yield of 37.6% followed by hydro alcoholic extract of 29.9% and water with 29.5% with least extractive yield of 1.5% with benzene respectively. The powdered drug subjected to qualitative phytochemical analysis with 18 solvents, of which methanolic extract of *Flueggea leucopyrus* leaves showed maximum phytoconstituents of all categories.

Conclusion

The present investigation was carried out to understand the pharmacognostic, physical, extractive yield and qualitative phytochemical analysis of leaves of *Flueggea leucopyrus* collected from Wayanad regions of Kerala. The powdered drug m*et al*l the standard specifications met by the official body and acetic acid extract showed highest extractive yield and benzene with lowest yield. Further, methanolic extract showed to be with maximum phytoconstituents as compared to any other solvent examined. These study lays the pharmacognostic monograph for leaves of *Flueggea leucopyrus* collected at high altitude regions and further detailed activity guided pharmacological experiments should

be carried out to disclose the traditional claim.

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