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Pharmacognostic evaluation, fluorescence and TLC analysis of *Peltophorum pterocarpum* (Dc.) backer ex heyne leaves

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Abstract
Present study is to evaluate the Pharmacognostic, Florescent Analysis and Thin Layer Chromatography of *Peltophorum pterocarpum* leaf. Fresh and shade dried leaves were selected and powdered for this study. Physicochemical parameters were performed as per WHO guidelines. The powder was investigated by morphology and Florescent Analysis (FA), Thin Layer Chromatography (TLC) was analysed according to method described by Chase and Pratt (1949) and Kokoshi *et al.,* (1958). The results of physico-chemical parameters such as determination of total ash, water soluble ash, insoluble ash, loss on drying, alcohol soluble extractive values are tabulated. This crude extract (leaf powder) showed the characteristic physicochemical values like 10.27% (total ash), 6.53% (water soluble ash), 12.5 % (acid insoluble ash), and 0.15% (moisture content). Methanolic extract of *Peltophorum Pterocarpum* was used for the fluorescent and TLC analysis. Fluorescent characters was observed varied colours like green, Yellowish green, dark green, Reddish brown and Yellowish brown, Blackish brown, Bluish brown, Dark brown, Light brown under different chemical treatments on methanolic extract used for the study. TLC showed nine different compounds with different R_f values, confirmed the presence of different compounds in the plant. From the results the *Peltophorum Pterocarpum* leaf have potential bioactive constituencies.

Keywords: *Peltophorum pterocarpum*, pharmacological evaluation, fluorescence, thin layer chromatography

1. Introduction
Plant based studies have been used in the treatment of various ailments ranging from common cold to cancer from long back [1]. *Peltophorum Pterocarpum* (belonging to Fabaceae family) is a deciduous tree usually reaching a height of about 15-24 m, although it may attain 50 m and a diameter of 50 -100 m. The bark is smooth and grey and has a dense, spreading crown. It has a deep root system, making it very wind firm [2, 3] and regarded as one of the most significant plant species in traditional system of medicine. The plant is used in different parts of the world for the treatment of several ailments like stomatitis, insomnia, skin troubles, constipation, ringworm, insomnia, dysentery, muscular pains, sores, and skin disorders and is the source of a diverse kind of chemical constituents such as aliphatic alcohols, fatty acids, amino acids, terpenoids, phenolics, flavonoids, alkaloids, steroids etc [4, 5]. Stem is used as antimicrobial, antioxidant, tooth powder, muscular pain dysentery for gargles [6] leaves for alzheimers, skin disorders [7, 8]. The isolated phytochemicals as well as different extracts exhibited numerous biological activities including antimicrobial, antioxidant, cytotoxic, aldose reductase inhibition and antiglycaemic activities. Flowers are used in treatment of Insomnia treatment, anti-inflammatory [9, 10]. Bark for Dysentery, eye lotion, embrocating for pains sores [10]. Root for heal wounds, toothache and throat sores, in eye treatment Literature survey indicated that very little physicochemical Work had been carried out on leaves of this plant. Exhaustive and up to date survey of literature on botanical and ethanobotanical profile earlier this formed the basis of my research work.

The effectiveness of herbal remedies, their easy availability, low cost and comparatively being devoid of serum toxic effects popularized them. The most important bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolics compounds. Many of the indigenous medicinal plants are used as spices and food plants. They are sometimes added to foods meant for pregnant women and nursing mothers for medicinal purposes as reported by Okwu, D. E. and Hill A.F [11-13]. Different parts of the plants like bark, roots, leaves, exudates etc. are used as per medicinal properties proposed by Perumal Samy R. and Gopala Krishnakone P [14].

Peltophorum pterocarpum Baker ex Heyne is regarded as one of the most significant plant species in traditional system of medicine.

The plant flower extract is known to be a good sleep inducer and used in insomnia treatment [15-17]. Its bark is used as medicine for dysentery, as eye lotion, embrocating for pains and sores. The traditional healers use the leaves in the form of decasan for treating skin disorders. Stem infusion of *Peltophorum pterocarpum* Baker ex K. Heyne used in dysentery, for gargles, tooth powder and muscular pain [18]. Flowers are used as an astringent to cure or relieve intestinal disorders after pain at childbirth, sprains, bruises and swelling or as a lotion for eye troubles, muscular pains and sores [19].

It is the source that of chemical constituents such as aliphatic alcohols, fatty acids, amino acids, terpenoids, phenolics, flavonoids, alkaloids, steroids etc [20, 21]. It is having biological activities including antimicrobial [22], antioxidant [23], cytotoxic [24], aldose reductase inhibition [25] and antiglycaemic activities [26]. Literature survey indicated that fluorescence analysis and running of TLC has not been carried out on this plant [27, 28]. This formed the basis of my research work.

2. Materials and Methods

2.1. Chemicals and Reagents

All the reagents used were of analytical grade obtained from Sigma Chemical Co. St. Louis, USA and Fine Chemicals Ltd., Mumbai, India.

2.2. Collection and Authentication of Plant:

The plant material was collected on 19th April 2014 from Andhra University campus, Andhra Pradesh, India and authenticated by Dr. B. S. Padal, taxonomist, Department of Botany, Andhra University, Visakhapatnam, Andhra Pradesh. The Voucher specimens A.U. (B.D.H), NO.21917 were deposited in the herbarium, A.U. College of Pharmaceutical Sciences, Andhra University.

2.3. Preparation of plant powder

The leaves were collected and washed with tap water cleaned and shade dried until the leaves are free from moisture content for 2 ½ months and subjected to milling to get powdered and sieved using mesh no 80 (0.177 mm). It was then homogenized to fine powder, weighed and stored in air tight container kept in a dark place. The powdered plant material was then used to perform the physicochemical analysis.

2.4. Extraction process

The freshly collected leaves were shade dried and powdered. The powdered material then subjected to Soxhlet extraction process with methanol

2.5. Soxhlet Extraction

The dried powdered materials of the plant were extracted successively three times with methanol. The methanolic extract is used for fluorescent analysis method. The extracts thus obtained were concentrated under vacuum at temperature of 43 ^{0}C by using rotary evaporator, dried completely, weighed and stored in desiccators [29].

2.5.1. Physicochemical Screening

Fig 1: Photographic image of *Peltophorum pterocarpum* plant parts

Table 1: Organoleptic characters of *Peltophorum Pterocarpum* leaf powder

Characters	Flower	Fruits	Stems	Leaves
Colour	Yellow	Light brown	brown	Green
Odour	Odour less	Characteristic	Characteristic	Characteristic
Taste	Sweetish bitter	Bitter	Bitter	Bitter
Texture	Smooth	Smooth	Title bit rough	Very smooth
Shape	Small longitudinal	Oval	Straight cylindrical	Small lanciolate

The various physicochemical parameters were studied as per standard protocols [11] which include determination of ash contents (total ash, water soluble and acid insoluble), extractive values and moisture content.

a) Determination of Total ash

20 g leaf powder was taken in a silica crucible (which allows rapid heating and cooling with virtually no risk of thermal breakage and it can stand on high temperature and chemical corrosion compare to porcelain crucible) previously ignited and weighed. It was incinerated by gradually increasing the heat not exceeding dull red heat (450 ^{0}C) until free from carbon, cooled and weighed. The percentage of ash was calculated. The procedure was repeated five times to get constant weight [30].

b) Determination of water soluble ash:

The Total ash was boiled with 25ml of water for 5 minutes and was filtered through an ash less filter paper (Whatmann No. 41). It was followed by washing with hot water. The filter

paper was ignited in the silica crucible, cooled and the water insoluble matter was weighed. The water soluble ash was calculated by subtracting the water insoluble matter from the total ash [31].

c) Determination of acid insoluble ash

The total ash obtained was boiled for 5 minutes with 10%w/v dilute hydrochloric acid and acid soluble ash was calculated as mentioned in above step.

$$\text{Ash Value} = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \text{X } 100$$

d) Determination of loss on drying

15g of the powdered leaves was weighed in a glass Stoppard weighing bottle which is previously dried for 30mins in the drier. Then, the sample was gently shaken side wise for even distribution and dried in an oven at 100 ^{O}C to 105 ^{O}C by removing the stopper. It was cooled and again weighed. The loss on drying was calculated [31].

e) Determination of alcohol soluble extractive

15g of coarsely powdered air-dried material and accurately weighed in a glass-Stoppard conical flask. A 300ml of water is added to flask and weigh to obtain the total weight including the flask. Shake well and allow standing for 1 hour. Attach a reflux condenser to the flask and boil gently for 6 hour; cool and weigh and filter rapidly through a dry filter. Then dry the extracted powder in oven till the weight is constant [27-31].

$$\text{Extractive Value} = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \text{X } 100$$

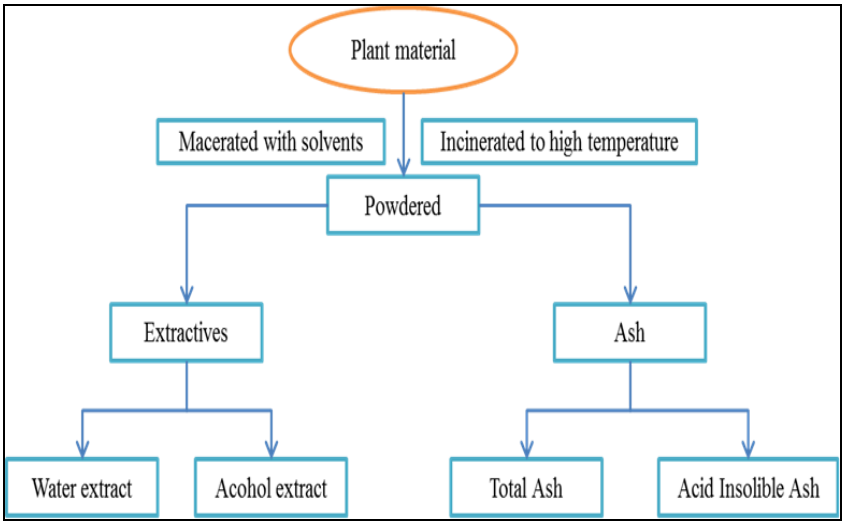

Fig 2: Plan of work

Table 2: Details of the Extraction

Plant material (g)	500
Solvent used	Methanol
Volume of the solvent (L)	3.2
Weight of the extract (g)	34

2.5.2. Fluorescent analysis

The fluorescent analysis of shade dried and powdered, methanolic extracted plant material of *peltophorum pterocarpum* was studied under UV light and daylight. Fluorescent analysis of plant powder was carried out according to the methods of Kokoshi *et al.*, (1958) and Chase and Pratt (1949) [32, 33] and Powder was subjected to different chemicals like, 1N NaOH, Acetic acid, 1N HCl, 1N HNO_3, 5% Iodine, 5 % $FeCl_3$, 1N NaOH in methanol. The fluorescence analysis of these leaf extracts were observed under ordinary visible light and also under UV light (245 nm) and recorded in Table 9.

2.5.3. Chromatographic characterization of the extract

Thin layer chromatography (TLC) is used for the separation of a mixture of compounds. TLC is performed on a sheet of aluminium foil which is coated with a thin layer of adsorbent silica gel, which are commercially available 60 F254 (Merck). Samples of leaf extracts prepared with methanol solvent. The extract was diluted to a great extent (1mg/1ml) and then filtered with a Whatmann filter paper no-1 and was spotted onto the TLC plate as a single spot with capillary tubes [23]. By the capillary action the solvent is drawn up by the plate and it is developed. The TLC was examined using UV chamber and 5% H_2SO_4 as visualizing agent for the detection of compounds. Qualitative evaluation of separated substance was carried out by calculating R_F values.

R_F =Distance travelled by the solute/Distance travelled by the solvent

3. Results and Discussion

Acid insoluble ash is a part of total ash and measures the amount of silica present, especially as sand and siliceous earth. The results of powdered ash *Peltophorum pterocarpum* was acid insoluble ash showed 12.5%, total ash content 10.27%, water soluble ash 6.53% water soluble ash is the water soluble portion of the total ash. More water soluble ash value appeared denotes that this plant powder ash is more soluble to water. Percent weight loss on drying or moisture content was found to be 0.15% Extractive values obtained using water and alcohol were recorded in table . It is useful for the evaluation of a crude drug as it gives an idea about the nature of chemical constituents present in it and is useful for estimation of chemical constituents, soluble in that particular solvent used for extraction. Alcohol extractive value of 14.667% showed and water extractive value of 10.33%. The result of percentage extractive yield for *Peltophorum pterocarpum* indicates that crude powder was highly soluble in alcohol than water.

Table 3: Total ash

Weight of the drug powder taken (g)	Weight of ash obtained	Percentage w/w total ash	Mean value
3.0	0.286	9.53	10.27
3.0	0.249	11.63	
3.0	0.29	9.66	

Table 4: Acid insoluble ash

Weight of powder taken(g)	Weight of acid in soluble ash (g)	%w/w acid in soluble ash	Mean value
2.0	0.266	13.3	12.5
2.0	0.235	11.75	
2.0	0.249	12.45	

Table 5: Water soluble ash

Weight of total ash (g)	Weight of water soluble ash (g)	%w/w of water soluble ash	Mean value
2.0	0.132	6.6	6.53
2.0	0.130	6.5	
2.0	0.130	6.5	

Table 6: Moisture content

Weight of the powder taken (g)	% Loss in weight (w/w)	Mean value
1.5	0.263	0.15675
1.5	0.161	
1.5	0.102	
1.5	0.101	

Table 7: Extractives

S. No	Solvent	Initial weight of sample (g)	Amount of solvent (ml)	Final weight of sample (g)	Extractive value (%)
1	Ethanol	15	150	12.80	14.667
2	Water	15	150	13.45	10.33

Table 8: Physicochemical parameters of *Peltophorum Pterocarpum* leaf

S. No	Physicochemical parameters	% yield
1.	Total ash	10.27
2.	Acid insoluble ash	12.5
3.	Water soluble ash	6.53
4.	Ethanol extractive	0.15675
5.	Water extractives	14.667
6.	Moisture content	10.33

The results of physico-chemical analysis of plant ash are given in Table No.1, 2, 3, 4, 5. Ash usually represents the inorganic part of the plant as ash contains inorganic material of the plant because ashing destroys all the organic material present in the plant sample. The inorganic elements play an important role in physiological process involved in human health.

The elements composition of ash may constitute sodium, potassium, calcium magnesium, phosphate. Trace elements play both curative and preventive role in combating diseases. High levels of Calcium overcomes the problems of high blood pressure, heart attack, premenstrual syndrome, colon cancer and imparts strength and rigidity to bones and teeth and reduces the risks of osteoporosis in old age. It also acts as an activator of the enzymes phospholipase, arginine kinase, adenosine triphosphatase and adenylkinase. Excess quantity of calcium ions in extra cellular fluid induces mental depression. At the other extreme, low levels of calcium causes spontaneous discharge of nerve fibers resulting in tetany [34]. Potassium is important for diuretic activity and is helpful in reducing hypertension and maintaining cardiac rhythm. High concentration of potassium in the medicinal Plants could be related to the diuretic action of drugs prepared from these plants [35]. Iron essential mineral to prevent anemia and cough associated with angiotension converting enzyme (ACE) inhibitors [36]. Phosphate ions are the major anions of intracellular fluids, phospholipids and the coenzyme NAD and NADP and especially of ATP and other high energy compounds. It helps in the process of ossification of bones by getting deposited in the form of calcium phosphate [37].

Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material. Some of the plant compounds show fluorescence in the visible range in daylight. The ultraviolet light produces fluorescence in many natural products (e.g. alkaloids like berberine) which do not visibly fluorescent in daylight. Some of the substances may be often converted into fluorescent derivatives by using different chemical reagents though they are not fluorescent, hence we can often assess qualitatively some crude drugs using fluorescence as it is the most important parameter of pharmacognostic evaluation [38, 39]. The results of fluorescent analysis of leaf powder of the medicinal plants viz., *Psidium guajava* and *Citrus aurantium* showed characteristic colouration in treatment with various chemical reagents. These results are supportive with *Cajanus cajan* leaf extracts [40].

Table 9: Fluorescence analysis of powdered leaves of *Peltophorum Pterocarpum*

Reagents	Visible	Short UV	Fluorescence
Powder + 1% glacial acetic acid	Yellowish green	Yellowish brown	Brownish black
Powder +10% NaOH	Yellowish green	Dark brown	Blackish brown
Powder + dil. NH_3	Dark green	Dark yellowish brown	Bluish brown

Powder + Conc. HNO_3	Green	Light brown	Brown
Powder+ dil.NH_3+Conc.HNO_3	Yellowish green	Blackish brown	Dark brown
Powder +1M HCl	Dark green	Light brown	Blackish brown
Powder +1M H_2SO_4	Dark green	Dark brown	Yellowish black
Powder + 10% $FeCl_3$	Brownish yellow	Brown	Dark brown
Powder +Acetone + Methanol	Reddish brown	Light brown	Brownish yellow
Powder +10% Iodine	Light brown	Brown	Black

This process of moving the compounds with the solvent is referred to as elution and the solvents used are eluting solvents. TLC technique is an important for phytochemical screening [41] hence, some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostic evaluation [42, 43]. The spots which were obtained after the evaluation of the TLC plate (methanolic extract) may contain the presence of alkaloids, saponins, glycosides, flavonoids, phenols and tannin derivatives. Quality parameters are carried out on plant samples in order to establish appropriate data that can be used in identifying crude drugs particularly those supplied in powder form.

Table 10: Thin layer chromatographic analysis of powdered leaves

Extracts	Mobile phase	R_f Values
Methanolic	Hexane (100%)	0.30
	Hexane (100%)	0.32
	Hexane (100%)	0.43
	Hexane: Chloroform (20:80)	0.75
	Hexane: Chloroform (20:80)	0.55
	Hexane: Chloroform (20:80)	0.40
	Hexane: Chloroform (20:80)	0.44
	Hexane: Chloroform (95:5)	0.46
	Chloroform (100%)	0.32
	Chloroform: methanol (20:10)	0.66

4. Conclusion

The plant *Peltophorum pterocarpum* having important role in the traditional Ayurvedic, Unani systems of holistic health and herbal medicine of the east. In the present study aerial part leaf of *Peltophorum pterocarpum* was thoroughly investigated for their physicochemical characters to analyze their quality, safety and standardization for their use. The information from the present study will provide data which is helpful in the correct identification and authentication of these medicinal plants and may help in preventing its adulteration. Further research is in progress regarding isolation, purification and characterization of therapeutically potent compounds from Methanolic extract, which could be subjected to pharmacological analysis to treat different ailments and in order to understand the exact action mechanism. WHO has emphasized the need to ensure the evaluations of TLC and Fluorescence properties are essential to standardize the various Unani and Ayurvedic formulations. In this study analysis of both Fluorescence and TLC of *Peltophorum Pterocarpum (DC)* leaves was carried out. The dried leaf powder of *Peltophorum Pterocarpum (Dc.)* was found to have fluorescent property. By the Analysis of TLC profile of the compounds with different R_F values we can isolate the compounds based on the polarity for further study. As the plant material shows fluorescence that may give an indication of presence of different compounds in the plant. The fluorescent analysis of powdered drug plays an important role in the determination of quality and purity of the drug.

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