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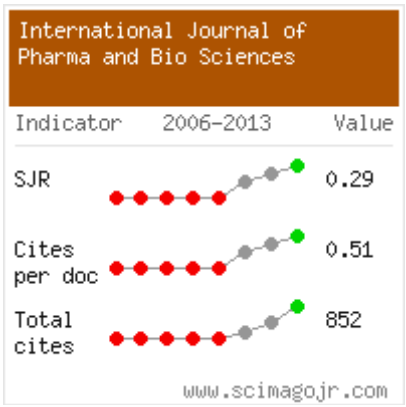

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PHARMACOGNOSTIC, PHYSICO-CHEMICAL AND PHYTOCHEMICAL EVALUATION OF *PACHYGONE OVATA* (POIR.) MIERS EX HOOK. F. ET THOMS LEAF

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ABSTRACT

Pachygone ovata being one of the unexplored species of the menispermaceae family has gained our attention to this study. Macroscopic features highlighted ovate-lanceolate leaves with an entire margin showing reticulate veination, microscopic analyses indicated anomocytic type of stomata on the abaxial surface, presence of starch grains in the parenchyma layer and unicellular and multicellular trichomes on the midrib. Physico-chemical parameters were analyzed for further standardization, which indicated low amounts of moisture (3.45) and low amounts of acid insoluble ash (2.01). Fluorescence and phytochemical studies helped in concluding the nature of the compounds and secondary metabolites present in the plant material, confirming the presence of flavonoids, triterpenoids, glycosides and alkaloids. Lowest phenolic content was recorded in the hexane extract (85 ± 4 µg GAE/mg dw) while the highest was found to be in the methanol extract of *P.ovata* (389 ± 4 µg GAE/mg dw). Only ethnopharmalogical evidences in this age cannot suffice for the usage of plants, it is now a prerequisite to authenticate the plants for safer health care, therefore the details included in this paper will help in authentication of this plant in terms of identification and quality assurance.

KEYWORDS: Pharmacognostic study, *Pachygone ovata*, phytochemical studies, physico-chemical analysis, total phenolic content

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INTRODUCTION

Pachygone ovata (Poir.) Miers ex Hook. f. et Thoms belongs to the Menispermaceae family [1,2,3]. This large evergreen climber commonly known as fish berry is native to Australia, Christmas Island, Sri Lanka, South India, Malaysia, Java, Borneo, Timor and New Guinea. The genus is very controversial and includes 33 species out of which only 5 are accepted species names and *ovata* is the only species found in India; mainly in Chennai, Mysore and Andhra Pradesh[4,5]. The dried fruits of the plant have been used traditionally by folklore to repel insects and as fish poison[6]. It is a decidious woody straggling shrub which can climb upto 15m or more, often found in sandy sea shores, foothills and in scrub upto an altitude of 900m. It has drooping branches which become yellowish with age, leaves are ovate and blunt, base is truncate to rounded, apex retuse, mucronate, coriaceous, turning yellow with age. It is a monoecious plant, with sweet scented flowers that lie 2-3mm across to each other. These flowers are yellow in color, highly fragrant and they aree arranged in 4-10cm long axillary racemes. The male flowers exhibit 6 stamens, 6-12 sepals; inner ones larger and imbricate, covered by 6 petals which are auriculate towards the base. The female flowers have sepals and petals similar to the male flower, with 3 carpels, glabrous, style reflexed, stigma entire with 6 staminodes[7]. The fruits are droopy and reniform, green in color when young and start turning orange to purple as it matures[6,8]. Dasgupta *et al.* analyzed the plant for its constituents and their pharmacological action; they were able to isolate N-methylcrotsparine, reticuline, reticuline N-oxide, quercitol, liriodenine, trilobine and coclaurine for the first time in this plant and as a consequence the first time in the Menispermaceae family[4]. From the roots of this plant a quaternary alkaloid with an erythrinane skeleton, pachygonine was isolated which showed negative chronotropic tendencies. Another two known alkaloids, magnoflorine and *O,O*-dimethylmagnoflorine were isolated and characterized from this plant[9]. Various other alkaloid compounds were isolated from the roots of *P. ovata* such as: stepholidine, coreximine, isoboldine, norjuziphine and notrilobine[10]. Another new dibenzo-*p*-dioxin biphenyl bisbenzylisoquinoline alkaloid, pachyovatamine was identified and isolated from the leaves and stems of Sri Lankan *P.ovata*[11]. In spite of all these discoveries of different benzyl- isoquinoline-derived alkaloids from *P. ovata* stems, leaves and roots based on the literature[9,10,11], yet a systematic standardization of the pharmacognostic and physico-chemical characterization has still not been reported. Hence, this study was taken up to lay down certain pharmacopoeial standards for the standardization of the natural product which represents a promising direction in phytopharmacy and this includes morphological and anatomical features and preliminary phytochemical screening of *P. ovata* along with images observed under the microscope to help in proper identification of the plant.

MATERIALS AND METHODS

Plant Material

Fresh, disease free leaves of *P.ovata* were collected from Chamundi hills of Mysore, Karnataka India. Dr. G.R. Shivamurthi (former Professor, Department of Botany, University of Mysore, Mysore, India) botanically identified and authenticated the plant. The collected plant material was brought to the laboratory, washed thoroughly under the running tap water in order to remove dirt, germs and other contaminants, shade dried and powdered. This fresh plant was subjected to microscopic and macroscopic studies, and for the phytochemical analysis powdered plant material was used. An authenticated voucher specimen of the plant (UMDB/SU/Po-5) is deposited in the herbarium of Department of Studies in Biotechnology, University of Mysore, Manasagangotri, Mysore for future reference.

Macroscopical And Microscopical Analysis

The macroscopic as well as microscopic studies were conducted according to the methods of World Health Organization[12]. Macroscopic evaluation is one of the simplest and quickest means to identify and classify a

particular sample. Macroscopic studies determines the color, shape, size, taste and odor of the sample[13].. However since macroscopic results are subjective, it is therefore required to substantiate the findings with microscopic and physico-chemical analyses. For microscopic studies cross sections of the plant sample were prepared and stained with phluoroglucinol, iodine solution, safranin and acetic acid using reported methods[14,15], to study and measure different components. The photomicrographs were taken using trinocular microscope (RCM-20XLT, Singhla, India). The natural products were sieved through moderately fine mesh with sizes ranging from 0.180 – 0.355 mm and analyzed.

Physico-Chemical Parameters

The physico-chemical parameters *viz.*, loss on drying, total ash value, water soluble ash value, acid insoluble ash value and water and alcohol soluble extractives value was evaluated as per WHO guidelines[16].

Fluorescence Analysis

Powdered material was analyzed under visible light and long ultra violet (UV) light after treatment with various organic/inorganic reagents such as: water, sodium hydroxide, methanol, hydrochloric acid, nitric acid and ammonia[17].

Phytochemical Screening

The crude powder of *P. ovata* was subjected to phytochemical analysis to determine the presence/absence of different phytoconstituents *viz.*, carbohydrates (Fehlings test), proteins (Ninhydrin test, Biuret test), alkaloids (Dragendroffs test), tannins (ferric chloride test), flavonoids (Shinoda test), glycosides (Baljets test), steroids and triterpenoids (Leibermann Burchard test) using reported protocols[17,18,19,20,21].

Soxhlet Extraction Of Plant Material

The ground plant powder was placed in a porous bag and extracted by various non-polar and polar solvents *viz.,* hexane, methanol and ethyl actetate. The solvent is heated and the vapours condense in the condensor, pouring onto the crude extract in the bag and extracting the compounds based on their polarity and solubility in the solvent system used. The extracts are then colleced and dried using a rotary evaporator and further used for phenolic studies[22].

Total Phenols Estimation

The total phenolic content was determined by Folin-Ciocalteu reagent following the method of Spanos and Worlstad (1990), with slight modification[23]. To 50 µl of each sample, 2.5 ml 1/10 dilution of Folin-Ciocalteau's reagent and 2 ml of Na_2CO_3 (7.5%, w/v) were added and incubated at 45º C for 15 min. The absorbance of all samples was measured at 765 nm using a Beckman Coulter DU 730 UV–visible spectrophotometer. Results were expressed as micrograms of gallic acid equivalent per miligram of dry weight (µg GAE/mg dw) through the calibration curve with gallic acid.

Thin Layer Chromatography

The sample was dissolved in methanol and applied with the help of micropipette on precoated silica gel G60 F254 TLC plates (Merck). Different combinations of solvent system were tried for best separation of constituents[24].

Statistical Analysis

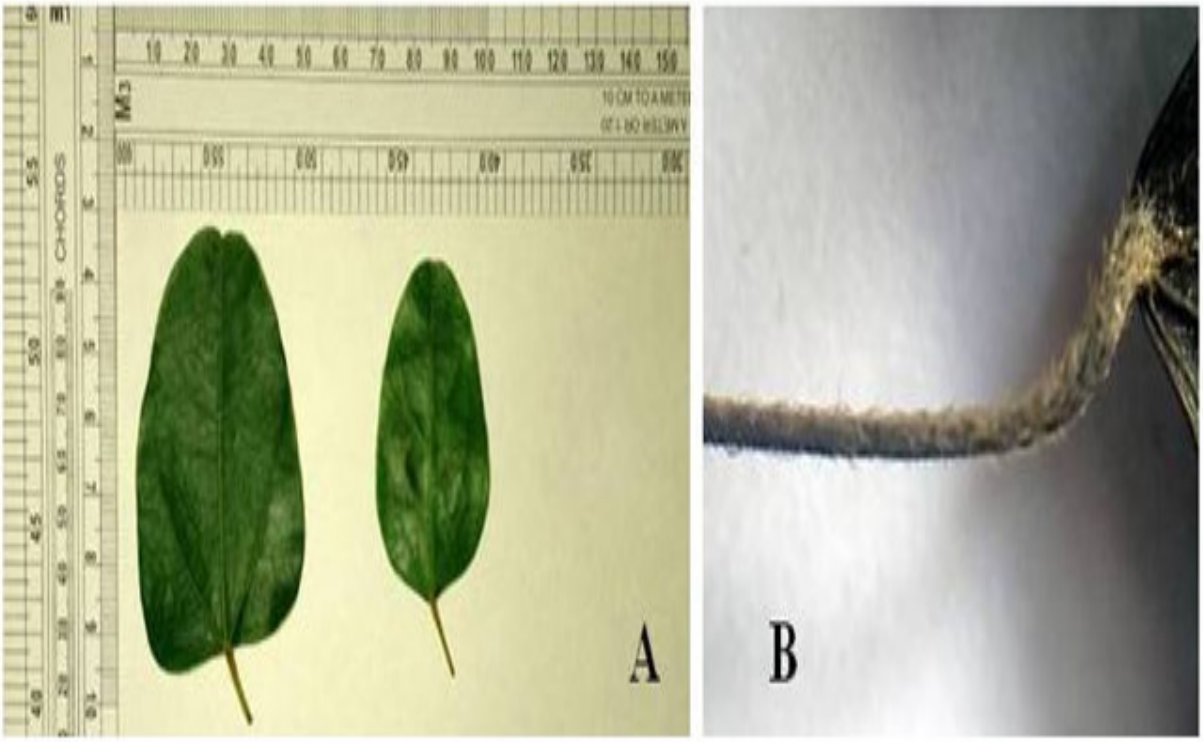
Experiments were performed in triplicates and repeated three times each. Results were reported as Mean ± S.E.M (Standard Error of Mean). *p* value <0.05 was considered significant.

RESULTS

Macroscopic Analysis

The fresh leaf of *P. ovata* was found to be dark green in color, having a length of 49mm and width of 29mm in case of young leaves, while mature leaves measured a length of 61mm and width of 45mm (Figure 1.A).

Figure 1
Macroscopical features of P.ovata leaf and petiole.



A. Comparison between young and mature leaf.
B. Petiole densely hairy

The powder of the leaves appeared dark green in color with characteristic odor and slightly bitter taste. The surface of the leaf was smooth with a retuse apex and the base is uneate with an entire margin. The veination was cross venulate and the leaf joins the stem with a densely hairy petiole (Table 1, Figure 2.B).

Table 1
Morphological characteristics of P. Ovata leaf

Parameter	Observation
Shape	Ovate-lanceolate
Apex	Emarginate retuse
Base	Truncate/rounded
Venation	Reticulate
Surface	Smooth
Margin	Entire
Petiole	Densely hairy
Color	Dark green
Odour	Peculiar/ characteristic
Taste	Bitter

Microscopic analysis

Transverse section of the leaf through the midrib consists of lamina and midrib region (Figure 2.A). The surface preparation of the leaves showed adaxial surface to be devoid of stomata (Figure 2.B) and abaxial surface with stomata (Figure 2.C). The abaxial surface had anomocytic type of stomata which was a little raised above the ground tissues. The outline of transverse section of the leaf shows its dorsiventral nature. The lamina exhibits upper and lower epidermis, the epidermal cells being rectangular and filled with chloroplast, the lower epidermis exhibiting many anomocytic stomata each of which is surrounded by 5-6 epidermal cells.

Mesophyll comprises of 2 layered palisade parenchyma cells (Figure 2.D) followed by spongy parachyma cells (Figure 2.E), these are filled with chloroplast and starch grains. The palisade layer is cylindrical columnar closely packed cells, while the spongy parenchyma are 2-3 layered irregular shaped cells mostly elongated with thin walls loosely arranged with inter cellular spaces mainly consisting of air spaces and excretory sacs. The lamina is traversed by vascular strands here and there. The midrib region is swollen and thicker than the lamina, in this portion both upper and lower epidermis were covered with thick cuticle. The midrib was composed of irregular-shaped epidermal cells that had uniseriate, long, ribbon shaped trichomes protruding from the lower surface (Figure 2.F). The trichomes were unicellular and multicellular in nature (Figure 2.G & H). This region is traversed by crescent shaped vascular bundles which consists of xylem and phloem. The xylem is towards the upper side and the phloem is towards the lower side. The vascular bundle on either side is surrounded by sclerenchymatous cells some having deposists of lignin, next to the sclerenchymatous bundle sheath lies the thin walled parenchymatous ground tissue and few layers of thick walled collenchymatous tissues. On either side the hypodermis consists of irregular shaped cells followed by a layer of epidermis (Figure 2.A). The outline of petiole was almost circular with undulating margin. It was covered all around by trichomes. The petiole is covered by a thick layer of cuticle all around. The transverse section shows the epidermis to be single layered, made up of thick walled rectangular cells, followed by hypodermis which consists of 3-4 layered collenchymatous cells continuing with cortex that consists of parenchymatous cells, these cells being hexagonal and have larger cells towards the inner region and smaller cells towards the outer region, these cells contain large number of chloroplast and sparsely distributed starch grains. A ring composed of sclerenchymatous cells was seen surrounding the vascular bundle. The vascular bundle consists of phloem elements which are made up of sieve tube and companion cells and are obliterated (Figure 3.A & B). Below this, xylem elements composed of vessels, tracheids and parenchyma cells were present. And eventually the Pith consisting of parenchyma cells and traversed with lignified fibers was seen in central portion (Figure 3.C & D).

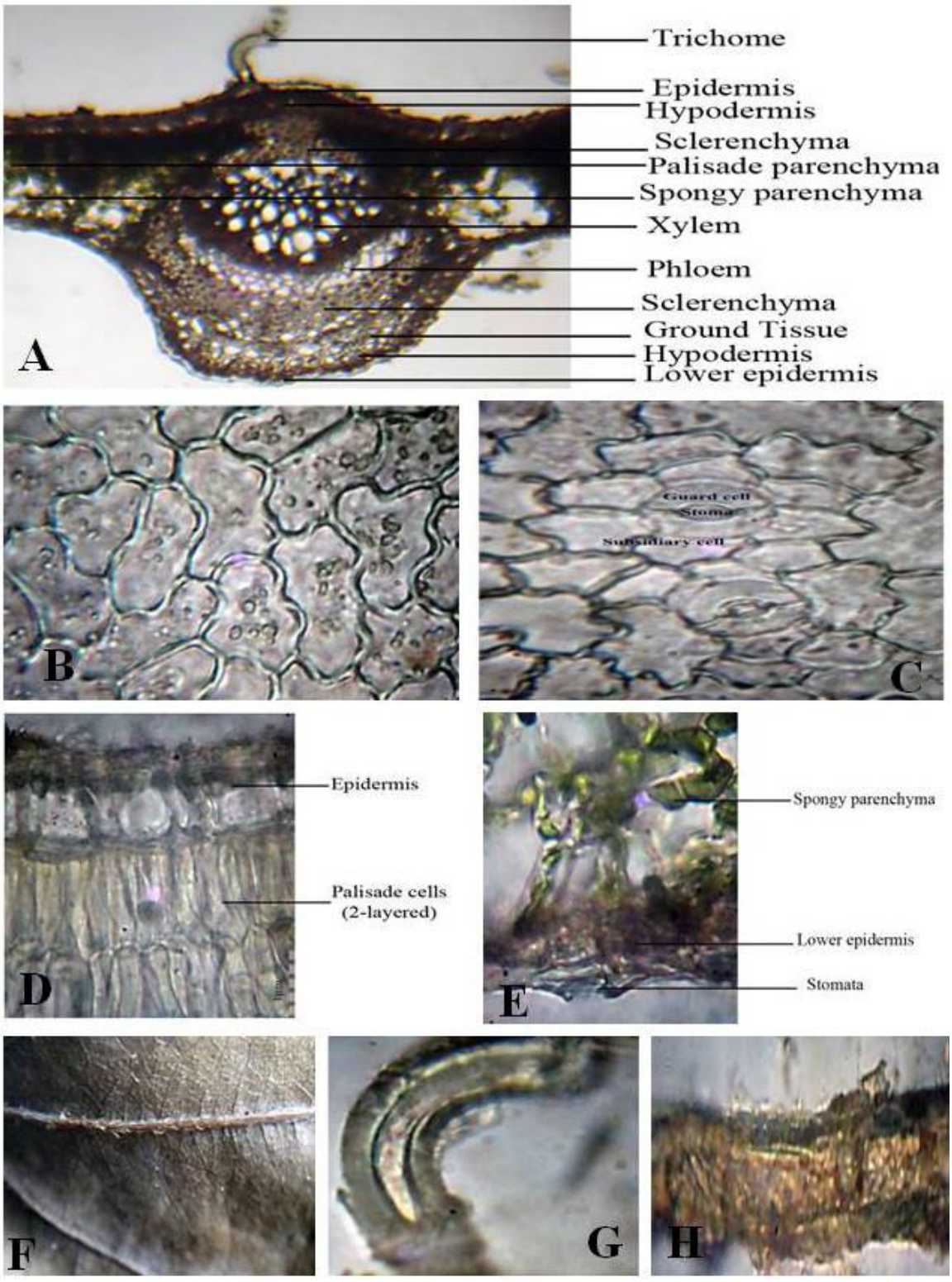


Figure 2

Midrib and lamina region.

A. Outline of TS (transverse section) of Leaf Through Midrib;
B. Adaxial surface without stomata;
C.Anomocytic type of stomata on abaxial surface;
D. Epidermis and palisade cells(the palisade being 2 layered);
E. Lower epidermis and spongy parenchyma (filled with chloroplast); F. Midrib with trichomes while lamina with no hair; G. Unicellular uniseriate trichome; H. Uniseriate multicellular trichome.

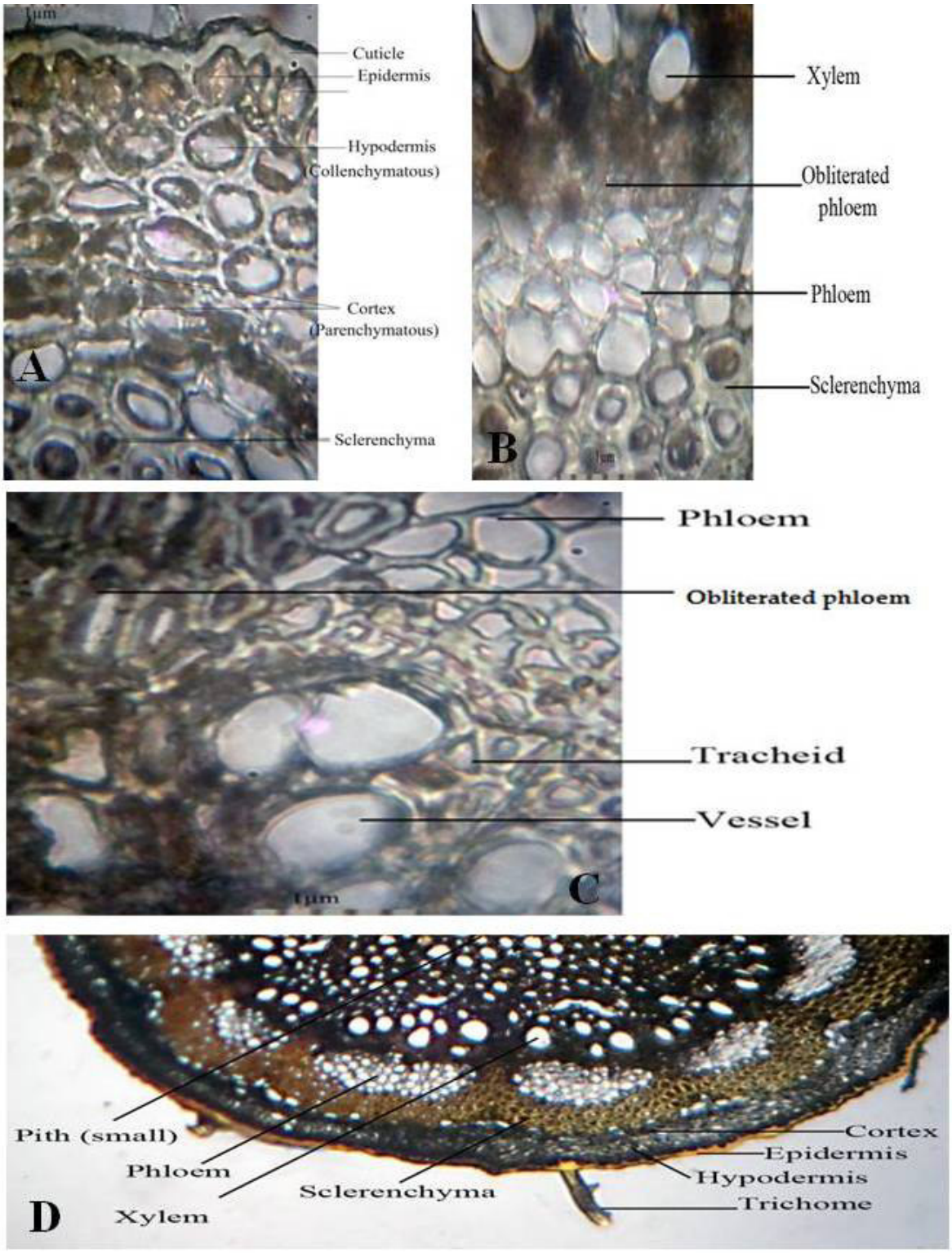


Figure 3

Transverse section of petiole of P.ovata leaf

A. Epidermis to sclerenchyma-petiole;
B. vascular bundle-petiole;
C. Phloem and xylem-petiole;
D. Outline of TS of petiole.

Physico-Chemical Screening

The physical content evaluation is an important parameter in detecting adulteration and improper handling. The moisture content of *P.ovata* leaf powder was found to be 5.45% which is low and would therefore discourage bacterial and fungal growth. The ash value was determined by three different methods (total ash= 6.5%, water soluble ash= 5.12% and acid insoluble ash= 2.01%). The alcohol and water soluble extractives were 3.56% and 6.23% respectively. The results obtained from physico-chemical studies have been compiled in table (Table 2).

Table 2
Physicochemical parameters of P.ovata leaf

No.	Parameters	Value (%) w/w*
1	Total ash	6.5
2	Acid insoluble ash	2.01
3	Water insoluble ash	5.12
4	Alcohol soluble extractives	3.56
5	Water soluble extractives	6.23
6	Loss on drying at 105°C	3.45

w/w : weight/weight

Fluorescence analysis

The color of the powder was green when viewed in visible light but this was changed to brown under the UV light. When treated with methanol, it changed from olive green to bright orange at 366nm. The most drastic change in color was observed when the leaf powder was mixed with 10% ammonia, it was seen as brick red and under the UV light it was seen as pale yellowish green. Plant powder in water was seen as pale green, while under UV light it was a darker shade of green. When mixed with 10% sodium hydroxide it was observed as reddish brown but under UV light it was only brown, the exact opposite was seen when mixed with 10% hydrochloric acid; where it was brown and under UV light it looked reddish brown. 10% nitric acid turned the sample color to pale yellow while at 366nm it was observed as brown. The results from fluorescence studies have been tabulated in the table (Table 3).

Table 3
Florescence analysis of powdered leaf of P.ovata

Treatment	Color observed	
	Visible light	*UV(long 366nm)
Powder without treatment	Dark green	Brown
Powder + water	Green	Dark green
Powder + methanol	Olive Green	Bright orange
Powder + 10% NaOH	Reddish-brown	Brown
Powder + 10% HCl	Brown	Reddish-brown
Powder + 10% HNO_3	Pale yellow	Brown
Powder + 10% NH_3	Brick-red	Pale yellowish-green

UV : Ultra violet

Phytochemical Screening

From the phytochemical studies it was apparent that there was an abundance of alkaloids, and moderate amounts of tannins, flavonoids, glycosides, carbohydrates and triterpenoids. Only traces

of steroid and protein were noted, while the amount of anthraquinone glycoside was significantly less. The results have been shown in table (Table 4).

Table 4
Qualitative phytochemical analysis of P.ovata leaf

Phytochemicals	Test	Results
Alkaloid	Dragendorff test	+ + + +
Carbohydrate	Fehling test	+ + +
Protein	Lowry assay	+
Cardiac glycoside	Keller- kiliani test	+ + +
Flavonoid	Shinoda test	+ + +
Tannin	$FeCl_3$ test	+ + +
Anthraquinone glycoside	Spectral analysis	+ +
Steroid	Liebermann-Burchard tet	+
Triterpenoids	Liebermann-Burchard tet	+ + +

+ : Traces, ++ : less, +++ : moderate, ++++ : abundant

Total Phenols Estimation

Variations in the quantity of total phenolics in the three extracts (hexane, ethyl actete and methanol) are presented (Figure 4). With all extracts there was a prominent concentration-dependency, wherein; 0.25mg concentration of all extracts showed higher phenolic content compared to their 0.05mg concentration. Quantitative estimation indicated that the methanol extract with a concentration of 0.25mg/ml, possessed higher concentration of phenolic compounds compared to the other solvents used (389 ± 4 µg GAE/mg dw), ethyl acetate extract having the next highest phenolic content with a concentration of 0.25 mg/ml (267 ± 5 µg GAE/mg dw) and hexane having the least amount of phenolic content at 0.25mg/ml (85 ± 4 µg GAE/mg dw) through the calibration curve with gallic acid.

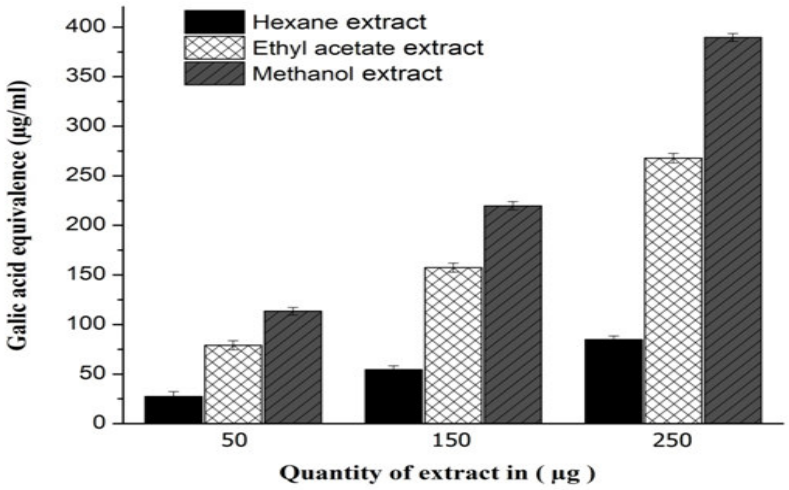


Figure 4
Total phenolic content of leaf extracts of P.ovata expressed as µg/ml of GAE (Gallic Acid Equivalence). Value expressed as ± S.E.M., n=3, p < 0.05.

Thin Layer Chromatography

The solvent system consisting of (chloroform 9: acetone 1: formic acid 0.1: water 0.02) produced only one very light band at 251 nm (Figure 5.A) while 4 bands were seen at 365 nm (figure 5.B), another solvent system using

(chloroform 7.7: methanol 0.873: acetic acid 1.165: water 0.194) gave 2 dark and 3 light bands at 251 nm (figure 5.C) and 11 colored bands were seen at 365nm (Figure 5.D). The retention factor of all the spots have been calculated in Table 5. The best of the solvent systems was chosen and preparative TLC was done (Figure 6), this produced several bands where each band was separated for further analysis (next topic of communication).

Table 5
TLC profile of methanolic exctracts of P.ovata

Sl. No.	Solvent Systems	wavelenth	No. of spots	R_f Values
1.	chloroform 9: acetone 1: formic acid 0.1: water 0.02	251 nm	1	0.65
2.	chloroform 9: acetone 1: formic acid 0.1: water 0.02	365 nm	4	0.1, 0.43, 0.58, 0.7
3.	chloroform 7.7: methanol 0.873: acetic acid 1.165: water 0.194	251 nm	5	0.09, 0.23, 0.33, 0.46, 0.55
4.	chloroform 7.7: methanol 0.873: acetic acid 1.165: water 0.194	365 nm	11	0.05, 0.08, 0.11, 0.13, 0.16, 0.18, 0.22, 0.25, 0.33, 0.42, 0.59

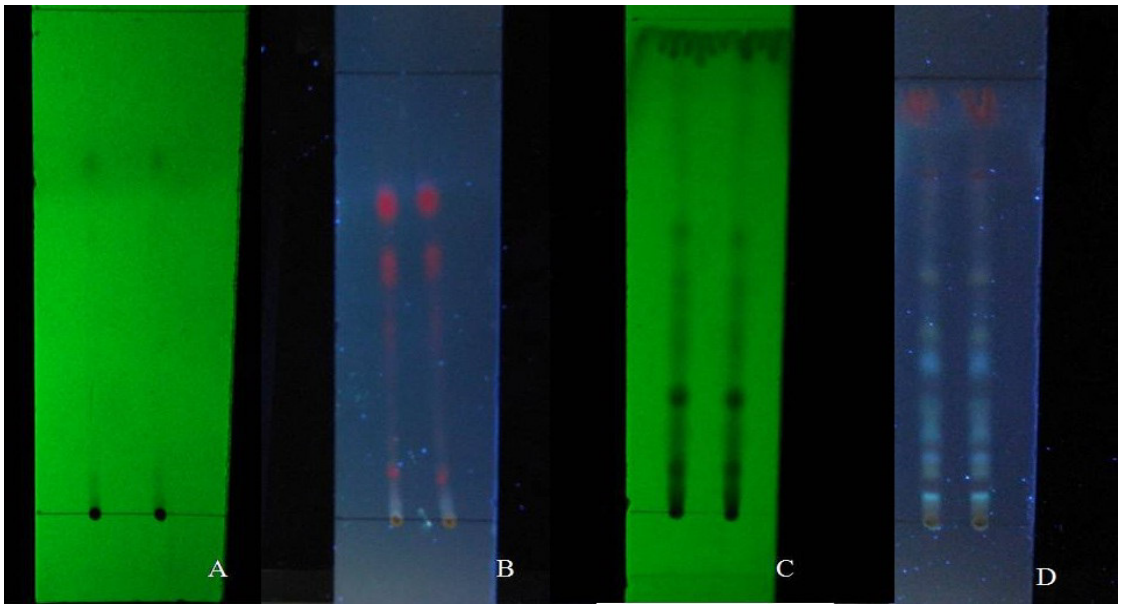


Figure 5
TLC plates showing different banding patterns.

A: 251 nm with one spot,
B: 365 nm with 4 spots (chloroform 9: acetone 1: formic acid 0.1: water 0.02),
C: 251nm with 5 spots, D: 365 nm with 10 spots (chloroform 7.7: methanol 0.873: acetic acid 1.165: water 0.194)

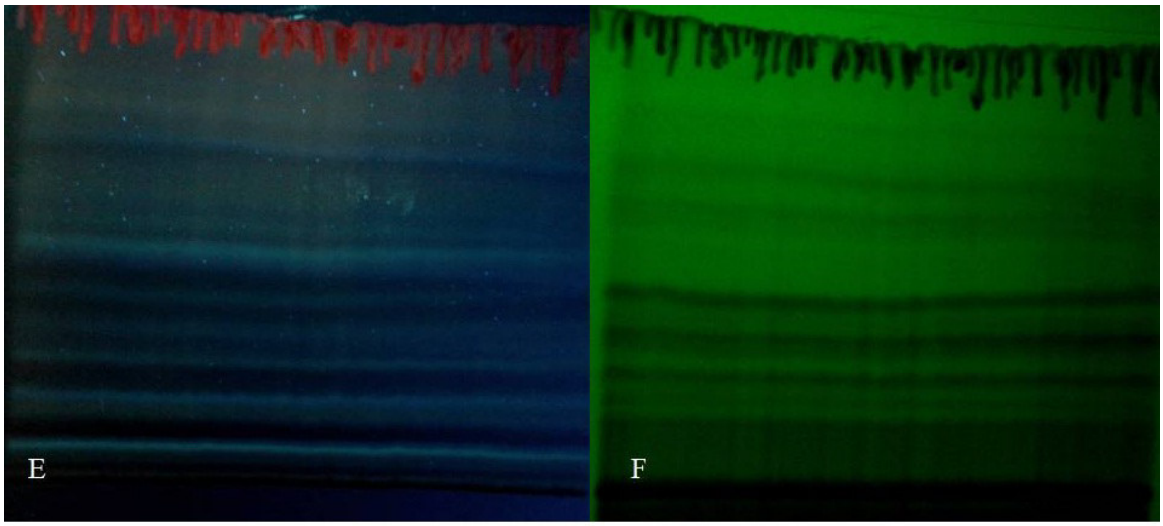


Figure 6
Preprative TLC observed under UV light (251 and 365nm)
E: observed under long wavelength (365nm),
F: observed under short wavelength (251nm)

DISCUSSION

Standardization of a plant material is one of the crucial steps in detection of the sample quality and purity. As no standardization of this plant material has been recorded, therefore a detailed pharmacognostic evaluation is an essential prerequisite for the correct identification of this plant. Macroscopy is one of the quickest and simplest methods of establishing purity and identity; it is based on shape, size, color, surface characteristics, texture, smell and appearance of the material under investigation. Microscopy is indispensable and comes along with macroscopy studies to authenticate the results from external investigation. The morphological features reveales ternate leaves varying from ovate- lanceolate to obtuse in shape which measures 49-61 mm in length and 29-45mm in width, with a rounded to truncate base which is 3 or 5 nerved and this is quite common in the Menispermaceae family as also seen in *Cocculus hirsutus*[25], giving it a multicostate appearance due to reticulate veination. The margin of the leaf is entire, featuring alternate phyllotaxy, emarginated retuse apex and short to medium sized petiole bearing trichomes all around. The abaxial and adaxial surface were almost similar in color expressing a shade of dark green, however as the leaves mature the green color is more intensified. The inflorescence is axillary raceme type, presenting whitish yellow flowers. The fruits are seen in clusters and are green in color turning orange to purple as they ripen. Certain macroscopic features like flowers, fruits and hairy midrib and petiole resembling that of *Cyclea peltata* leaves of the Menispermaceae family[26].

The microscopic studies of *P.ovata* revealed the presence of the stomata to be only on the lower surface and indicated its dorsiventral nature this being a common feature of the menispermaceae family. The stomata was anomocytic in nature and the lower and upper surface of the lamina was covered by thick cuticle; the abaxial surface being heavily cuticular, this similar morphology was seen in the *Cocculus hirsutus*[27]. The midrib was fairly prominent and planoconvex in sectional view. It was flat on the adaxial side and hemispherical on the abaxial part. The epidermal layer of the midrib was irregular shaped followed by sclerenchyma cells which were deposited with lignin. The four layered ground tissue of the midrib consists of a wide circular zone of angular, compact, thin walled parenchyma cells (spongy and palisade) forming a thick bundle sheath surrounding crescent shaped

vascular bundles (xylem and phloem) which were surrounded by sclerenchyma cells. The paranchyma cells of lamina appeared palisade in nature; signifying more chloroplast which explains the dark green color of the upper side of the plant. These characters can be used for standardization and identification of the plant. Similar work on other plants have been reported by Rakholiya *et al.* on *Mangifera indica* [28] Vohra *et al.* on *Lens culinaris medikus* seeds [29] and Patil *et al.* on *Thespesia populnea* roots [30].

The physico-chemical studies are done to ensure uniformity in quality of formulations; it is a parameter in quality control therefore avoiding adulteration of herbal drugs[31]. The percentage of loss of moisture on drying is crucial to be determined as excess water in the medicinal plant material will encourage bacterial and fungal growth, which will lead to deterioration of the material. The moisture content was within acceptable range thus implying the formulation can be stored for a long period and would not easily be attacked by microbes. Determining the ash is useful for knowing the purity and grade of product. Dry ash procedures use high temperatures to convert minerals to oxides, sulfates, phosphate, chlorides or silicates. Acid insoluble ash indicates the amount of silica present in the sample as the ash is mainly composed of non-volatile inorganic components (metallic salts and silica) and this is an indicator of the impurity level[30]. From the results obtained, acid insoluble ash showed a lower percentage (2.01%) in comparison with the acid insoluble ash value of *Heterofragma quadriloculare* leaves (3 %) [32] and much lower value compared to *Mangifera indica* leaf (7%)[28], hence indicating lower amounts of silica; but in comparison with some ferns from the *Adiantum* species it showed a very slight difference in the percentage of acid insoluble ash[33]. The percentage extractives in different solvents indicate the nature and quantity of the constituents in the extract and also help in estimation of specific constituents soluble in a particular solvent[32,34]. A study conducted on *Lens culinaris medikus* seeds showed a value between 1-9% depending on cold and hot extraction [29] while research conducted by Madhavan *et al.* showed much higher value of 13.6% in the case of *Ophiorrhiza mungos* leaves[27]. In our study the less percentage of alcohol soluble extractives (3.56%) indicated low amounts of organic matter present in *P.ovata* almost similar values was seen from *Momordica tuberosa* leaves[35] and *Adiantum capillus-veneris*[33]. Florescence analysis is another rapid method to identify doubtful specimen and are important to check adulteration of the sample. Some substances may not show fluorescence in visible light but by adding some reagents they can be converted to fluorescent derivatives and this can be used to qualitatively assay the sample, similar work has been conducted by Ramesh *et al.* [36]. In case of the Malaysian traditional medicine, Ajisamat, florescence studies were carried out to distinguish between the two different species[37]. The phytochemical studies reveal the chemical nature of the plant, from this research work the major phytoconstituents detected were alkaloids, tannins, flavonoids, triterpenoids and glycosides as also seen with *cocculus pendulus* leaf [38]. However, the plant extract in our study showed relatively less traces of protein and steroids. The presence of alkaloids in this plant has also been confirmed from the work conducted by Dasgupta *et al.* on the leaf of *P.ovata* [4]; similar work has been seen by Reddy *et al.* on *Hymenodictyon orixence* leaf [39].

Phenolic compounds embraces a wide range of plant substances and are very important plant metabolites which commonly posses an aromatic ring bearing one or more phenol groups. Some of these have the capacity to neutralize free radicals by donating a hydrogen atom or an electron in aqueous solutions [40]. From this point of view, phenolic phytochemicals have gained the public attention due to their therapeutic effect which has also led us to investigate on the content of phenols present in *P.ovata.* The phenolic content of *P.ovata*, was recorded to be the highest in the methanol extract (389 ± 4 µg GAE/mg dw) and the lowest in the hexane extract (85 ± 4 µg GAE/mg dw) both having a concentration of 0.25mg/ml; indicating that the solvent used to extract the plant material has a significant correlation with the phenolic content as also seen with

Marrubium peregrinum extract [41]. Phenols show high solubility in polar solvents, therefore increasing the concentration of phenols in the extract [42], which is also evident from our study. Previously some phenolic studies have been conducted on lamiaceous plants such as *Mentha piperita, Melissa officinalis*, *Rosmarinus officinali* [43] Basil (*Ocimum basilicum*) [23], Amaranthaceae (*Amaranthus spinosus*) roots [44] and Menispermaceae plant (*Tinospora cordifolia*) stem[45]. The TLC profile will help in further isolation of the bioactive compound, also using these R_f values the compounds with similar known R_f values can be distinguished and used for further analysis; similar work has been conducted on *Curcuma pseudomontana* rhizome[46]. Therefore, pharmacognostical evaluation provides diagnostical characters which would be useful in identification and authentication of the drug in the herbal industry. The anatomical parameters allows for those who are handling the plant to keep the quality in control. The morphological as well as microscopic studies are the primary steps in establishing botanical standards. The physico-chemical and phytochemical evaluation can be useful in identifting the authentecity, all of these together serve as a standard data for distinguishing *P. ovata* from other similar plants.

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