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Preliminary phytochemical screening and TLC fingerprinting profile of *Physalis angulata*

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Abstract
Background: *Physalis angulata linn.* belonging to family Solanaceae, is wastelands herbal plant, native to north America and south America, widely distributed in India and Nepal. The aim of the present study is to perform macroscopy, microscopy, preliminary phytochemical screening and TLC fingerprinting profile of whole plant of *Physalis angulata*.
Materials and Methods: All the preliminary phytochemical studies, Macroscopy, Microscopy, TLC fingerprinting of *P. angulata* has been performed as per WHO guidelines for herbal drug evaluation.
Results: Preliminary phytochemical screening including physicochemical parameters, macroscopy and microscopy of whole plant of *P. angulata* was performed and significant results observed. TLC fingerprinting profile of *P. angulata* was also developed and phytochemicals were evaluated qualitatively and quantitatively.
Conclusion: On the basis of macroscopy, microscopy and physicochemical evaluation, TLC fingerprinting of selected plant species *P. angulata*, the present study concluded that all the standardization parameters could be helpful for developing plant monograph and correct identification of plant species as well as differention of plant species, to any other adulterant substance or adulterant plant species.

Keywords: *P. angulata*, phytochemicals, pharmacology, pharmacognostical evaluation

Introduction
Physalis angulata Linn., an annual or perennial herb belonging to family Solanaceae. *P. angulata* is native to north America and south America [1]. Several species of the genus have been widely introduced into cultivation in various parts of the world [2]. In India, six species of the genus physalis have been reported, inhabiting to tropical and temperate regions and they are growing naturally in sunny to somewhat shaded fields, pastures, roadsides & wastelands [3]. *P. angulata* is also growing naturally in Nepal specially monsoon season in fields, pastures, roadsides, and wastelands of Terai regions.
P. angulata is widely used in traditionally medicine to cure inflammatory related illness such as dermatitis, asthma, malaria and hepatitis and it is also utilized to treat the analogous sickness in Indonesia, Peru, Mexico and Brazil [4]. *P. angulata* is medicinally important plant used in traditional medicine in Nigeria as anti-pyretic, anti-diuretic and cervicitis treatment [5].
P. angulata, an herbaceous plant possesses several pharmacological activities like immunosuppressive [6], immunomodulatory [7], anti- inflammatory [8], anti-cancerous activity [9], anti-bacterial [2], anti-leishmanial, diuretic, antimycobacterial, antispasmodic, anti-coagulant and anti-hyperglycaemic activity [10, 11, 12].
The significant phytochemicals reported for anticancer and immunosuppressive activity were withanolides; physagulide P and physalin B. similarly other pharmacological activities like anti-parasitic, immunomodulatory activity, anti-inflammatory, anti-mycobacterial, anti-asthmatic, diuretic, anti-leishmanial and hypotensive activity were also reported. Phytochemical constituents reported in *P. Angulata* were seco- steroids [Physalin (D, I, G, K, B, F, E)], withanolides [Physagulins E, F and G], Glucocorticoids, flavonoids, alkaloids & labdane type diterpenoids [13, 14, 15]. It also contains phytosterols, carbohydrate, vitamins, minerals and lipid.

Materials and Methods
The intended material for the study of *P. angulata* was procured from herbal garden of shalom institute of Health and allied sciences, SHUATS, Allahabad, U.P. with the help of field botanist. Healthy plants were selected and uprooted carefully and identified as *P. angulata* by

Department of Pharmacognosy, SIHAS, SHUATS. The leaves, roots and stems of *P. angulata* were collected and washed with water properly and dried in sunlight for 2 hours and further dried in shade. The dried leaves, stems and roots were powdered with the help of grinder.

Chemicals and instruments

Microscope, camera lucida, stage and eye piece micrometre and other basic equipment's like blade, brush, forceps, watch glass, glass slide and coverslips were used for microscopy. Tarred silica crucible, ash less filter paper, Muffle furnace, Digital balance used for determination of ash values. Solvents like conc. HCL, Phloroglucinol, chloroform, conc. Sulphuric acid and dil. HCL were used. The chemical and solvents used for the study were analytical grade.

Pharmacognostical evaluation

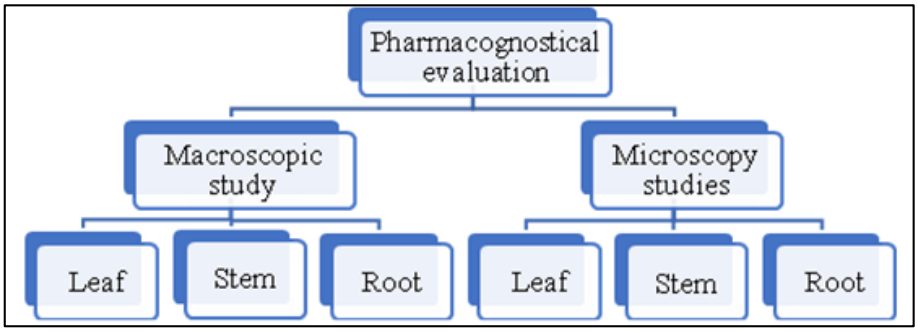

Fig 1:

Macroscopic studies

The morphological characters like shape, size, apex, margin, venation, base, petiole, surface texture (stem & root), colour, odour (Powdered), and taste (Powdered) of *P. angulata* leaves, stem and roots were studied. The macroscopic studies were performed as per Khandelwal [16].

Microscopic studies

Collection of specimens: Healthy plant of *P. angulata* was selected and collected carefully from its natural habitat. The required sample of *P. angulata* leaf, root and stem were cut and removed from the plant and washed with water.

Sectioning: The fresh leaf, stem and root from healthy plant of *P. angulata* were collected and washed with water. T.S of leaf, stem and root were cut with the sharp blade and sections were stained with Phloroglucinol and dil. HCL, mounted with coverslip by using glycerine. The above procedures were performed as per Khandelwal [16].

Photomicrographs: Photographs of the T.S of *P. angulata* leaf, stem and root at a different magnification were taken with microscopic unit. Resolutions 10x and 45x were used for the magnification of the detail view of T.S as well as surface preparation.

Quantitative microscopy

Stomatal number: A stoma is a minute epidermal opening with a central pore and two kidney shaped similar cells containing chloroplasts known as guard cells and varying number of subsidiary cells covering the guard cells. The primary and most important function of stomata is gaseous exchange while transpiration is the secondary function. The average number of stomata per square millimetre of epidermis is termed as stomatal number. Upper and lower epidermis of leaf of *P. angulata* were peeled out separately with the help of forceps and kept it on slide and covered it with coverslip by mounting in glycerine water. After that the slide was kept on stage of microscope for observation and counting of stomata. The result recorded and calculated the stomatal index. Stomatal number were explained as per C.K Kokate [17].

Stomatal Index: The percentage proportion of the ultimate divisions of the epidermis of a leaf which have been converted into stomata is termed the stomatal index. The procedure for stomatal index where performed as per Trease and Evans [18].

$$\text{i.e. } I = S \times 100/E+S \quad \text{.....Eq. (1)}$$

Where, I= stomatal index, S= No. of stomata and E= the number of epidermal cells or subsidiary cell.

Physicochemical evaluation

The powder of whole plant of *P. angulata* was used for the determination of Ash values, loss on drying, extractive value and foaming index. Physicochemical evaluation was performed as per procedure given in WHO guidelines [19].

Determination of ash values

Total ash value: The residue remaining after incineration is the ash content of the drug which simply represents inorganic salts, naturally occurring in drug or adhering to it or deliberately added to it as a form of adulteration. Total ash usually consists of carbohydrates, phosphate, silica and silicates. Weighed accurately about 1 gm of air dried crude powdered drug of *P. angulata* and was taken in a tarred silica crucible. The tarred silica crucible was kept in a muffle furnace and incinerated by gradually increasing the temperature to 500-600 °C until it was white which indicate the absence of carbon. The content cooled and weighed. The percentage of total ash was calculated with reference to the air-dried drug. The above procedure was performed as per WHO guidelines [19].

Water soluble ash: Previously incinerated 1gm of crude powered drug of *P. angulata* was taken and dissolved in a beaker containing 25ml of water and boiled it for 5 minutes. The insoluble matter was filtered out with ash less filter paper and ignited for 15minutes at a temperature not exceeding above 450 °C. The weight of insoluble matter was subtracted from the weight of total ash. The difference in weight represents the water-soluble ash and percentage of water-

soluble ash was calculated. Water soluble ash value was calculated as per WHO guidelines [19].

Acid insoluble ash: Accurately weigh 1gm of powered drug of *P. angulata* which was previously incinerated was boiled with 25ml of 2N HCL for 5 minutes. The insoluble matter was filtered out with ash less filter paper, washed with hot water, ignited and weighed. Then, percentage of acid insoluble ash was calculated. Acid insoluble ash was calculated as per WHO guidelines [19].

Determination of solvent extractive values: 1 gm of powered drug of *P. angulata* was macerated with 20ml of different solvents (90% alcohol, 90% chloroform) in closed flask for 24 hours. It was shaken frequently during first 6 hours and then allowed to stand for 18 hours. Thereafter it was filtered and 5ml out of filtrate was evaporated to dryness in tarred flat bottom dish at 105 °C and weighed. The solvent extractive value was calculated with reference to the air-dried drug. Solvent extractive values were determined as per WHO guidelines [19].

Moisture content determination: About 3gm of powered drug of *P. angulata* was accurately weighed and was taken in a tarred porcelain dish. The powered drug was dried at 105 °C using hot air oven until constant weight was achieved. The percentage loss of drying was calculated by using difference in weight with the reference to air dried drug.
Preparation of Solvent Extracts

Method of extraction: Successive solvent extraction (Continuous hot percolation) technique was employed using Soxhlet apparatus as per WHO Guidelines.
Solvents and Materials:

- Petroleum ether
- Chloroform
- Ethanol
- Set of Soxhlet apparatus
- Dried seeds of *P. angulata* fruits.

Extraction Procedure

Preparation of petroleum ether extract: The dried seeds of *P. angulata* fruits weighed approximately about 50gm were extracted with pet. ether (65-85 °C) until maximum extracts were obtained. The solvent was recovered by distillation at 60-80 °C. The reddish-brown colour extract obtained, reflects the colour of its marc. The percentage yield of the extract was noted and extracts were finally stored for subsequent analytical use.

Preparation of chloroform extract: The marc left after pet. ether extraction was separately dried and then subjected for extraction with chloroform (54-58 °C). After the extraction was finished, the chloroform solvent was recovered by distillation. The extract was brown in colour and stored for future use. Percentage yield were recorded.

Ethanol extract: Similarly, the dried seeds left behind marc of chloroform extraction were further extracted with ethanol 99.9%v/v (75-78 °C). After the completion, the solvent was recovered by distillation process. Yellow colour extract was obtained and the percentage yield was recorded.

Thin layer chromatography (TLC) fingerprinting studies

The principle of separation in TLC is basically adsorption, particularly when silica gel G is used as stationary phase, and partly with partition. Mobile phase solvent typically flows through the stationary phase because of capillary action. The components move and get separated according to their affinities towards the adsorbent. The component having more or less affinity towards the stationary phase travels slower or faster respectively. TLC constantly proves useful in studies, and assessing quality and purity, of herbal medicines, generally due to its simplicity, cost effectiveness, and speed. TLC studies carried out for different ratios help to precisely acknowledge the solvent system capable of showing best showing best resolution in separating the various components of the sample plant under investigation.

Preparation of slurry and plates

The slurry, a mixture of silica gel G (150gm) and water (250ml) was prepared. The prepared slurry was poured on to TLC plates and spread to get uniform thickness. The plates were allowed for air dried, approximately 10min after that the plates were kept inside Hot-air oven at 100-105 °C for 1hour for activation of plates.

Preparation of sample: Small quantity of Pet. ether, C.E & E.E extracts were taken and dissolved in their respective solvents as per required concentration of the samples.
The above prepared sample extracts solutions were applied on the TLC plates by using capillary tube and immersed in the TLC jar containing suitable solvents system (Mobile Phase), covered and allowed to develop. The developed TLC plates were allowed for air dried and then kept inside UV-inspection chamber for visualization of the separated bands. Then, the R_f values were calculated for each different spot observed.

R_f Value = Distance travelled by solute / Distance travelled by solvent……Eq. (4)

In some cases, the spot was not clearly separated and might be large in size such that direct use of the above formula could not give the actual R_f value. Therefore, average R_f values of the upper spot and lower spot were calculated using the below modified formula;

R_f Value= (Upper spot distance + Lower spot distance)/ Distance travelled by solvent.

A number of combination of solvents in different proportion were tried by trial and error method in attempt to develop standard solvent system capable of separating the components best in perfect resolution.

Results

Macroscopy of *P. angulata* Leaf

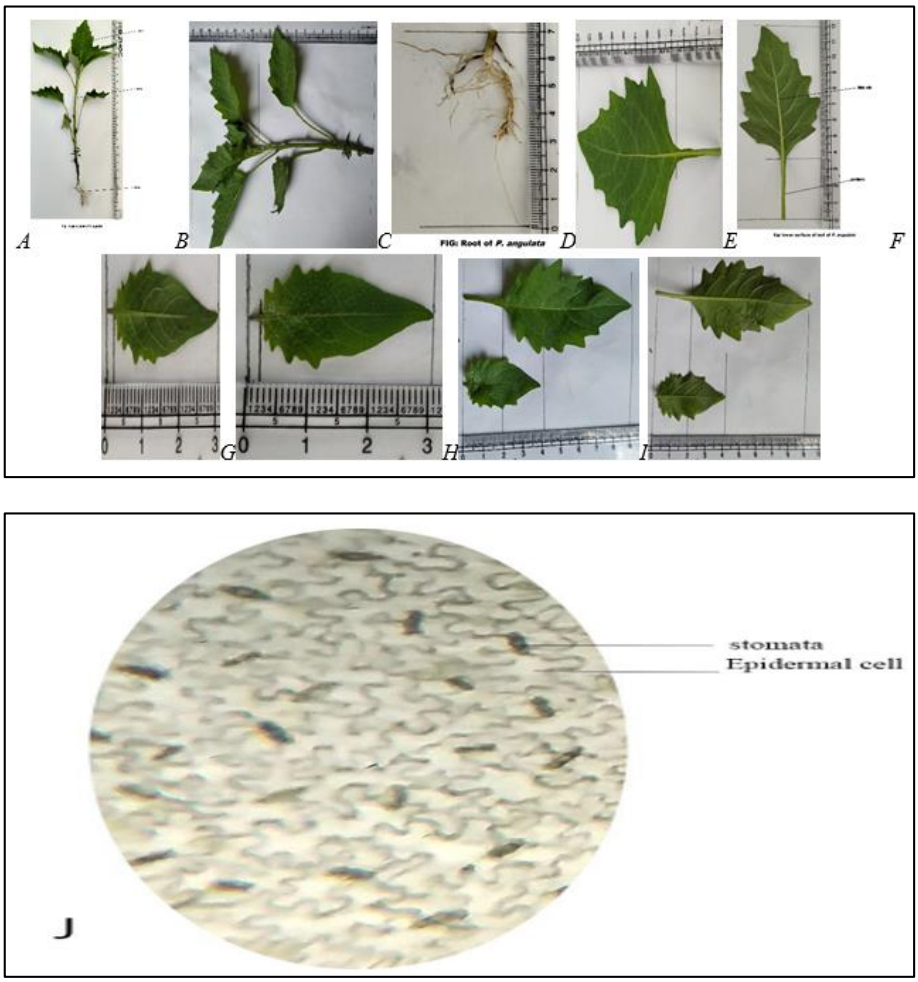

Fig 1: A is whole plant, B is upper part of *P. angulata*, C is root, D and E is upper and lower surface of leaf, F and G is upper and lower surface of leaf, H and I is comparison of upper and lower surface of leaf of *P. angulate*, J Stomata present in lower epidermis.

Table 1: Macroscopically characters of *P. angulata* Leaf

Parameter	Observation
Type Shape	Simple Ovate
Size	Length: 2.9cm -8.4cm
Apex	Acute
Margin	Irregularly toothed
Venation	Reticulate
Base	Cuneate
Petiole	0.2-3.6cm
Color	Upper surface was slight dark green than lower surface. Lower surface: light green

Microscopy of *P. angulata* Leaf

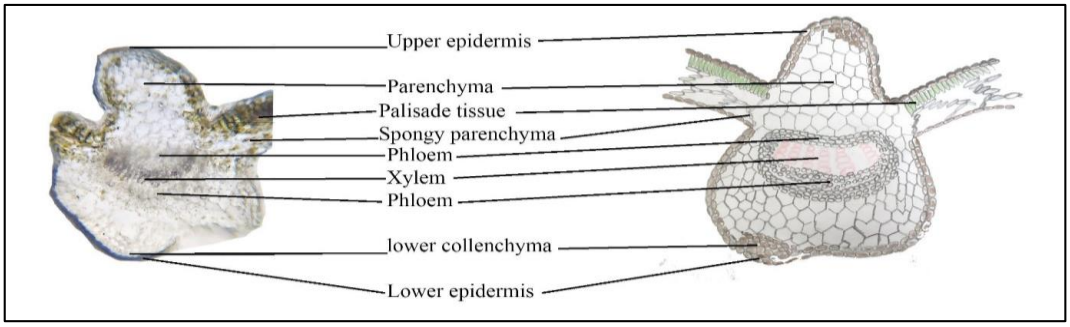

Fig 2: T.S of leaf of *P. angulata*

Microscopy of Leaf (T.S) passing through Mid-Rib

Epidermis: It consisted of single layered upper and lowered epidermis made up of parenchyma cell. Trichomes were absent. The Parenchyma closely packed without any intercellular space, thin layer of cuticle.

Collenchyma: Collenchyma 2-3 layered was observed below upper epidermis and above lower epidermis. The rest of the portion occupied by unequal layer of parenchyma. There was 4-7 layers of parenchyma cells towards the upper epidermis side and 2-4 layers of parenchyma towards lower epidermis.
Vascular Bundle: Centrally situated vascular bundle which was c-shaped, bi-collateral (phloem-xylem-phloem) and consisted of well-developed xylem vessels. Metaxylem observed towards the lower epidermis side while protoxylem towards the upper epidermis side. Piths were absent.

T.S of mesophyll section of leaf
Epidermis: It consisted of upper epidermis and lower epidermis, single layered parenchymal cell, upper and lower epidermis closely packed without any intercellular space. Numbers of stomata were more in lower epidermis as compared to upper epidermis and type of stomata reported as anomocytic and each guard cells were surrounded by 2-5 number of subsidiary or epidermal cells. The outline of epidermal cells was wavy in nature. Upper epidermis cells were less wavy as compared to lowered epidermis.

Palisade Tissue: single layered palisade cells were observed towards upper epidermis cells. Cells of epidermis filled with chlorophyll, closely packed without any intercellular space, more in length in comparison to breadth. Palisade layer discontinued in the mid-rib section of the lamina.

Spongy Parenchyma: Mesophyll portion of the leaf occupied by spongy parenchyma. Cells were oval in shape with intercellular space.

Table 2: Macroscopy of *P. 52 ngulate* stem

Parameter	Observation
Type	Herbaceous
Shape	Cylindrical
Size	10-60cm
Surface texture	Slightly segmented
Colour	Green

Microscopy of stem of *P. angulate*

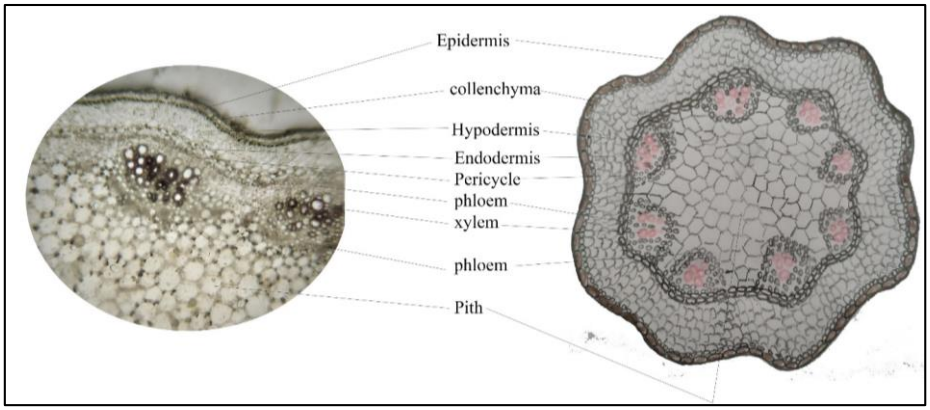

Fig 3: T.S of stem of *P. angulata*

Microscopy of T.S of stem (From periphery to center)
Epidermis: Single layers made up of parenchyma cell which was oval or rectangular in shape, closely packed without any intercellular space trichome were absent. Outer line of the epidermis was wavy in nature and consists of grooves. The outer surface of stem covered with thin layer of cuticle.

Hypodermis: collenchyma, 1-2 layer made up of collenchymatous cell and closely packed. Hypodermis cells were made up of parenchyma, 4-6 layered, cells were oval in outline, smaller in size towards the peripheral region and bigger towards the inner size.

Endodermis: Endodermis was single layered, wavy and made up of barrel shaped parenchyma cells.

Pericycle: Pericycle was just below endodermis and made up of single layered.

Vascular bundle: The no. of vascular bundle ranges 6-8 in number and each vascular bundle was located below grooves of the stem. Well-developed bi-collateral type of vascular bundle was observed (phloem-xylem-phloem). Metaxylems were present towards peripheral region while protoxylem towards central axis, well developed xylem vessel with deposition of lignin was observed. All elements of the xylem were observed.

Pith: It occupied major portion of stem. It consisted of large size oval or spherical shaped cells, closely packed with very less intercellular space.

Table 3: Macroscopy of *P. angulata* Root

Parameter	Observation
Type	Tapped root with small rootlets
Shape	Irregular, cylindrical
Size	5-9cm
Fracture	Short
Colour	Yellowish white (outer surface), creamy white (inner surface)

Microscopy of *P. angulata* root

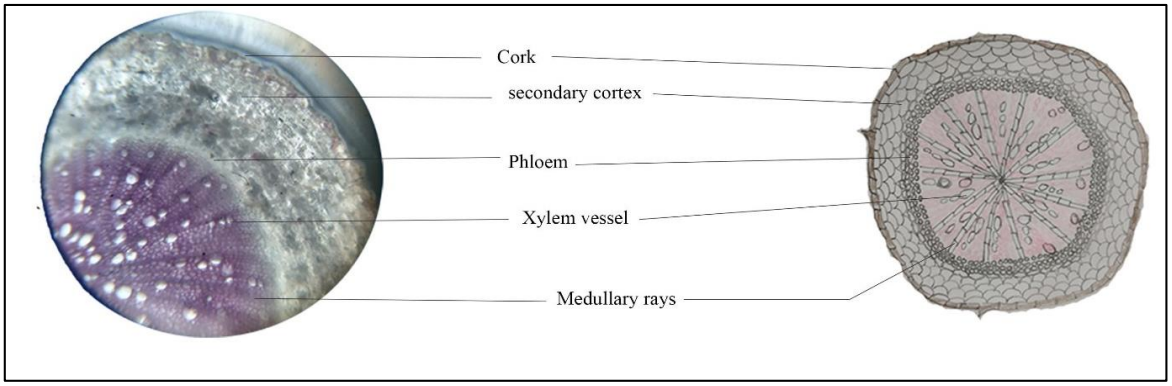

Fig 4: T.S of root of *P. angulata*

Microscopy of root of *P. angulata*

Cork: It was the outermost single layered with several unicellular root hairs. It consisted of thin walled, compactly arranged parenchyma cells. It also consisted of thin cuticle which prevents plant surfaces from becoming wet and helps top prevent plants from drying. Unicellular root hairs help in absorption of water and minerals from soil.

Cortex: cortex consist of 4-5 layered parenchymatous cells without inter-cellular spaces. It did not contain chloroplasts but contained leucoplasts for storage of starch grains. Cortex is responsible for transportation of water and salts from the root hairs to the centre of the root.

Endodermis: Endodermis was single layered and compactly arranged. It was also indistinctly arranged with phloem.

Vascular bundle: Vascular bundle was well developed. It occupied 2/3rd of the whole transverse section of root. Metaxylem was observered towards center and protoxylem towards periphery (Exarch). It had well developed xylem vessels, parenchyma. The medullary rays radiating from center to periphery.

Powder microscopy

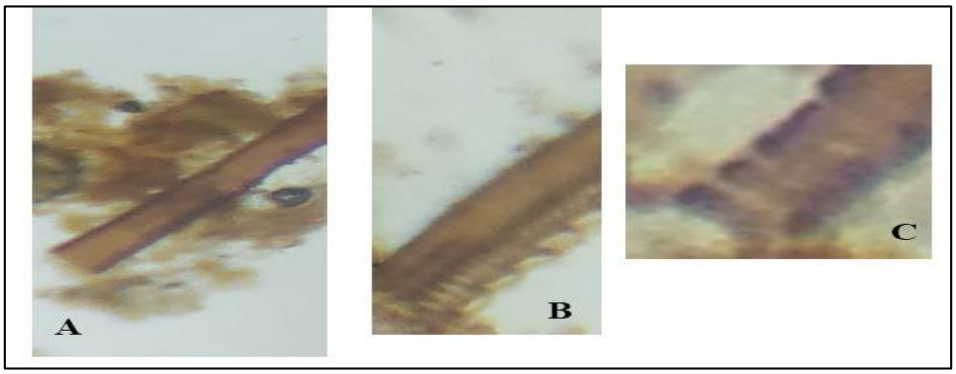

Fig 5: T.S of Powder Microscopy

In powder microscopy of *P. angulata*, bordered pits were observed in xylum vessel as shown in fig 5A. In fig 5B, bordered pit, spiral xylem vessel and oval shaped parenchyma were compactly arranged. Fig 5C represented the spiral shaped xylem vessel.

Quantitative microscopy of leaves of *P. angulata* includes stomatal number and stomatal index. Results shown in table 4 and physicochemical parameters shown in table 5.

Table 4: Leaf constant for *P. angulata sp.*

Leaf constants of lower surface	Value
Stomatal number	4-6
Stomatal index	28.57

[Note: In upper surface, they were very few stomata. Stomatal number in upper surface of leaf was 1 to 2]

Table 5: Physicochemical constant of *P. angulata sp.*

Analytical parameter	Observation
Total ash value	21.835%w/w
Water soluble ash	46.89%w/w
Acid insoluble ash	17.11%w/w
Solvent extractive value (Chloroform + ethanol)	11.13%w/w
% Moisture content	7.49%w/w

Table 6: Percentage yield of successive extraction of seeds of *P. angulata.*

Analytical parameter	Percentage Yield
Pet. ether extract	2.492%w/w
Chloroform extract	1.516%w/w
Ethanol extract	2.03%w/w

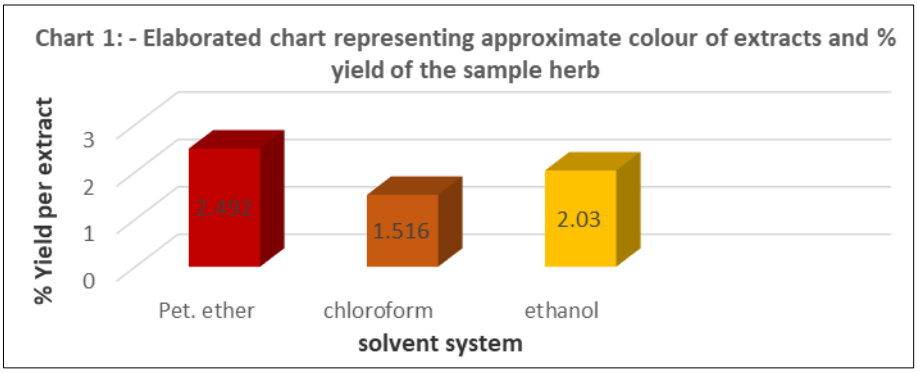

Fig 6: Graphical representation of percentage yield of extracts shown below

Development of solvent system

Various solvent systems were repetitively adjusted in different proportions in order to develop standard suitable solvent system; capable of separating individual components from the respective solvent extract of *P. angulata* seeds.

For Pet. ether extract; firstly Toluene: ethyl acetate: formic acid [7:3:1] was the solvent system used as mobile phase. Three spots were observed but the spots were not clearly separated from each other. Then same solvent system but in different ratio 7:2:1 was used. Again 3 spots were observed and cleared enough than first one. Again, the same solvent system but in the ratio 7:1:1 was used. Only 2 spots were observed.

For Chloroform extract; different solvent system such as Benzene: ethyl acetate: methanol: formic acid [12:4.5:2:1.5], chloroform: methanol [6:4], and Pet. ether: chloroform [2:2] were used. All the solvent system except Pet. ether: chloroform [2;2] were unsatisfactory because no spots were observed there; some of them only showed the movement while some did not. only one spot was observed in the Pet. ether: chloroform [2:2] solvent system.

For Ethanol extract; solvent system chloroform: ethanol was used in different ratio i.e. [9:1], [9:2] & [9:3].

After performing the TLC of Pet. ether, chloroform and ethanol extracts of *P. angulata*, different spots were observed and its R_f values were reported and represented in the table 8.

Table 7: Representing R_f values and no. of spots separated in the respective extracts.

Extracts	No. of spots	R_f Values
Pet. ether	3	0.93, 0.78, 0.72
Chloroform	1	0.67

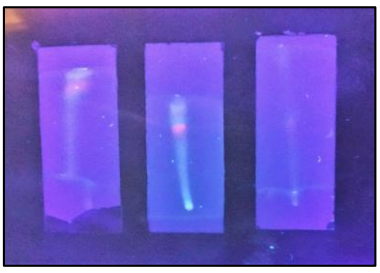

Fig 7: TLC plates showing separation of spots in the solvent system, Toluene: ethyl acetate: formic acid in different ratio [7:3:1], [7:2:1] & [7:1:1]

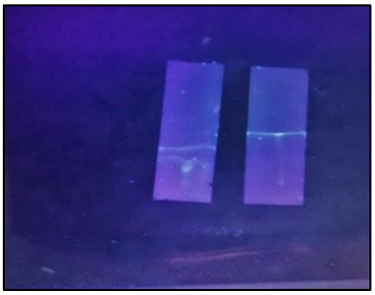

Fig 8: TLC plates showing separation spots of chloroform extract, in solvent system Pet. ether: chloroform [1:1].

Conclusions

On the basis of macroscopy, microscopy and physicochemical evaluation, TLC fingerprinting of selected plant species *P. angulata*, the present study concluded that all the standardization parameters could be helpful for developing plant monograph and correct identification of plant species as

well as differention of plant species, to any other adulterant substance or adulterant plant species.

The current study of this plant limited towards preliminary phytochemical analysis and herbal drug standardization. Futuristic approach may be focused towards activity guided isolation of potent phytochemicals and development of suitable novel dosage form, for treatment of wide spectrum of challenging diseases.

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