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Pharmacognostic profile of *Pogostemon auricularis* (L.) Hassk

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Abstract
Herbs are rich source of primary and secondary metabolites which are vital for plant growth as well helpful to other living being with additional medicinal values. *Pogostemon auricularis* plant with historical background of medicinal status selected for further research work. Plant's Macroscopical, Chemo Microscopical study conducted as a proof reading of its identity. Moisture free pulverized leaves of *Pogostemon auricularis* evaluated for physical nature like: extractive value, ash value, moisture content, swelling index. On basis of obtained results methanolic extract were screened for phytochemical study. Microscopy study revealed the presence of covering trichome on ventral surface, dorsal lateral with glands like out growth, parenchymatous tissue in mesophyll, collateral type of vascular bundle at the midrib. *Pogostemon auricularis* leaves Methanolic extract found with presence of Proteins & Amino acids, saponins & cardiac glycoside, tannins, phenolic, flavonoids, alkaloids and sterols. Alcohol Soluble macerates, Water soluble macerates, Methanol soluble were correspondingly 7.2 % w/w, 8.2% w/w, 24.7% w/w. Water soluble ash, Acid insoluble ash, Total ash, 7.82 % w/w, 1.45 % w/w, 20.78 % w/w respectively. present moisture content was 2.71 % v/w. calculated swelling index for PA was 2.6 % v/v. The results of TLC disclosed the phenolics retained at R_f value 0.50 comparable with gallica acid reference standard at 0.53. Tannins presence mirrored on calculating R_f value on 5 % $FeCl_3$ spray 0.64 with Tannic acid run along with it and retained at 0.67 on the same plane. For flavonoids observed R_f value 0.36 in comparison to Rutin as standard moiety reflected its presence at 0.35 treating with Anisaldehyde – H_2SO_4 spray. Quantitative estimation revealed the present Total phenol 56.45% w/w, Total flavonoids 52.33 % w/w, Total tannins 13.17 % w/w.

Keywords: *Pogostemon auricularis*, macroscopical, microscopical, swelling index, preliminary analysis

Introduction
Traditional health care practice using herbs has served new direction to militarize quality life and good health. Medicines prepared with herbals have added valuable attribute to man's tough journey to counter ill condition as well preventing more damage. Not so long-ago people have developed more interest in the use of herbal preparations. Growing interest → demand → production → consumption of herbal preparations as medicines have elevated considerations for the making governing provisions and actions [1].
Evidence of herbal medicinal plant was found in 1960 around the coffin of Neanderthal man. Coffin was surrounded with eight plant species, which are still used in modern system of medicine. It is said that 60,000 years ago it came in practice. Different culture and system have their own belief about use of medicinal plant. Initially by 3500 BC it was found as magic in Egyptians. In 2700 BC Chinese system started providing scientific base to use of plants for its curing action. In 1550 BC remnants was found with 700 formulations named Ebers papyrus. Gradually more information found about plant formulation and its mechanism of treatment. "Father of Medicine," - Hippocrates, categorized 300-400 herbs on basis of its nature hot and cold, moist and dry.

Pogostemon auricularis
The plant was selected from the ancient available literature. The Indian medicinal plants have mention about *Pogostemon auricularis* (L.) hassk species (S.N yoganarsimhan, 1996). Synonyms of mentioned plant are *Mentha auricularis* L. Mant., *Dysophylla auricularis* (L.) Blume belongs to family Lamiaceae. Its known with Various names in different region of world like: Bengali: Acha-kamsen, Chinese: Shui zhen zhu cai, Indonesian: Ke kucing, Malaysian: Kekucing, Thai: Saapraeng saapkaa.

As per botanist; Taxonomic position of *Pogostemon auricularis* is Kingdom: Plantae, Phylum: Magnoliophyta, Class: Angiospermae, Order: Lamiales, Clade: Asterids, Family: Lamiaceae, Sub family: Lamioideae, Genus: *Pogostemon*, Species: *auricularis.*

In India it is habitat in Bellary, Mysore, Chikamagalur, Bihar, Assam, south India, Kodagu, Sikkim, Maharashtra, west Bengal, Khasia, Assam, Kerala, Garo Hills) Sambalpur, Shimoga, Godavari District and fully-fledged naturally in wastelands. Worldwide it is found at South East Asia, Bangladesh, Pakistan, Afghanistan, Japan, Malaysia, China. Plant fluorescence and fruit setting happens in February - July. Medicinally it was involved in various Ayurvedic preparations like; paste with lime applied to infants and kids' abdomen to get rid of stomach disturbance. Hot decoction given in Rheumatism. Traditionally used to relieve stomach ache, Fever, cough, wound healing, sedatives, heart diseases, digestive aid, appetizers, chicken pox, anticonvulsant, carminative, dysmenorrhea, headaches, diuretic and tetanus. Leave juice used in hysteria, eye infection. *Pogostemon auricularis* traditionally used to treat snake bite. In current scenario it is safe alternative anti-diarrheal agent.

Collection and authentication of herbs

Pogostemon auricularis (PA) was identified and collected from its natural occurrence. Forest area near government college of science, kolli hills, Tamil Nadu, India, is the natural habitat for taken species. It was found in chemical reach stage of its growth. Government botanist Dr. V Nandagopalan helped in collection and verifications of plant. Research scholar Mrs. Kamleshwari form the same department extended help for Identification of different microscopical characteristics of PA leaf.

Materials and Methods

After the collection and deposition of specimen copy to herbarium the treatment was given to collected aerial parts of plant. Leaves were made moisture free in bloom. Shade dried leaves were reduced to its small pieces and again dried. Powder pass through sieve 60# to 40#. powered samples stored in moisture free, fungus free and tightly closed container. Dried powder material stored at normal room temperature.

Macroscopical evaluation

Entire collected plants were exposed to sensory evaluation; Colour, odour, taste and appearance. leaves characteristics including shape, size, margin, and venation were also checked.

Chemo microscopical evaluation of leaves [2]

Fresh leaf of selected species PA was studied for their micro arrangement of various tissue. Detailed arrangement of tissues and cell were seen under 100 and 400 magnification power. Powered characters were also added in.

Prepared Microscopical section was cleaned and kept hydrated in small watch glass. It stained with Phloroglucinol and conc. HCL (2:1) and observed for lignified tissues. Treatment with Iodine, picric acid, Conc. H2SO4, Sudan III. (Department of botany, university of hawai, manoa). Sections were kept in hydrating medium to prevent the air entrapment. Edges were sealed with freezing material.

Physical assessment

Extractive value [3]

Requirements: Ethanol, Methanol, Distilled water, Stoppered glass flask, Glass funnel, evaporating bath, measuring cylinder, Desiccator, weight balance, Evaporating dish, Mechanical stirrer.

Alcoholic extractive value

Extractive value was performed by using cold maceration with Ethanol and Methanol. 5 gm of air-dried powdered sample was socked with 100 ml of Ethanol and Methanol in two different assembly. For first 4 to 6 hours often it was shaken and then kept steady for next 16 to 18 hours. On completion of 24 hours of maceration, assembly was bought out of dark room and stirred vigorously. Quickly Entire solvent was strained and mark was discarded. 25 ml of filtrate was transferred to evaporating plate. Solvent evaporation continued till it bought to semisolid consistency residuals. Residue was kept in desiccator immediately in hot condition only. % extractive amount was calculated relating with air dried powdered test samples.

Aqueous extractive value

As its mentioned above extractive value was calculated for water soluble content. Here as extractive solvent 100 ml water mixed with 10 ml chloroform. Solvent evaporation was kept at 105 ℃, in context to vaporized temp of solvent, water.

Ash value

Requirement: dil. Hydrochloride acid, silica, platinum crucible, Beaker, Muffle furnace, strainer, Desiccator, Tripod stand, Sand bath, Sand bath, Ash less filter paper (what no. 41), Microwave protective hand gloves

Total Ash value

2 gm of powdered test sample was dried and placed in silica crucible and lid was closed. Assembly placed in muffle furnace at temp set was 410 ℃. Once it reached desired temperature, sample was charred for next 4 hours continuously. Difference in weight is answer to total ash content.

Acid in soluble Ash value

Total ash in gms dissolved in 25 ml concentrated HCL with continuous stirring. It transferred to silica crucible and reheated for next 15 mins at same above-mentioned temp. condition. It was filtered through Watman filterer and residual was weighed gave the quantity of acid insoluble ash.

Water soluble Ash value

Total Ash transferred to 25 ml to mild hot water and stirred to dissolve added material. Again, assembly was heated and filtered. Filtrate was kept aside to see any sedimentation and residuals were dried and weighed. Final amount of residuals were subtracted from total taken ash and that is the answer of water-soluble part of ash

Moisture content determination (LOD)

Requirements: Glass plates, Glass marker, Hot air oven, Desiccator, Weigh balance It was done by gravimetric method. 2-3 gm of dried pulverized sample was placed in preweighed, clean flat bottom glass plate. Evenly spreaded Sample was transferred in hot air oven sustained at 105 ℃ for the duration till got constant mass of placed assembly.

$$\% \text{ Moisture Present} = \frac{\text{Initial weight of assembly} - \text{final weigh of assembly}}{\text{Initial weight of assembly}} \times 100$$

Assembly = Weight of powered sample + empty glass plate
Initial weight = Before placing in hot air oven
Final weight = Weight of the assembly at the end of study

Swelling index
Requirements: Calibrated stoppered measuring cylinder, Calibrated scale, Stirrer, Weigh balance Placed 1 gm of sample to the cleaned and dried measuring cylinder. water was poured up to the mark 20 and stir gently. Give occasional stirring in first 4 to 6 hours. Allowed it to steady up to 24 hours on flat surface. On completion of 24 hours measured the rise in volume in reference to the volume maintained before 24 hours.

$$\text{Swelling Index} = \frac{\text{Final volume} - \text{Initial volume}}{\text{final volume}} \times 100$$

(Value in ml)
Initial volume = 1gm sample + 20 ml water
Final volume = Rise in volume after 24 hours

Extracts preparation
Requirements: Soxhlet assembly, RBF, measuring cylinder, Glass funnel, Heating mantel, Rotary evaporator, Wide mouth bottle, Evaporating dish, Desiccator, Methanol Around 300 gm of PA powdered sample was used for preparing extract. In hot continuous extraction process mentioned quantity of drug sample and 3 cycle of Methanol as extracting solvent used at temperature of heating mantle maintained NTM 45 ℃. Till complete accumulation of active desired metabolite process continued. Solvent evaporated on hot water bath and residual extractives stored in air tight contains.

Preliminary chemical scrutiny of extracts [4]
Qualitative analysis
Prepared methanolic extract of *Pogostemon auricularis* was treated with various reagents to check the presence of chemical constituents belonging to particular category of primary and secondary metabolites. performed test are mention below.
Requirements: Glass test tubes, test tube holder, pipette, beaker, separating funnel, UV chamber, Tripod stand, glass rod, Hot air oven, water bath, glass marker, match box, Standard literature, Analytical scale reagents

Test for carbohydrates: Molisch's test, Fehling's test, Benedict's test, Barfoed's test, (for Non-Reducing Polysaccharides) Iodine test, Tannic acid.

Protein: Millons Test, Biuret Test, lead acetate test

Amino Acids: Millons Test, Biuret Test, lead acetate test, Ninhydrin Test, glyoxylic acid test.

Alkaloids: Dragendorff's, Mayer's, Hager's, Wagner's tests. (Common extract treatment) In one test tube Methanolic extract liquefied with equal quantity of 1% hydrochloric acid and put it on burner for heating. Test tube was Cooled down and used further for alkaloid presence.

Glycosides: General test to distinguish glycone and carbohydrate moieties, Test for Cardiac glycoside: Baljet's test, Raymond test, Legal's test, Keller killiani test, Libermann's test (bufadenolid).

Anthraquinone glycoside: Borntrager's test, modified Borntrager's test

Tannins and Phenolics: Lead acetate, 5 % Ferric chloride, Bromine water, Gelatine

Flavonoids: Shinoda test, Chloroform and H_2SO_4, zinc dust and conc. HCL, NaOH.

Vitamins identification: Vit A, Vit D.
Phyto sterols ant triterpenoid: Salkowski reaction, Libermann's, Libermann-Burchard test

Chromatography Study:
After preliminary phytochemical investigation *Pogostemon auricularis* evaluated further to confirm the presence of secondary metabolites. if it performed precisely Thin layer chromatography helped best to ensure the presence of chemical moieties.

Requirements: Glass plate 10 x 20 cm, Glass beaker, Glass rod, vaporizing chamber, Hot air oven, glass spreader, Glass development chamber, spraying bottle, Small glass capillaries, micro pipette, Test tube, distilled water, Silica Gel.

Thin layer plate preparation
TLC plate was prepared by using silica gel G. G stands for gypsum which help to make bond between silica and glass plate. Slurry was prepared of spreadable consistency with water. Slurry was poured to one end of clean glass plate, kept on slanted manner. The position help slurry to move down by covering entire surface and further with Glass applicator help in spreading and achieving thinness of 0.4 mm. coated plate was kept at 110 ℃ for an hour for activation.

TLC chamber preparation
Simultaneously TLC developing chamber made ready by adding mobile phase in set proportion. TLC chamber lid was placed and allowed for saturation with mobile phase vapours. The process checked with placing small strip of paper, wetting in upwards direction confirms readiness of chamber.

Spotting
Dried plate was brought out for spotting. Small amount of extract mixed with methanol (to liquefy) and strained. With help of micropipette band were placed on activated coated plates. Spotting was done around at the height of 2 cm from bottom.

Development of chromatographic plate
Lid was open and activated plate was placed in the chamber and immediately lid was replaced tightly. Mobile phase run on capillary force, as it reached to 80 % height of the plate process stopped and TLC plate was removed and kept for drying.

Detection
Dried plate was sprayed with detecting reagent and Visualized

for any colour change of spot.

Phenolic compounds [5]
Study was done to check phenolics present in selected plant species by using silica coated plates and various chemicals.
Parameter:
1. Stationary phase: Silica gel GF254 (Adsorbent)
2. Reference standard: Gallic acid
3. Plate dimension: 10 x 20 cm
4. Thickness: 0.4 mm
5. Stimulation temperature: 110 ºC for one hour
6. Mobile phase: Toluene, Acetone and Acetic acid (3:2:1)
7. Detection: Vaniline-sulphuric acid
8. Absorbance / Wavelength 366 nm

Tannins compounds [6]
Study was done to check phenolics present in selected plant species by using coated plates and various chemicals.
Parameter:
1. Stationary phase: Silica gel GF254 (Adsorbent)
2. Reference standard: Tannic acid
3. Plate size: 10 x 20 cm
4. Breadth of prepared plate: 0.4 mm
5. Instigation temperature: 110 ºC - one hour
6. Mobile phases: water-methanol-chloroform (8:35:67, v/v/v)
7. Detection: 5 % $fecl_3$ / 0.5 % Vanilin (methanol) – 4% HCl

Flavonoid compounds: Study was done to check phenolics present in selected plant species by using coated plates and various chemicals [7].
Parameter:
1. Stationary phase: Silica gel GF254 (Adsorbent)
2. standard: Rutin
3. Plate size: 10 x 20 cm
4. Thickness: 0.4 mm
5. Plate promoting temperature: 110 ºC - one hour
6. Mobile phase: Formic acid: Ethyl acetate: Water: glacial acetic acid (11:8:100:27: 8)
7. Detection: anisaldehyde-sulphuric acid / iodine vapor
8. Colour: yellow, green or blue fluorescent

Quantitative analysis
Estimation of total phenolic content (TPC) [7]
Method: Spectrophotometric Apparatus required: volumetric flask (100 ml, 50 ml, 10 ml), Glass rod, Pipette, Beaker Reagent required: Folin Ciocalteu reagent, NaHCO3 solution, Gallic acid (Calibration curve)

Procedure for test sample
To measure the TPC, 1 mg/ml stock solution was prepared. 0.5 ml aliquot transferred to small volumetric vessel and mixed with 2.5 ml Folin Ciocalteu's reagent (10 % medium water) and 2.5 ml NaHCO3 (8-10 % aqueous solution). Preparation was incubated at 45 ̊C for 50 Mins in thermostat. To confirm the present amount of TPC, absorbance was checked at 765 nm wavelength.

Standard solution: Standard solutions of ranging concentration (2,4,6,8,10,20,50,100 µg/ml) of gallic acid prepared. From prepared extract 0.5 ml transferred to all labelled test tubes and blend prepared by adding rest of the reagents. Incubated for 1 hr and checked for their abs. readings are plotted to establish calibration curve. Outcome of the test sample was extrapolated on graph of std. gallic acid. Unit followed as (mg of gallic acid /g of extract).

Appraisals of total flavonoid content [8]
Estimation method: Spectrophotometric Requirements: volumetric flask (100 ml, 50 ml, 10 ml), Glass rod, Pipette, Beaker, Rutin (Reference standard), (Analytical grade reagents; AlCl3.6H2O solution, Aqueous NaNO2 solution, Potassium acetate, Methanol.

Preparation of Test solution
1 ml aliquot of PA Methanolic extract placed in six separate volumetric flasks. 3 ml of methanol, Aqueous $NaNO_2$ solution 0.3 ml (1:20, w/v). 3 ml of $AlCl3.6H_2O$ solution was also added to each six-test tube. For next 5-7 mins it was allowed to complete reaction. 2 ml of NaOH was mixed in it and capacity was made up to 10 ml by adding methanol. Abs of test preparation was checked at 510 nm with UV-VIS spectrophotometer.

Standard solution
TFC of test sample was calculated from the Rutin calibration curve. 100 mg Rutin initially solubilized in 10 ml distil water and marked up to 100 ml for Stock solution preparation. Aliquot was taken and different concentrations were prepared with range of dilution series. For each prepared concentration abs. was checked and standard calibration curve was prepared. abs of the test sample were extrapolated to calibration of std. Rutin. The equivalent conc. of Rutin mentioned as (mg of Rutin /g of extract).

Estimation of total tannin content [9]
Estimation method: Titrimetric method
Requirements: Burette (50 ml) with stand, Glass flask (250 ml), White tile, Beaker, Glass funnel, petroleum ether (Defatting agent), (Analytical grade) Indigo carmin, potassium permanganate, Double distilled water.

Procedure for test sample
2 gm of drug powder defatted with Petroleum ether for 12 hours. Filtered and mark was dried. Dried powder set for hot percolation with 50 ml distilled water in iodine flak. Filtered was down further with double refined water up to 150 ml. 10 ml extract was withdrawn and transferred to glass flask. 10 ml indigo carmine dye added and volume marked up to 100 ml (Double concentrated H_2O). Prepared solution was kept on heating at maintained temp 60-70 °C. titrate the solution with 0.1N KMnO4. Procedure continues till blue colour changes to greenish and final bright shiny yellow.

Standard solution: Same procedure was repeated without adding Plant extract and titration was done. Reading noted for end point colour change.
- Each ml 0.1 N KMnO4 solution ˜ 0.0004157 g of total Tannin.

Results and Discussion
Macroscopical evaluation
As per human tendency if have to remember something we visualize it immediately. It means that visual impact is best identification of anything in this universe. Macroscopical characteristics help in Identification of plant belonging to particular genus to most extent.

Fig 1: *Pogostemon auricularis* plant

Observed sensory parameters *Pogostemon auricularis* leaves are mentioned in table no. 1.

Table 1: Anatomical features of selected plant species

Sr. No.	Observed features	*Pogostemon auricularis* leaves
1	Colour	Bright fresh green for both ventral
2	Odour	Not specific
3	Taste	Bitter
4	Appearance	Strigose on upper surface, villous on lower ventral,
5	Shape	Ovate - Bracts Lanceolate, Bracteoles, Base round Apex: acute to acuminate
6	Size	5.5-6 cm × 1.7 cm
7	Margin	Creanulate
8	Venetation	Retticulate
9	Touch	Immature- Glabrous smooth Mature – rough
10	Extra features	Punctuation on leaf blade

Chemo microscopical evaluation

There can be morphological resemblance amongst the species under same genus. To find out exact about its identification, microscopy is quite useful technique. To confirm the identity of each of present tissue present in midrib and lamella portion, taken T.S was stained with different reagents and below mentioned layers were observed.

i. Epidermal layer ii. Mesophyll iii. Vascular bundle.

Epidermal layer

It is dorsiventral leaf. Outermost protecting line made with cuticle found to both the lateral of section. It was wavy because of epidermal outgrowth. Below to it continuously arranged palisade cells with tint of chlorophyll was present.

Epidermal opening

Water conservation center in leaf surface are stomata. Here diacytic structure found with pore at the center surrounded with guard cells and around to it subsidiary structure was found with specifically wavy walled cells with parallel arrangement.

Epidermal guard

during microscopy, leaf blade was found with both type of protecting structure. Few number of straight covering trichomes were noted on ventral surface. Dorsal lateral was found with 2 glands like out growth. Glandular trichomes were having diffused bottom grounded in epidermal layer. It has small single cell stock and single cell head.

i. Mesophyll

Layer between to epidemis identified as mesophyll. As it is visible in captured figure it was arranged with parenchymatous tissue in beautiful manner. Upper most and lower line of rounded tissue recognized as collenchyma to give protection from friction. Middle part of midrib was filled with tightly arranged thick walled parenchyma. Entire preparation was treated with IKI.

ii. Vascular bundle

Vascular structure is continuous long pipe like structure. They transport food and water across the different plant parts. At the middle of midrib this delicate structure was existed with Seth. Arrangement of xylem and phloem was radial. Meta and proto xylem were also seen during the study. It was confirmed with collateral type. Blue coloured stained starch grain was present.

Lamella: lamella was also stained with IKI and Phloroglucinol + Hcl. The lining was spreaded with palisade cells and spongy parenchyma.

The same is shown in captured section in figure no: 2 with labelling.

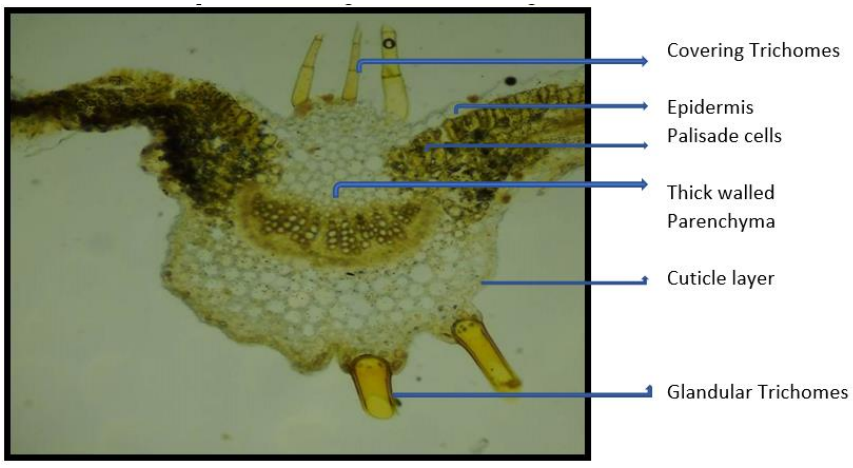
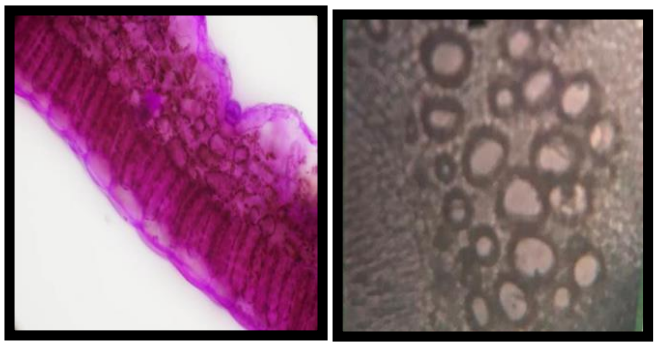


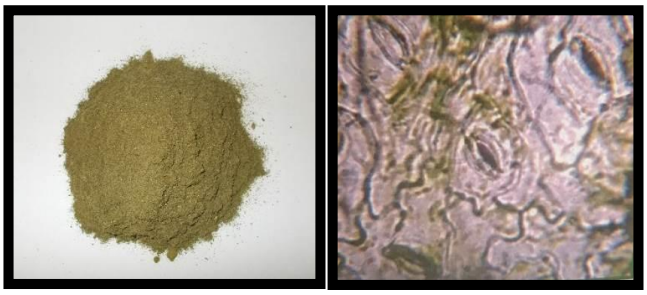
Fig 2: Magnified midrib section



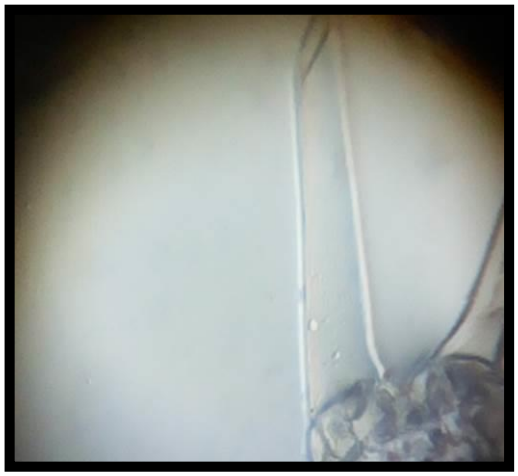
a. Stained lamella part **c.** vascular bundle

Fig 3: Transverse section of *Pogostemon auricularis* leaf

Powder microscopy of *Pogostemon auricularis* performed. Observed characteristics were stomata, trichomes, distinctive cuticle layer, epidermal cells arrangement, vessels, starch grains.



a. Pulverized leaves b. Stomata



c. Trichomes supported by cuticle and epidermal cell

Fig 4: Powder microscopy of pulverized *Pogostemon auricularis* leaf

Physical Evaluation

Present metabolites and their exact pattern of liberation form intact plant part is best known from extractive value study. Generally, from the scale of non-polar to polar solvents of extraction are chosen and study end point helps to derive the nature of present metabolites. Here few values of further use were displayed with solvent Ethanol, Methanol and water.

Ash value is the measure of impurity or faulty collection of plant parts. Higher the ash value more the carbenous part present or adhered soil impurities.

Least moisture content is advisable for longer storage as well retardation of microbial growth of crude drugs with medicinal impurities. Here it meets the requirement with reported value of 2.71 %v/w. Swelling index is measure of present gummy material as an excretory product of plant metabolic process.

Table 2: Measure of Physical characteristics of *Pogostemon auricularis* leaves

Evaluation parameters	Study Outcomes
	Pogostemon auricularis
Extractive value	
1) Alcohol Soluble macerates	7.2 % w/w
2) Water soluble macerates	8.2 % w/w
3) Methanol soluble	24.7% w/w
Ash value	
1) Water Soluble Ash	7.82 % w/w
2) Acid Insoluble Ash	1.45 % w/w
3) Total Ash	20.78 % w/w
Moisture content	2.71 % v/w
Swelling Index	2.6 % v/v

Extract preparation for the study

From the outcomes of, it was clear that maximum chemicals constituents are soluble in Methanol. Nature of present constituents was determined but could not identify here. Further study progressed with methanolic extract of *Pogostemon auricularis* leaves.

Table 3: Physical nature of investigational test methanol extracts

Methanolic Extracts	Quantity	Colour	Odour	Consistency
Extraction of leaves				
PA	250 gm	Bottle green	NS	Semi solid

Preliminary chemical analysis of methanolic extracts

Qualitative analysis

As its mentioned above in physical evaluation study, it gave idea about solubility pattern of metabolites accumulated in plant species. but the which particulars are exactly present in investigational samples are confirmed in preliminary investigation. On treatment with different reagents, specific functional group of present metabolite structure reacts and gives the change as an endpoint process. Investigational species PA found with its chemical nature described in table no. 4

Table 4: Qualitative scrutiny of metabolites present in Methanolic extracts of *Pogostemon auricularis* leaves

Sr. No.	Tests of Phytoconstituents	Methanolic extracts of leaves
		Pogostemon auricularis
1	Carbohydrate	
	a) Molisch's test	- ve
	b) Fehling's test	-ve
	c) Benedict's test	-ve
	d) Barfoed's test	-ve
	(Non-Reducing Polysaccharides)	
	e) Iodine test	-ve

	f) Tannic acid	-ve
	g)	
2	Proteins & Amino acids	
	Millons Test	+ve
	Biuret Test	+ve
	lead acetate test	+ve
	Ninhydrin Test	+ ve
	Glyoxylic acid (Tryptophan)	+ve
3	Alkaloid	
	a) Dragendorff's reagent	+ve
	b) Mayer's reagent	+ve
	c) Hager's reagent	+ve
	d) Wagner's reagent	+ve
4	General test for glycoside (1 % ferric sulphate + 5 % glacial acetic cid) Cardiac glycoside	
	a) Baljet's test	+ve
	b) Raymond test	+ve
	c) Legal's test	-ve
	d) Keller killiani test	+ve
	e) Libermann's test (bufadenolid)	- ve
5	Saponins glycoside	
	Froth test	- ve
	Hemolytic test	+ve
6	Anthraquinone glycoside	
	a) Borntrager's test	-ve
	b) Modified Borntrager's test	- ve
7	Tannins and Phenolics	
	a) Lead acetate	+ve
	b) 5 % Ferric chloride	+ ve
	c) Bromine water	+ ve
	d) Gelatine	+ ve
8	Flavonoids	
	a) Shinoda test	+ve
	b) chloroform and H2SO4	+ve
	c) zinc dust and conc. HCL	+ve
	d) NaOH	- ve
9	Vitamin	
	Vit A	-ve
	Vit D	-ve
10	Phytosterols and Triterpenoid	
	a) Salkowski reaction	+ ve
	b) Libermann's test	+ ve
	c) Libermann-Burchard test	+ ve

+ve = Present -ve = Absent

Pogostemon auricularis leaves Methanolic extract found with presence of Proteins & Amino acids, saponins & cardiac glycoside, tannins, phenolic, flavonoids, alkaloids and sterols.

Chemical fingerprinting

Chromatography is said to be finger printing technique. It is cooperative technique to find purity of test sample. It helps direct visualized observation between reference standard and test compound. It provides check on various chemical process like distillation to check end point separation. It plays good role to analysis multi components test sample like: crude herbs, food product and pharmaceutical preparation.

TLC for phenolic metabolite

Table 5: Calculated R_f values for the phenolic compounds

Sr No.	Spotting Samples	Extract	In UV light	After Spraying
			R_f value	
1.	Gallic acid	Reference standard	0.55	0.53
3.	*Pogostemon auricularis*	Methanolic extract	0.52	0.50

TLC for tannins metabolite

Table 6: Observed R_f values for the tannins with spraying reagents

Sr No.	Spotting samples	extracts	5 % $fecl_3$ spraying	Vanillin + Hcl spraying
			R_f value	
1.	Tannic acid	Reference standard	0.67	0.69
3.	*Pogostemon auricularis*	Methanolic extract	0.64	0.67

TLC for flavonoid metabolite

Table 7: Calculated Rf values for the flavonoid with spraying reagents and Iodine chamber

Sr No.	Spotting Samples	Extracts	Anisaldehyde – H_2so_4 spraying	Iodine vapour Chamber
			R_f value	
1.	Rutin	Reference standard	0.35	0.36
2.	*Pogostemon auricularis*	Methanolic extract	0.36	0.35

Quantitative analysis

After confirmation about presence of vital secondary plant metabolites, the amount in what they were present was done for, Phenols, flavonoids and tannins. It is important to know plan further study like pharmacological study, formulation preparation etc.

Estimation of total phenolic content (TPC)

Table 8: Calibration curve of standard reference sample Gallic acid

Sr No.	Gallic acid conc. (µg/ml)	Absorbance
1	2	0.163
2	4	0.213
3	6	0.239
4	8	0.257
5	10	0.298
6	20	0.312
7	50	0.379
8	100	0.517

Slope : 0.0031
Intercept : 0.22
Correlation coefficient
$R^2 = 0.908$

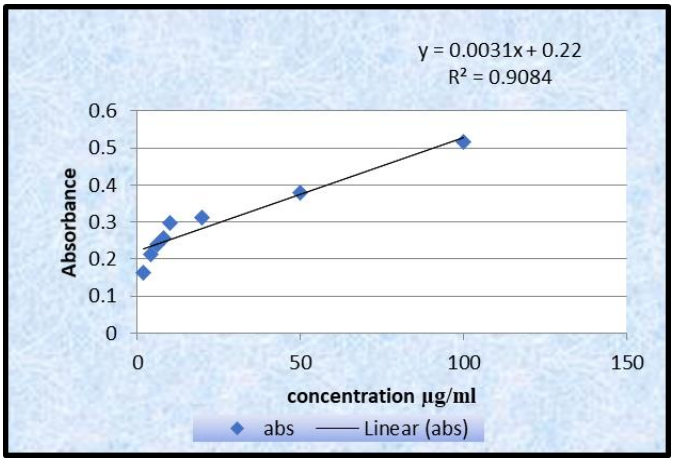


Fig 2: Standard curve of gallic acid for assessment of total phenolic content

Gallic acid as a standard reference compound. Resulted absorbance of test sample was extrapolated on liner curve of it and from the derived equation value of Y is answer to phenols present in test leaves. Results are shown in table no. 9.

Table 9: % Phenolic content in leaves investigational extracts

Sr. no.	Samples of investigation	% Phenolics present
1.	*Pogostemon auricularis* leaves extract	56.45%

Assessment of total flavonoid content

Table 10: Calibration curve of standard reference Rutin

Sr No.	Rutin conc. (µg/ml)	Absorbance
1	1	1.068
2	2	2.035
3	4	2.135
4	6	2.82
5	8	3.135
6	10	3.965

Slope : 0.2796
Intercept : 1.0815
Correlation coefficient:
$R^2 = 0.9424$

Rutin calibration curve was prepared to measure the flavonoid part present in PA leaves extract. On extrapolating the absorbance of PA leaves extract it showed the 52.33 % w/w of flavonoid present in sample.

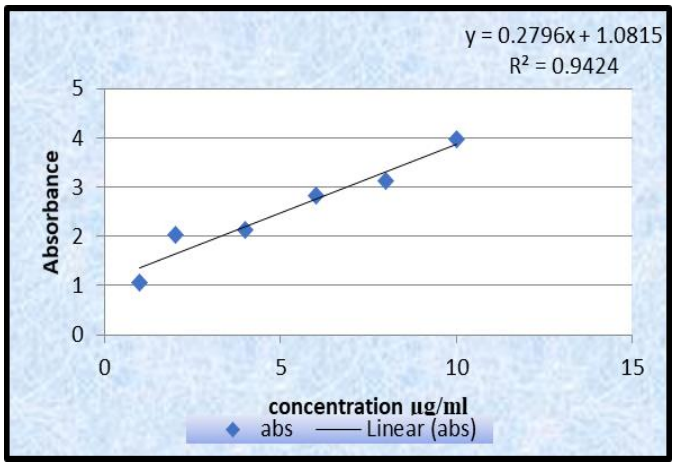


Fig 3: Typical linear graph of Rutin for estimation of total flavonoids content

Table 11: TFC of leaves extracts samples

Sr. no.	Samples of investigation	% Flavonoid present
1	*Pogostemon auricularis* leaves extract	52.33%

Estimation of total tannin content

Total tannins were calculated by performing titration with 0.1N $KMnO_4$. Endpoint multiplication with dilution factor gave the result of tannins present in it. Each ml 0.1 N KMnO4 solution˜ 0.0004157 g of total Tannin.

Table 12: TTC of leaves methanolic extracts

Sr. No.	Samples of investigation	% Tannins present
1	*Pogostemon auricularis* leaves extract	13.17 %

Conclusion

Microscopical study of Rostellularia *procumbens* and *Pogostemon auricularis* leaves helped to understand detail midrib arrangement of transversely arranged tissues. The dried and crushed test sample of *Pogostemon auricularis* leaves better yield in Methanol extraction as compared to ethanolic and aqueous extraction. It determined that the methanol soluble content is more in the plant extracts. From phytochemical investigations, it can be established that inorganic and calciferous matter present in the test extract. Extractive values signify the phyto constitution of plants and more of the nature of it. High amount of methanol soluble constituents was found as compared to alcohol and aqueous medium. Both the selected plant extract contains Phyto moieties such as good amount of phenolic contents. Polyphenols have numerous positive impacts on human wellbeing, like restraining the oxidization of proteins with low density, thereby decreasing the risk of heart disease. All phenolic mixes have calming and hostile to cancer causing properties. Excellent amount of tannis, which shows various therapeutic effects as antioxidants, antimicrobials, anthelmintics, and antivirals as well for the cancer treatment. Smart presence of flavonoid also noted during study. Flavonoids are responsible for beautiful colors of plants and shows benefit as anti-viral, anti-cancer. Particularly quercetin helps to alleviate sinusitis, asthma and heart disease. From the qualitative chemical investigation, it can be concluded that the leaves extracts contain alkaloids, carbohydrate, glycoside, flavonoids, diterpenes, phenols, tannins, protein and amino acid. Further chromatographic study confirmed plant extract with the Tannins, flavonoids, alkaloids, and phenolic compounds. Desired clinical effects are observed with plant accumulates when they are dispensed in right dosage form and administered in required quantity. So, it is important to know the quantity of present secondary compounds in collected samples.

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Abbreviations

TPC: Total phenolic content, TFC: Total phenolic content, TTC: Total tannin content, PA: *Pogostemon auricularis.*

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