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Pharmacognostical standardization of *Hyptis capitata* Jacq. Lamiaceae

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Abstract
Objective: The present study focused on pharmacognostic evaluation of root, stem and leaves of the medicinal herb *Hyptis capitata* Jacq belonging to the family Lamiaceae.
Methods: The pharmacognostic parameters viz., microscopic, macroscopic, organoleptic characters of fresh and dried plant parts were studied as per the guidelines of W.H.O. The physicochemical parameters like moisture content, total ash, acid insoluble ash, water soluble ash, alcohol and water soluble extractive values were estimated.
Results: Organoleptic and microscopical studies revealed diagnostic characters such as highly branched stem upto 60-70 cm height having slightly aromatic odour, glandular and non-glandular trichomes, and well developed vascular bundles. Powder microscopic studies showed the presence of phloem fibres, periderm tissues and xylem vessels having spiral thickening. The crude drug powder of root, stem and leaves showed wide range of colours or fluorescence when treated with different reagents. Physico chemical analysis of crude drug powder of root, stem and leaves revealed significant values of moisture (12.5 ± 0.05,10 ± 0.10, 8.07 ± 0.03%), total ash (17 ± 0.10,15.72 ± 0.10,12.88 ± 0.07%), acid insoluble ash (2.21 ± 0.03,1.46 ± 0.005,1.69± 0.005%), water soluble ash (8.93 ± 0.02, 4.55 ± 0.03, 7.86 ± 0.05%) and maximum extractive values in water soluble extraction.
Conclusion: The pharmacognostic studies and physicochemical analysis may help the identification, authentication and standardization of the crude drug.

Keywords: Pharmacognosy, organoleptic studies, powder analysis, fluorescence, physicochemical analysis

Introduction
Ancient people were fully aware of the rich potential of herbs for curing different ailments. The Indian system of medicine viz., Ayurveda, Siddha, Unani and Homeopathy prominently trust on the plant based products for their preparations and formulations Traditional medicine is attracting more attention in the health care sector because they are rich in the powerful source of biologically active compounds. The knowledge of active constituents in indigenous drugs may lead to the substantial improvement of drugs. The increasing acceptability of herbals is due to the minimal side effect and better compatability with human body. According to World Health Organisation, more than 80% of the world's population relies on traditional medicines for their primary health care needs. Natural drugs are considered as green medicine which is always supposed to be safe and economic. Plant based products and their formulations were employed in s. Due to the increase of the demand of crude drugs in pharmaceutical industries, they are usually adulterated by organic matters resembling to standard drugs or substituted by inferior quality of crude drugs. The increase in the requirement of naturally derived drugs and unavailability of materials cause adulteration with low quality and cheap organic materials which are very close resemblance to the genuine drugs. The misuse of medicinal plant and their product starts with wrong identification and adulteration. The pharmacognostical studies ensures the scientific validation of the medicinal plants.
Lamiaceae or mint family is well known for medicinal properties. *Hyptis capitata* is a slightly aromatic folk medicinal perennial herb belongs to Lamiaceae, commonly called buttonweed, knob weed, false, ironwort and is a native to Florida, Mexico, Central America and South America but naturalized in Australia, South East Asia and some tropical islands. The plant has some folkloric advantages such as anti-cough, anti-spasmodic, anti-inflammatory and wound healing properties. The plant is also known to contain the chemical constituents responsible for cytotoxicity and anti-HIV. The traditional knowledge on this folk medicinal information is not scientifically validated. In this context, it is an urgent necessity to authenticate and standardise *Hyptis capitata* Jacq by using pharmacognostic specifications. Moreover, the pharmacognostic studies on *Hyptis capitata* Jacq remained few and fragmentary that prompted the present

Investigation. The study focussed on pharmacognostical parameters of root, stem and leaf of *Hyptis capitata* to confirm its scientific validation.

2. Materials and methods

2.1 Collection and authentication of the plant material

Hyptis capitata Jacq. belongs to Lamiaceae was collected from natural habitat of Thiruvananthapuram district, Kerala. The plant was identified and authenticated by Department of Botany, University of Kerala. The voucher specimen (KUBH-6166) was deposited in the herbarium of same department.

2.2 Methods

The collected plant material was washed thoroughly and separated. The fresh bioparts- root, stem and leaves were used for the macroscopical, organoleptic and histological studies. The shade dried bioparts were powdered and stored in air tight container protected from sunlight. The powder was used for powder microscopy and physicochemical studies.

2.3 Macroscopic studies

The morphological studies are very important in plant identification and crude drug standardization. The macroscopical or morphological studies helpful in the identification of the plant material and also plays a very important role in standardization. The plant parts - root, stem and leaves were subjected to macroscopic and organoleptic studies such as colour, odour, shape, texture, fracture of the crude drug.

2.4 Microscopic studies

The anatomical studies were conducted on transverse sections of root, stem and leaves. The sections were stained with safranin [1] and diagnostic characters were studied [2, 3]. Photomicrographs of sections were taken with the help of Digital Microscope (model LABOMED, USA). Quantitative microscopic characters such as stomatal frequency, stomatal index, palisade ratio and vein islet number were studied [4].

2.5 Powder microscopy

The powder microscopy helps to study about the structure and type of tissues present in the crude drug powder. Powder analysis of fine dried powder of root, stem and leaf were conducted by mixing fine powder with safranine or 5% NaOH and examined under binocular research microscope. The characteristic features of powder were recorded and photographed. The measurement of cell components was done with the help micrometer.

2.6 Fluorescence Analysis

A small quantity of dried leaf and stem powder was placed separately on grease free clean microscopic slide and 1-2 drops of freshly prepared reagent solution was added, mixed by gentle tilting the slide for few minutes. The slide was viewed inside the UV chamber and observed the colour in day light and UV light. The colour in different reagents was recorded [5]

2.7 Physico-chemical Analysis

Physico-chemical parameters such as, moisture content, total ash, water soluble ash, acid insoluble ash, water and alcohol soluble extractive were determined using standard methods [4]

2.7.1 Determination of moisture

Determination of moisture content in crude drug powder by loss of weight on drying (LOD) method [6]. About 5gm of powdered drug material of root, stem and leaf taken in a petridish and dried in hot air oven at 105 ^{0}C for 4 Hrs. After cooling the amount of moisture content in the sample was calculated with reference to the air dried sample.

2.7.2 Determination of Total ash

About 2gm of powdered drug was weighed accurately into a tarred silica crucible. It was incinerated at 600 °C in a muffle furnace until free from carbon. The crucible was cooled and weighed. Percentage of total ash was calculated with reference to air-dried substance.

2.7.3 Determination of acid insoluble ash

Ash obtained from the total ash was boiled with 25ml of 2N HCl for a few minutes. It was then filtered through an ash less filter paper. The filter paper was transferred into a tarred silica crucible. Ash is then incinerated at 450°C in a muffle furnace until free from carbon. The crucible was cooled and weighed. Percentage of acid insoluble ash was calculated with reference to air-dried substance.

2.7.4 Determination of water soluble ash

Ash obtained from the total ash was boiled with 25 ml of distilled water for a few minutes. It was then filtered through an ash less filter paper. The filter paper was transferred into a tarred silica crucible. Ash is then incinerated at 450°C in a muffle furnace until free from carbon. The crucible was cooled and weighed. Percentage of water-soluble ash was calculated with reference to air-dried substance.

2.7.5 Determination of extractive values

The solvents used for the extraction were ethanol and water. About 5 gm of the crude drug powder subjected for maceration with 100 ml of the above mentioned solvents for 24 hrs, shaking frequently and allowed it to stand for 18 hrs and filtered rapidly. 25 ml of the filtrate was transferred to a beaker, evaporated to dryness on water bath and stored in an oven at 105 ^{0}C for 6 hrs. The filtrate was cooled, weighed and the percentage of extractive value was calculated with reference to air- dried drug.

3. Results

3.1 Morphological characteristics

The macroscopical or morphological studies helps in the identification of the plant specimen and is the primary step in the characterization of the crude drug The plant *Hyptis capitata* (Fig:1A) is a perennial herb which ranged from 60-70 cm height with quadrangular stem. Leaves are obovate and opposite decussately arranged, margin is irregularly dentate, leaf apex is acute, axillary globose capitulum like inflorescence with numerous sessile white fragrant flowers. Floral characters posess companulate calyx, bilipped tubular corolla, didynamous stamens with purple coloured anthers. Nutlets are black coloured, ovoid and found inside the persistent calyx

3.1.1 Organoleptic study

Organoleptic characteristics of root, stem and leaf of *Hyptis capitata* Jacq showed in Table-1

Table 1: Organoleptic Characters of *Hyptis capitata* Jacq.

Characteristics	Root	Stem	Leaf
Shape	Cylindrical	Quadrangular	Obovate
Colour	Brown	Green	Green
Odour	Slightly aromatic	Slightly aromatic	Slightly aromatic
Taste	Characteristic	Characteristic	Characteristic
Fracture	No exudation	No exudation	No exudation

3.2. Microscopical studies

The microscopical chracterization of the crude drug material provides a proper identification of a particular plant species

3.2.1 Stem: The T.S of the stem is quadrangular in outline with ridges and furrows having glandular and non-glandular trichomes. Collenchymatous cells are seen at the ridges and chlorenchyma at the furrows. Vascular bundles are 10 in number, conjoint, collateral and open arranged in a ring like fashion. A semilunar shaped bundle cap and a large parenchymatous pith is present (Fig: 1B).

3.2.2 Trichomes: Both glandular and non-glandular trichomes are present in young stem and leaf. Non-glandular trichomes are unbranched, multicellular having 6-9 cells and 0.14 mm in length possess two basal cells. Glandular trichome are rounded and unicellular (Fig: 1C& D).

3.2.3 Leaf: The leaf is dorsiventral and hypostomatic. The stomata is diacytic type. The trichomes are abundant on the upper epidermis. The non-glandular trichomes are seen at the midrib region and glandular trichomes are at lamina region. Epidermis consists of box like larger cells with cuticle. Lower epidermis is found to be illdefined with and small sized cells. Mesophyll comprises unilayered palisade and four layered spongy tissue. The midrib is bowl shaped with a single conjoint vascular bundle (Fig 1E).

3.2.4 Petiole: Transverse section of petiole is more or less circular in outline. Epidermis is made up of small sized cells with cuticle. Both glandular and non-glandular trichomes are present. Epidermis is followed by collenchymatous hypodermis. chlorenchyma patches are present on two lateral sides. There are 4-6 conjoint vascular bundles distributed in parenchymatous cortex of which two are large and median while the remaining are comparatively smaller (Fig:1F).

3.2.5 Root: Secondary growth of root showed more xylem vessels and phloem fibres, reduced primary xylem ,4-5 medullary rays, periderm and cork cells (Fig:1G).

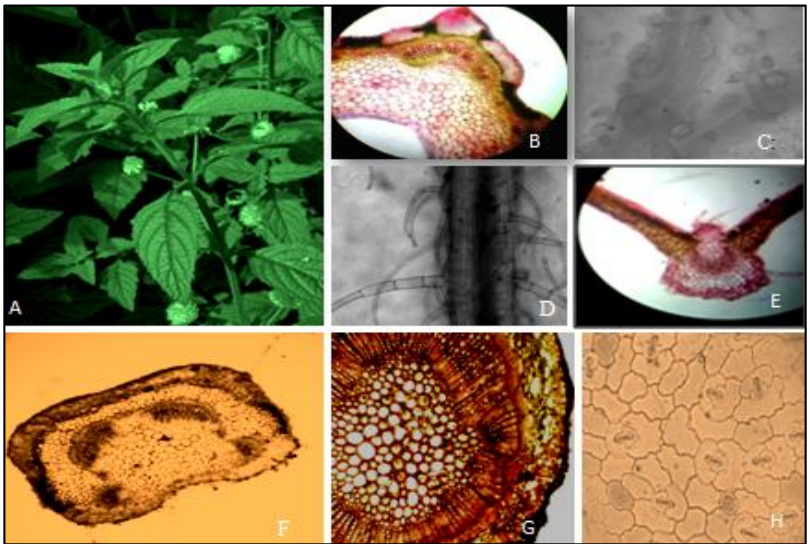

Fig 1: Habit and microscopic studies. (A) Habit of *H.capitata* ; (B) T.S of stem ; (C) Glandular trichomes ;(D) Non-glandular trichomes ; (E) T.S of leaf ; (F) T.S of petiole ; (G) T.S of root ; (H) diacytic stomata

3.3 Quantitative Microscopy

The leaf of *Hyptis capitata* exhibited diacytic type of stomata with two unequal subsidiary cells. The guard cells are oval in shape (Fig.1H).The various leaf constants stomatal frequency, stomatal index, palisade ratio and vein-islet ratio were determined (Table-2).

Table 2: Quantitative Leaf Microscopy.

Leaf constants	Results
Type of stomata	Diacytic
Stomatal frequency	112.5
Stomatal index	23.2
Vein-islet ratio	22mm^2
Palisade ratio	5.73 Palisade/epidermal cell

3.4 Powder Microscopy

Powder microscopic inspection is an indispensible evaluation for the identification of characteristic cellular inclusions in broken and powdered form (Fig: 2 A-G). The organoleptic characteristics of powder and quantitative micrometric results were shown in Table-3&4

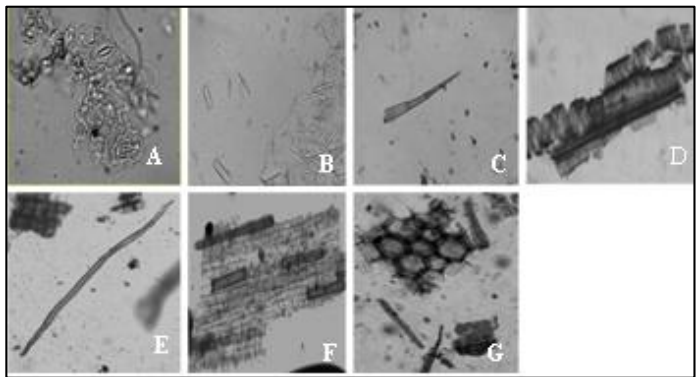

Fig 2: Powder microscopy of Hcapitata crude drug powder: (A) stomata; (Et) Fiagnents of xylem vessels ; (C) Broken non glandular trichome; (D) Spiral thickening ; (E) Tiachied ; (F) Pldoen fibres ; (G) Perideim tissues

Table 3: Organoleptic characteristics of powder of *H. capitata* Jacq

Powder characters	Colour	Odour	Texture
Root	Brown	Slightly aromatic	Smooth
Stem	Light green	Slightly aromatic	Coarse
Leaf	Dark green	Slightly aromatic	Smooth

Table 4: Quantitative powder microscopy of *H. capitata* Jacq

0	Cell elements	Results	
		Length	Breadth
Root	Xylem vessel	0.9mm	0.28mm
Stem	Tracheid	0.45mm	0.15mm
	Xylem vessel	0.83mm	0.23mm
	Trichome	0.22mm	0.008mm
Leaf	Nonglandular trichome	0.14mm	0.003mm
	Xylem vessel	0.02mm	0.1mm

3.5 Flourescence Analysis

Behaviour of crude drug powder of leaf and stem of *H. capitata* upon treatment with different reagents showed different colours both in day light and UV light (Table-5).

Table 5: Flourescence Analysis of *Hyptis capitata* Jacq

Reagents	Day light			UV light		
	Root	Stem	Leaf	Root	Stem	Leaf
Powder+1N NaOH	Dark brown	Brown	Dark	Dark	Black	Black
Powder+50% H_2SO_4	Light brown	Dark green	Dark	Black	Black	Dark
Powder+methanol	Brown	Greenish yellow	Dark	Dark	Flourescent green	Green
Powder +benzene	Reddish brown	Dark green	Dark green	Dark	Bluish dark	Red orange
Powder+petroleum ether	Dark brown	Dark	Dark	Black	Dark	Dark
Powder +ethylacetate	Dark brown	Dark	Dark	Black	Orange red	Orange red
Powder + iodine	Dark brown	Yellowish brown	Yellowish green	Black	Black	Dark
Powder +ammonia	Dark brown	Dark green	Brown	Dark	Black	Dark
Powder+picric acid	Yellowish brown	Yellow	Yellowish green	Dark	Black	Dark
Powder +acetic acid	Brown	Yellow	Dark green	Dark	Orange	Orange

3.6 Physico-chemical Analysis

Physico-chemical parameters mainly remain constant for the drugs which can be used for specific identification thereby assessing the quality of the crude drug. Various physicochemical parameters like moisture content, total ash, acid insoluble ash, water soluble ash, and extractive values were investigated and reported in the Table- 6

Table 6: Physico-chemical Analysis of *Hyptis capitata* Jacq

Parameters (%)	Root	Stem	Leaf
L.O.D	12.5 ± 0.05	10 ± 0.10	8.07 ± 0.03
Total Ash	17 ± 0.10	15.72 ± 0.10	12.88 ± 0.07
Acid insoluble ash	2.21 ± 0.03	1.46 ± 0.005	1.69 ± 0.005
Water soluble ash	8.93 ± 0.02	4.55 ± 0.03	7.86 ± 0.05
Alcohol soluble extractive	4.08 ± 0.01	2.28 ± 0.07	2.8 ± 0.01
Water soluble extractive	5.25 ± 0.03	4.49 ± 0.08	3.03 ± 0.02

4. Discussion

Majority of human population trust and depend on medicinal plants based on the traditional knowledge from their ancestors to cure various ailments. Most of these traditional medicinal plants used from folk information is not scientifically proved. A scientific validation is necessary to standardize and proper handling of crude drug. It is necessary to check the identity and ensure the quality of the crude drug. The pharmacognostic evaluation is the primary requisite for the standardization and proper handling of the medicinal plants.

According to W.H.O., the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity should be carried out before any tests are undertaken[7] .The macroscopic and sensory characters showed diagnostic features like slightly aromatic highly branched less hairy stem, winged petiole with

obovate leaf, irregularly dentate margin, globose head like axillary racemose inflorescence with numerous white coloured sessile flowers, purple coloured anthers, simple pointed stigma and black coloured ovoid nutlets. These morphological characters help distinguish the study material *H. capitata* from the allied species *H. suaveolens* [8]. The microscopical sections revealed the presence of glandular and non glandular trichomes, bowl shaped midrib, diacytic stomata with unequal subsidiary cells, crescent shaped arrangement of vascular bundles in petiole. The organoleptic evaluation is a qualitative evaluation technique based on the sensory profiling of the crude drugs. Organoleptic characteristics helps in detection of adulterated or substituted drug. The root, stem and leaf emit a slightly aromatic smell and leaf has little sour taste. The texture of root and stem powder was smooth where as the powder of stem was coarse.

The powder microscopy highlighted a number of characteristic cell elements like non-glandular trichomes and diacytic type of stomata frequently seen in leaf powder. The powder of stem and root displayed small vessel elements with spiral thickening, phloem fibres and periderm tissues. These characteristic features helps to identify the correct source material in powdered form.

Flourescence study is an essential parameter for the standardization of crude drug [9] Fluorescence seen in UV light mainly exhibit the flourescent nature of many natural products which is lacking in natural day light. If the substance themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents. Hence this method can be used to assess the crude drugs qualitatively and it is an important technique of pharmacognostic evaluation [10]. Variable colours were obtained when the crude drug powder of leaf, stem and root were treated with solvents of different polarity and chemical reagents.

Physico-chemical parameters are constant for the drugs which showed significant values and used for specific identification of the plant. The requirement of moisture content for herbal drug is less than 14% [11]. The loss on drying indicated the moisture content of the drug powder to be in the desirable value (12.5 ± 0.05,10 ± 0.10, 8.07 ± 0.03%) in root, stem and leaf of *H. capitata* respectively. Low moisture content recorded in the present study indicated less chance of microbial contamination during storage. Ash value is a criterion to assess the identity of the crude drug especially in the powder form [12]. Moreover, the total ash of a crude drug also reflected the care taken in drug preservation and the purity of crude and the prepared drug. Total ash value gives an idea of earthern materials or inorganic salt usually consists of carbonate, oxides, phosphates, silicates and silica in the drug or adhering to it. The total ash value of root, stem and leaf powder of *H. capitata* were found to be 17 ± 0.10, 15.72 ± 0.10, 12.88 ± 0.07% respectively. The results obtained in the present study remained in agreement with the reports on *Leucas cephalotes* [13]. The acid insoluble ash is a part of total ash and it measures the amount of silica present, especially as sand and siliceous earth. Water soluble ash is the water soluble portion of the total ash. The acid insoluble ash and water soluble ash were present in appreciable amount. The acid insoluble ash value of a crude drug less than the total ash value of the same drug indicated that very small amount of earthy matters present in it. More water soluble ash in root, stem and leaves denoted that the plant powder ash is more soluble to water compared to other. It is useful for the evaluation of crude drug as it gives an idea about the nature of the chemical constituents present in it and is useful for the estimation of chemical constituents, soluble in that particular solvent for extraction [14] . It also gives an indication whether the crude drug is exhausted or not [15]. Water soluble extractive values of root, leaf and stem were found in significant quantity (3.04%. and 4.56%) which denoted that water permeates the cells of aerial parts. The water soluble extractive values 5.25 ± 0.03,4.49 ± 0.08, 3.03 ± 0.02% of root, stem and leaves were more than alcohol soluble extractive value 4.08 ± 0.01, 2.28 ± 0.07, 2.8 ± 0.01% indicated that the crude drug powder contained more amount of water soluble chemical constituents. These results were in agreement with the reports on *Hyptis suaveolens* [16].

5. Conclusion

The present study envisages evaluation of a set of pharmacognostic parameters of *H. capitata* as per W.H.O guidelines. The results of the study revealed the proper morphological identifying characters of fresh plant as well as in powder form, significant amount of moisture and ash values, presence of water soluble chemical constituents. The results obtained in this study would serve as a standard reference for the botanical identification and confirmation which may be useful for the future analysis and preparation of the drug from the tribal medicinal herb *Hyptis capitata* Jacq.

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