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PHARMACOGNOSTIC AND PHYSICOCHEMICAL ANALYSIS OF *HOLIGARNA ARNOTTIANA* HOOK. *F*.

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ABSTRACT
Objective: The main objective of the study was to evaluate the pharmacognostic and physicochemical analysis of *Holigarna arnottiana*. **Methods:** Dried leaf and bark samples were analyzed for powder analysis, fluorescence analysis and fresh samples were microscopically analyzed by using accepted criteria. **Results:** In anatomical studies stem and leaves showed the presence of resin ducts. Anomocytic stomata were observed. The results of physicochemical analysis showed that the powder forms and all other parameters are within the accepted range to maintain as a drug. This is the first report of *Holigarna arnottiana* regarding the physicochemical analysis. **Conclusion:** The plant part has the quality to maintain as a drug according to the accepted criteria.

KEY WORDS: *Holigarna arnottiana*, Pharmacognosy, Physicochemical, Fluorescence analysis.

INTRODUCTION
In all countries and cultures, trees are the main source of medicine from time immemorial. Medicine, in several developing countries using local traditions and beliefs, is still the mainstay of health care. Traditional and folklore medicine bequeathed from generation to generation is rich in domestic recipes and communal practice.[1] Many of the pharmaceutical drugs in use are derived from plants directly or indirectly, it is obvious that at least some plants contain compounds with pharmacological activity that can be harnessed as medical agents. However the major drawback in promoting the use of medicinal plants is the lack of standardization as well as the misperception in the identification of the plant and their adulterants. To avoid such situations authentication is a must. In Ayurveda, dried and powdered form of plant parts are using and the actual medicinal plant is difficult to identify in such medicines.

Pharmacognosy dealing with the knowledge of drugs, its identity, purity and preservation and are important for the identification and standardization of the crude drug. Physicochemical and pharmacognosic parameters are highly essential for the plant part used for the preparation of phytomedicines.[2] The present study dealt with some important diagnostic characters including macroscopic, microscopic, powder analysis with chemicals and fluorescence analysis. It may be useful in determining authenticity and identifying adulteration of the crude drug.

Anacardiaceae family, under the order Sapindales comprises of 70 genera and 600 species which include trees, shrubs and vines. *Holigarna arnottiana*, is a large evergreen tree grows up to 35 meter height, is one of the allergic plants in this family and are endemic to Western Ghats, from Central and South Sahyadris. The latex oozing out from the bark and leaves produce contact dermatitis, by intense pruritus and erythema appeared within 24 hours after the contact. In this context the objective of the study was to validate the drug characteristics of this plant.

MATERIALS AND METHODS
Fresh bark and leaves of *H. arnottiana* were collected from local areas of Pathanamthitta district, Kerala and air dried and then powdered. The specimen is deposited in the herbarium, Department of Botany, University College, Thiruvananthapuram.

Physico Chemical Analysis
The parameters such as determination of foreign matter, moisture content, pH, water soluble and alcohol soluble extractive, total ash, water soluble and Alcohol soluble ash were analyzed according to standard procedure.

Sample Preparation
Fresh samples of *H. arnottiana* were brought laboratory in polythene cover and washed thoroughly in running water to get rid of dirt. The samples were then shade dried, powdered and used for further studies.

Determination of Foreign Matter: One gram of sample was Weighed and then carefully separated the foreign matter by differentiating colour and texture. The separated matter was weighed calculated the percentage.[3]

Determination of Moisture Content: Five gram of powder was weighed and dried at 80°C for 24 h in hot air oven. After 24 hour, the powder was weighed again and the difference in the weight was determined. The percentage of moisture was calculated.[4]

% Moisture = (Fresh weight - Dry Weight) ×100

Determination of pH: 5% (w/v) (5g in 100ml of water) powder was kept on shaker for 5h with 140 rpm and filtered. The filtrate was analyzed for pH by using pH meter (Elico, India).[5]

Determination of Water Soluble Extractive

Five gram of powder was weighed and taken into 100ml conical flask. 25ml of distilled water was added into it and kept on a rotator shaker (140 rpm) for 24h and then dried in hot air oven at 80°C for 24h and weighed again. The difference in weight was determined and percentage of water soluble extractive was calculated.[6]

$$\text{Percentage of Water soluble extractive} = \frac{\text{Difference in weight} \times 50 \times 100 \times 100}{\text{Initial weight} \times 5\ (100\text{- Loss on Dryness})}$$

Determination of Alcohol Soluble Extractive

Five gram of powder was weighed and taken into 100 ml conical flask. 25 ml of absolute alcohol was added into it and kept on a rotator shaker (140 rpm) for 24 h. After 24 h it was filtered and dried in hot air oven set at 80°C for 24 h and weighed again. The difference in weight was determined and percentage of water soluble extractive was calculated.[6]

$$\text{Percentage of Water soluble extractive} = \frac{\text{Difference in weight} \times 50 \times 100 \times 100}{\text{Initial weight} \times 5\ (100\text{- Loss on Dryness})}$$

Determination of Total Ash Content: 10 g of powder was weighed in crucible and powder was kept in a muffle furnace, the powder was heated up to 300°C for 3-4 h until the whole powder turns into ash. The crucible was cooled and weighed again. The difference in weight was noted and percent of total ash was calculated as per The standard procedure.[7,8]

$$\text{Percentage of Total ash} = \frac{\text{Difference in weight}}{\text{Initial weight} \times 100}$$

Determination of Acid Insoluble Ash: Five gram of ash was weighed and mixed with 2M HCl (25ml) and boiled for 5 min. The insoluble matter was collected in ashless filter paper and then washed with hot water, ignited and weighed.

$$\text{Percentage of Acid insoluble ash} = \frac{\text{Difference in weight}}{\text{Initial weight} \times 100}$$

The percentage acid soluble ash was calculated by taking difference between total ash and acid insoluble ash.

Percentage of acid soluble ash = (Total ash – Acid insoluble ash) ×100

Pharmacognosy: This includes the macroscopic and microscopic characteristic of the plant, powder behavior and florescent analysis. The morphological and taxonomical characteristics were studied and described in technical terms.[9]

Macroscopic Characteristics: Leaf, bark, flowers and fruits were collected and compared with the characteristics described in flora by Benthem and Hooker.

Microscopic Characteristics

Microscopic studies were carried out by taking free hand sections of leaf, petiole and stem of *H. arnottiana* then stained in safranin and mounted with glycerin. Photographs were taken by using image analyzer.[10]

Stomatal Index: Lower side of the leaf was pealed and the number of stomata was counted in each unit area under the simple microscope where each stoma was counted as one cell. Stomatal index is the percentage of the number of stomata to the total number of epidermal cells. Stomatal index was calculated by using following formula given.[11]

$$\mathbf{I = \frac{S \times 100}{E + S}}$$

I = Stomatal index, S = No. of stomata per unit area,
E = No. of epidermal cells in the same unit area

Powder Behavior and Fluorescence Analysis

Powdered leaves and barks were selected for powder Behavior and treated with different combinations of chemical. The fluorescent characteristics were observed under the long and short wavelength of UV light.

PHYTOCHEMICAL ANALYSIS

Preparation of Plant Extract

The collected fresh leaves and bark of *H. arnottiana* were dried under the shade and ground into fine powder.

The powder was extracted using Soxhlet apparatus with petroleum ether (30- 40ºC bp), chloroform (60-62ºC bp) and ethanol (60ºC bp) based on their polarity. The serial extraction was done in duration of 12h for each solvent. The extract was filtered and concentrated using a rotary evaporator and stored at 4ºC for further phytochemical screening.[12]

Extractive Values: A known volume of petroleum ether, chloroform and ethanol extracts were taken and weighed. Then the extract was allowed to dry in reduced pressure and ambient temperature. The final weight was measured after complete dryness. The yield of each of three extracts were calculated.

Phytochemical Screening

The phytochemical screening includes both qualitative and quantitative analysis of the three extracts.

Qualitative Analysis: The presence of secondary metabolites such as flavonoids, tannins, coumarins, saponins etc. were analysed.

Test for Reducing Sugar: 1ml of water was added to 0.5ml of extract and 5-8 drops of Fehling's solution was added at hot and observed for brick red precipitate.

Test for Glycosides

0.5g of plant extract was diluted with 5ml of distilled water and adds 2ml of glacial acetic acid containing one drop of ferric chloride solution. Pour 1ml of concentrated sulphuric acid along the side of the tube. Brown ring at the interface indicates the presence of glycosides.

Test for Flavonoids: 1ml of extract was added to Magnesium. Add concentrated HCl drop by drop. Development of pink colouration indicates the presence of flavonoids.

Test for Tannins: Two drops of 2% of ferric chloride was added with 1ml of extract, dirty green colour confirms the presence of tannins.

Test for Coumarins: 1ml extract dissolved in methanol. Add a few drops of alcoholic NaOH into it. Concentrated HCl added through the sides of test tubes. Appearance and disappearance of yellow colour confirms the presence of coumarins.

Test for Saponins: 5ml of extract shaken with 5ml of distilled water and then heated to the boiling point, frothing indicating the presence of saponins.

Test for alkaloids: About 0.5g of extract was warmed with 10 ml of 2% H_2SO_4 for 2 min. and filtered. To 1ml of this aliquot a few drops of Dragendorff's reagent was added. Presence of orange brown precipitate indicates the presence of alkaloids.

Quantitative Analysis: Based on the result of qualitative analysis, the ethanol extract was selected for quantification. Total phenol content in the methanol extract was quantified and then RP-HPLC has been carried out to quantify each component.

Total Polyphenol Content: Folin – Ciocalteau reagent was diluted ten times. 100µl of extract was added with 2.5ml of Folin – Ciocalteau reagent. The mixture was incubated at room temperature for one hour and the absorbance at 725nm was measured using a spectrophotometer (Schimadsu, Japan). The total polyphenol content was expressed as mg g^{-1} in gallic acid equivalents (GAE).[13]

RESULTS AND DISCUSSION

Physicochemical characteristics of *H. arnottiana* have been done which helps to evaluate the physical and chemical quality of the plant. The bark and leaf powder has no characteristic odour. The percentage of foreign matter was very low and the moisture content was 6.8% for bark and 5.6% for leaf. The amount and composition of ash remaining after combustion of plant material varies considerably according to the part of the plant, age, treatment etc. The constituents of the ash also vary with time and from organ to organ.[13] The total ash value was 7.6% for bark and 8.6% for leaf, indicating that the crude plant has low inorganic components. The water content in the plant determines the deterioration time of that plant. The moisture content was 6.8% for bark and 5.6% for leaf and the recommended range of moisture content 8-14% of plant drug.[14] The results are enlisted in Table 1& 2.

Table: 1. Physico Chemical Analysis of *H. arnottiana* leaf

No	Parameters	Results (% w/w)
1	Foreign matter	3.8
2	Moisture content	5.68
3	pH	6.0
4	Water soluble extractive	15.5
5	Alcohol soluble extractive	7.48
6	Total ash content	8.6
7	Water soluble ash	23.0
8	Acid insoluble ash	74

Table: 2. Physico Chemical Analysis of *H. arnottiana* bark

No	Parameters	Results (% w/w)
1	Foreign matter	2.1
2	Moisture content	6.8
3	pH	6.1
4	Water soluble extractive	16.5
5	Alcohol soluble extractive	9.24
6	Total ash content	7.6
7	Water soluble ash	26.0
8	Acid insoluble ash	80

The macroscopic study of leaf and bark was carried out. The plant is a large tree grown up to 35-40 feet height. Bark is fissured. Latex present which is white and when in contact with air become black. Leaves are simple, alternate, clustered at the ends of twig and arranged spirally in the branchlets. Leaf blade is 8-25cm long and width of lamina is 2-7.5cm and is leathery. The leaves are obovate, tip is slightly tapering and the base is narrow. Margins are entire with raised midrib and reticulate venation. Primary nerves vary from 10-15 with secondary nerves of about 10-25 pairs. Flowers are polygamous, borne on panicles, golden yellow colored and are found at the branch ends. Fruits are smooth rounded, one seeded, greenish when young and reddish when ripened (Table 3).

Table: 3. Organoleptic and Macroscopic Characteristic *of H. arnottiana* Leaf and Bark

Sl. No.	Specification	Leaf	Bark
1	Colour	Dark green	Dark brown
2	Odour	Characteristic	Characteristic
3	Size	Lamina 8- 25cm long and width 2- 7.5cm.	-
4	Shape	Obovate	-
5	Surface	Leathery	Fissured and pustular
6	Margin	Entire	-
7	Apex	Obtuse to slightly acuminate	-
8	Base	Narrow	-
9	Venation	Reticulate	-

The transverse section of leaf showed the presence upper epidermal cells. Palisade cells are just beneath the epidermis. In the mid rib region collenchymatous cells are arranged beneath the epidermis. Xylem bundles in are arranged and within this phloem cells. Above the bundles sclerenchyma and parenchyma cells were arranged. Resin ducts are also found in the petioles. Anomocytic stomata are present at the lower surface and the stomatal index was 15.46/sq.mm. (Fig. 1).

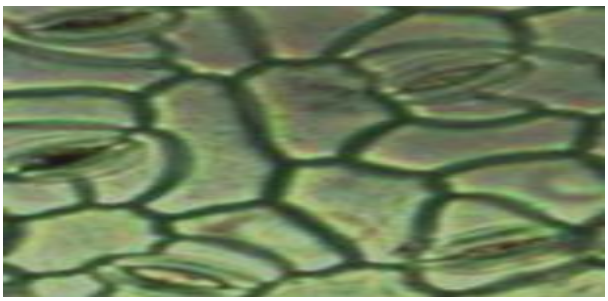


Fig. 1. Anomocytic Stomata, A portion enlarged

The transverse section of stem showed the presence of Epidermis and epidermal hairs. Below this are the spongy parenchymatous cortical cells. Xylem cells and Phloem cells are at the centre portion and have medullary rays. Presence of resinous ducts was observed. (Fig. 2).

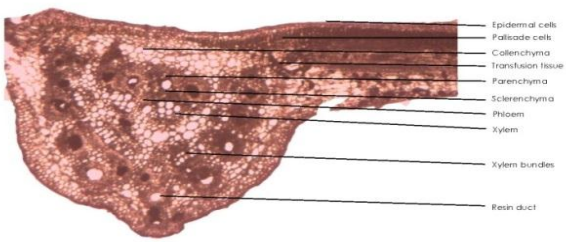


Fig. 2. Leaf anatomy of *H. arnottiana*

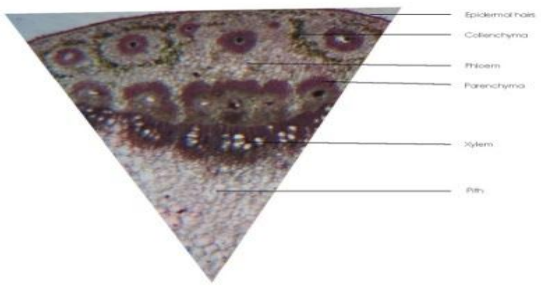


Fig. 3. Stem anatomy of *H. arnottiana*

In the powder behavior of both leaf and bark with chemicals along with 1N NaOH, the powder appeared as dark yellow indicating the presence of flavonoids. Presence of alkaloid is observed when treated with Mayers reagent. Tannins are present when the powder treated with 40%NaOH+Lead acetate and 5%$FeCl_3$. The results are same when treated the leaf and bark powder. No change was observed when the powder treated with Con.H_2SO_4, 5%Aq.KOH, Conc. HNO_3+ Ammonia and Aqueous 1 % $AgNO_3$ (Table 4).

On treatment with NaOH, the bark and leaf powder showed wide variety in characteristics. Under 254nm the bark powder showed dark brown colour while leaf powder showed pale green colour. On 365nm, the former showed black and latter showed dark brown coloration. The results are listed in the Table 5. Fluorescence study is an essential parameter for first line standardization of crude drug and this fluorescence phenomenon can be used for the qualitative examination of herbal drugs.[14]

Table: 4. Powder Behaviour of *H. arnottiana* Bark Powder with Chemicals

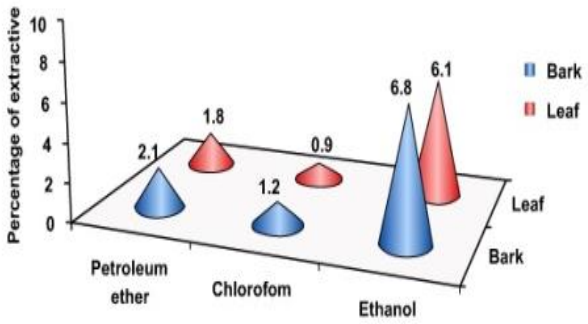
Reagent	Color developed	Inference	Color developed	Inference
	Bark		Leaf	
Powder as such	Brown	-	Color developed	Inference
Powder +1N NaOH	Dark yellow	Flavonoids	Dark green	-
Powder +Mayers reagent	White precipitate	Alkaloids	Dark yellow	Flavonoids
Powder + 40%NaOH+Lead acetate	White precipitate	Tannins	White precipitate	Alkaloids
Powder +5%$FeCl_3$	Black	Tannins	White precipitate	Tannins
Powder +Con.H_2SO_4	Brown	-	Black	Tannins
Powder +5%Aq.KOH	No change	-	No change	-
Powder+ Conc. HNO_3+ Ammonia	No change	-	No change	-
Powder + Aq. $AgNO_3$ (1 %)	No change	-	No change	-

Table: 5. Fluorescence analysis *of H. arnottiana* Bark Powder

Treatment	Visible light	245nm	365nm	Visible light	245nm	365nm
	Bark			Leaf		
Powder as such	Brown	Dark brown	Black	Dark green	Dark green	Brown
Powder + NaOH in water	Brown	Dark brown	Black	Greenish brown	Pale Green	Dark brown
Powder + 1 N HCl	Brown	Dark brown	Black	Brown	Dark Brown	Black
Powder + H_2SO_4	Black	Dark brown	Black	Black	Dark Brown	Black
Powder + NaOH in Alcohol	Brown	Dark brown	Black	Brown	Dark Brown	Black
Powder + 10 % HCl	Brown	Brown	Brown	Dark green	Dark green	Brown
Powder + 5 % KOH	Black	Black	Black	Dark green	Black	Black

The phytoconstituents in each solvent were quantified as yield of extraction.

The relative yield from the petroleum ether, chloroform and ethanol extracts was quantified and expressed in percentage of yields. The results revealed that yield was highest for the bark ethanol extract of *H. arnottiana* (6.8%) (Graph 1). Preliminary phytochemical analysis of petroleum ether, chloroform and ethanol extracts of leaf and bark was carried out. All the three extracts of *H. arnottiana* were serially undergone for preliminary phytochemical analysis. The ethanolic extract of leaf and bark contains all the tested phytochemicals except saponins. Reducing sugar and tannins were present in the chloroform extract whereas no chemicals were obtained in petroleum ether extract of both leaf and bark (Table 5).Similar study was reported in *Holigarna grahamii*.[15,16] The alcoholic and ethanolic extracts showed the presence of bioactive compounds like tannin, phenols, alkaloids and catechin in *Anacardium occidentale* and *Mangifera indica*.[17,18] Similarly *Semecarpus anacardium* possesses several bioactive compounds.[19,20]



Graph 1. Extractive value of *H. arnottiana*

Table: 8. Qualitative Analysis of *H. arnottiana* Leaf and Bark Extracts

	Leaf			Bark		
Phytochemicals	**Petroleum ether**	**Chloroform**	**Ethanol**	**Petroleum ether**	**Chloroform**	**Ethanol**
Reducing sugar	-	+	+	-	+	+
Glycosides	-	-	+	-	-	+
Flavonoids	-	-	+	-	-	+
Tannins	-	+	+	-	+	+
Coumarins	-	-	+	-	-	+
Saponins	-	-	-	-	-	-

CONCLUSION

To identify the correct authenticity of a medicinal plant, the crude drug standardization is essential. Ash value determination is very useful to identify the purity and give an idea of the earthy matter or any other impurities in a crude drug. The extractive values are used to evaluate the chemical constituents in the crude drug and for the estimation of constituents soluble in particular solvents. The water soluble extractive value will be higher because of the presence of highly polar chemical substances. The determination of foreign organic matters gives idea about contamination of plant parts with insect, moulds or other animals. And the high content of foreign organic matters indicates that the material is not coming from the original plant part. According to World Health Organization, the microscopic study of medicinal plants is the first step toward establishing its identity and purity and should be carried out before any tests. The anatomical characteristic of leaf and stem has been analyzed.

CONFLICTS OF INTERESTS

All authors have none to declare

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