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Research Article

PHARMACOGNOSTIC STUDY AND PHYTOCHEMICAL INVESTIGATION OF *PLECTRANTHUS HADIENSIS*

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ABSTRACT

Plectranthus is a genus which belongs to the family of Lamiaceae. Even though numerous studies have been carried out on plants belonging to this family, Plectranthus remains a genus in which a lot of incongruity exists. Even though *Plectranthus hadiensis* is used as a medicinal herb, the information regarding it is scanty and not well documented. Our study focuses on the pharmacognostic studies and the phytochemical screening of *Plectranthus hadiensis*, previously known as *Plectranthus zeylanicus* or *Coleus zeylanicus*. The study includes macroscopy and microscopy, anatomy and phytochemistry of the stem of *Plectranthus hadiensis*. Phytochemical screening of the plant revealed the presence of phenolics, flavonoids, tannins, alkaloids, proteins and carbohydrates. The detailed pharmacognostic account of *P. hadiensis* which includes macroscopic and microscopic characters will be helpful for the correct botanical identification of the plant. The study scientifically validates the use of the plant in traditional medicine.

Keywords: *Plectranthus hadiensis*, Pharmacognosy, Total phenolic content, Total flavonoids.

INTRODUCTION

Medicinal herbs play a major role in the treatment of diseases and infections. The curative properties of medicinal plants are mainly due to the presence of various complex chemical substances of different compositions which occur as secondary metabolites[1]. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds[2]. Medicinal plants form a large group of economically important plants that provide the basic raw materials for indigenous pharmaceuticals[3].

Plectranthus hadiensis is reportedly cultivated in Tamil Nadu on river banks and sandy loams. The root and stem of this plant has a quite distinct and specific aroma. The herb accepted as the source of Hribera (Iruveli) in Kerala is *Coleus Zeylanicus* (Benth.) Cramer (syn. *Plectranthus zeylanicus* Benth)[4]. This species is reportedly an endemic taxon of Sri Lanka, where it is known by the Sinhalese name *Iruveriya,* the juice of stem and leaves of which mixed with honey is taken as a remedy for diarrhoea and is not known in the wild. This has been introduced in this part of the country since long and probably owes its Vernacular name to its original Sinhalese. Rheede has illustrated this herb in his *Hortus Malabaricus* (IX t, 74)[5]. This plant belongs to the Lamiaceae family and is used in Ayurveda. In the present study, the pharmacognostic characteristics and phytochemical analysis of *P. hadiensis* was performed. In this paper, we report the macromorphological, micromorphological, physiochemical and phytochemical analysis of the stem of *Plectranthus hadiensis*.

MATERIALS AND METHODS

Collection and identification of plant material

The specimen was collected from Kanjikode, Kerala and authenticated by Dr. Kunhikannan, Scientist E, IFGTB, Coimbatore, India. The shoot part of the plant (*Plectranthus hadiensis*) collected was dried and pulverized. 10g of this powdered sample was refluxed with hexane, chloroform, methanol and water in the ratio 1:10(w/v). The crude extracts were collected in amber coloured sample bottles and stored. All chemicals and reagents used including the solvents were of analytical grade.

Macroscopic and microscopic analysis

The macroscopic characters such as colour, odour, taste, nature, texture were studied for morphological investigation. For microscopic studies, uniform and thin free hand section were taken from the fresh stem, dehydrated, double stained and finally mounted and viewed by following the micro techniques method of Johansen (1940)[6]. Macro and microscopic characters were studied as per Wallis (1976) and Trease and Evans (1982)[7,8]. The powder was treated with phloroglucinol solution and glycerol for microscopic analysis.

Phytochemical analysis

Chemical analysis was carried out in the hexane, chloroform, methanolic and water extracts of the shoot of *Plectranthus hadiensis* using standard procedures to identify constituents, as described by Harborne (1984), Trease and Evans (1979), and Sofowara (1993)[9-12].

Determination of total phenolic compounds

Total phenolic content was determined by the method described by Lister and Wilson (2001)[13,14]. 50 µg, 100 µg, 250 µg, 500 µg and 1 mg of the extracts were made up to 0.5 mL with distilled water. 2.5 mL of Folin-Ciocalteau reagent (1:10 dilution) and 2 mL of sodium carbonate (7.5% w/v) were added and the tubes incubated at 45 ºC for 15 min. The absorbance was read at 765 nm. Gallic acid was used as a standard, and results were expressed in terms of gallic acid equivalence (GAE) in µg.

Determination of total flavonoids

The flavonoids contents in the extracts were determined spectrophotometrically using the method of Ordon-Ez *et al*. based on the formation of a flavonoid-aluminum complex[15,16]. An amount of 2% ethanolic $AlCl_3$ solution (0.5 mL) was added to 0.5 mL of sample. After 1 h at room temperature, the absorbance was measured at 420 nm. A yellow color indicated the presence of flavonoids. Extract samples were evaluated at a final concentration of 0.1 mg/mL. Total flavonoids contents were calculated as quercetin (mg/g extract).

RESULTS AND DISCUSSION

Macroscopic and microscopic analysis

The herb *Plectranthus hadiensis* (Figure 1) is a semi-succulent, strongly aromatic, soft tomantose herb with fibrous roots, fleshy leaves, ovate or orbicular, acute, retuse or emarginated at apex. The base is rounded or truncate; margin is crenate, serrate or bullate. Flowers are small, blue or purplish in the terminal part; there are two calyxes that are lipped and glandular; two corollas lipped with four upper lips which are lobed, lower entire and boat shaped. Closely similar to *C. ambonicus* Lour., but can readily be recognized by its characteristically different odour and less succulent leaves. Transverse section of the stem is almost rectangular in outline. A very thin cuticle covers the epidermis and is uniseriate. Below the epidermis is the cortex, a distinct endodermis with casparian strips is absent. Pericycle is represented by scattered groups of stone cells in a complete ring. The stele is represented by closely arranged

vascular bundles. Cambium is 3 to 4 layered. Medullary rays are uniseriate and narrow. Intraxylary phloem present at the periphery of the pith, in the form of separate strands.

Organoleptic property and Powder microscopy

The color of the pulverized stem powder was brownish. Coarse powder in appearance. Highly aromatic, that is characteristic of the plant. Astringent in taste. Fibers are few, lignified well developed sclerenchymatous fibers from the vascular bundle region, thin, and isolated fibers measure 200 - 600 microns in length and 10 - 20 microns in breadth. Fragments of mesophyll tissue containing vascular strands are seen good many in number. The anatomic characteristics are visualized using the glycerol staining and phloroglucinol stains the lignin content of the powder specimens (Figure 2-5). The xylem vessels are clearly visible using glycerol at 40x magnification (Figure 6).

Fig. 1: ***Plectranthus hadiensis***

Fig. 2: Powder microscopy of ***P. hadiensis*** **(glycerol) (4x)**

Fig. 3: Powder microscopy of ***P. hadiensis*** **(glycerol) (40x)**

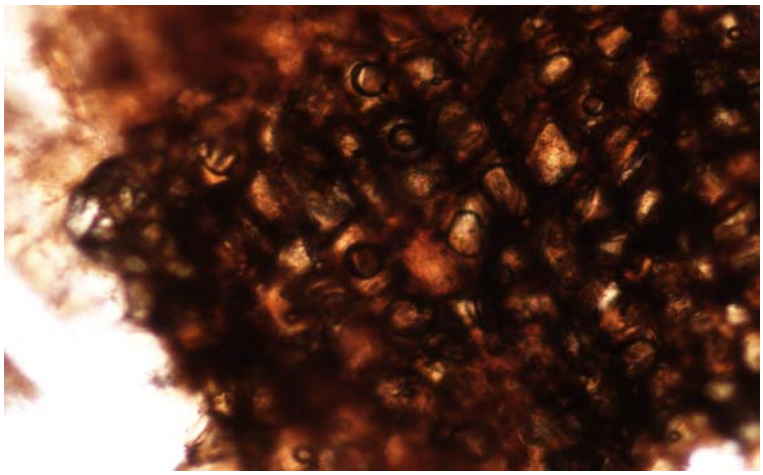

Fig. 4: Powder microscopy of *P. hadiensis* (phloroglucinol) (10x)

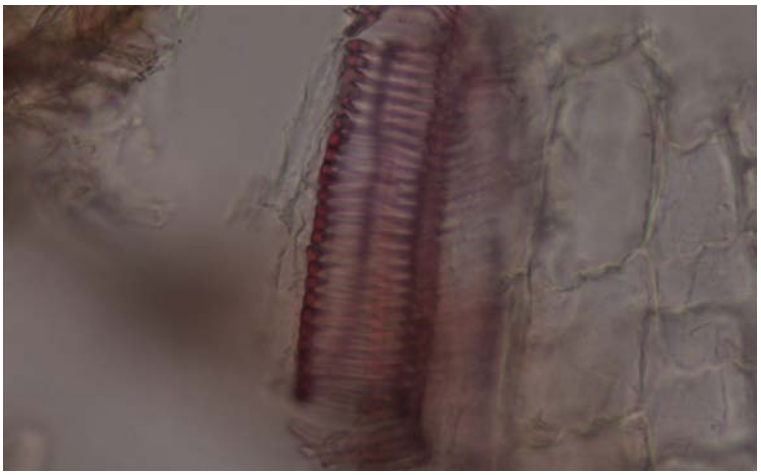

Fig. 5: Powder microscopy of *P. hadiensis* (phloroglucinol) (40x)

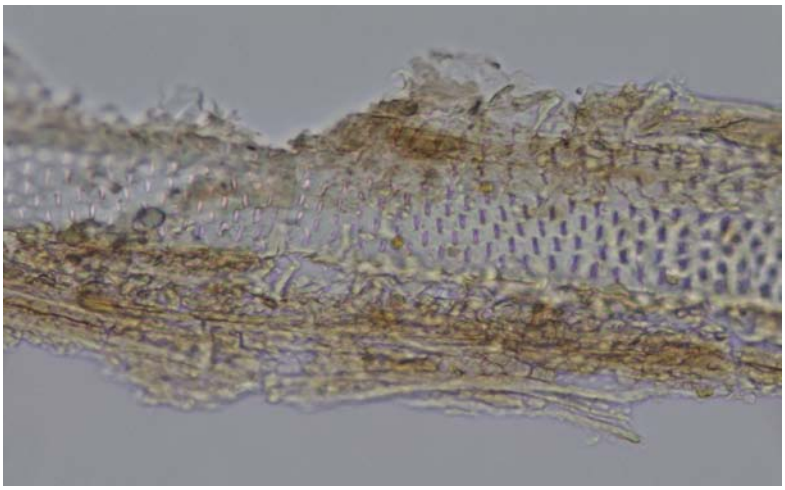

Fig. 6: Xylem vessels of *P. hadiensis* (glycerol) (40x)

Phytochemical analysis

Phytochemical-screening results (Table.1) of the powdered sample extracted in water, hexane, chloroform and methanol showed the presence of all the constituents in aqueous and methanol, whereas the chloroform extract showed the presence of proteins, carbohydrates and cardiac glycosides and the hexane extract was positive for cardiac glycosides, indicating that water and methanol were able to extract most of the phytochemicals present in the shoot.

Total phenolic content

Total phenolic contents in the extracts were expressed as GAE and are presented in Table.2. The methanolic extract had high phenolic content followed by aqueous, chloroform and hexane. Polyphenols are a group of chemical substances found in plants, characterized by the presence of more than one phenol group per molecule. Polyphenols are generally further subdivided into tannins, and phenylpropanoids such as lignins and flavanoids. In human health

these compounds, numbering over 4000 distinct species, are thought to be instrumental in combating oxidative stress, a syndrome causative of some neurodegenerative diseases and some cardiovascular diseases.

Total flavonoids

Based on the absorbance values of the various extract solutions, and compared with the standard solutions of quercetin equivalents, the results obtained in this study revealed that the level of flavonoids in the methanolic extract of *Plectranthus hadiensis* stem extract was higher (Table 3). Aqueous extract, chloroform extract and hexane extract all contained less total flavonoids than the methanolic extract. Plants often contain substantial amounts of antioxidants, including tocopherols (vitamin E), carotenoids, ascorbic acid, flavonoids and tannins[17]. Although there is presence of flavonoids in the extracts, it is considerably lower than total phenolics present and thus the medicinal property of the plant could be attributed to the higher content of phenolics.

Table 1: Phytochemical analysis of extracts

Phytochemicals	Water	Methanol	Chloroform	Hexane
Flavonoids	+	+	-	-
Alkaloids	+	+	-	-
Phenols	+	+	-	-
Tannins	+	+	-	-
Proteins	+	+	+	-
Carbohydrates	+	+	+	-
Saponins	+	+	-	-
Glycosides	+	+	-	-
Cardiac glycosides	+	+	+	+

+ present, - absent

Table 2: Total phenolic content of the extracts

	Total phenolic content			
Amount (µg)	**Hexane extract** GAE±SD(µg)	**Chloroform extract** GAE±SD(µg)	**Methanolic extract** GAE±SD(µg)	**Aqueous extract** GAE±SD(µg)
50	12.37±0.17	8.94±0.09	21.89±0.32	14.46±0.04
100	18.74±0.61	13.47±0.73	35.51±0.34	29.70±0.09
250	35.29±1.02	27.04±0.26	63.03±1.01	48.19±0.13
500	48.83±0.38	33.64±1.16	79.83±1.38	67.78±1.07
1000	60.61±1.07	45.38±1.08	86.74±1.43	79.44±1.14

GAE ± SD at 95% confidence interval (n=3)

Table 3: Total flavonoids in the extracts

	Flavonoid content (mg/g extract) QE			
Amount (µg)	**Hexane extract** Mean±SD	**Chloroform extract** Mean±SD	**Methanolic extract** Mean±SD	**Aqueous extract** Mean±SD
50	6.62±0.04	7.27±0.08	13.03±0.15	16.83±0.09
100	15.56±0.53	12.33±0.65	19.27±0.26	23.87±0.16
250	24.48±1.16	18.37±0.74	35.11±1.05	38.63±0.24
500	29.94±1.16	23.37±1.02	39.64±1.13	45.33±0.94
1000	34.73±1.42	27.55±1.34	42.39±1.21	48.95±1.16

Mean ± SD at 95% confidence interval (n=3), QE- Quercetin

CONCLUSION

There is a need for biologically active compounds with low profiles of adverse reactions compared to pharmacological drugs and this has triggered an extensive investigation of herbal phytochemicals and their mechanisms of action. The net biological activity is determined by the outcome of the multiple cellular effects exerted by phytochemicals and a better understanding of these cellular effects is vital to properly utilize the phytochemicals, as promising agents for promoting health and preventing disease. Results of phytochemical evaluation reveal the presence of polyphenols, alkaloids, tannins, cardiac glycosides, saponins and terpenoids. The high content of polyphenols can account for the use of this plant in traditional medicine and further studies have to be performed to isolate and characterize the biomolecules present in the plant extract.

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