Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2013; 5(3); 227-231

ISSN: 0975-4873

Research Article

Phytochemical Evaluation of Coleus Vettiveroids K.C. Jacob

*[1]Beesha S. Kamal, [2]Padmaja V

[1]*College of Pharmaceutical Sciences, Govt: T.D. Medical College, Alappuzha, Kerala, India.*
[2]*College of Pharmaceutical Sciences, Medical College, Thiruvananthapuram, Kerala, India.*

ABSTRACT
Coleus vettivereoids K .C. Jacob belonging to the family Labiatae is a widely accepted plant known for its traditional uses such as antipyretic, antibacterial, anti-inflammatory etc. Evaluation of its physicochemical parameters, preliminary phytochemical screening and HPTLC studies was done to establish its phytochemical standards. Histochemical studies revealed the distribution of starch, lignins, phenolic substances and alkaloids. Physicochemical parameters such as ash values, extractive values were also evaluated. Preliminary phytochemical screening indicated the presence of carbohydrates, proteins, steroids, alkaloids, flavanoids and tannins in detectable amounts. HPTLC profiles were recorded with the petroleum ether extract. The results generated can be utilized for its identification, authentication and prevention and detection of adulteration of the plant Coleus vettivereoids.

Keywords: Coleus vettiveroids, Labiatae.

INTRODUCTION

The demand for crude drugs has undergone a considerable change in recent years due to aggressive marketing of the crude drugs. Standardized extracts from them or pure phytopharmaceuticals need to be studied extensively for their quality, purity, potency, safety and efficacy[1].

Labiatae is a large family that occurs worldwide and has species that are adapted to almost all habits and altitudes. It comprises about eighty species worldwide. Taxonomically Coleus lour is the closest to Plectranthus.

Botanical information:

Latin name: Pavonia odorata

Regional names:

Hindi - Valak

Kannada - Muchivala, Lavanchi

Malayalam - Iruveli

Sanskrit -Valakam,Hriberam

Tamil - Kuruver, Vettiver

Telungu - Vettiveru, Kuriveru

Synonyms: Amara cites Balam, Hriberam, Varhistam, Kesam and Ambu as synonyms for Coleus vettiveroids

Ayrvedic Formulary of India, published by the Government of India has identified Hriberam as Coleus vettiveroids. In south India Coleus zeylanicus is invariably used as Hriberam. Both these appear to posses more or less identical properties. The plant is cultivated in south India through vegetative cuttings and the whole plant is used in medicine. The plant is a small profusely branched succulent aromatic herb with stems and branches and deep straw coloured aromatic roots, leaves glandular hairy broadly ovate with dentate margins and prominent veins.

Coleus vettiveroids K. C. Jacob (Plectranthus vettiveroids) is an important plant in the Indian system of medicine. The plant is a bitter cooling, diuretic, tricogenous and antipyretic. It is useful in hyperdipsia, vitiated conditions of pitta, burning sensation, leprosy, skin diseases, leucoderma, fever, vomiting, diarrhoea and ulcers. It has antibacterial and antiinflammatory action also, and used in a number of ayurvedic formulations like Iruveli kashaya, Amritati kashaya, Vilambudadi kashaya, Dasamoolarishtam etc.

Inspite of the numerous medicinal uses attributed to the plant, there is no data available on the physicochemical standards required for the quality control of its crude drug. Hence the present investigation was undertaken to determine physicochemical and phytochemical standards for authenticating the plant material of Coleus vettiveroids.

MATERIALS AND METHODS

Collection and authentication of plant material: The plant material for the proposed study was collected from the Botanical Garden, Poojappura, Thiruvananthapuram, Kerala, India. The species of the proposed study was identified as Coleus vettiveroids by, Pharmacognosy Unit, Ayurveda Research Institute, Poojappura, Thiruvananthapuram, Kerala, India

Preparation of plant material for extraction: Washed the plant material thoroughly to remove soil and other impurities, dried in shade, and pulverized.

Successive solvent extraction: 100g of the powdered drug was subjected to Successive solvent extraction using petroleum ether, chloroform, ethyl acetate, ethyl alcohol and water. Each extract was dried in a vacuum drier.

Preliminary phytochemical screening: Preliminary phytochemcal screening of each extract were done to determine the presence of primary and secondary metabolites. Study also includes chemical tests for detection of inorganic constituents.

Quantitative microscopy: Diameter of starch grains, length and breadth of fibers, vein-islet and vein termination number, stomatal number, stomatal index and palisade ratio were determined.

*Author for correspondence: E-mail: beeshak@yahoo.co.in

Table 1: Results of *Histochemical studies*

Experiment	Observations		
	Leaf	Root	Stem
1. Section mounted in water	Shows epidermis palisade cells, spongy Tissue and vascular bundle	Shows cork, cortex and v. bundle	Shows epidermis, cortex, v. bundle and pith
2. Section mounted in Phloroglucinol and con. HCl	Lignified xylem vessels	Lignified xylem and sclerenchy matous tissue.	Lignified xylem vessels and peri Cyclic sclerenchy matous tissue
3. Section mounted with dilute Iodine solution	Dense bluish spots scattered around the region of palisade cells and v.bundle	Very few bluish spots scattered the cortex.	Dense bluish spots scattered around The cortex,above and below pericycle and few In the pith region.
4. Section mounted in 5% FeCl3 solution	Greenish spots in the region of palisade Cells and vascular Bundle.	greenish spots in the epidermis, cortex upto Vas. bundle	Greenish spots in the epidermis cortex up to v. bundle.
5. Section mounted In a drop of dragen-Droff"s reagent	Dark reddish colour in the region of palisade and vascular Bundle.	Dark reddish colour in the cortex and phelloderm.	Dark reddish colour in the Epidermis, Phelloderm and pale in pith.
6. Section mounted in a drop of H_2SO_4	No change	No change	No change

Table 2: Results of Phytochemical Screening

Chem. Tests	Pet ether	Chloroform	E.acetate	ethanol	water
1. Carbohydrate					
Molisch's	-	-	-	+	+
Fehling's	-	-	-	-	-
Benedict's	-	-	-	-	+
2. Alkaloids					
Mayer's	-	-	+	+	+
Dragendroff's	-	-	+	+	+
Hager's	-	-	+	+	+
Wagner's	-	-	+	+	+
3. Steroids					
salkowiski Liebermsnn	+	+	+	-	-
Burchard	+	+	+	-	-
4.Tannins and Phenols					
$FeCl_3$ test	-	+	+	+	+
Lead acetate	-	+	+	+	+
Diute iodine	-	+	+	+	+
Pot dichromate	-	+	+	+	+
5.Flavanoids					
shinoda test	-	-	-	-	-
6. Fats and oils					
Spot test	-	-	-	-	-
saponification	-	-	-	-	-
7. Proteins and Amino acids					
xanthoprotein	-	-	-	+	+
Millon's	-	-	+	+	+
Biuret test	-	-	+	+	+
Ninhydrin	-	-	+	+	+

Table 3: Quantitative Microscopic Parameters

Sl No	Parameters	Values
1.	Diameter of starch grains	8.3-33.2 - 49.8µm
2.	Length of fibres	315.4-464.8 - 1859.2µm
3.	Breadth of fibres	15.2 - 33.4 - 50.2µm
4.	Quantitative Microscopic analysis of leaf	
	Vein-islet number	12-13.5-15
	Vein-termination number	5-7-9
	Stomatal number	Upper 75-82
		Lower 33-45
	Stomatal index	Upper 36.5 - 38.3
		Lower 25.3-26.8
	Palisade ratio	Upper l0-12

Figure A

Figure B

Coleus vettiveroids Fig A:Root system Fig B:Aerial part with inflorescence

Table 4: Physicochemical constants of *Coleus vettiveroids* K. C. Jacob

Parameters	values (%w/w)
1. Extractive values	
Alcohol soluble extractive value	14.4
Water soluble extractive value	11.2
Ether soluble extractive value	2.4
2. Ash values	
Total ash value	11
Acid insoluble ash value	6.06
Water soluble ash value	1.85
Sulphated ash value	4.16

Table 5: Fluorescence analysis of powder of *Coleus vettiveroids*

Treatment	Day light	U.V.light
Powder as such	Greyish blue	No flourescence
Powder+ 1N HCl	Yellow	Yellowish green
Powder+ aqous 1N NaOH	Brownish black	Green
Powder+ alcoholic 1N NaOH	Orange brown	Green
Powder+50%H_2SO_4	WINE red	Green
Powder+methanol	Green	Green

Histochemical studies: Performed by adding a drop of specific chemical reagent to the histological section of leaf, stem and root to find out the localization and presents of various active constituents in the section itself [4,5,6,7.]

Physicochemical analysis: Physicochemical parameters such as the percentage of ash values and extractive values

Table 6: Results of High performance Thin Layer Chromatography.

Solvent system	No of peaks	HRf values
Toluene: chloroform (70:30)	6	6,12,17,4,56,95
Petroleum ether: ethyl acetate (90:10)	8	3, 7, 11, 2, 36, 4, 55, 93
Cyclohexane: ethyl acetate (90:10)	7	5, 13,22,31,56,8,96

Table 7: Results of HPTLC of compound 1 in different solvent systems

HRF	Max.Wl	Solvent systems
31	259	Cyclohexane: ethyl acetate (90:10)
55	260	Toluene: chloroform (70:30)
54	257	Petroleum ether: ethyl acetate (90:10)

Table 8: Results of HPTLC of compound 2 in different solvent systems

HRF	Max.Wave length	Solvent systems
95	200	Cyclohexane: ethyl acetate (90:10)
80	200	Toluene: chloroform (70:30)
92	200	Petroleum ether : ethyl acetate(90:10)

Table 9 : Results of HPTLC of compound 3 in different solvent systems

HRF	Max.Wave length	Solvent systems
22	200	Cyclohexane: ethyl acetate (90:10)
17	200	Toluene: chloroform (70:30)

were performed according to the official methods prescribed in Indian pharmacopoeia and the WHO guidelines[9] on quality control methods for medicinal plant materials.

Determination of crude fiber content: Crude fiber content was calculated according to the official method.[8]

Fluorescence analysis: Fluorescence study was performed by treating drug powder with different chemical reagents[9, 10]

HPTLC study: Precoated Silica Gel,G 60 F254 plates were used for application of the sample.A small quantity of the extract was dissolved in petroleum ether and applied on the precoated plate with the help of linomat applicator. Solvent system optimized for TLC was chosen for HPTLC. On a 2.8X10 cm TLC plate an aliquot of the sample solution was applied as 5mm band at 8.00mm from the base of the plate. Then it was developed in twin trough glass chamber up to 75.00 mm using the mobile phases petroleum ether: ethyl acetate (90:10) toluene : chloroform (70:30) cyclohexane: ethyl acetate (90:10).The developed plate was dried in air and subsequent photography at U.V 200-360 nm were taken.[11,12,13.]

RESULTS AND DISCUSSION

Histochemical studies: Reults of histochemical studies are shown in the table1.

The study revealed the distribution of starch (using dilute iodine solution), localization of lignins (using phloroglucinol-HCl), phenolic substances (5%FeCl2), alkaloids (using dragendroff's reagent), Con.H2SO4 (triterpinoid saponins) in the histological sections of leaf root and stem. The results showed that starch grains are present in the palisade cells and vascular bundle region of leaf, cortical region of root, cortex, pericycle and pith region of stem. Phenolic substances are present in the region of palisade cells and vascular bundle in the case of leaf, epidermis, cortex and vascular bundle region in the case of stem and root. Alkaloids are distributed in the region of palisade cells and vascular bundle region of leaf, cortex and phelloderm of root, epidermis, phelloderm and pith regions of stem. No triterpinoid saponions seen in these sections.

Quatitative microscopic studies: Results are shown in table 2.

Physicochemical constants: Results of ash values and extractive values are shown in table no: 3

Determination of crude fibre content: Crude fibre content=12.92%w/w

Flourescence analysis: Results are given in table: 4

Preliminary phytochemical screening of whole plant of Coleus vettiveroids: Results are shown in table: 2 Petroleum ether extract upon phytochemical screening showed the presence of steroids and phenolic compounds, Chloroform extract gave positive tests for steroids, alkaloids and for phenolic compounds and tannins. Ethyl acetate extract gave positive tests for proteins amino acids, alkaloids, tannins and phenolic compounds and Water extract showed positive tests for carbohydrate mainly reducing sugars, starch, proteins, amino acids, alkaloids, phenolic compounds and tannins.

Chemical tests for detection of inorganic constituents: The test solution did not give response to the chemical tests for the detection of inorganic constituents such as calcium, magnesium, potassium, sulphate and chloride.

Results of HPTLC: Results are shown in table 6.by using the solvent system Toluene: Chloroform (70:30) we got a total of 6 peaks, 8 peaks for Petroleum ether: Ethyl acetate (90:10) and 7 peaks for Cyclohexane: Ethyl acetate (90:10). Out of the compounds separated by 3 different solvent systems , 2 compounds show identical spectral parameters in the 3 systems and one compound showed identical spectral parameters in Toluene: Chloroform (70:30) and Cyclohexane: Ethyl acetate (90:10) .

CONCLUSION

The plant C. vettiveroids was ethno-medically used by local people to treat various ailments without standardization. The standardization of a crude drug is an essential part to establish the correct identity and authenticity of this medicinally useful plant. The preliminary phytochemical screening showed the presence of carbohydrates, steroids, proteins, aminoacids, phenolic compounds,tannins and alkaloids in various extracts.Flourescence analysis of various extracts gave valuable information of the identification of plant. Physicochemical constants such as ash values, extractive values, determination of crude fibre content and histochemical studies and HPTLC study will help for identification of plant for future reference. The results of these investigations could form the basis for proper identification, collection and investigation of the plant

REFERENCE

1. Dr. Vinod. D .Rangari, Pharmacognosy and Phytochemistry, ,Edn:2, Vol.1, Career publications,Pune,2002,56
2. M.Abdel-mogib,H.A.Albar and S.M.Batterjee, Chemistry of the genus Plectranthus. Molecules 2002, 7(2), 271-301.
3. Vaidyratnam.PS, Indian Medicinal Plants: A Compendium of 500 species.Edn 1, Vol.4, Orient Longman Ltd,1997, 318-320
4. Trease G .E, Evan.W .C, Pharmacognosy, 12th edn, Churchill and Livingstone, 1983, 98-99,485-86,538-547,137-141
5. Kokate C.K. Practical Pharmacognosy. Edn 1, Vallabh Prakashan, New Delhi, 1986, 111.
6. Indian Pharmacopoeia. Edn 4, Vol. I, Government of India, Ministry of Health Welfare, Controller of Publications, New Delhi, 1996, A-53-A-54., 947, A-124, A-70-71, A-89, A-74, A-76, A-105.
7. WHO/PHARM/2.559/rev.1. Quality Control Methods for medicinal plants Materials. Organization Mondiale De La Sante, Geneva, 1992, 9-34.
8. Maynarda. J, Methods in food analysis, Academic press, Newyork,1970,176
9. Chase.C. R and Pratt, R.S, Fluorescence of powdered vegetable drugs with particular reference to the development of a system
10. Harborne JB. Methods of Extraction and isolation. In: Phyotchemical Methods. Chapman & Hall, London, 1998, 60-66.
11. Kokoshi C J, Kokoshi R J and Sharma F.J; Fluorescence of powdered vegetable drugs under ultraviolet radiation.J Pharm Assos.1958; 47:715-717.
12. P.D.Sethi,HPTLC,Quantitative Analysis of Pharmaceutical Formulations,1st edn,1996
13. HPTLC Analysis of Medicinal Plants. HPTLC,Selvino/Bargmo,Italy,1987,113