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Original Research Article

Preliminary Pharmacognostic studies and Chromatographic fingerprinting of *Coleus aromaticus*

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Abstract: Nature has been a basis of medicinal agents for past era and an remarkable number of current drug have been isolated from natural sources, many based on their use in traditional medicine. Plants from the genus *Coleus* have been used in conventional medicine by many cultures. Flavonoids, glycosides, phenolic compounds and volatile constituents have been reported as the major phyto-constituents of the *Coleus aromaticus*. In the present work an attempt had been made for assess the pharmacognostic parameters, total flavonoid content along with chromatographic fingerprinting of aqueous extract of *C.aromaticus via* TLC and HPLC analysis. The results revealed the presence of rosmaranic acid and chlorogenic acid present in the aqueous extract of the *C.aromaticus*.

Keywords: *C.aromaticus*, Flavonoid content, TLC & HPLC.

INTRODUCTION

Botanicals are of great significance to the health of individuals and communities [1]. India is recognized as the "*Emporium of Medicinal Plants* [2]". Due to their great consequence, demand of medicinal plants has increased several folds. The genus *Coleus* was first described by De Loureiro (1970). The name *Coleus* is derived from the Greek word *Koleos*, which means sheath around the approach[3]. *Coleus aromaticus* Benth. syn. C. amboinicus Lour, Plectranthus amboinicus (Lour.) Spreng. English: Country borage, Indian borage; Sanskrit: Karpuravalli, Sugandhavalakam; Hindi: Patharchur; Bengali: Paterchur; Tamil: Karpuravalli[4]. The leaves are valuable in cephalagia, otalgia, anorexia, dyspepsia, flatulence, colic, diarrhoea, cholera, halitosis, convulsions, epilepsy, cough, asthma, hiccough, bronchitis, strangury, hepatopathy and malarial fever [5].

Fig-1: Coleus aromaticus

EXPERIMENTAL WORK

Procurement and authentication of Crude drugs

The crude drug were procured from Botanical Garden from SBMNIPSR, Asthal Bohar (Rohtak) and authenticated from ICAR-National Bureau of Plant and Genetic Research, Delhi. The drugs were then allowed to dry in air and crushed in small pieces for extraction and extractive values.

Evaluation Parameters [6-8]

Macroscopic examination

- **Color**

Untreated samples were examined under diffuse day light. An artificial light source with wavelength similar to those of day light may also be used. The color of sample was recorded.

- **Surface characteristic, texture and fracture characteristics**

Materials were touched to determine if it is soft or hard bend and ruptured it to obtain information on brittleness and the appearance of the fracture plane-whether it is fibrous, smooth, rough, granular etc.

- **Odor**

A small portion of the sample was placed in the palm of the hand and slowly and repeatedly, the air was inhaled over the material.

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- **Taste**

A small amount of drug powder was kept over the tounge and the taste was observed.

Physical evaluation

Determination of foreign matter

About 10 gm of the sample was weighed and spread on a white tile uniformly without overlapping. Then the sample was inspected by means of 5x lens and the foreign organic matter was separated. After complete separation the matter was weighed and percentage w/w was determined.

Determination of solvent extractive value

Determination of water soluble extractive value

Five gm of powdered crude drug was macerated with 100ml of water in closed flask for 2hr and was occasionally shaked for 6hr time period and was allowed to stand for 18hr. After filtration the 25ml of the filtrate evaporated to dryness in a tarred flat bottom shallow dish. Dried at 105°C and weighed. Percentage of water soluble extractive value was calculated with reference to the air dried drug.

Determination of alcohol soluble extractive value

Alcohol is an ideal solvent for extraction of various chemicals like tannins, alkaloids, resins etc. Ethyl alcohol (95% v/v) was used for determination of alcohol soluble extractive.

Five gm of powdered drug was macerated with 100ml of ethanol closed flask for 24hr and was occasionally shaked with 6hr time period and was allowed to stand for 18hr. After filtration the 25ml of the filtrate evaporated to dryness in a tared flat bottomed shallow dish. Dried at 105°C and weighed. Percentage of ethanol soluble extractive value was calculated with reference to the air dried drug.

Determination of Moisture Content

The percentage of active constituents in crude drug is mentioned on air dried bases. Hence, the moisture content of the crude drugs should be determined and should also be controlled. The moisture content should be minimized in order to prevent decomposition of crude drugs either due to chemical changes or microbial contamination. .

Procedure

The powdered samples weighed 5gm accurately and kept in IR moisture balance. The loss in wt. was recorded as percentage (%) moisture with respect to air dried sample of crude drug.

Determination of Ash value

The residue remaining after incineration is the ash content of the drug, which simply represents inorganic salts, naturally occurring in drugs or adhering to it or deliberately added to it as a form of adulteration. Many times the crude drugs are admixed with various mineral substances like sand, soil, calcium oxalate, chalk powder or other drugs with different inorganic content. Ash value is a creation to judge the purity of crude drugs. Generally either total ash value or acid-insoluble ash value or both is determined. Total ash usually consists of phosphates, silicates and silica. On the other hand, acid-insoluble ash, which is a part of total ash insoluble in dilute hydrochloric acid, contains adhering dirt and sand.

Determination of total ash

Total ash was determined by weighing 2 gm of the air dried crude drug in the tared platinum or silica dish and incinerated at a temperature not exceeding 450°C until free from carbon and then was cooled and weighed.

Determination of acid insoluble ash

The ash obtained from the previous process was boiled with 25ml of 2M HCI for 5 min. and the insoluble matter was collected on ash-less filter paper and was washed with hot water, ignited, cooled in a dessicator and weighed. Percentage of acid insoluble ash was calculated with reference to the air dried drug.

Determination of water soluble ash

The ash was boiled with 25ml of water for 5 min. and the insoluble matter was collected on ash-less filter paper and was washed with hot water, ignited for 15min. at a temperature not exceeding 450°C. The weight of the insoluble matter was subtracted from the weight of the ash and this represents the water soluble ash. Percentage of water soluble ash was calculated with reference to the air dried drug.

Preparation of extracts

The powdered plant material (200gm) was extracted successively with redistilled, analytical grade petroleum ether (40-60^{O}C), chloroform, ethanol, methanol and water.

Fig-2: Extraction Process

Qualitative Phytochemical analysis [8]

The extracts obtained were subjected to various qualitative tests to reveal the presence or absence of common phytopharmaceuticals.

Determination of Total Flavonoids Content

The total flavonoids content was determined by $AlCl_3$ colorimetric method. The content of flavonoids was determined as quercetin equivalent. 10 mg/ml of plant extract in respective solvent (stock solution SS) was mixed with 2 ml $AlCl_3$ (2% w/v) in methanol and the solution was made up to 25ml with methanolic solution of acetic acid (0.5% v/v) (Probe solution PS). 1ml of SS was made up to 25ml with methanolic solution of acetic acid (contrast solution CS).The absorbance of PS and CS was measured at 420nm after 30 minutes. The result expressed as % of total Flavonoids content [9].

%TFC = Absorbance at 420 x dilution x 100 / $E^{1\%}_{1\ cm}$ x wt. of extract in gms

Chromatographic Finger printing of Plant extracts

TLC analysis of plant extracts

TLC fingerprinting of aqueous concentrate and oil of leaves of *C. aromaticus* was exposed to thin layer chromatography contemplates, to discover the presence of number of mixtures on help of compound test [10].

HPLC analysis of plant extracts

The HPLC examination was performed utilizing a LC-100, CyberlabTM, Salo Torrace, Millburry, MAO 1527, USA with LC-UV-100 UV finder. A CAPCELL (C-18) HPLC-stuffed segment (4.6 mm I.D.X 250 mm), type MG 5 µm, number AKAD/05245 was utilized for the chromatographic partitions. The mobile phase comprised of The mobile phase consisted of a mixture of water and phosphoric acid [999: 1] (v/v) (solvent A) and acetonitrile (solvent B).The stream rate was 1.5 mL/min, and a section temperature of 25°C. The infusion volume of aqueous extract of C.aromaticus was 25µl, and UV identification was affected at 310 nm [11, 12].

RESULT AND DISCUSSION

Morphological studies of the leaves will enable to identify the crude drug (Table:1). Ash values and extractive values (Table:2) can be used as reliable aid for detecting adulteration. These simple but reliable standards will be useful for society in using the drug as a home remedy. Also the manufacturers can utilize them for identification and selection of the raw material for drug production. Standardization plays a significant role in the production of phytopharmaceuticals of standard quality as the quality standards are based on proper selection of raw materials. As very little specific standards are mentioned in the official monographs evaluation of the crude drugs is of great importance for the pharmaceutical industries. This involves the determination of identity, purity & quality. Many organic & inorganic contaminations which are virtually impossible to avoid while collecting crude drugs affect the purity of any crude drug which needs proper assessment & detection based on different pharmacognostic & phytochemical parameters. The preliminary phytochemical screening showed presence of various bioactive molecules like polyphenols and flavonoids. The quantitative assay of the total flavonoid contents revealed that %TFC is 2.50(table 4).

Qualitative analysis of aqueous leaves extract of *C.aromaticus* was carried out by TLC. Finger printing. Thin layer chromatography is typically accomplished for a superior distinguishing proof of the bioactive mixtures. In the present study the TLC profiling showed the presence of various metabolites, for example, flavonoids and phenolic and tannins.

The results revealed that aqueous extract *C. aromaticus* showed 3 and 1 spots in n-Butanol:Acetic

acid:water (10:1:1) and n-Hexane:Formic acid:Ethyl acetate:water (5:4:3:1) respectively .R_f value were tabulated in table 5. Different R_f value of the mixtures gives a thought about their extremity that may likewise help in choosing a specific dissolvable framework for additional disconnection of any compound from the plant separates utilizing chromatographic furthermore, spectroscopic procedures.

HPLC separations of aqueous leaves extract of *C.aromaticus* were performed on a Cyber Lab C-18 column (250 x 4.0 mm, 5µ). The HPLC chromatogram of aqueous leaves extract of *C.aromaticus* showed RT = 7.221 & 9.98 min respectively (Fig 3 & Table 6).The result revealed the presence of flavonoid in aqueous leaves extract of *C.aromaticus* showed presence of plant phenolic (Chlrogenic and rosmarinic acid) as compared with standard moieties.

CONCLUSION

The present work aids in the standardization and identification of the plant material. The result obtained from the phytochemical screening showed the presence of the various active constituents in the leaves of the plant. Further chromatographic finger printing was carried out by TLC and HPLC showed the presence of Chlorogenic acid and Rosmarinic acid as active content in the aqeous leaves extract of *C.aromaticus*.

Table-1: Morphological Parameters

Characteristics	*Coleus aromaticus*
Size	Length: 5-8cm Width:3-5
Shape	simple, opposite, broadly ovate,crenate and fleshy
Color	Light green
Odor	Aromatic
Taste	Aromatic

Table-2: Physico-chemical Parameter

S.No.	Parameter	*C.aromaticus*
1.	Foreign organic matter	1.02
2.	Ethanol soluble extractive	8.7
3.	Water soluble extractive	26.3
4.	Total ash	17.01
5.	Acid-insoluble ash	1.70
6.	Water soluble ash	29.4
7.	Loss on drying	7.8

Table-3: Phytochemical Screening of *Coleus aromaticus* leaves

Test	Pet.ether	Chloroform	Ethanolic	Methanolic	Aqueous
Carbohydrate					
Molish	(-)ve	(-)ve	(-)ve	(-)ve	(-)ve
Benedict	(-)ve	(+)ve	(-)ve	(+)ve	(-)ve
Starch	(-)ve	(-)ve	(-)ve	(-)ve	(+)ve
Hexose sugar	(-)ve	(-)ve	(-)ve	(+)ve	(-)ve
Tannin					
$FeCl_3$	(-)ve	(-)ve	(+)ve	(-)ve	(-)ve
Protein					
Biuret	(-)ve	(-)ve	(-)ve	(-)ve	(-)ve
Xanthoprotein	(-)ve	(-)ve	(-)ve	(-)ve	(-)ve
Amino acid					
Ninhydrin	(-)ve	(-)ve	(-)ve	(-)ve	(-)ve
Alkaloids					
Dragnodroff	(-)ve	(+)ve	(-)ve	(-)ve	(+)ve
Mayer	(-)ve	(+)ve	(-)ve	(-)ve	(+)ve
Steroid					
Salkowski	(-)ve	(+)ve	(+)ve	(-)ve	(+)ve
Libermann –Bucher	(-)ve	(+)ve	(+)ve	(-)ve	(+)ve
Flavonoids					
Shinoda	(-)ve	(-)ve	(-)ve	(-)ve	(+)ve
NaOH	(-)ve	(-)ve	(-)ve	(-)ve	(+)ve
Lead acetate	(-)ve	(-)ve	(-)ve	(-)ve	(+)ve
Coumarin	(-)ve	(-)ve	(-)ve	(-)ve	(+)ve
Glycosides					
Baljet	(-)ve	(+)ve	(-)ve	(-)ve	(+)ve
Legal	(-)ve	(+)ve	(-)ve	(-)ve	(+)ve
Killer-Killani	(-)ve	(-)ve	(-)ve	(-)ve	(-)ve

(+)ve = Present (-)ve Absent

Table-4: Total Flavonoids Content

S. No	Sample	%TFC
1.	Aqueous extract of *C.aromaticus*	2.50

Table-5: TLC Fingerprinting of plants extract

S.No.	Extract	Solvent System	Number of spot	R_f value
1.	Aqueous extract *C. aromaticus*	n-Butanol:Acetic acid:Water (10:1:1)	3	0.36, 0.72, 0.82
2.	Aqueous extract *C. aromaticus*	n-Hexane:Formic acid:Ethyl acetate:Water (5:4:3:1)	1	0.79

Table-6: HPLC Analysis of Plants extract

S.No	Sample	Height	Area	Conc.	RT	Inference
1.	Aqueous extract of *C.aromaticus*	58566	1014983	61.4524	7.221	Chlorogenic acid
2.	Aqueous extract of *C.aromaticus*	52566	914983	59.342	9.98	Rosmarinic acid

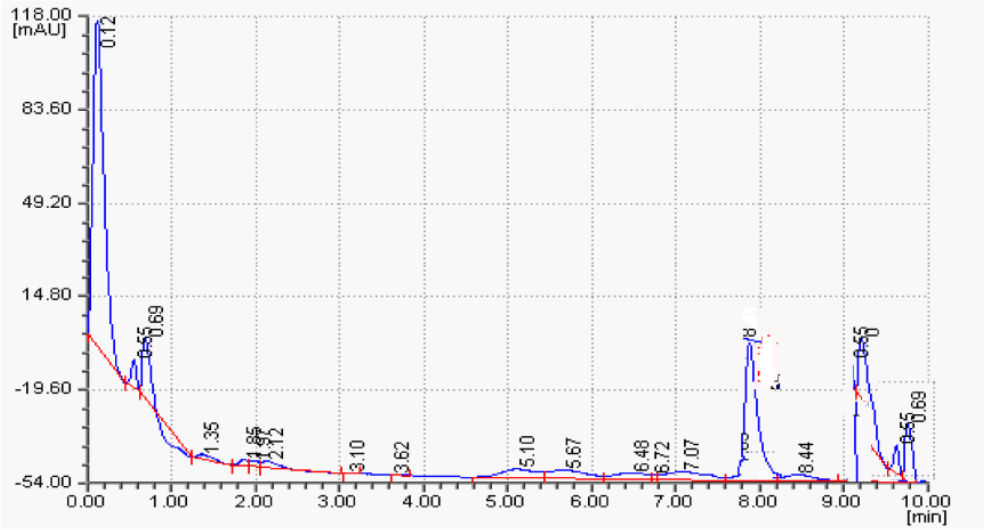

Fig-3: HPLC chromatogram of aqueous leaves extract *C.aromaticus*

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