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STUDIES ON PRELIMINARY PHYTOCHEMICAL SCREENING OF DIFFERENT EXTRACTS OF *CENTRATHERUM PUNCTATUM* Cass. - A TRADITIONAL WOUND HEALER

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ABSTRACT

Present study aims at investigating the phytochemical constituents present in different extracts of *Centratherum punctatum*. In the current study, solvents such as petroleum ether, chloroform, ethanol, and water were respectively used to identify the bioactive compounds from the aerial parts of *Centratherum punctatum* and screened for their phytochemical constituents. Fluorescence analyses of the plant powder as such and also for different successive extracts were observed in day light as well as under UV light. Different physicochemical constants such as, ash value, extractive value was determined as per WHO recommended physicochemical methods. Preliminary qualitative chemical test for different extracts revealed the presence of alkaloids, phenols, quinones, flavones, sterols and proteins in *C. punctatum.* The present investigation reveals the presence of many phytochemical constituents which could be used as a standard data for determining the identity, purity and strength of this drug, for checking the quality of the drug. These major chemical constituents may also contribute in the management of various diseases for which it is used as anti cancer, analgesic and wound healing agents.

Keywords: *Centratherum punctatum,* Successive solvent extraction, Physicochemical, Fluorescence, Phytochemical analysis.

INTRODUCTION

Plants have been in use as spices, food and folk remedies. The world health organization (WHO) estimates that in developing countries, about 80% of the population relies on plant based preparations for their primary Health care [1]. Nowadays herbal drugs are in great demand due to their safety and efficacy and are free from serious side effects [2]. To ensure the reproducible quality of the herbal products, proper identification and authentication of the plant drug be very much essential [3]. The medicinal values of plants lie in some chemical substances so as to produce a definite physiological action on the human. *Centratherum punctatum* a traditional drug belonging to the family Asteraceae. *Centratherum punctutam* Cass. is one among 33 species of the type genus *Centratherum* and is a perennial bushy plant of 45-60 cm height. It has a well branched stem with revitalizing scented foliage and purple flower heads. Recently an vital oil containing nearly 59 different compounds has been isolated from the leaves of this plant [4]. Centratherin, sesquiterpene lactone, has been isolated from *C. punctatum* but its medicinl properties have not yet been established conclusively [5]. An interrelated species *C. anthelminticum* is known for anti-filarial [6] and anti-hyperglycemic properties [7].

The nearby work deals with the phyto-chemical characterization of *Centratherum punctatum* C. The present article attempts to standardize the drug on the origin of chemical parameters which could be useful in evaluating the therapeutic potentials of this ornamental plant.

MATERIALS AND METHODS

Plant Materials

Aerial parts of the chosen plants were scatterbrained from in and around Trichy, identified using Flora of Presidency of Madras and authenticated by comparing with the sampling deposited at RAPINAT Herbarium, St. Joseph`s College, Trichy, Tamil Nadu, India.

Powder Analysis

Fresh Aerial parts of *C. punctatum* were collected and washed under running tap water, to remove adhered filth. The plants were then washed with distilled water, blotted and shade dried. The samples were pulverized in a mixer, sieved with a fine mesh sieve and used for organoleptic study, fluorescence analysis, physico-chemical characterization.

Organoleptic Evaluation

Organoleptic evaluation refers to the assessment of the selected plant drug by color, odor, taste and texture, etc. The organoleptic characters of the samples were evaluated accordingly to the textual methods [8].

Fluorescence analysis

Fluorescence analysis is an fundamental parameter for the first line standardization of crude drug. Both the plant powder and different successive extracts were treated as such with eight different reagents/solvents and kept for 10 min before the analysis**.** The Fluorescence analysis was carried out according to the reported method [9,10]. Where the color of the plant powders and successive extracts were studied under ordinary and ultra-violet light at 366 nm.

Physico-chemical characterization

Different physico-chemical parameters were determined according to the official methods and as per strategy on quality control for medicinal plant materials [11, 12]**.** The average percentage w/w of the ash content and the extractive values were determined.

Extract prepararion

Aerial portion of the selected plant drug was subjected to successive solvent extractions using polar to non-polar solvents. 100 gms of powdered plant material was placed in soxhlet extraction for 8 hrs with 250 ml of the various solvents starting from petroleum ether, chloroform, ethanol, and water. The extracts obtained were later reserved for evaporation to remove the excessive solvents. These extracts thus obtained were stored in a cool dry place. Later used for preliminary phyto-chemical screening and fluorescence analysis. The yield of the crude extracts were measured.

Phytochemical screening

Different phytochemical constituents present in both the powder and extracts were screened**.** The primary metabolites like; proteins, carbohydrates and fixed oils and fats were tested for their presence as per the standard procedures [13,14]. Similarly, the secondary metabolites like, alkaloids, flavonoids, saponins, phenolics, tannins, volatile oils, terpeniods and glycosides were also assessed in the extracts.

RESULTS AND DISCUSSION

Plant identification and documentation is the most essential part of any pharmacognostical study. After proper identification and authentication the plant drug was subjected to preliminary phytochemical and analytical studies. The data of the results obtained were presented and discussed.

Table 1: Organoleptic Study of the Aerial Plant Powder

Character	Observation
Colour	Green
Odour	Strong Aromatic
Taste	Bitter

Powder drug Analysis

Organoleptic study

Organoleptic study forms an important part of powder analysis and is a technique for the qualitative evaluation based on the study of morphological and sensory profiles of whole drugs. Its utility in plant identification has been reported in many studies [15-17]. The present investigation on organoleptic study of aerial plant powder of *Centratherum punctatum* indicated the characters like colour, odour and taste. The data are presented in **Table.1**.

Fluorescence analysis

Fluorescence analysis of the drug powder with different reagents/solvents is an important pharmacognostic tool in finding out the various chromophores of the chemical constituents present in the drug under study [18]. This method is adequately sensitive and enables the precise and accurate determination of the analyte over a satisfactory concentration range [19]. The pharmaceutical and neutraceutical industries are nowadays confronted with adulteration and cheating [20]. The establishment of the salient features of the plant is inevitable in this field to avoid adulteration and substitution. On treatment with different reagents/ solvents colour changes noticed in this plant were given in **Table 2**. The fluorescence features observed of the different extracts were tabulated in **Table 3**.

Table 2: Flourescence features of *Centratherum punctatum* powder

S. No.	Particulars of the treatment	Under ordinary light	Under UV light (366 nm)
1.	Powder as such	Light Green	Light Green
2.	Powder + 1N NaOH (aqueous)	Light Green	Greenish Brown
3.	Powder +1N NaOH (alcoholic)	Yellow	Green
4.	Powder + 50% HCl	Dark Green	Dark Green
5.	Powder + 50% H_2SO_4	Brownish Black	Black
6.	Powder + 50% HNO_3	Brick Red	Brick Red
7.	Powder + Ammonia	Green	Green
8.	Powder + Iodine	Cream	Cream
9.	Powder + 5% $FeCl_3$	Black	Black
10.	Powder + Acetic acid	Cream Colour	Fluorescent Green
11.	Powder + Petroleum ether	Greenish Yellow	Fluorescent Green
12.	Powder + Chloroform	Green	Green
13.	Powder + KOH	Yellowish Brown	Yellowish Brown
14.	Powder + H_2O	Brownish Yellow	Cream
15.	Powder + Ethyl Acetate	Light Green	Light Green
16.	Powder + n-Hexane	Light Green	Light Green
17.	Powder +Picric acid	Yellowish Green	Light Green
18.	Powder + Methanol	Light Green	Flourescent Green

Table 3: Flourescence features of extract of *Centratherum punctatum*

S. No.	Extract	Under ordinary Light	Under UV Light
1.	Water	Brown	Black
2.	Ethanol	Dark Green	Green
3.	Petroleum ether	Dark Green	Blackish Green
4.	Chloroform	Dark Green	Red

Physicochemical parameters

The physicochemical constant is an important parameter in detecting adulteration or improper handling of drugs. Deterioration time of the plant material depends upon the amount of water present in plant material. If the water content is high, the plant can be easily deteriorated due to contamination by fungal colonies. Ash value represents the inorganic salts naturally occurring in the drug and also those adhering to it. The ash value is determined by four different methods, which measures total ash, acid-insoluble ash, water soluble ash and sulphated ash. The loss on drying at 105°C in leaf was found to be 5.9 %. The total ash is particularly important in the evaluation of purity and identity of drugs mainly the presence or absence of foreign inorganic matter such as metallic salts and silica [21]. The percentage of total ash was 10.2% in *C. punctatum*. Acid-insoluble ash is the part of total ash which is insoluble in dil. HCl and measures the amount of silica present. Water soluble ash is the water soluble portion of total ash [17]. The ash values revealed a high percentage of acid soluble ash. The percentages of total ash, acid soluble ash, water insoluble ash and alcohol soluble ash are carried out and results are as tabulated in the **Table.4** .

Table 4: Ash values of *Centratherum punctatum*

WHO Parameters	Average values (%w/w Leaves)
Loss on drying	5.9%
Total ash	10.2%
Acid soluble ash	7.35%
Water soluble ash	1.6%
Alcohol soluble ash	2.35 %

Preliminary phytochemical screening

The preliminary phytochemical screening of *C.punctatum* was carried out wherein the consistency was found to be sticky in the non-polar whereas the polar solvent extracts were found to be non-sticky **(Table 5)**. The percentage yield w/w of the extracts was also determined wherein the highest yield was found to be in the water extract (22.35%). **Table 6.**

Table 5: Phytochemical analysis of different extracts and powder of *Centratherum punctatum*

S. No.	Test	Procedure	Observation	PP	WE	EE	PEE	CE
1	Saponin	Drug+water+shaking+	Honey comb like froth	-ve	-ve	-ve	-ve	-ve
2	Protein	Drug+ Picric acid	Violet color	+ve	+ve	+ve	+ve	+ve
3	Tannin	Drug + lead acetate+ water	Formation of white ppt	-ve	-ve	-ve	+ve	+ve
4	Sterol	Liebermann Test	Bluish green	+ve	-ve	+ve	-ve	-ve
5	Terpenes	Salkowski Test	Bluish green	-ve	-ve	-ve	-ve	+ve
6	Sugar	Anthrone Test	Purple color	+ve	+ve	+ve	-ve	+ve
7	Flavones	Magnesium turnings +HCL	Magenta color	+ve	+ve	+ve	-ve	+ve
8	Quinone	Drops of conc HNO_3	Red color	+ve	+ve	+ve	+ve	+ve
9	Phenol	Ferric Chloride test -	Intense color	+ve	+ve	+ve	+ve	+ve
10	Alkaloid	Drug+Dragondroffs reagent	Orange color	+ve	+ve	+ve	+ve	+ve

+ve = Presence, -ve = Absence. PP-Plant Powder, WE- Water Extract, EE-Ethanol Extract, PEE-Petroleum Ether Extract and CE-Chloroform Extract.

Table 6: Preliminary phyto-profile for the extracts of aerial parts of *Centratherum punctatum*

S. No.	Used Solvent	Color	Consistency	% Yield w/w
1.	Water	Brown	Non Sticky	22.35g
2.	Ethanol	Dark Green	Non Sticky	12g
3.	Petroleum ether	Dark Green	Sticky	3.4g
4.	Chloroform	Dark Green	Sticky	6.3g

Determination of phytochemical profiles of plants is a suggestion of the class of compounds present in the plant. A variety of pharmacological activities are expressed by medicinal plants based on the type and amount of secondary metabolites [22] present in the plant. Preliminary phytochemical results revealed the presence as well as absence of certain photochemical constituents in the extracts. Phytochemical analysis discovered the presence of terpene, flavonoids, steroids, protein, sugar, quinone phenol, alkaloids and tannins. Protein, phenol and alkaloids were present in all the extracts and powder **(Table 5)**. Flavonoids and proteins were absent only in petroleum ether extract and present in the other extracts. Flavonoids serve as a health promoting compound in anti-inflammatory, oestrogenic, enzyme inhibition, antimicrobial, anti-allergic, antioxidant, vascular and cytotoxic antitumour activities [23-25]. Phenols were present in both the extracts and powder. Phenols have been found to be of great importance as they protect the human body from the oxidative stress, which cause many diseases, including cancer, cardiovascular and age related diseases [26]. Steroids were present in both powder and EE.Steroids are known to be useful in pharmacy due to their relationship with sex hormones. Triterpenoids are also recognized for analgesic and anti-inflammatory activities [27]. Tannins have usual considerable attention in the field of nutrition, health and medicine, due to their antioxidant, antimicrobial and anti-inflammatory properties [28]. Coumarins are known remarkably for their antioxidants [29]. Thus preliminary phytochemical analysis revealed the presence of various chemical constituents which possess a broad spectrum of biological activity. Hence the plant could be used as an effective drug in the management of various diseases.

CONCLUSION

Phytochemical screening can serve as a supporting evidence for proper credentials and validation of a plant. Ahead of any drug is included in the pharmacopoeia, these principles must be established. In the current study attempts were made to determine the chemical for the selected drug *C. punctatum.* Chemical standard determined in the present work can contribute in checking the adulteration or substitution of this plant drug besides providing chemical evidence for the therapeutic action of this traditional drug source

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