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Phytochemical and Pharmacognostical Stud- Ies of Centratherum Punctatum

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ABSTRACT: Centratherum punctatum is a medicinal plant belonging to the Family Aster- aceae. The plant extensively used in traditional systems of medicine. This review aims to pro- vide a comprehensive overview of the phyto- chemical and pharmacognostical studies con- ducted on Centratherum punctatum. The Phyto- chemical analysis Centratherum punctatum has revealed the presence of various bioactive compounds including flavonoids, terpenoids, phenolic acids, alkaloids.. Pharmacognostical studies have focused on establishing the macro- scopic, microscopic, and physicochemical characteristics of Centratherum punctatum, providing valuable information for its authenti- cation of plant material Additionally, the evalu- ation of various pharmacognostical parameters, such as ash values, extractive values . that en- sures the safety and efficacy of Centratherum punctatum preparations. Fluorescent analysis of Centratherum punctatum was carried out by using different chemical reagents .In conclusion studies of Centratherum punctatum provide valuable scientific information about the plant chemical composition, medicinal property. These studies contribute the understanding of its therapeutic potential and facilitate the devel- opment of herbal formulations.

KEYWORDS: Centratherum punctatum, phy- tochemicals, pharmacognostical studies, bioac- tive compounds, flavonoids, terpenoids, alkloids physicochemical properties, Fluores- cent analysis

I. INTRODUCTION

Phytochemical and pharmacognostical studies play a pivotal role in unraveling the intricate composition and potential therapeutic properties of medicinal plants. Among these, Centratherum punctatum, commonly known as "Kesavardhini ," has garnered significant attention due to its rich phytochemical profile and traditional usage in various folk medi- cine systems.

Phytochemical analysis of Centratherum punctatum involves the identification and quantification of bio- active compounds present in its different plant parts. These compounds encompass a diverse range of secondary metabolites, including alkaloids, flavo- noids, terpenoids, phenolic compounds, and essen- tial oils. Such a comprehensive analysis provides invaluable insights into the plant's chemical compo- sition, aiding in the identification of potential bioac- tive agents responsible for its therapeutic effects.

Pharmacognostical studies delve into the macro- scopic and microscopic characteristics of Centrath- erum punctatum, aiding in its proper identification and authentication. Macroscopic examination in- volves observing the plant's external features, such as its size, shape, color, and texture, while micro- scopic analysis delves into the cellular structure of the plant, revealing details about its epidermal cells, vascular tissues, and secretory structures. These studies are crucial for distinguishing Centratherum punctatum from other similar species and ensuring the quality and authenticity of herbal products de- rived from it.

In conclusion, the phytochemical and pharmacog- nostical studies of Centratherum punctatum shed light on its chemical composition, structural fea- tures, and potential therapeutic applications. This comprehensive understanding serves as a foundation for further research into harnessing the medicinal potential of this plant, contributing to the develop- ment of new herbal medicines and nutraceuticals

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Figure1: Centratherum punctatum plant

II. MATERIALS AND METHODS

Pharmacognostical studies

Powder Characteristics

Macroscopic evaluation of plant materialOrganolep- tic evaluation can be done by means of organs of sense. This refers to theevaluation of drug by colour, odour, size, shape, taste and special featuresinclud- ingtouch, texture etc. for this purpose authentic specimen of the material under study andsample of Pharmacopoeial quality should be available toserve as a reference. However,the judgement based on the sensory characteristics like odour, taste, etc. may vary fromperson to person and time to time based on individual's nature. No preliminarytreatment is nec- essary for evaluating the sample in this manner.

Color

The untreated samples were examined under difused sunlight or an artificial lightsource with wavelength similar to day light.

Size

Size was measured using graduated ruler in millime- tres.

Odour and taste

Samples were crushed by gentle pressure and exam- ined by repeated inhalation of airover the material.

Texture and fracture

The texture was examined by taking small quantity of material and rubbed in betweenthe thumb and fore finger. Bent and rupture caused to the sample provided informationof the brittleness and appear- ance of the fractured plane as fibrous, smooth,rough, granular etc.2

Microscopic evaluation

Stains and reagents

Saffranin: Dissolve 1 gm saffranin in 100 ml dis- tilled water

Glycerol: Mix equal amounts of glycerol and dis- tilled water

Microscope

Leica DM 1000 LED.

Trinocular 'Leica'microscope attached with 'Leica DFC 295' digital camera connectedto the computer and Leica ApplicationSoftware LAS Version 3.6.1.

Microtome

Plant microtome, Automatic MT3

Methodology

Free hand sections and microtome sections of the materials were taken. Thin sectionswere selected, stained with saffranin, mounted in glycerin. Ob- served through were transferred to computer.

The microscopic examination of powdered leaf ma- terial was performed to detect andto establish vari- ous peculiar microscopic characters in order to dif- ferentiate betweenthe adulterated and the substituted powdered or intact leaves supply. Slides of pow- deredleaf material was prepared using formalin, glycerin and water (8:1:1 v/v/v) and werethus em- bedded and seen under microscope on different magnifications at 10x,40x, 100xafter staining with Phloroglucinol and HCL.3

Physico- chemical parameters Determination of ash value

Used to determine quality and purity of a crude drug and to establish the identity of it. Ash contains inor- ganic radicals like phosphates, carbonates and sili- cates of sodium, potassium, magnesium, calcium etc. these are present in definite amount in a particu- lar crude drug hence, quantitative determination in terms of various ash values helps in their standardi- zation. Used to determine foreign inorganic matter present as an impurity. The ash remaining following ignition of herbal materials is determined by three different methods which measure total ash, acid insoluble ash and water- soluble ash.

Total Ash Value

It is the total amount of material remaining afterig- nition. this includes both "physiological ash", which is derived from the plant tissue itself, and "non- physiological" ash, which is the residue of the ex- traneous matter.

Procedure:

Place about 2-3 g of the ground material, accurately weighed, or the quantity specified in the monograph, in a suitable tared dish previously ignited, cooled and weighed. Incinerate the material

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by gradually increasing the heat, not exceeding 450 °C, until free from carbon; cool, and weigh. Calculate the content in mg of ash per g of air-dried material. If carbon- free ash cannot be obtained in this manner, cool the crucible and moisten the residue with about 2 ml of water or a saturated solution of ammonium nitrate

R. Dry on a water- bath, then on a hot-plate and ignite to constant weight. Allowthe residue to cool in a suitable desiccator for 30 minutes, and then weigh without delay. Calculate the content of total ash in mg per g of air-dried material

Acid-Insoluble Ash Value

The residue obtained after boiling the total ash with dilute hydrochloric acid, and igniting the remaining insoluble matter.

Procedure:

Transfer the crucible containing the total ash into a 100 ml beaker; add 25 ml of dilute hydrochloric acid. Place mere gauze over a Bunsen burner and boil gently for 5 minutes. Collect the insoluble mat- ter on an ashless filter-paper and wash with hot wateruntil the filtrate is neutral. Ignite a crucible in the flame, cool and weigh. Transfer the filter- paper containing the insoluble matter to the weighed emp- ty crucible, ignite to constant weight. Allow the res- idue to cool in a suitable desiccator for 30 minutes, thenweigh without delay. Calculate the content of acid-insoluble ash with reference to the air-dried sample of the crude drug.

Water-Soluble Ash Value

Water-soluble ash is the difference in weight be- tween the total ash and the residue after treatment of the total ash with water.

Procedure:

To the crucible containing the total ash, add 25 ml of water and boil for 5 minutes. Collect the insolu- ble matter in a sintered-glass crucible or on an ash less filter paper. Wash with hot water and ignite in a crucible for 15 minutes at a temperature not exceed- ing 450 °C. Subtract the weight of this residue in mg from the weight of total ash. Calculate the content of water- soluble ash in mg per g of air-dried material.

Fluorescence Analysis

An amount of 1–2 mg powdered plant materials was placed on a microscope slide, and treated with dif- ferent chemicals such as 1N NaOH (aqueous and alcoholic), 1N HCl, ammonia, 5% FeCl3, 5% io- dine, acetic acid, 1N HNO3, and 1N H2SO4 and observed under daylight, short wave (254 nm) and long wave (366 nm) UV lights using a UV cabinet.5

Figure 2: Fluorescence Spectrometer Extraction of leaves of Kesavardhini by cold maceration.

The word 'maceration' means 'softening' andem- ployed in the preparation of tinctures,extracts and concentrated infusions. This is a smooth method of crude drug extractionand is official in Indian Phar- macopoeia. In this process, the dried powder of Keshavardhini leaves to be extracted is placed in a closed vessel and suitablemenstruum is added and left for 7days with occasional shaking. The liquid is thenstrained off and the solid residue (Marc) is pressed to remove the solution as much aspossi- ble.The liquids are mixed and cleared up by filtra- tion. The extract obtained wasthen subjected to qualitative phytochemical analysis. The percentage yield ofextractwas calculated.6

Figure 3: Cold MacerationQualitative phytochemical analysisChemicaltestsfor alkaloids

Chemical tests for alkaloids

A small portion of dried alcoholic extract was shak-en (acidified) with dilute hydrochloric acid and fil-tered. The acidified filtrate was tested with the fol-lowing reagents, to detect the presence of alkaloids.

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a. **Mayer's test:** The acidified extract (two ml) was treated with 1 ml of Mayer's reagent (potassium mercuric iodide), shaken and noted for the presence of a creamy precipitate.
b. **Wagner's test:** The acidified extract (two ml) was treated with a few mlof Wagner's reagent (solu- tion of Iodine in potassiumiodide) and observed for the presence of reddish-brown precipitate
c. **Hager's Test:** The acidified extract (two ml) was treated with 1 ml of Hager's reagent (saturated pic- ric acid solution) and observed for the presence of yellow precipitate.
d. **Dragendorff's test:** The acidified extract (two milliliters) was treated with a few ml of Dragen- dorff's reagent (Potassium bismuth iodide) and ob- served for the presence of orange red precipitate.

Chemical tests for Glycosides

A small portion of the extract was hydrolysed with dilute hydrochloric acid for few hours on a water bath and the hydrolysate was later subjected to fol- lowing tests to detect the presence of glycosides.

a) Legal's Test: The residue (dry extract) left after evaporation was dissolved in a few milliliters of pyridine. Two milliliters of freshly prepared sodium nitro prusside solution was added to it and then made alkaline with sodium hydroxide solution. It was observed for the formation of pink red Color.

b) Baljet's test: The few ml of the extract was treated with 1ml sodium picrate solution and a yel- low to orange color reveals the presence of cardiac glycosides.

c) Liebermann-Burchard's Test: The five ml of the hydrolysate taken in a test-tube was evaporated, the residue taken in dry chloroform (one ml) and then it was mixed with two ml of specially distilled acetic anhydride followed by a few drops of concen- trated sulphuric acid through the sides of the test tube. It was then observed for the development of a deep red color in the lower portion and green color in the upper portion which changed to blue and vio- let.

d) Borntrager's test: A little of the residue obtained from the hydrolysate was mixed with water and shaken with equal volume of chloroform**.** The chlo- roform layer was separated to which dilute ammonia solution was added and shaken well and noted whether any pink color was present in the ammonia- cal layer.7

Chemical tests for tannins

a) **Ferric chloride test:** A small quantity of the ex- tract diluted with water was treated with dilute ferric chloride solution (5%) and observed for the pres- ence of blue color.

b) **Gelatin test:** The extract dissolved in water was filtered. To the filtrate, 2% solution of gelatin con- taining 10% sodium chloride was added. Noted for the presence of milky white precipitate.

c) **Lead acetate test:** The extract dissolved in water was treated with 10% lead acetate solution. Noted for the presence of bulky white precipitate.

Chemical tests for flavanones and flavonoids

a) **Aqueous sodium hydroxide test:** Aqueous sodi- um hydroxide solution was added to the few ml of the extract and the presence of yellow coloration of the solution was noted

b) **Filter paper test:** The filter paper was wetted with small quantity of alcoholic solution of the ex- tract. That filter paper was exposed to ammonia vapours and noted the yellow colour.

Chemical tests for carbohydrates

A small quantity of ethanolic extract was mixed with water or alcohol and filtered. The filtrate was subjected to the following tests to detect the pres- ence of carbohydrates.

a) **Molisch's Test:** The filtrate (two ml) was treated with a few drops of Molisch's reagent and two ml of concentrated sulphuric acid was added through the sides of the test tube without shaking. Observed for the presence of violet ring at the junction of two solutions.

b) **Fehling's Test:** The filtrate (one ml) treated with 1 ml each of Fehling's solution A and B and boiled on a water bath for half an hour, then observed for the presence of red residue at the bottom of test tube.

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c) **Benedict's Test:** The filtrate (few drops) was treated with two ml of Benedict's reagent. Then the mixture was heated on a boiling water bath for two min and the presence of red precipitate was noted.

Chemical tests for proteins

a) **Million's Test:** The extract (two ml) was treated with few drops of Million's reagent (1g of mercury+ 9ml of fuming nitric acid) and observed for the presence of white precipitate, which on warming turn into a red colored solution.

b) **Biuret Test:** The extract (two ml) was treated with one drop of 2% copper sulphate solution. To this 1ml of 95% ethanol was added followed by excess of potassium hydroxide solution and Ob- served for the presence of violet colored solution. c)Ninhydrin Test: The extract (few ml) was treated with two drops of ninhydrin solution and heated on a water bath and then the presence of violet color was noted.9

III. RESULTS AND DISCUSSION

Table1: Macroscopic features

Features	Observation
Colour	Leaves: Light green Flowers: purple
Size	Typically grows to a height of 6-90cm
Odour	Smelling like a pine-apple upon being crushed
Taste	Bitter
Texture	Smooth

Microscopic studies

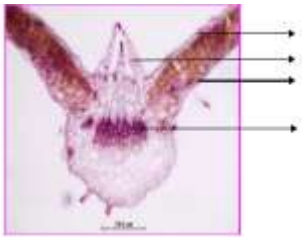

Figure4: T S of centratherum punctatum

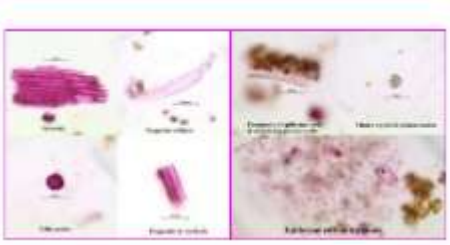

Figure 5: Powder analysis of Centratherum Punctatum

Physico- chemical parameter

Total Ash value

Wt. of empty crucible (g)	Wt. of crucible + sample (g)	Wt.of crucible + ash (g)	Wt of ash (g)	Percentage yield (%w/w)
36.51g	38.51g	36.55g	0.004g	0.2
	38.53g	36.51g	0.003g	0.15
	38.52g	36.51g	0.0035g	0.17
	Average (%w/w)			0.17%w/w

Table2: Total Ash value of C.punctatum Acid in solu-bleash value

Acid insoluble ash value

Wt. of empty crucible (g)	Wt. of crucible + sample (g)	Wt.of crucible + ash (g)	Wt of ash (g)	Percentage yield (%w/w)
36.51g	38.51g	36.55g	0.004 g	0.2
	38.53g	36.51g	0.003g	0.15
	38.52g	36.51g	0.0035g	0.17
	Average (%w/w)			0.17%w/w

Table3: Acid in soluble Ash value of C.punctatum

Water soluble ash value

Wt. of empty crucible (g)	Wt. of crucible + sample (g)	Wt.of crucible + ash (g)	Wt of ash (g)	Percentage yield (%w/w)
31.59g	33.59g	31.73g	0.03g	1.5
	33.56g	31.74g	0.05g	2.5%w/w
	33.54g	31.76g	3%w/w	3%w/w
	Average (%w/w)			2.3%w/w

Table4: Water soluble Ash value of C.punctatum

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Fluorescence Analysis

Fluorescence Analysis of leaf powder of Centrath- erum punctatum were examined in day-light, short and long-UV to detect he fluorescent compounds by the standard method and the results are shown in table no5.

Solvent used	Visible light	UV light	
		At short (254nm)	At long (366nm)
Distilled water	Green	Green	Dark green
1N NaOH	Green	Dark Green	Black
1N HCl	Pale brown	Green	Black
50% HNO3	Brown	Dark Green	Black
FeCl3	Dark green	Black	Black
CHCl3	Green	Green	Black
Picric acid	Green	Dark green	Black

Table5: Fluorescence Analysis of powder of Centratherum punctatum

Phytochemical studies

Percentage yield of cold maceration of leaves of Centratherum punctatum obtained as tabulated be-low in table no 6.

Extract	Method of extraction	Physical nature	Percentage yield (%w/w)
Ethanol	Cold macera-tion	Solid	5.25

Table6: Percentage yield of extracts

Qualitative phytochemical analysis

Phytochemical analysis of extracts was carried out to identify various phytoconstituents and the results were summarized in Table 7 after conducting chem- ical tests.

Chemical constituent	Keshavardhini
Alkaloids	+
Glycoside	-
Carbohydrate	+
Flavonoids	+
Tannins	-

Table 7: Qualitative phytochemical analysis of Keshavardhini

Figure6:Qualitative phytochemical analysis

IV. SUMMARY AND CONCLUSION

The present study involves the pharmacognostical and phytochemical studies of Centratherum puncta- tum.it provides valuable insights into the plant's botanical features and chemical composition. The presence of diverse bioactive compounds suggests its potential for various medicinal applications. Fur- ther research is needed to fully elucidate the mecha- nisms of action and therapeutic potential ofCen- tratherum punctatum's phytochemical constituents. This plant holds promise as a natural source of bio- active compounds that could contribute to the de-velopment of new pharmaceuticals or nutraceuticals. However, rigorous scientific investigation and clini- cal trials are necessary to validate its safety and effi- cacy for specific medicinal uses.

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