International Journal of Research Instinct
(Peer-reviewed Journal)

ISSN ONLINE 2348-2095
Research Article

PHYTOCHEMICAL SCREENING AND GC-MS ANALYSIS OF METHANOL EXTRACT OF FLOWER OF *CENTRATHERUM PUNTATUM* CASS

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ABSTRACT

The present study was designed to investigate the phytochemical constituents present in methanol extract of flowers of *Centratherum punctatum*. Fluorescence analyses was carried out in the flower powder and were observed in day light as well as under UV light.In order to understand the role of phytochemicals, the present investigation is been focused on separation of phytoconstituents by GC-MS technique using Perkin-Elmer Gas Chromatography– Mass Spectrometry. GCMS provides the information about the amount of each chemical present in a sample by comparing to a standard, a pre-measured known amount of the chemical also measured on the GCMS. The compounds which were identified by GC-MS in methanolic flower extract perhaps have medicinal value and acquire various pharmaceutical applications. The identified phytocomponents needs further research on toxicological aspects to develop safe drug.

Keywords:

Centratherumpunctatum Cass, Phytochemical, Fluorescent, Antimicrobial activity and GC-MS.

1. INTRODUCTION

Currently herbal drugs are in great stipulate than ever before. The communities were also of general awareness in regarding the safety and efficacy of herbal drugs. Hence, development of new drugs without any side effect is the urgent need of the society [1]. Quality control studies on plant material are essential to ensure the reproducible quality of the herbal products. The initial step to ensure quality of any starting material is authentication [2]. *C.punctatum* belongs to the family Asteraceae. Although cultivated in subtropical and warm temperate regions for its ornamental

qualities, C.*punctatum* is valued as a medicinal plant as it is effective against number of ailments. *Centratherum punctatum* Cass., is rich in bioactive molecules that could be useful as anti cancer, antimicrobial, analgesic and wound healing agents. The acetone extract of *Centratherum punctatum* Cass exhibited the antimicrobial activity against a number of bacterial and fungal species [3]. Besides they also depicted antioxidant and antiproliferative capabilities. Its purple flower essence is being used in the preparation of many herbal medicines. The plant extract was proved to possess some antimicrobial effects [4]. It is used in hair oil preparations and as skin whitening and antiageing agents. An attempt was made to study proteases in floral extracts of *C.punctatum* which may play a role in wound healing property of the plant [5].Traditionally it is used to increase libido, used as pain killer, used as an antidote for snake bite and tiger bite [6]. Using different solvents such as petroleum ether, chloroform, ethanol, and water, the active biocompounds were identified from the aerial parts of *C. punctatum* [7]. The micromorphological features of *C. punctatum* were analyzed to localize the active molecules histochemically [8]. The antimicrobial activity of ethanol and aqueous extracts of *C. punctatum* Cass.was also evaluated [9]. The aqueous extract of aerial parts of *Centratherum punctatum* Cass.was subjected to HPTLC [10]. Wound healing activity was also evaluated in the ethanol and aqueous extract of *Centratherum punctatum* Cass. (asteraceae) in rats[11]. As there was no availability of literature on the flowers of *C. punctatum* Cass. The phytochemical constitutents, fluorescent assay and separation, identification and structural determination of phytochemicals by GC-MS was evaluated in the floral extracts of C. *punctatum*.

2. MATERIALS AND METHODS

2.1. COLLECTION OF PLANT MATERIALS

Plant parts of *Centratherum punctatum* Cass. Flower was collected from the herbal garden maintained by the Department of Botany at St. Joseph`s college, Trichy, Tamil Nadu, India. The plant was identified and authenticated by botanist Rev.Fr. John Britto, Director of RAPINET Herbarium, St. Joseph`s college, Trichy, Tamil Nadu, India.

2.2. PREPARATION OF EXTRACTS

The plant material was thoroughly washed to remove all the impurities and foreign organic matter and then shade dried at room temperature and powdered with the help of blender. The powdered material was then sieved and stored in plastic container for further use. Extraction of *Centratherum punctatum* was carried out by both cold and hot extraction process. 20 gram of flower powder was subjected to 200ml of methanol. Then the extracts obtained were filtered in fine muslin cloth and left for evaporation to remove the excess solvent. For aqueous extraction same quantity of

plant powder was taken and subjected to 120ml of water (1:6 ratio). The extract was filtered with the help of fine muslin cloth and reduced to dryness and stored in freeze condition for further use.

2.3. PHYTOCHEMICAL SCREENING [12]

Methanol extract of flower was subjected to phytochemical screening for the analysis and observation of various phytoconstituents like carbohydrates, tannins, phenolic compounds, quinones, terpenoids, phytosteroids, coumarins and saponins.

2.4. FLUORESCENT ANALYSIS [13]

The fluorescent analysis was carried out with the powder of flower of *C.punctatum*. The flower powder was treated with solvents such as 1N methanolic and aqueous sodium hydroxide, 50% hydrochloric acid, 50% sulphuric acid, 5% ferric chloride and picric acid and also studied under ordinary and ultra-violet light.

2.5. ANTIBACTERIAL ACTIVITY OF METHANOL EXTRACT OF FLOWER OF *C.punctatum*

Preparation of extract

The stock solution of methanol extract of flower of *C.punctatum* was prepared in 1% Dimethyl sulfoxide (DMSO).

Microorganism

Staphylococcus aureus, *Escherichia coli* and *Pseudomonas aeruginosa* were selected for antibacterial studies. The bacterial isolates were first sub cultured in a nutrient broth and incubated at 37°C for 18hr.

Preparation of standard bacterial Suspensions

Bacterial strains were maintained on Nutrient agar slants (Hi media) at 4°C. These suspensions were prepared immediately before screening. Chloramphenicol was used as the standard antibiotics (1 µlg/ml))

Antibacterial susceptibility testing using Kirby Bauer agar well diffusion assay

To assess the antibacterial activity of the prepared extracts 0.6ml of standardized bacterial stock suspension (10^5-10^6) colony-forming units per ml was thoroughly mixed with 60ml of /sterile nutrient agar. 20ml of the inoculated nutrient agar were distributed into sterile petri dishes. The agar was left to set. Wells (6mm diameter) were made with the help of cutter. Test materials both aqueous and methanol extract at 4 different concentrations namely 100 µg/ml, 200 µg/ml, 300 µg/ml and 400 µg/ml were placed on agar well and were allowed to stand for 1 h at room temperature so as to allow the plants extracts to diffuse into medium and then incubated at 37°C for 24 hours. Well diffusion tests were performed in triplicates for all strains and the antibacterial

activity was expressed as average mean of inhibition diameter (mm) produced by various extracts.

2.6. GC-MS INVESTIGATION OF FLOWER EXTRACT OF *Centratherum punctatum* Cass.

A Volume of 1µl of clear extract was injected into GC-MS (PerkinElmer Clarus 500) with a oven programming of 50ºC (1min) @10 ºC/min to 150 ºC (1 min) @8ºC/min to 250ºC (1min)@15 ºC/min to 300 ºC (5 min). The injector temperature was maintained at 280ºC. The split ratio was set as 1:8. The carrier gas used in the analysis was helium which had the flow rate of 1ml/min. A 30 m Capillary column of elite 5ms, with a Column id of 250 µm was used. The compounds were detected in the range of 40- 450amu by matching with NIST library.

3. RESULT AND DISCUSSION

3.1. PRELIMINARY PHYTOCHEMICAL ANALYSIS

Qualitative analysis carried out on methanol extract of flower showed the presence of phytochemical constitutent and the results are tabulated in **Table.1.**

S.N	Test	Observation	ME of Flower
1	Terpenoid	Reddish brown	+
2	Phenols	Blue green	+
3	Saponins	Foam	+
4	Anthraquinones	Pink precipitate	-
5	Steroids	Browning	+
6	Tannins	Green black/dark blue	+
7	Cardiac glycosides	Brown ring	-
8	Carbohydrate	Purple /red	+
9	Quinines	Red	+
10	Glycoside	Pink	-
11	Phlobatannins	Red colour precipitate	+
12	Coumarins	yellow	+
13	Gum/mucilage	Pink colour	-

+ = Positive, - = Negative, ME = Methanol extract

Table.1. Preliminary Phytochemical analysis of methanol extract of Flower of *C.punctatum*

Phytochemical analysis revealed the presence of terpenoids, phenols, saponins , steroids, tannins, carbohydrate, quinines and coumarins in methanol extract of flower and phytochemicals such as anthraquinones, cardiac glycoside, gum and mucillage were absent in methanol extract of flower of *C.punctatum*.

Many of the terpenoids are commercially fascinating because of their use as flavours and fragrances in foods and cosmetics examples menthol and sclareol or because they are important for the superiority of agricultural yield, such as the flavour of fruits and the fragrance of flowers like linalool [14] . Many plants produce volatile terpenes in order to attract specific insects for

pollination or otherwise to drive out certain animals using these plants as food. Less volatile but strongly bitter-tasting or toxic terpenes also protect some plants from being eaten by animals (antifeedants) [15]. Last, but not least, terpenes play an important role as signal compounds and growth regulators (phytohormones) of plants, as shown by preliminary investigations. Terpenoids have been used as anticarcinogenic (e.g. perilla alcohol), antimalarial (e.g. artemisinin), anti-ulcer, hepaticidal, antimicrobial or diuretic (e.g. glycyrrhizin) activity and the sesquiterpenoid antimalarial drug artimisinin and the diterpenoid anticancer drug taxol [16,17].

Phenolic compounds have been studied mainly for their properties against oxidative damage leading to various degenerative diseases, such as cardiovascular diseases, inflammation and cancer. Indeed, tumour cells, including leukaemia cells, typically have higher levels of reactive oxygen species (ROS) than normal cells so that they are particularly sensitive to oxidative stress [18].

Saponins are plant compounds that crop up either as steroid alkaloids, glycosides of triterpenoids or steroids. They posseshypocholesterolaemic, immunostimulant, hypoglycemic effect and anticarcinogenic properties [19]. The hypoglycemic effect of saponins is due to stimulation of pancreatic β-cells, inhibition of glucose transport across the brush border cells of the small intestines and suppression of transfer of glucose from the stomach to the small intestines. They inhibit gastric emptying in a dose dependent manner [20]. They lower cholesterol level by forming large micelles that are then excreted in bile. These compounds are said to lower serum levels of low density lipoproteins-cholesterol and decrease absorption of cholesterol in the intestines [21].

Steroids are used as the main treatment for certain inflammatory conditions, such as systemic vasculitis (inflammation of blood vessels) and myositis (inflammation of muscle). They may also be used selectively to treat inflammatory conditions such as rheumatoid arthritis, lupus syndrome or gout. Steroids are used as the main treatment for certain inflammatory conditions, such as systemic vasculitis (inflammation of blood vessels) and myositis (inflammation of muscle). They may also be used selectively to treat inflammatory conditions such as rheumatoid arthritis, lupus and gout.

Tannins are polyphenols found in plenty in the tree bark, wood, fruit, fruit pod, leaves and roots and also in plant gall. They are classified into two broad groups – hydrolysable tannins and condensed tannins. The tannin epigallo-catechin-3-gallate is reported to exhibits anti-diabetic activity [22]. In clinical terms, all forms of tannins might participate in the management of glucose level in blood. Tannin is used to excite the receptor cells to utilize carbohydrate. Ellagic acid and quercetin act synergistically to reduce viability, proliferation and trigger apoptosis of MOLT-4 human leukemia cells [23]. Ellagic acid and resveratrol inhibit skin tumorgenesis in mice [24].

Quinine is frequently used as anti-malarial agent along with some other uses as a flavor in carbonated beverages [25], skeletal muscle relaxant, treats hemorrhoids and varies vein and also used as oxytoxic agent [26].

Humans have used cardiac-glycoside-containing plants and their crude extracts as arrow coatings, homicidal or suicidal aids, rat poisons, heart tonics, diuretics and emetics. In modern times, purified extracts or synthetic analogues of a few have been adapted for the treatment of congestive heart failure and cardiac arrhythmia. Therapeutic uses of cardiac glycosides primarily involve the treatment of cardiac failure.

3.2. FLUORESCENT ANALYSIS [27]

Fluorescence studies showed the presence of chemical constituents by emitting characteristic fluorescence. The plant powders showed various shades of green, yellow and red colour under visible light and brown colour under ultraviolet light after treatment with different chemical reagents and data obtained were tabulated in **Table 2.**

Test	Ordinary light	Under UV light (Flower Powder)
Powder + 1N NaOH in methanol	White colour	Brown
Powder + 1N NaOH in water	White colour	Brown
Powder + 50% HCl	White colour	Light brown
Powder + 50% H_2SO_4	White colour	Brown
Powder + Picric acid	Yellow colour	No change
Powder + 5% Ferric chloride	brown colour	No change

Table 2.Florescence features of powders of flower of *Centratherum punctatum*

3.4. ANTIBACTERIAL ACTIVITY OF METHANOL EXTRACT OF FLOWER OF *C. punctatum*

The plant extracts generally, inhibit gram-positive bacteria and gram-negative bacteria. The antibacterial activities of *C.punctatum* extracts were tested against three bacterial strains. The antibacterial activity of *C.punctatum* was investigated by well diffusion method. The results were tabulated in **Tables. 3**. All the tested bacteria responded differently against different extracts and the

effect of the extracts was concentration dependent. Maximum zone of inhibition was observed in the methanol extract of flower against *Staphylococcus aureus* when compared with *E.coli* and *Pseudomonas*. The zone of inhibition was greater in *E.coli* when compared with that of *Pseudomonas* species.

Name of Organisms	Zone of inhibition (diameter in mm/ µl)				
	10µl	20µl	30µl	40µl	control
Eschericia coli	10	15	20	28	25
Staphylococcus aureus	15	16	19	22	25
Pseudomonas aeruginose	10	13	18	20	25

Table 3. Antibacterial activity of methanol extract of flower of *C.punctatum*

3.5. GC- MS

GC-MS analysis of methanol extract of flower of *C.punctatum* was carried out to detect the possible chemical components present in the plant drug. The active principles with their peak name, retention time and % peak area are presented in **Table 4.** The chromatogram and the double mass spectrum of the methanol extract of the flower are shown in **Figure.1.** The results obtained showed the presence of various important phytochemical constituents such as Cyclohexasiloxane, dodecamethyl, Myretene acid bromide, 2-$C_{14}H_{26}$, 7-Hexadecyne, Bicyclo[3.3.0]octan-3-one,6-hydroxy-6-methy,7-Oxabicyclo [4.1.0] heptanes,1-methyl-4(2- ethyl, Cyclopropaneacetic acid,2-hexy-, 1-Octadecyne, trans-2-Dodecen-1-o1,trifluoroacetate, octadecanoic acid, methylester, Bicyclo [4.1.0] heptan-2-ol,(1,alpha.,2beta.,6, 9-octadecenoic acid(Z)-, Phosphonic acid, dioctadecylest, Phytol, 9,12-Octadecadienoic acid (Z,Z), 9,12,15-Octade catrienoic acid,(Z,Z,Z), Octadecanoic acid, (E)-13-Docosenoic acid, 3,7,11,15-tetramethyl-2,6,10,14-hexa, Trifluoroacetyl-lavanduol in methanol extract of flower. Compounds such as cyclohexasiloxane are used as cosmetics, deodorant, defoamers, lubricants, and soaps to soften, smooth, and moisten. 9,12-octadecadienoic acid are used as antiinflammatory, hypocholesterolemic cancer preventive, hepatoprotective, nematicideinsectifuge, antihistaminic, antieczemic, antiacne, 5-alpha reductase inhibitor antiandrogenic, antiarthritic, and as an anticoronary .

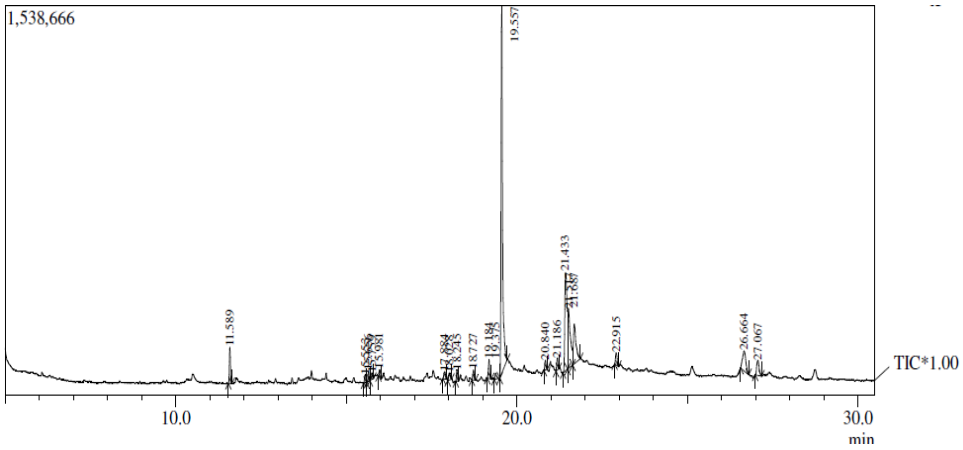

Figure.1 Chromatogram and the double mass spectrum of the methanol extract of the flower

S.No	Peak Name	Retention time	% peak Area
1	Cyclohexasiloxane, dodecamethyl	11.589	2.81
2	Myretene acid bromide	15.553	0.67
3	2-$C_{14}H_{26}$	15.656	0.85
4	7-Hexadecyne	15.759	0.61
5	Bicyclo[3.3.0]octan-3-one,6-hydroxy-6-methy	15.981	0.65
6	7-Oxabicyclo[4.1.0]heptanes,1-methyl-4(2-methyl	17.884	0.91
7	Cyclopropaneacetic acid,2-hexy-	18.028	0.83
8	1-Octadecyne	18.245	0.94
9	trans-2-Dodecen-1-ol,trifluoroacetate	18.727	0.98
10	Octadecanoic Acid,Methylester	19.184	1.66
11	Bicyclo[4.1.0]heptan-2-ol,(1,alpha.,2beta.,6	19.375	0.62
12	9-octadecenoic acid(z)-	19.557	41.03
13	phosphonicacid,dioctadecylest	20.840	0.89
14	Phytol	21.186	2.24
15	9,12-Octadecadienoic acid (Z,Z)-	21.433	27.09
16	9,12,15-Octade catrienoic acid,(Z,Z,Z)-	21.517	11.24
17	Octadecanoic acid	21.687	8.03
18	(E)-13-Docosenoic acid	22.915	1.36
19	3,7,11,15-Tetramethyl-2,6,10,14-Hexa	26.664	4.96
20	Trifluoroacetyl-lavanduol	27.067	2.72

Table. 4. Principle components present in methanol extract of flower of *C.punctatum*

4. CONCLUSION

There is an urge to introduce new and biologically safe and active drugs eco-friendly drug in nature and effective as antimicrobial agents. Habitually medicinal plants posses several phytochemical compounds, which are very much necessary to control the growth of the micro organisms. The present study characterized the phytochemical profile of the methanol extract of flower of *C.punctatum* using GC-MS. The chromatogram shows the comparative concentration of different components getting eluted as a purpose of retention time. The heights of the different peaks indicates the relative concentration of the compounds exist in the methanolic extract of *C.punctatum*. The Chromatogram depicted the analysis of the compounds which were eluted at different time intervals to recognize the nature and structure of the compounds. These spectrum are finger print of the compound which can be identified from the NIST library. The identification of various bioactive compounds confirms the therapeutic application of *C.punctatum* for a variety of diseases. Further research is in progress for the pharmacological evaluation of *C.punctatum*

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