Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 3, 2013

Research Article

IN- VITRO CYTOTOXIC, ANTIOXIDANT AND GC-MS STUDIES ON *CENTRATHERUM PUNCTATUM* Cass.

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Received: 27 Mar 2013, Revised and Accepted: 19 May 2013

ABSTRACT

Objective: Herbal medicine is as ancient as mankind and has significantly contributed to the health care of the human society. The present study is carried out to investigate the cytotoxic and antioxidant potentials of Ethanol extract of aerial parts of ***Centratherum punctatum* Cass.** (Family - Compositae).

Methods: Antioxidant activity measured using DPPH assay and evaluating cytotoxic effect using Trypan blue dye exclusion method and analysis of active extract using GC-MS.

Results: Cytotoxic effect was evaluated employing Trypan blue dye exclusion method using Ehrlich Ascites Carcinoma cell lines which depicted the cytotoxic potentials of the selected drug source. Besides the extract also showed significant antioxidant activity comparable with that of standard ascorbic acid in the DPPH free radical scavenging assay. Gas chromatography mass spectrometry (GC-MS) analysis of the Ethanolic extract which revealed potential cytotoxic effect revealed the presence of anticancerous compounds such as Eugenol, Spathulenol, Viridiflorol, Hexadecanoic acid, Phthalic acid, bis(7-methyloctyl) ester, Eicosane and Squalene.

Conclusion: Present findings indicated promising anticancer and antioxidant potentials of the Ethanol extract of aerial parts of ***Centratherum punctatum* Cass.**

Keywords: In-vitro Cytotoxicity; In-vitro Antioxidant; GC-MS; DPPH Assay; *Centratherum Punctatum* Cass.

INTRODUCTION

The traditional medicine systems recommend the usage of different parts of herbs in the form of tinctures, powders, decoctions or extracts. Though these systems are effective, they need to be standardized and validated for better acceptability and adaptability. This can be achieved only through standardization and validation studies which involve botanical, chemical *In-vitro and In-vivo studies*. In the present work attempts were made to evaluate the cytotoxic potentials of the ethanol extract of aerial parts of an Asteraceae drug source ***Centratherum punctatum* Cass.** as it is used to control various ailments like Cancer, Inflammation, intestinal disorders, fever and pain. The extract is subjected to both cytotoxic and antioxidant studies employing in-vitro methods. After establishing the efficacy extract was assessed for its potential bioactive molecules using GCMS method.

MATERIALS & METHODS

Plant Material

Fresh plants of Centratherum punctatum Cass. were collected from the Herbal Garden of Srimad Andavan Arts and Science College, Tiruvanaikovil, Trichy - 620005 during the month of February. Identified with the help of Flora of Presidency of Madras[1] and confirmed by comparing with the Herbarium specimen deposited at Royal Botanics Garden, Kew (Voucher specimen number K000373089).

Chemicals

All the reagents used were of analytical grade obtained from Merck-India.

Preparation of Ethanol Extract

After proper identification and authentication aerial parts of the plant were cleaned, shade dried and coarsely powdered. 250 gms of powder was weighed and transferred to Stoppered flask, and treated with 1000 ml of Ethanol until the powder is fully immersed. The flask was shaken every hour for the initial 6 hrs and then it was kept aside and again shaken after 24 hrs. This process was repeated for 3 days and then the extract was filtered. The extract was collected and the solvent was distilled off.[2] The final residue obtained was subjected to In-vitro cytotoxic activity, DPPH Assay and after confirming the selected therapeutic potentials the extract was subjected to GC-MS analysis.

In-vitro Cytotoxicity

Trypan blue Exclusion method

Short term in-vitro cytotoxic potential of selected drug was assessed using Ehrlich Ascites Carcinoma cell lines by incubating with different concentrations of the Ethanolic extract of the drug under study at room temperature for 3 hours. The tumour cells were aspirated from peritoneal cavity of tumour bearing mice using an Insulin syringe and transferred to a test tube containing isotonic saline. The cells were then washed in normal saline and cell number was determined using a Haemocytometer and adjusted to 1 X 10^6 cells/ml. For the cytotoxic assay, different concentrations of the Ethanol extract of drug was added to each tubes and the final volume was adjusted to 1 ml with normal saline. Control tubes were kept with saline, tumour cells and without the drug. All the tubes were incubated at 37ºC for 3 hours. After incubation, 0.1 ml of 0.4% trypan blue dye in isotonic saline was added to each tube and the number of viable (unstained) and dead (stained) cells were counted using haemocytometer.[3]

In-vitro Antioxidant Assay

DPPH Radical Scavenging Assay

Free radical scavenging capacity of the Ethanol extract of ***Centratherum punctatum* Cass.** was determined using DPPH. DPPH solution (0.004%w/v) was prepared in 95 % methanol. Five different concentrations of drug under study were taken in test tubes. 1 ml of freshly prepared DPPH reagent was added to the test tubes and incubated in dark. After 10 minutes of incubation, the absorbance was measured at 517 nm using spectrophotometer (Systronics UV:Visible spectrophotometer 119, India). Ascorbic acid was used as a positive control. % scavenging activity of the DPPH free radical was measured using the following equation

$$\text{Inhibition (\%)} = (A_0 - A_1 / A_0) \times 100$$

Where; A_0 is the absorbance of control and A_1 is the absorbance of test.[4]

GC-MS investigation of ethanol 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.[5,6,7]

RESULTS AND DISCUSSION

In-vitro Cytotoxicity

Trypan blue Exclusion method

The drug under study was revaluated for its cytotoxic potential against EAC cell lines using Trypan blue method and the data of the results obtained were encouraging. The extract must be having considerable membrane damage potential and might have activated the apoptotic pathway inside the cancer cells as reported by earlier workers in the same family.[8]

In-vitro Antioxidant Assay

DPPH Radical Scavenging Assay

The DPPH assay is one of the most widely accepted method for assessing antioxidant potential of plant extracts. Table – 2 clearly depicts the scavenging effect of **Ethanol extract of *Centratherum punctatum* Cass.** which is comparable with that of ascorbic acid positive control. [9]

GC-MS investigation of Ethanol extract of *Centratherum punctatum* Cass.

The data of the results obtained through GC-MS analysis of ethanolic extract of the aerial parts of ***Centratherum punctatum* Cass.** is presented in **Figure 1 & 2**.

Table 1: Cytotoxic effect of Ethanol extract of *Centratherum punctatum* Cass. on EAC cells

Concentration of Drug (µg/ml)	Viable cells	Viable Cells (%)	Death Cells	Death Cells (%)
Control	130	96.3	5	3.7
25	86	81.9	19	18.1
50	89	72.6	34	27.6
100	79	66.9	39	33.1
200	61	47.7	67	52.3
500	24	22.6	82	77.4

Table 2: DPPH Assay of Ethanol extract of *Centratherum punctatum* Cass.

S. No.	Concentration of Extract (mg/ml)	Scavenging of DPPH free radical (% activity of Ethanol extract)
1.	0.1	15.32±2.72
2.	0.2	29.65±3.21
3.	0.3	36.17±4.58
4.	0.4	49.35±5.66
5.	0.5	54.26±5.22
6.	Ascorbic acid (0.5mg/ml)	82.45±4.26

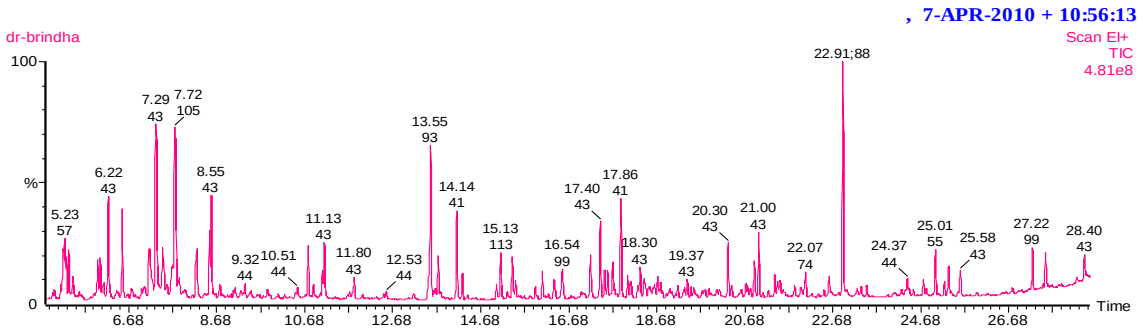


Fig. 1: Chromatogram of Ethanol Extract of *Centratherum punctatum* Cass.

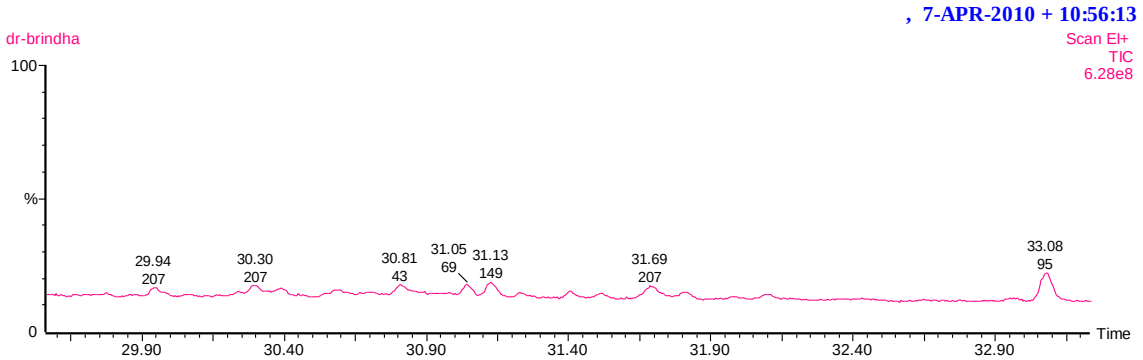


Fig. 2: Chromatogram of Ethanol Extract of *Centratherum punctatum* Cass.

Thirty compounds identified through GCMS analysis in the Ethanol extract of ***Centratherum punctatum* Cass.** are given in the **Table 4**.

Table 3: List of Chemical fractions identified in the Ethanol Extract of *Centratherum punctatum* Cass.

S. No.	Peak name	Molecular Formula	Molecular Weight	Retention time	% Peak Area
1.	Cyclopentanol	C6H12O	100	4.39, 4.44, 5.23	4.497
2.	2,3-Dioxabicyclo[2.2.1]heptanes	C6H10O2	114	7.29	1.500
3.	Hemimellitene	C9H12	120	7.72,7.75	1.801
4.	Cyclohexanol	C12H20O2	196	13.55	1.034
5.	Eugenol	C10H12O2	164	13.65,13.71	0.318
6.	Limonene oxide	C10H16O	152	15.28	0.038
7.	2H-1-Benzopyran-2-one	C9H6O2	146	15.40	0.282
8.	Bicyclo[3.1.1]hept-2-en-4-ol, 2,6,6-trimethyl-, acetate	C12H18O2	194	15.48	0.107
9.	Nonanoic acid, 9-oxo-, ethyl ester	C11H20O3	200	16.08	0.156
10.	2-Butenedioic acid (Z)-,	C12H20O4	228	16.54	0.284
11.	Acetic acid, 1-methyl-1-(4-methyl-5-oxo-cyclohex-3-enyl)ethyl ester	C12H18O3	210	17.17	0.329
12.	Spathulenol	C15H24O	220	17.57	0.110
13.	Caryophyllene oxide	C15H24O	220	17.68	0.237
14.	Viridiflorol	C15H26O	222	17.86, 18.03,18.77	0.884
15.	1,2-Cyclohexanediol, 1-methyl-4-(1-methylethenyl)-	C10H18O2	170	18.30	0.233
16.	7-Oxabicyclo[4.1.0]heptane, 1-methyl-4-(2-methyloxiranyl)-	C10H16O2	168	18.39	0.145
17.	1á-Cadin-4-en-10-ol	C15H26O	222	18.51	0.094
18.	1,3-Bis-(2-cyclopropyl,2-methylcyclopropyl)-but-2-en-1-one	C18H26O	258	19.03	0.033
19.	2-Pentadecanone, 6,10,14-trimethyl-	C18H36O	268	21.00	0.388
20.	Vinyl decanoate	C12H22O2	198	21.82, 21.95,22.48	0.214
21.	Hexadecanoic acid, ethyl ester	C18H36O2	284	22.91	1.463
22.	Hexanedioic acid, mono(2-ethylhexyl)ester	C14H26O4	258	27.52	0.265
23.	1,2-Benzenedicarboxylic acid, diisooctyl ester	C24H38O4	390	28.40	0.225
24.	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	C16H22O4	278	28.82	64.382
25.	Docosanoic acid, ethyl ester	C24H48O2	368	29.14	0.189
26.	Phthalic acid, bis(7-methyloctyl) ester	C26H42O4	418	30.30, 31.13, 31.40,31.52	0.438
27.	Eicosane	C20H42	282	30.81	0.279
28.	Squalene	C30H50	410	31.05	0.115
29.	2,5,5,8a-Tetramethyl-4-methylene-6,7,8,8a-tetrahydro-4H,5H-chromen-4a-yl hydroperoxide	C14H22O3	238	31.69	0.208
30.	Azulene	C15H24	204	33.08	0.324

Among 30 compound fractions identified following were proven for their anticancer and antioxidant activity. (TABLE IV)

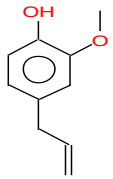
Table 4: List of Compounds

S. No.	Compound Name	Nature of Compound	Activity Established
1.	Eugenol	Phenylpropene	Kills human colon cancer cell lines *in vitro*.[10]
2.	Spathulenol	Diterpene	Immuno modulatory effect.[11]
3.	Viridiflorol	Sesquiterpénols	Anticancer activity against human amelanotic melanoma cell line C32, Renal cell adenocarcinoma, Hormone-dependent prostate carcinoma and Human breast cancer cell line MCF-7 .[12]
4.	Hexadecanoic acid, ethyl ester	Palmitic acid ester	Antioxidant.[13]
5.	Phthalic acid, bis(7-methyloctyl) ester	Aromatic dicarboxylic acid ester	Antitumour activity against mice sarcoma 180 cell lines.[14]
6.	Eicosane	Alkane	Antitumour activity against the human gastric SGC-7901 cell line.[15]
7.	Squalene	Triterpene	Antioxidant, Antitumor and Immunostimulant.[16]

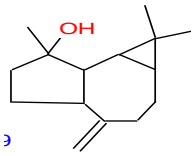
CONCLUSION

The greatest lacuna existing in herbal-based medicines are lack of standards and validation studies. Considering the immense potentials of existing herbal drug for human health care in the present work attempts were made in the present work to scientifically validate and chemically standardize the potential anticancer drug ***Centratherum punctatum* Cass.**

Chemical standardization studies revealed that ***Centratherum punctatum* Cass.** is rich in secondary metabolites which possessed wide range of biological activities such as anticancer, anti inflametory, anti pyretic and analgesic. The anti-oxidant activity against DPPH free radicals and in-vitro cytotoxic activity against EAC cell lines exhibited by the selected drug can be attributed to the presence of anticancer fractions such as Eugenol, Spathulenol, Viridiflorol, Hexadecanoic acid, Phthalic acid, bis(7-methyloctyl) ester, Eicosane and Squalene. Following structures of the Fractions which possessed proven anticancer activity were identified through GC-MS in the ethanolic extract of ***Centratherum punctatum* Cass.** screened for In-vitro Cytotoxic effect. Further In-depth studies can result in the development of a human friendly anti cancer drug from this plant source.

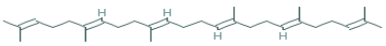


Eugenol

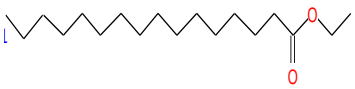


Spathulenol

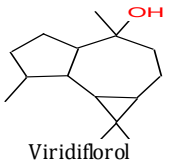
Eicosane



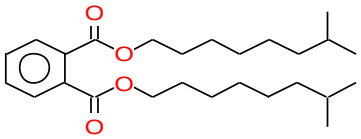
Squalene



Hexadecanoic acid



Viridiflorol



Phthalic acid, bis(7-methyloctyl) ester

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