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GC-MS characterization of *n*-hexane soluble compounds of *Cyperus rotundus* L. rhizomes

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ABSTRACT

Cyperus rotundus L., popularly known as nutgrass or nagarmotha, is commonly used in the traditional medicine for inflammatory disorders. In the present study, n-hexane extract from rhizomes of *C. rotundus* (HCR) was analyzed for its constituents using GC-MS technique. The rhizomes were collected, washed, shade dried and powdered. N-hexane extract was prepared by cold percolation method and preliminary phytochemical screening was carried out. It was subjected to Gas Chromatography coupled with Mass Spectroscopy (GC-MS) for the identification of components thereon. Preliminary phytochemical screening of HCR revealed the presence of phenolics, sterols and terpenoids. GC-MS data indicates the presence of twenty seven low polar components in HCR. The major identified molecules include hentriacontane (7.15%), triacontane (6.12%), nonacosane (5%), octacosane (4.38%), octadecane (2.35%), hexadecane (2.32%), eicosane (1.56%), pentatriacontane (1.43%), 9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione (1.37%), Heneicosane, 3-methyl- (1.27%), *α*-cyperone (1.25%), heptadecane (1.15%) and gamma-Sitosterol (1%). As some of these constituents are known to possess anticancer activity, HCR could be used as an active therapeutic ingredient.

INTRODUCTION

Plants harbor several chemical constituents such as phenolics, terpenes, flavonoids and alkaloids, which are known to possess many pharmacological activities (Zheng and Wang, 2001). In recent years, there has been a tremendous interest in the field of natural product research as a source of potential drug substances (Rout *et al.,* 2009). Phytochemicals form the basis of many pharmaceutical formulations used in the treatment of health disorders. Most of the clinical anticancer drugs use phytochemicals as their precursors (Russo *et al.,* 2010). Certain bioactive phytocomponents have been known for their anticancer properties. Some of these include curcumin, genestein, resveratrol, lycopene, rosemarinic acid and sulforaphane (Gutheil *et al.,* 2011). Cyperaceae represents a family of monocotyledonous sedges with grassy resemblance. The ecological significance of Cyperaceae members lie in their riverside vegetation habitat contributing to erosion control and water purification (Babu and Savithramma, 2014). They are known to possess a number of biological activities, including antimicrobial (Bisht *et al.,* 2011), antimutagenic (Kilani *et al.,* 2005), antimalarial (Thebtaranonth *et al.,* 1995), anticonvulsant (Mohsen *et al.,* 2011) and wound healing activities (Puratchikody *et al.,* 2006). *C. rotundus* L. is a common perennial weed belonging to the family Cyperaceae. The tubers are blackish in color and have a specific odor. *C. rotundus* typically grows tropical and temperate countries (Jeyasheela *et al.,* 2014). In traditional medicine, the roots and rhizomes of *C. rotundus* from Asian and African continent are used in the treatment of digestive ailments, dyspepsia, epilepsy, ophthalmia, inflammatory disorders and fever (You *et al.,* 2004).

There are reports on clinical studies with 2 % aqueous extract of *C. rotundus* wherein the extract showed potent anti-inflammatory activity in conjunctivitis (Singh *et al.,* 2012). The plant has been reported to contain alkaloids, saponins, flavonoids, essential oils, glycosides, sesquiterpenes and epoxides (Aslam, 2002).

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The rhizome oils of *C. rotundus* have been shown to possess different compositions. Hitherto, there are no reports on the evaluation of phytoconstituents from *n*-hexane extracts of this plant. Hence, in this study, composition of *n*-hexane extracts from rhizomes of *C. rotundus* (HCR) is reported.

MATERIALS & METHODS

Plant material

Dried rhizomes of *C. rotundus* were collected from local Ayurvedic pharmacy, Mangalore, India. The plant material was authenticated by Dr. Sunil Kumar, Senior Research officer, Department of Pharmacognosy, SDM Research center, Udyavara, Udupi and Voucher specimen (No.11110101) was deposited. The rhizomes were coarse powdered using a kitchen blender and stored at -20^{0}C until further analyses.

Extraction

HCR was prepared according to the procedure explained by Raaman[13]. One gram of powdered sample was extracted with 10 ml of *n*-hexane by cold percolation for 24 h.

Preliminary phytochemical analysis

Preliminary phytochemical screening was carried out to detect the various constituents such as alkaloids, phenolics, coumarins, flavonoids, sterols and terpenoids in the extract by performing qualitative tests (Raman, 2006).

GC-MS Analysis

GC-MS analysis was carried out using Perkin Elmer Turbo Mass Spectrophotometer (GC-MS-5975C, AGILENT, USA) equipped with an auto sampler XLGC. The column used was Perkin Elmer Elite - 5 capillary column (dimethyl polysiloxane, 30m × 0.25mm) with a film thickness of 0.25mm. The carrier gas used was Helium at a flow rate of 1.5ml/min. 1µl sample injection volume was utilized. The inlet temperature was maintained as 250°C. The oven temperature was programmed initially at 70°C for 3 minutes and then programmed to increase to 300°C at a rate of 10°C. Total run time was 35 minutes. The MS transfer line was maintained at a temperature of 240°C. MS was recorded using electron spray ionization at 70eV and data was evaluated using total ion count (TIC) for compound identification and quantification. The spectra of the components were compared with the spectral database of known components in the GC-MS library (NIST-11). Measurement of peak areas and data processing were carried out by Turbo-Mass-OCPTVS-Demo SPL software (Adams *et al.,* 2004).

RESULTS AND DISCUSSION

Phytochemical screening HCR indicated the presence of phenolics, sterols and terpenoids in abundance as the major constituents (Table 1). GC-MS of HCR indicated the presence of 50 low polar constituents. Out of fifty constituents 23 could not be identified as the mass fragmentation showed similarity below 80%. A major compound eluted at RT 29.9 min could not be identified though it accounted for 25.22%. Out of 27 identified constituents, 12 compounds such as hentriacontane (7.15%), triacontane (6.12%), nonacosane (5%), octacosane (4.38%), octadecane (2.35%), hexadecane (2.32%), eicosane (1.56%), pentatriacontane (1.43%), 9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione (1.37%), heneicosane,3-methyl- (1.27%), α-cyperone (1.25%), heptadecane (1.15%) and gamma-sitosterol (1%) were the major constituents.

Table 1: Phytochemical screening of *n*-hexane extract of *Cyperus rotundus* rhizomes.

Phytoconstituents	Results
Alkaloids	–
Carbohydrates	–
Carboxylic acids	–
Coumarins	–
Flavanoids	–
Phenolics	+
Quinones	–
Resins	–
Steroids	+
Saponins	–
Tannins	–
Terpenoids	+

+ : Presence, - : Absence

The remaining 15 constituents were found in trace amounts (Fig. 1, Table 2). Various constituents identified from GC-MS analysis including phenolics (cyperone), terpenoids (eicosane, hentriacontane, triacontane, pentatriacontane) and steroids (gamma sitosterol), which correlate well with the results of phytochemical screening. Certain phenolics are known to possess cytotoxic activity on various cancer cell lines by activation of caspase mediated apoptosis. The antitumor efficacy of phenolic compounds is mainly attributed to their free radical scavenging and pro-oxidant activities (Nandi *et al.,* 2007). Several plant based sterols are well known for their anticancer activity. Beta sitosterol and campesterol isolated from sterol fraction of red algae *Porphyra dentata* showed significant antitumor activity on 4T1 breast cancer cells *in vitro* and *in vivo*. Phytosterols exert anticancer activity by inhibition of cancer cell proliferation, angiogenesis and induction of apoptosis/necrosis (Kazlowska *et al.,* 2013). Terpenoids constitute an important class of phytochemicals with antioxidant and anticancer activities. There are reports on the anticancer activity of terpenoids isolated from *Clerodendrum infortunatum* (Sannigrahi *et al.,* 2012), *Baccharis trimera* (De-Oliveira *et al.,* 2013) and *Curcuma longa* (Guo and Wang, 2014). Certain terpenoids such as D-limonene, perillyl alcohol and salvicine have shown interesting antitumor activity in pre-clinical studies with minimal cytotoxicity on normal cells (Seidenia, 2015). The extract has shown a potent antioxidant and free radical scavenging which further may contribute to its anticancer activity (Hema *et al.,* 2013). The basis for this activity could be clearly explained by the presence of sterols, terpenoids and phenolics in *C. rotundus* extract.

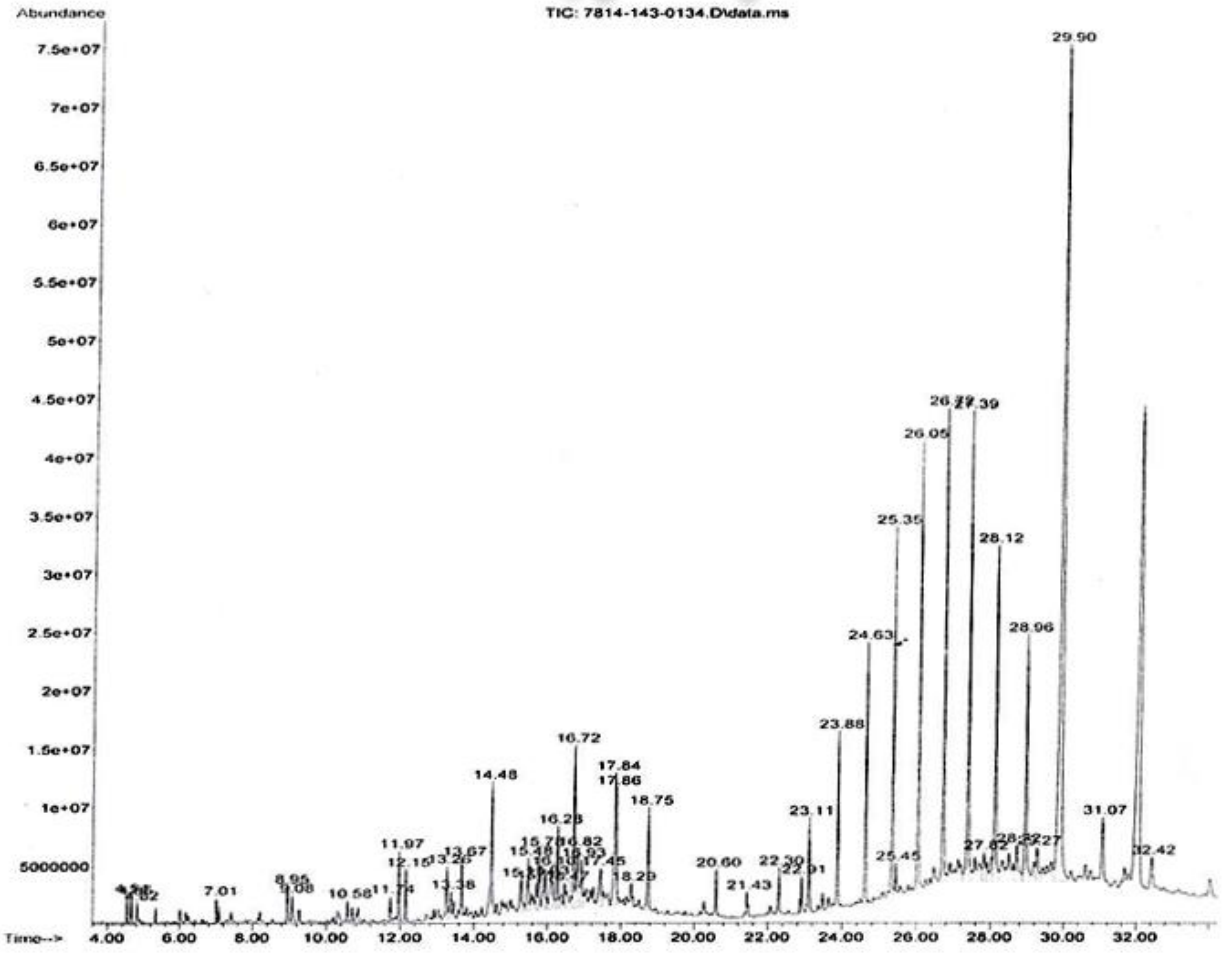

Fig. 1: Total Ion Chromatogram (TIC) of *n*-hexane extract of *C. rotundus* rhizomes.

GC-MS analysis revealed that phenolic hydrocarbons and sterols were found predominantly in *C. rotundus* extract. Among the major components identified, hentriacontane was known to possess anti-inflammatory (Kim *et al.,* 2011) and cytotoxic on lymphoma cells (Licea *et al.,* 2012) via suppression of caspase-1 activation and anti-apoptotic activities respectively. There are reports on the antimicrobial and anticancer activity of n-triacontane isolated from ethanol extracts of *Acanthospermum hispidum* (Chakraborty *et al.,* 2012). Gamma sitosterol is reported to possess antidiabetic, anti-inflammatory, anticancer and antiangiogenic properties (Raman *et al.,* 2012). The presence of these compounds make *C. rotundus* extract as a probable good therapeutic entity for cancer treatment. There are reports on the essential oil composition of *C. rotundus* rhizomes. Volatile oils of *C. rotundus* rhizomes from South Africa indicated the presence of α-cyperone (11 %), myrtenol (7.9 %), caryophyllene oxide (5.4 %) and β-pinene (5.3 %) as the major constituents (Lawal and Oyedeji, 2009). Four chemotypes of essential oils of *C. rotundus* from different regions of Asian continent have been identified as H, K, M and O. The H-type from Japan consisted of α - cyperone (36.6%), ß-selinene (18.5%), cyperol (7.4%) and caryophyllene (6.2%). The M-type from China, Vietnam and Hong Kong was found to contain α -cyperone (30.7%), cyperotundone (19.4%), ß-selinene (17.8%), cyperene (7.2%) and cyperol (5.6%).

The O-type from Japan, Taiwan, Thailand, Hawaii and the Philippines was rich in cyperene (30.8%), cyperotundone (13.1%) and ß-elemene (5.2%). In addition, the Hawaiian O-type contained cyperene (20.7%) and cyperotundone (25.0%) as the major compounds. The K-type, native of Hawai was known to possess cyperene (28.7%), cyperotundone (8.8%), patchoulenyl acetate (8.0%) and sugeonyl acetate (6.9%) as the major constituents (Sivapalan, 2013). These studies clearly indicate the influence of geographical location on chemical composition of *C. rotundus* oils.

Table 2: Compounds identified from *n*-hexane extract *Cyperus rotundus* rhizomes.

Peak	RT	Area %	Name of the compound
1	4.541	0.26	*α*-Pinene
2	4.656	0.30	--
3	4.821	0.23	--
4	7.010	0.24	--
5	8.950	0.56	Naphthalene
6	9.077	0.44	Dodecane
7	10.579	0.27	--
8	11.737	0.33	*α*-Copaene
9	11.966	0.85	Tetradecane
10	12.150	0.69	Cyperene
11	13.264	0.88	*α*-Selinene
12	13.378	0.55	Phenol,2,4-bis(1,1-dimethylethyl)
13	13.671	0.81	--
14	14.479	2.32	Hexadecane
15	15.287	0.80	Azulene,1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1- methylethenyl)-,[1R-1.alpha.,3a.beta.,4.alpha.,7.beta.)]-
16	15.477	0.99	--
17	15.700	0.48	--
18	15.776	0.92	--
19	15.929	0.69	--
20	16.101	1.15	Heptadecane
21	16.279	1.25	*α*-Cyperone
22	16.476	0.35	--
23	16.724	2.35	Octadecane
24	16.820	1.41	--
25	16.928	0.99	--
26	17.450	1.10	--
27	17.838	2.33	--
28	17.863	1.37	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione
29	18.289	0.24	Heptacosane
30	18.748	1.56	Eicosane
31	20.599	0.63	Docosane
32	21.432	0.07	--
33	22.304	0.67	Tetracosane
34	22.915	0.41	9-Octadecenal,(Z)-
35	23.112	1.35	--
36	23.882	2.04	--
37	24.632	3.25	--
38	25.351	4.38	Octacosane
39	25.447	0.31	Squalene
40	26.051	5.78	--
41	26.725	6.12	Triacontane
42	27.387	7.15	Hentriacontane
43	27.826	0.79	--
44	28.125	5.54	--
45	28.717	1.27	Heneicosane,3-methyl-
46	28.958	5.00	Nonacosane
47	29.270	1.00	Gamma Sitosterol
48	29.900	25.22	--
49	31.071	1.43	Pentatriacontane
50	32.426	0.87	--

RT - Retention time, -- Unidentified compounds

CONCLUSION

Even though there are reports on certain major constituents of *C. rotundus*, the complete phytochemical profile of *n*-hexane extracts of plant rhizomes is not available till date. Results of the present study indicated the presence of certain pharmacologically important constituents in *n*-hexane extracts of *C. rotundus* rhizomes. The identified components can be further isolated and confirmed for their bioactivities.

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