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PHYTOCHEMICAL CHARACTERISATION AND CHEMICAL FINGERPRINTING OF *CYPERUS ROTUNDUS* L. RHIZOME

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ABSTRACT

Cyperus rotundus L. commonly called Nutgrass is a highly potential traditional herbal drug used for the treatment of a number of ailments. The present study was aimed to standardize the drug by phytochemical evaluation and chemical fingerprinting using HPTLC. The chemical constitution of the diethyl ether extract was analyzed by GC-MS. The preliminary phytochemical screening of various extracts showed the presence of terpenoids, phenols, steroids, alkaloids, flavonoids, sugars etc. HPTLC fingerprinting of the ethanol extract exposed various peaks with different R_f values. GC-MS analysis of the diethyl ether extract revealed the presence of Asarone (2.86%), Caryophylleneoxide (3.81%), 3-Cyclohexene-1-carboxaldehyde (5.00%), Naphthalene (2.59%), 2H-Cyclopropa[a]naphthalen-2-one (2.13%), 2(3H)-Naphthalenone (6.98%), 2, 2, 7, 7-Tetramethyltricyclo [6.2.1. (1,6)] undec-4-en-3-one (2.60%), Octadec-9-enoic acid (5.09%), n-Hexadecanoic acid (2.11%), Rotundene (1.29%), β.-Elemenone(1.78%), cis-Z-.alpha.-Bisabolene epoxide (1.95%), Nonacosane (1.72%), α-Copaene (1.14%) and 1-Formyl-2,2,6-trimethyl-3-(3-methyl-but-2-enyl)-6-cyclohexene (1.18%) as the major components.

KEYWORDS: GC-MS analysis, HPTLC Fingerprinting, Qualitative phytochemical analysis

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INTRODUCTION

Cyperus rotundus L., a noxious weed as well as a traditional herbal medicine belonging to the family Cyperaceae is a plant of interest with respect to its pronounced therapeutic value. This plant commonly referred as Nutgrass or Purple Nutsedge has a worldwide distribution and found across tropical, sub-tropical and temperate regions. It is a perennial sedge with short stoloniferous rhizome and small dark tubers which are reddish white inside with a characteristic odour. The root tubers of *C. rotundus* are extensively used for the treatment of a number of ailments and is used in more than hundred pharmaceutical preparations of Ayurveda [13].The important bioactive chemical constituents identified in *C. rotundus* include cyperone, selinene, cyperene, cyperotundone, patchoulenone, sugeonol, kobusone, isokobusone etc whose pharmacological activities reveal the therapeutic value of this herb and its use in various systems of medicine[7].Infusion of the *C. rotundus* rhizome has been used in the treatment of fever, diarrhoea, dysentery and other intestinal problems and also to relieve pain. It is also used as a home remedy for treating indigestion, gastric disorders and irritation of bowel. The ethanolic extract of *C. rotundus* exhibit anti-inflammatory activity in carrageenan induced oedema and formaldehyde induced arthritis in rats[3]. The methanol extract of *C. rotundus* rhizome at the doses of 250 and 500 mg/kg b.w. showed significant antidiarrhoeal activity in castor oil induced diarrhoea in mice[14]. The hydroalchoholic extract of *C. rotundus* is reported to have an anticonvulsant effect on PTZ-induced kindling in mice[9]. *C. rotundus* is also studied in detail to analyse its hepatoprotective[6], anti-cancerous[8], anti-malarial[15], Wound healing[10]activities etc. Thus *C. rotundus* is a plant of great interest for researchers all over the world because of its medicinal value. The present study aims at the phytochemical evaluation as well as standardization and authentication of this potential herbal drug by HPTLC and GC-MS analysis.

MATERIALS AND METHODS

Sample Collection

Fresh rhizomes of *C. rotundus* were collected from a medicinal plant field around Kollam, Kerala and the plant materials were authenticated by Dr. Sunil Kumar, Senior Research officer, Department of Pharmacognosy, SDM Research center, Udyavara, Udupi. Voucher specimen of the plant was deposited at the Dept. of Botany, Nesamony Memorial Christian College with voucher specimen number 0032. The rhizomes were cleaned, shade dried, coarse powdered and stored at -20^{o}C until further analyses.

Qualitative phytochemical analysis

A known quantity of powdered root tubers of *C. rotundus* was taken in a thimble and placed it in a Soxhlet extractor. The sample was extracted successively using Petroleum Ether, Chloroform, Ethyl Acetate and Ethanol and the extracts were concentrated by distillation and dried under vacuum[4]. The percentage of dried extract with reference to the sample taken was recorded.The qualitative phytochemical tests were done according to standard procedures[11].

HPTLC Fingerprinting

One gram of powdered sample was soaked in 10 ml ethanol and kept for cold percolation. After 24hours the content was filtered and concentrated using a vacuum flash evaporator. Five and ten microlitre of the extracted sample was applied on a pre-coated silica gel F254 on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was then developed in Toluene: Ethyl acetate solvent system (8: 1). The plate was kept in Photo-documentation chamber andcaptured the images at white light, UV 254 nm and UV 366 nm. The developed plate was sprayed with vanillin sulphuric acid reagent anddried at 120^{0} C in hot air oven. The plate was photo-documented in visible light mode using Photo-documentation chamber. Before derivatization, the plate was fixed in scanner stage and scanning was done at 254 nm and 366 nm. The peak table, peak display and peak densitogram were recorded[12].

GC-MS Analysis

One gram of sample was extracted with 100 ml of diethyl ether using Soxhlet apparatus. The extract was concentrated to dryness and analyzed for composition by GC-MS.The study was carried out on a 5975C Agilent system equipped with a DB-5ms Agilent fused silica capillary column (30 × 0.25 mm ID; film thickness: 0.25 µm), operating in electron impact mode at 70 eV. Pure helium (99.9995%) was used as carrier gas at a constant flow of 1.5 mL/min and an injection volume of 1 µL was employed (split ratio is 10: 1). Mass transfer line and injector temperature were set at 230°C and 250°C, respectively. The oven temperature was programmed from 70^{o}C (isothermal for 2 min), with an increase of 10^{o}C/min, to 300^{0}C/min, ending with a 9 min isothermal at 300^{0}C. The total running time for GC was 35 min. Mass spectra were taken at 70eV; with a scan range 40-700 m/z. Ion source temperature set to 230^{0}C and interface temperature being 240^{0}C.To identify the compounds, the extract was assigned for comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with the published literature. National Institute of Standards and Technology library sources (NIST II) were used for matching the identified compounds from the plant materials. Turbo-Mass-OCPTVS-Demo SPL software as used for the measurement of peak area and data processing[1].

RESULTS AND DISCUSSION

Four different extracts of the *C. rotundus* root tubers were analysed for the presence of phytochemicals. Sugars, flavonoids and steroids were seen in the petroleum ether, chloroform, ethyl acetate as well as ethanol extracts.The

chloroform extract showed coumarins and ethanol extract showed the presence of phenols. Tannins were present in both the ethyl acetate and ethanol extracts. Except in ethanol extract, terpenoids were present in all the other three extracts (Table. 1). The yield of extract from *Cyperus rotundus* was higher in ethanol and lower in ethyl acetate (Table.2). Qualitative phytochemical analysis gives primary information about the presence of bioactive phytochemicals in the sample. This study revealed the presence of sugars, flavonoids, steroids, coumarins, phenols and tannins in *C. rotundus*.

Table 1
Preliminary phytochemical tests of C. rotundus successive extracts

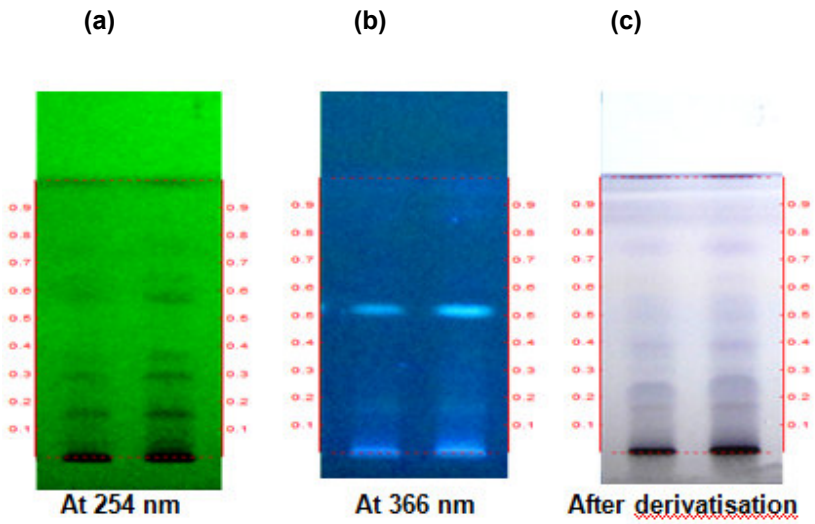
Test	Pet ether	Chloroform	Ethyl Acetate	Ethanol
Alkaloids	+	+	–	–
Carbohydrates	+	+	+	+
Carboxylic acid	–	–	–	–
Coumarins	–	–	–	–
Flavanoids	–	–	+	+
Phenol	–	–	–	–
Quinones	–	–	+	–
Resins	–	–	–	–
Steroid	+	+	+	+
Saponins	–	–	–	+
Tannins	–	–	–	+
Terpenoids	+	+	+	

Table2
Yield of extract from C. rotundus

Sample (g)	Solvents	Yield (g)	% w/w
40	Petroleum Ether	0.809	2.022
	Chloroform	0.816	2.040
	Ethyl acetate	0.162	0.405
	Ethanol	1.657	4.142

HPTLC fingerprinting of ethanol extract of *C. rotundus* yield the following results. Photodocumentationunder 254 nm gives 7 spots (Fig. 1a), 8 spots under 366 nm (Fig 1b), and 12 spots under 620 nm post-derivatisation with vanillin sulphuric acid spray reagent (Fig 1c) and the $R_f$values are tabulated (Table.3.).Densitometric scan at 254 nm revealed 9 peaks corresponding to 9 different compounds in the ethanol extract with R_f0.19 (24.79%), 0.65 (19.42%), 0.34 (17.60%), 0.86 (12.54%), 0.42 (11.39%) and 0.73 (10.42%) being the major peaks (Fig 2; Table.4). Densitometric scan at 366 nm showed 6 peaks, and peaks with R_f 0.60 (38.96%), 0.03 (34.79%), 0.70 (8.57%), 0.15 (8.63%) and 0.24(8.09%) were the major peaks detected (Fig 3; Table.5). Densitometric scan at 620 nm (derivatised) showed 9 peaks, peaks with R_f 0.45 (24.97%), 0.57 (14.14%), 0.21 (13.47%), 0.03(11.07%), 0.84(10.70%) and 0.64 (9.85%) being the major ones (Fig 4; Table.6). The developed HPTLC chromatogram of the root tubers of *C. rotundus* is specific with the selected solvent system, Toluene: Ethyl acetate (8: 1) and can be used for standardization of the drug. HPTLC fingerprinting of a particular plant species help not only in the identification and quality control of a particular species but also provide basic information regarding the isolation, purification, characterization and identification of marker chemical compounds of the species[2].

Figure 1
HPTLC photo documentation of Ethanol extract of C. rotundus rhizome



Track 1- C. rotundus - 5 µl
Track 2- C. rotundus – 10 µl

Table 3
R_f values (At 10 µl)

At 254 nm	At 366 nm	After derivatisation
0.06(L Green)	0.06(F L Violet)	0.06(L Violet)
0.10(L Green)	-	0.10(L Violet)
-	-	0.14(L Violet)
0.16(Green)	0.16(F L Violet)	-
-	-	0.18(Violet)
-	0.20(F L Violet)	-
-	0.28(F L Violet)	0.28(L Violet)
0.30(Green)	-	0.30(L Violet)
0.37(L Green)	0.37(F L Violet)	-
-	-	0.39(L Violet)
-	-	0.49(L Violet)
-	0.52(F Aqua)	-
0.57(L Green)	-	0.57(L Violet)
-	0.60(F L Violet)	-
0.64(L Green)	-	0.64(L Violet)
-	-	0.75(L Violet)
-	0.86(F L Violet)	0.86(L Violet)

****L-Light, D-Dark, F-Fluorescence***

Figure 2
Densitometric scan of Ethanol extract of C. rotundus rhizome at 254 nm

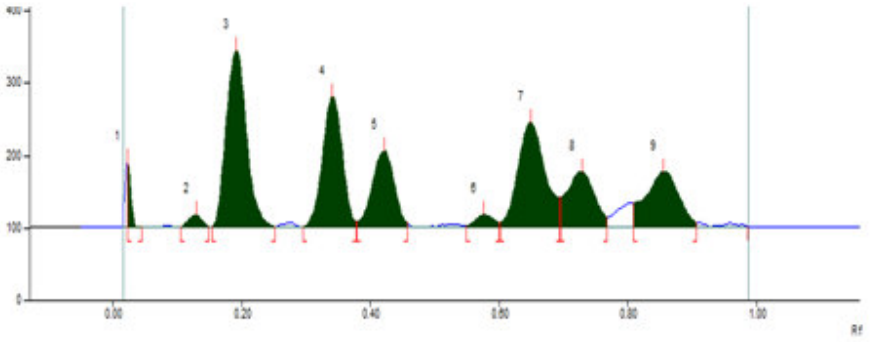


Table4

Peak list and R_f value of the chromatogram at 254 nm

Peak	Max R_f	Area %
1	0.03	1.51
2	0.13	1.11
3	0.19	24.49
4	0.34	17.60
5	0.42	11.39
6	0.58	1.52
7	0.65	19.42
8	0.73	10.42
9	0.86	12.54

Figure 3

Densitometric scan of Ethanol extract of C. rotundus rhizome at 366 nm

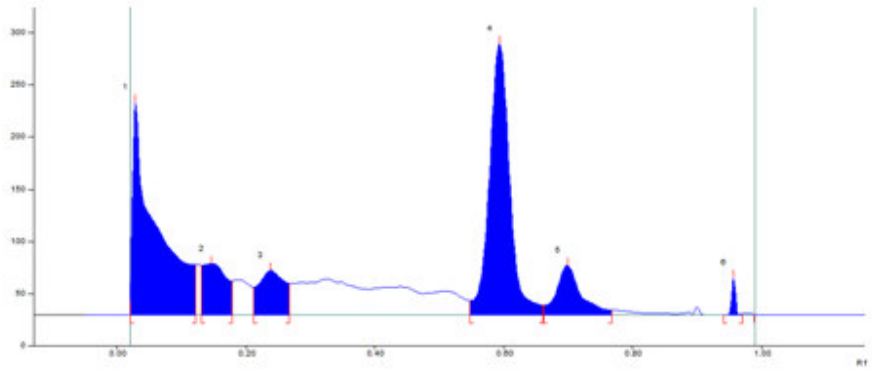


Table 5

Peak list and R_f value of the chromatogram at 366 nm

Peak	Max R_f	Area %
1	0.03	34.79
2	0.15	8.63
3	0.24	8.09
4	0.60	38.96
5	0.70	8.57
6	0.96	0.96

Figure 4

Densitometric scan of Ethanol extract of C. rotundus rhizome after derivatisation

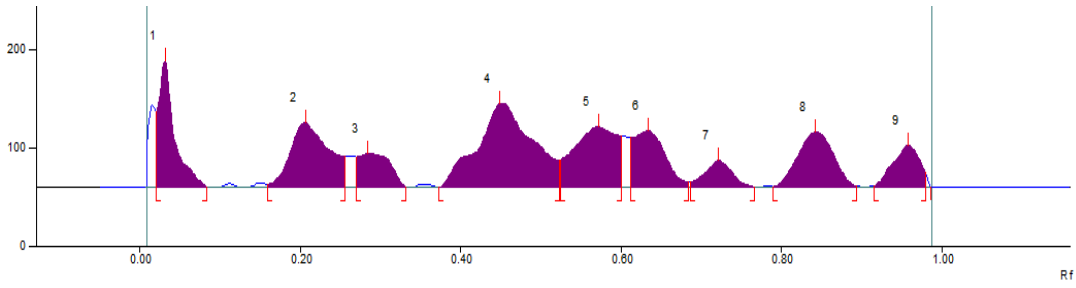


Table 6
Peak list and R_f value of the chromatogram after derivatisation

	Max R_f	Area %
1	0.03	11.07
2	0.21	13.47
3	0.29	5.84
4	0.45	24.97
5	0.57	14.14
6	0.64	9.85
7	0.72	3.94
8	0.84	10.70
9	0.96	6.03

GC-MS chromatogram of the diethyl extract of *Cyperus rotundus* tubers revealed the presence of 65 chemical constituents (Fig. 6.).Among these, 28 constituents were identified as their mass fragmentation showed similarity above 80%. The major components present in the extract include β- Asarone (2.86%), Caryophyllene oxide (3.81%), 3-Cyclohexene-1-carboxaldehyde (5.00%), Naphthalene (2.59%), 2H-Cyclopropa[a]naphthalen-2-one (2.13%), 2(3H)-Naphthalenone (6.98%), 2, 2, 7, 7-Tetramethyltricyclo [6.2.1. (1,6)] undec-4-en-3-one (2.60%), Octadec-9-enoic acid (5.09%), n-Hexadecanoic acid (2.11%), Rotundene (1.29%), β.-Elemenone(1.78%), cis-Z-.alpha.-Bisabolene epoxide (1.95%), Nonacosane (1.72%), α-Copaene (1.14%) and 1-Formyl-2,2,6-trimethyl-3-(3-methyl-but-2-enyl)-6-cyclohexene(1.18%) while the rest of the components were found in trace quantity(Table.7). GC-MS results of *C. rotundus* indicated the presence of a number of terpene derivatives. The rhizome oils of *C. rotundus* from India were reported to have α-copaene and caryophyllene oxide as major components which were absent in the species from other countries[5]. This indicates the difference in chemical constituents of *C. rotundus* according to changes in environmental conditions.

Figure 5
Gas Chromatogram of the diethyl ether extract of C. rotundus rhizome

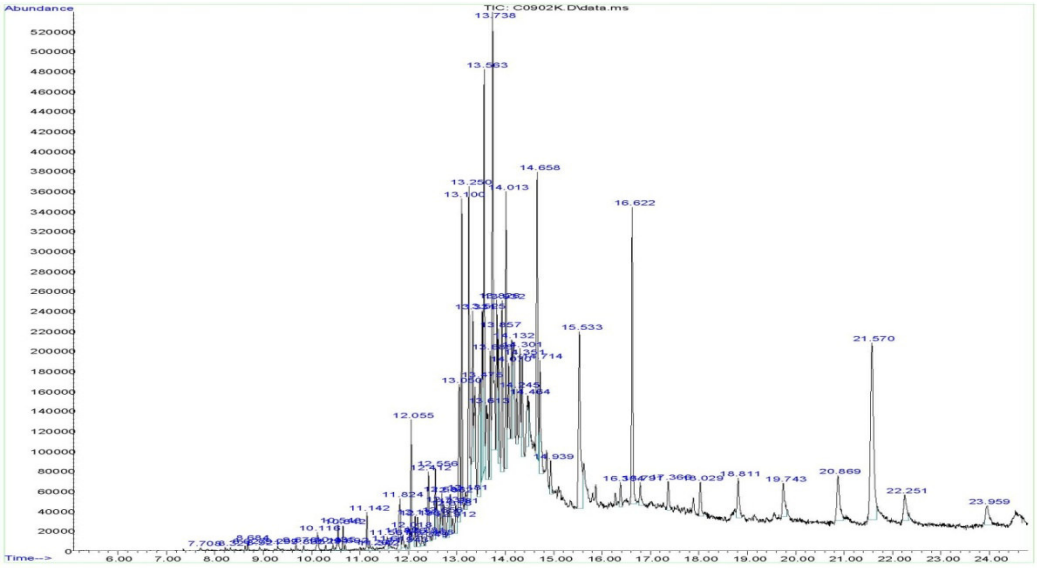


Table7

Phytochemicals identified by GC-MS of diethyl ether extract of C. rotundus rhizome

Peak	RT	% Area	Name
1.	7.708	0.07	-
2.	8.321	0.05	-
3.	8.627	0.06	-
4.	8.684	0.09	Decane
5.	8.921	0.06	-
6.	9.297	0.08	-
7.	9.678	0.09	Nonane
8.	9.835	0.07	-
9.	10.116	0.36	-
10.	10.291	0.08	-
11.	10.435	0.10	-
12.	10.548	0.44	(-)-Myrtenol
13.	10.642	0.31	Bicyclo[3.1.1]hept-3-en-2-one
14.	10.692	0.07	-
15.	11.142	0.50	Thymol
16.	11.267	0.06	-
17.	11.342	0.05	-
18.	11.561	0.32	-
19.	11.611	0.08	-
20.	11.824	1.14	α-Copaene
21.	11.886	0.26	-
22.	11.943	0.07	-
23.	12.018	0.24	-
24.	12.055	1.35	3H-3a,7-Methanoazulene
25.	12.137	0.35	Bicyclo[7.2.0] undec-4-ene
26.	12.193	0.54	1,14-Tetradecanediol
27.	12.281	0.19	-
28.	12.349	0.09	-
29.	12.412	1.29	Rotundene
30.	12.556	1.04	1H-Cycloprop[e]azulene, decahydro 1,1,7-trimethyl-4 methylene-Naphthalene
31.	12.656	0.18	γ -Muurolene
32.	12.731	0.60	β -Panasinsene
33.	12.818	0.65	-
34.	12.862	0.50	-
35.	12.912	0.19	-
36.	12.981	0.60	-
37.	13.050	2.86	β- Asarone
38.	13.100	3.81	Caryophyllene oxide
39.	13.181	0.36	-
40.	13.250	5.00	3-Cyclohexene-1-carboxaldehyde
41.	13.525	2.59	Naphthalene
42.	13.563	5.27	-
43.	13.613	1.78	β.-Elemenone
44.	13.688	2.13	2H-Cyclopropa[a]naphthalen-2-one
45.	13.738	6.98	2(3H)-Naphthalenone
46.	13.826	1.95	cis-Z-.alpha.-Bisabolene epoxide
47.	13.857	1.76	-
48.	13.932	2.60	2, 2, 7, 7-Tetramethyltricyclo [6.2.1. 0(1,6)]undec-4-en-3-one
49.	14.013	4.04	-
50.	14.070	1.09	-
51.	14.132	1.59	-
52.	14.245	0.73	Nootkatone
53.	14.301	1.18	1-Formyl-2,2,6-trimethyl-3-(3-methyl-but-2-enyl)-6-cyclohexene
54.	14.351	1.79	-
55.	14.464	1.27	-
56.	14.658	4.73	-
57.	14.714	2.11	n-Hexadecanoic acid
58.	14.939	0.59	-
59.	15.533	5.09	Octadec-9-enoic acid
60.	16.384	0.42	-
61.	16.622	5.18	-
62.	16.791	0.46	-
63.	17.366	0.59	-
64.	18.029	0.79	Hexacosane
65.	20.869	1.72	Nonacosane

\- = ***unidentified***

CONCLUSION

In the present investigation preliminary phytochemical characterization of *C. rotundu*s root tubers were performed and occurrence of various bioactive compounds of medicinal value was found. The ethanol extract was subjected for chemical fingerprinting using HPTLC analysis and diethyl ether extract was subjected to GC-MS analysis which revealed the presence of 65 different compounds of which 28 are identified.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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