International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 21-10-2021, Accepted: 06-11-2021, Published: 22-11-2021
Volume 6, Issue 6, 2021, Page No. 460-465

Phytochemical profiling and antioxidant activity of tuber extracts of *Cyperus rotundus* L

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Abstract
The plant selected for the present investigation was *Cyperus rotundus L*. The extraction of *C. rotundus* tubers was made through sequential extraction method using petroleum ether, chloroform, ethanol and water. The extracts were tested for the presence of alkaloids, flavonoids, steroids, saponins, tannins, glycosides, phenols and terpenoids. Alkaloids, phenols, Tannins and terpenoids were present in all the four extracts of tuber, but the flavonoids were available with petroleum ether alone. Steroids showed their presence only with the water extract. Saponins found in all the extracts except aqueous tuber extract. While glycosides were identified in the petroleum ether and ethanolic extracts. UV-VIS and FTIR spectra of petroleum ether extract confirmed the presence of many phytochemical compounds. The petroleum ether extract was subjected to GC-MS analysis and the spectrum showed the presence of more than 50 compounds. Antioxidant activity was estimated in terms of the free radical scavenging ability of these extracts by DPPH. While the ethanolic extracts of tubers show significant activity and petroleum ether extract showed moderate activity.

Keywords: *Cyperus*, phytocompounds, GC-MS, antioxidant, UV, FTIR

Introduction
Plant resources have been used from the beginning days of human civilization. Plants synthesize hundreds of chemical compounds for functions including defence against insects, fungi, diseases and herbivorous mammals. Therapeutic properties of medicinal plants are very useful and the traditional medicinal plants have been used many years to heal various diseases and the advantage of these medicinal plants being 100% natural [1]. Phytochemical analysis is a valuable step, in the detection of the bioactive principles present in medicinal plants and subsequently may lead to drug discovery and development [2]. The attractive colours and fragrance produced by the plants is due to specific phytochemicals present in them [3]. They may be tannins, flavonoids, glycosides, saponins, steroids and alkaloids. Phenolic and polyphenolic are the other group of secondary metabolites.
These include phenolic acids, phenylpropanoids, flavonoids, flavones, aurones, flavonones, dihydrochalcones, isoflavones, xanthones and stilbens, hydrolysables and condensed tannins and quinines [4, 5]. These compounds also act as defense mechanisms of plants under different environmental stress conditions such as wounding, infection, excessive light or UV irradiation. Phenolic phytochemicals are known to exhibit several health beneficial activities such as antioxidant, anti-inflammatory, antihepatotoxic, antitumor and antimicrobial [6, 7, 8]. The presence of phytochemical constituents determine the medicinal properties of plants. Gas Chromatogram Mass Spectrometric method (GCMS) is used to disclose phytochemical profiling of plants and this method segregates chemical mixtures and recognises numerous components available in a sample at a molecular level. It is one of the most widely used tools for exploring environmental samples.
Cyperus rotundus L. also known as Nagarmotha, purple nut sedge or nut grass, is a common perennial weed plant. It has wide range of medicinal and pharmacological applications, due to which it is widely used as traditional medicine across worldwide to treat various diseases and ailments such as indigestion, constipation, dysentery, abdominal distention, neurogenic gastralgia, chest pains, irregular as well as painful catamenia, skin diseases, furuncle infections, staphylococcal infections, leprosy, sprains and bruises, fever and animal tissue stomach ache. It has numerous range of pharmacological properties. It also has good nutritive values. Phytochemical screening is very important in identifying new sources of therapeutically and industrially important compounds like alkaloids, flavonoids, phenolic compounds, saponins, steroids, tannins, terpenoids. Hence an attempt was made to identify bioactive compounds present in *C. rotundus* L.

Materials and Methods
Collection of sample
The plant selected for the present investigation was *Cyperus rotundus L*. The tubers of *Cyperus* were collected. The tubers were washed thoroughly under running tap water to remove dirt and soil adhered to them and finally rinsed with distilled water.
Then they were shade dried, weighed, ground into fine powder and stored at 20^0C until extraction.

Preparation of plant extracts
The extraction of *C. rotundus* tubers was made through sequential extraction method using the solvents petroleum

ether, chloroform, ethanol and water. 10 g of powdered tubers were taken and extracted with 100ml of the above said solvents in 1:10 ratio, respectively. The extractions were obtained through continuous shaking for 48 hrs; they were then filtered through Whatman no.1 filter paper.

Preliminary phytochemical analysis

Preliminary Phytochemical screening for phytoconstituents like alkaloids, flavanoids, glycosides, phenols, saponins, steroids, tannins and terpenoids was carried out by the standard procedures [9, 10, 11, 12, 13] (Table -1).

Table 1: Procedure for preliminary phytochemical tests

Phyto compounds	Phytochemical tests	Procedure	Result
Alkaloids	Iodine test	3ml extract + few drops of Iodine	Appearance of blue colour
	Wagner's test	2ml extract + few drops of Wagner's reagent	Appearance of reddish brown precipitate
Flavonoids	Pew's test	2ml extract + zinc powder+ drop wise Conc.Hcl	Appearance of purple red
	Shinoda test	2ml extract + magnesium metal+ drop wise Conc.Hcl	Appearance of magenta colour
	NaOH test	2ml extract + few drops of NaOH+ few drops of dil.Hcl	Appearance of intense yellow colour
Glycosides	Keller Killane test	2ml extract + few drops of glacial acetic acid+ one drop of 5% $Fecl_3$ and conc.H_2SO_4	Reddish brown colour appears at the junction of the two liquid layers and the upper layer of bluish green
	Glycosides test	1ml extract + 1ml water+ few drops of aqueous soln. of NaOH	Appearance of yellow colour
Phenol	Ellagic test	1ml extract + few drops of 5 % glacial acetic acid + 5 % NaOH	The solution turned muddy or higher brown precipitate
Saponin	Foam test	2ml extract + 20ml dis H_2O + shake in a graduated cylinder for 15 min.	Presence of foam about 1 cm
Steroids	Salkowski's test	2ml extract + 2ml chloroform + 2 ml conc.H_2SO_4 + shake well.	The layer of red chloroform and acid shows greenish yellow fluorescence
Tannins	Gelatin test	2ml extract + 1% gelatin+ 10% NaOH	Formation of white precipitate
	Lead acetate test	5ml extract + few drops of 10% lead acetate	Formation of yellow or red precipitate
Terpenoids	Terpenoids test	5ml extract + 2ml chloroform + 3 ml of conc. H_2SO_4	Appearance of reddish brown colour

Characterization of bioactive compounds

UV and FTIR analysis

Based on the results obtained from preliminary phytochemical analysis, petroleum ether extract of tubers was selected for further analysis. It was examined under visible and UV light. For UV and FTIR spectrophotometer analysis, the extract was centrifuged at 3000 rpm for 10 min and filtered through Whatmann No. 1 filter paper. The sample is diluted to 1:10 with the same solvent. The extract was scanned in the wavelength ranging from 300-1100 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected. FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks and their functional groups. The peak values of the UV and FTIR were recorded. Each and every analysis was repeated twice for the spectrum confirmation.

Gas chromatography-mass spectrometry analysis

The GC-MS analyses were carried out for petroleum ether extract in a Shimadzu GC-MS-QP 2010 gas chromatograph fitted with a DBI (Methyl phenyl siloxane, 30 m x0.25 mm id. d) capillary column. Carrier gas, helium with a flow rate of 0.7 ml/min; column oven temperature 70^0C, 5 min in 180^0C, 180-260^0C at 3^0C/min, 5 min in 60^0C, 260-280^0C at 0.2^0C/min and finally 5 min in 280^0C; injector temperature, 280^0C detector temperature 290^0C, volume injected, 1 µL of TMS ether derivatives in *n*-hexane (2%); split ratio, 3:0. The MS operating parameters were as follows: ionization potential 70 eV; ion source temperature 200^0C; quadrupole 100^0C amu, eV voltage 3000 volts.

The concentrated extract is injected into the GC-MS instrument (Hewlett Packard 5890 GC-MS with Mass Selective Detector with an HP-1 glass capillary column. Each chemical component in a sample had a distinct retention time measured in minutes, shown in a peak on a graph which measured abundance on the ordinate against retention time on the abscissa. The integrated peak was correlated to the concentration of the chemical. The resulting mass spectrum was unique to that chemical. This mass spectrum formed a "Fingerprint" that could identify the compound by a computer search of mass spectra.

Compound identification

Components of the petroleum ether extract were identified by comparison of their mass spectra and retention indices with those published in the literature and contained in the NIST '98 MS computer library (Wiley).

Antioxidant activity

The free radical scavenging activity of these extracts was determined by using 1, 1-diphenyl-2-picrylhydrazyl or DPPH [14]. Briefly, 1ml Solution of the flesh extract at a proper concentration was mixed with 2 ml of 10mg/L methanolic solution DPPH. The mixture was shaken vigorously and allowed to stand at room temperature for 5min and absorbance (ΔA) was recorded at 517nm by using a spectrophotometer. Lower absorbance of sample indicated the higher free radical scavenging activity. The control consisting of methanol and reagent solution without extracts was prepared. The scavenging ability (SA) was calculated as follows

$$\text{DPPH scavenging effect (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

Where A_0 is the absorbance of the control at 30 min. and A_1 is the absorbance of the sample after 30 min.

Results and Discussion

Preliminary phytochemical analysis

Extracts from nut grass were made using petroleum ether, chloroform, ethanol and water. The extracts were tested for the presence of phytoconstituents like alkaloids, flavonoids, glycosides, phenols, saponins, sterols, tannins and terpenoids (Table-2). The phytochemicals such as Alkaloids, phenols, Tannins and terpenoids were present in all the four extracts of tuber, but the flavonoids were available with petroleum ether alone.

Steroids showed their presence only with the water extract. Saponins found in all the extracts except aqueous tuber extract. While glycosides were identified in the petroleum ether and ethanolic extracts.

Table 2: Preliminary phytochemical analysis of tuber extracts of *Cyperus rotundus*. L

S.No.	Test		Tuber extracts			
			Petroleum ether	Chloroform	Ethanol	Aqueous
1	Alkaloids	i) Iodine test	+	+	+	+
		ii) Wagner's test	+	+	+	+
2	Flavonoids	i) Pew's test	+	-	-	-
		ii) Shimoda	+	-	-	-
		iii)NaOH	+	-	-	-
3	Glycosides	i) Keller Killane	+	-	+	-
		ii) Glycosides	+	-	+	-
4	Phenols	Ellagic test	+	+	+	+
5	Saponin	Foam test	+	+	+	-
6	Sterols	Salkowski's test	-	-	-	+
7	Tannins	i) Gelatin	+	+	+	+
		ii) Lead acetate	+	+	+	+
8	Terpenoids	Terpenoid test	+	+	+	+

UV-VIS and FTIR spectroscopic studies

The tubers of the plant *Cyperus rotundus* L. were shade dried. Based on the results obtained from the preliminary phytochemical analysis, Petroleum ether and ethanolic extracts of tubers were prepared with the powdered material and were subjected for UV-VIS and FTIR analysis.

UV-VIS spectroscopic analysis

UV-VIS spectra for the petroleum ether extract of tubers are depicted in the Figure-1. The spectrum for petroleum ether extract showed a broad peak in the range of 291– 317 nm. It indicates the presence of compounds with C=O functional group. Another sharp peak at 641 nm denotes the presence of ethyl ether and the functional group N=O.

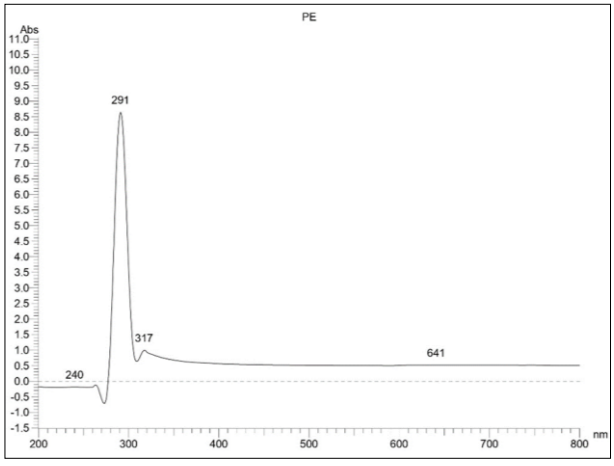

Fig 1: UV-Visible absorption spectrum recorded from petroleum ether extract of *Cyperus rotundus* L. tubers

Fourier-transform infrared spectroscopic analysis

The FTIR spectra for the petroleum ether extract shows various peaks (Fig. 2). The peaks between 2800-3000 cm^{-1} show the presence of N-H stretch (amides) and C-H stretches (alkanes). Another two peaks between 1300-1450cm^{-1} predicts the presence of C-H bending which means aldehydes might be present. The peaks between 3400-3500 cm^{-1} indicate heterocyclic amine and N-H stretches. The presence of isothiocyanate, C=C terminal alkyne, C =C medial alkyne, S-H stretches is confirmed by the peaks between 2000 – 2500 cm^{-1}. The FTIR spectra of the ethanolic extracts of tubers showed a sharp peak at 1043.49 cm^{-1} is meant for the presence of C-N stretching (amines) and the peak at 877.61 cm^{-1} is the indication of C=O bending (alkenes). A sharp peak is obtained at 3354.21 cm^{-1} which indicates the presence of imino compounds and N-H stretchs. S-S stretches found between 600-650 cm^{-1}. A broad absorption band in the range of 3250-3650 cm^{-1} representing hydrogen bond. It confirms the presence of hydrate, hydroxyl, ammonia and amino group. The FTIR spectra of petroleum ether extract shows that the tubers of *C. rotundus* are enriched with many significant compounds. These compounds could have been serving as the bioactive compounds which could be correlated with the medicinal properties of the plant.

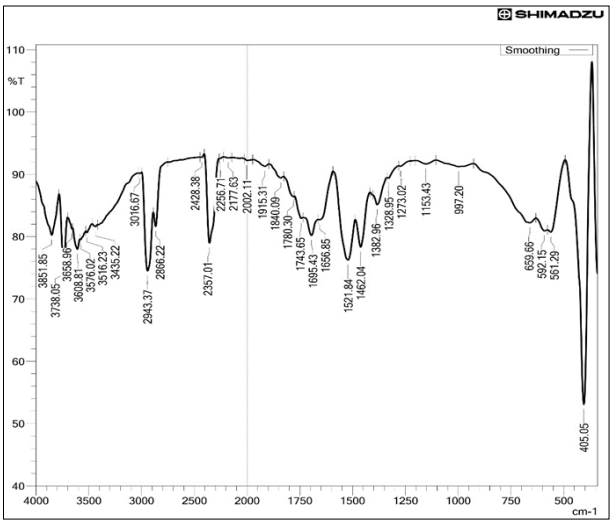

Fig 2: FTIR spectrum recorded from petroleum ether extract of *Cyperus rotundus* L. tubers

GC-MS

GC-MS spectrum of many different major peaks indicated the presence of more than fifty compounds. The name of the compounds, their retention time and other properties are shown in the table-3. The spectrum profile of GC-MS confirmed the presence of many major components with retention time from 2 to 15 minutes. Many compounds have been extracted by petroleum ether. The results show that the plant tuber is rich in many secondary metabolites which could be useful in identifying the lead compounds with the potentiality to be developed into the drugs in future (Fig. 3).

Table 3: GC-MS analysis of Petroleum ether extract of *Cyperus rotundus* L. tubers

Peak	Retention time	Area	Area %	Height	Height %	A/H	Name
1	6.639	67752	0.07	42600	0.23	1.59	1-decene
2	7.889	600302	0.58	253548	1.39	2.37	Benzaldehyde, 2-hydroxy-
3	11.34	335515	0.32	180881	0.99	1.85	1-dodecene
4	11.406	1980021	1.9	839382	4.6	2.36	Benzoic acid, 2-hydroxy-, methyl ester
5	15.918	641946	0.62	315831	1.73	2.03	1-pentadecene
6	16.094	65040	0.06	33450	0.18	1.94	Octadecane
7	18.368	997302	0.96	478304	2.62	2.09	Phenol, 2,4-bis(1,1-dimethylethyl)-
8	19.992	11105000	10.67	3851424	21.12	2.88	Diethyl phthalate
9	20.063	1031039	0.99	484824	2.66	2.13	1-hexadecene
10	20.21	71961	0.07	31959	0.18	2.25	Octadecane
11	21.684	144990	0.14	63326	0.35	2.29	1-tetradecanol
12	22.019	471737	0.45	218943	1.2	2.15	Oxalic acid, cyclohexylmethyl tridecyl ester
13	23.211	350332	0.34	122412	0.67	2.86	Tetradecanoic acid
14	23.789	1026454	0.99	482493	2.65	2.13	1-nonadecene
15	26.648	1294002	1.24	443942	2.43	2.91	N-Hexadecanoic acid
16	27.168	1374966	1.32	471560	2.59	2.92	1-nonadecene
17	27.274	77599	0.07	34863	0.19	2.23	Nonadecane
18	28.173	86962	0.08	32775	0.18	2.65	Procaine
19	29.318	464932	0.45	152291	0.84	3.05	9,12-Octadecadienoic acid (Z,Z)-
20	29.414	667800	0.64	202955	1.11	3.29	Oleic acid
21	29.49	161080	0.15	48246	0.26	3.34	(E)-13-Docosenoic acid
22	29.778	450278	0.43	166611	0.91	2.7	Octadecanoic acid
23	30.254	1049528	1.01	402272	2.21	2.61	1-heptacosanol
24	30.341	129404	0.12	48399	0.27	2.67	Heptadecane
25	30.445	823258	0.79	382230	2.1	2.15	Eicosyl acetate
26	31.78	129513	0.12	42193	0.23	3.07	Heneicosane
27	32.443	2952030	2.84	497165	2.73	5.94	2-imidazo[1,2-a]pyridin-2-ylphenol #
28	32.625	86816	0.08	54327	0.3	1.6	1,4,5,6-Tetrahydro-6-[phenylmethyl]-2H-1,2,4,5-tetrazine-3-thione
29	32.66	73544	0.07	36069	0.2	2.04	Naphtho(2,3-b)-1,4-diazabicyclo(2,2,2)octene
30	33.09	621542	0.6	270580	1.48	2.3	1-heptacosanol
31	33.162	157439	0.15	66751	0.37	2.36	Heneicosane
32	33.268	455290	0.44	181284	0.99	2.51	Octacosyl acetate
33	34.37	350233	0.34	57919	0.32	6.05	Hexanoic acid, undecyl ester
34	34.487	406965	0.39	121118	0.66	3.36	Tetratriacontane
35	34.619	1409433	1.35	384811	2.11	3.66	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
36	34.877	923282	0.89	371474	2.04	2.49	Bis(2-ethylhexyl) phthalate
37	35.707	467541	0.45	200176	1.1	2.34	1-hexacosanol
38	35.769	213382	0.21	112341	0.62	1.9	Tetracosane
39	36.083	425177	0.41	116757	0.64	3.64	1-(Piperidin-1-yl)hexadecan-1-one
40	36.182	901613	0.87	112956	0.62	7.98	Octadecanoic acid, ethenyl ester
41	36.455	323594	0.31	61348	0.34	5.27	Heptacosyl heptafluorobutyrate
42	36.569	592406	0.57	95581	0.52	6.2	Tetradecanoic acid, 2-hydroxy-1,3-propanediyl ester
43	36.745	1818047	1.75	365611	2	4.97	Ergosta-5,7,9(11),22-tetraen-3-ol, (3.beta.,22E)-
44	36.78	1204639	1.16	318936	1.75	3.78	Ergosta-5,7,9(11),22-tetraen-3-ol, (3.beta.,22E)-
45	36.995	2857819	2.75	356318	1.95	8.02	Tritriacontane
46	37.222	472506	0.45	132281	0.73	3.57	Octadecanoic acid, 2,3-dihydroxypropyl ester
47	37.494	739026	0.71	256030	1.4	2.89	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester
48	37.956	49387759	47.45	2410367	13.22	20.49	Ergosta-5,7,22-trien-3-ol, (3.beta.,22e)-
49	38.135	487077	0.47	232841	1.28	2.09	1-hexacosanol
50	38.187	675050	0.65	246703	1.35	2.74	Hexatriacontane
51	38.264	1547133	1.49	304998	1.67	5.07	5,6-dihydroergosterol
52	38.49	1103727	1.06	186119	1.02	5.93	Neoergosterol
53	38.646	315021	0.3	90834	0.5	3.47	1-(Piperidin-1-yl)octadecan-1-one
54	39.426	5830093	5.6	437440	2.4	13.33	Ergosta-5,8-dien-3-ol, (3.beta.)-
55	39.898	1689676	1.62	328314	1.8	5.15	.Gamma.-ergostenol

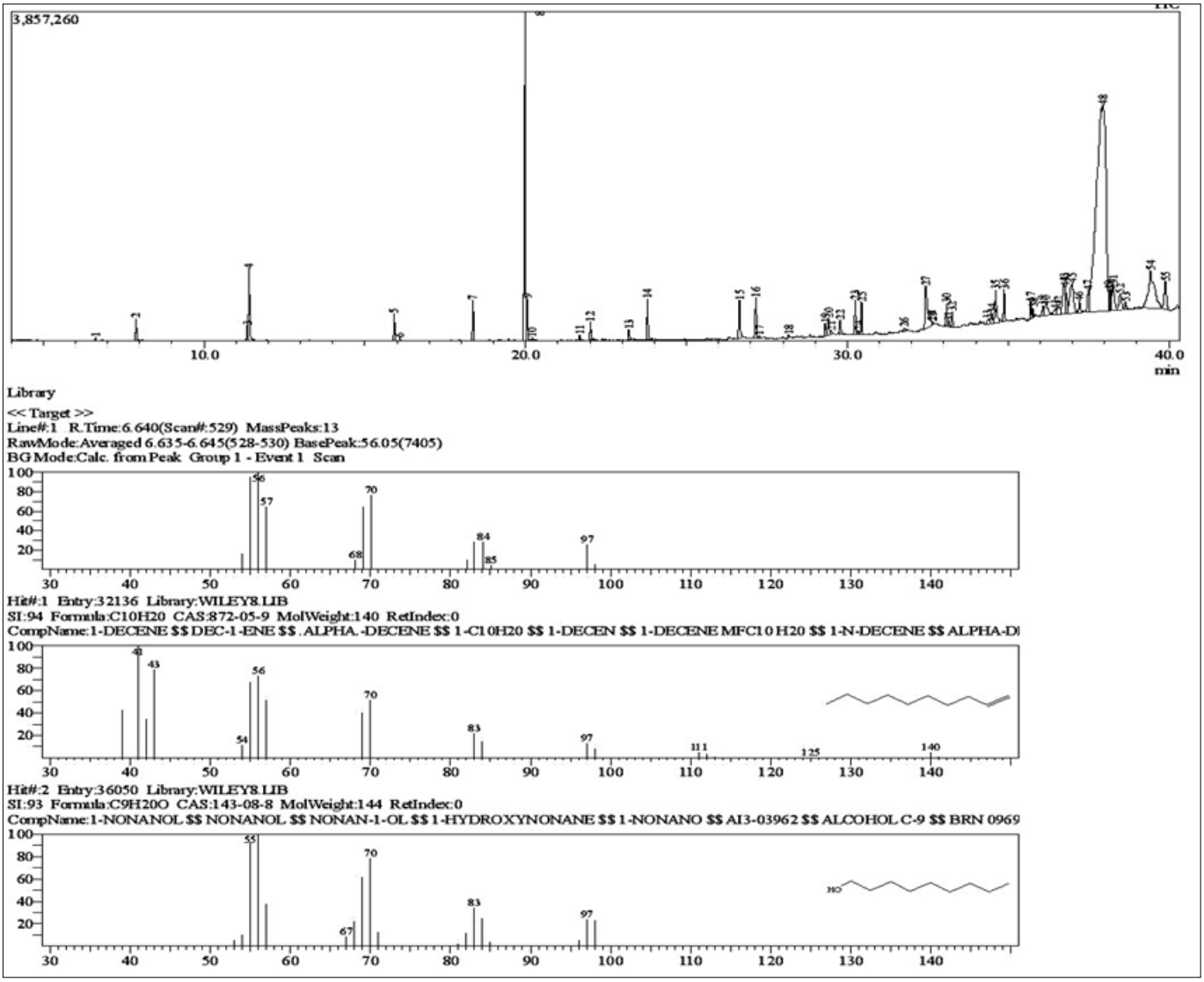

Fig 3: GC-MS analysis of Petroleum ether extract of *Cyperus rotundus* L. tubers

Antioxidant activity

Free radical scavenging activity on DPPH was studied with all the four extracts (petroleum ether, chloroform, ethanol and aqueous) at various concentrations. Highest activity was noticed with the ethanolic extract of tubers (65.4%) followed by aqueous extract (62.8%). As far as the extract with chloroform is concerned, the analysis showed poor results when compared to other extracts (46.2%). The tuber extract with petroleum ether showed free radical scavenging activity at moderate level (51.3%) (Table -4).

The results obtained from this study revealed that free radical scavenging activity of the tuber extract of *C. rotundus* is high with the polar solvents than non-polar solvents.

It was evident from the previous study reported that polar solvents extracts, which contained the highest amount of total phenolic contents exerted strong reducing power and scavenging DPPH radical activity [15].

This result suggests that the bioactive compound which is responsible for free radical scavenging activity might have been a strong electrophilic compound since they have been extracted with ethanol and water which are polar protic solvents. This might have been the reason why extracts with chloroform and petroleum ether show poor activity for this parameter.

Table 4: Antioxidant activity of tuber extracts of *Cyperus rotundus* L.

Plant extracts	Absorbance at 517 nm	% of scavenging activity
control	0.78	-
Petroleum ether	0.38	51.3%
Chloroform	0.42	46.2%
ethanol	0.27	65.4%
water	0.29	62.8%

Summary and Conclusion

New medicines are being developed for the treatment of complicated diseases but these medicines are themselves associated with a number of side effects that range from minor to sever intensity. Herbal medicines are phytochemical compounds used for the treatment of many diseases. In the present investigation, the fresh tubers of *Cyperus rotundus* were collected and shade dried and extracts were prepared using petroleum ether, chloroform, ethanol and water. Preliminary Phytochemical screening for phytoconstituents like alkaloids, flavonoids, glycosides, phenols, saponins, sterols, tannins and terpenoids was carried out by the standard procedures. The phytochemicals such as Alkaloids, phenols, Tannins and terpenoids were present in all the four extracts of tuber, but the flavonoids were available with petroleum ether alone. UV-VIS and

FTIR analysis were made and the presence of many phytochemical compounds was confirmed from the spectra. The petroleum ether extract of *Cyperus* tubers was subjected to GC-MS analysis and the spectrum showed the presence of more than 50 compounds. Antioxidant activity was estimated in terms of the free radical scavenging ability of these extracts by using 1, 1-diphenyl-2-picrylhydrazyl or DPPH. While the ethanolic extracts of tubers show significant activity, petroleum ether extract showed moderate activity. So it was concluded that the phytoconstituents responsible for antioxidant activities could have been polar in nature. Further efforts could be taken in future to purify the compounds and to synthesize new drugs as the derivatives using these compounds.

Acknowledgement
The authors thankfully acknowledge the University Grants Commission STRIDE component-I for financial assistance.

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