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ORIGINAL ARTICLE

Morphological and Chemoprofile (Liquid Chromatography-mass Spectroscopy and Gas Chromatography-mass Spectroscopy) Comparisons of *Cyperus scariosus* R. Br and *Cyperus rotundus* L.

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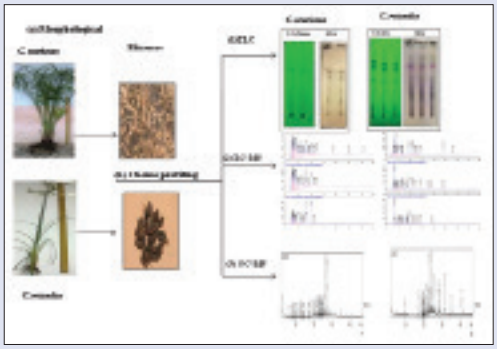
ABSTRACT

Background: *Cyperus scariosus* (CS) R.Br and *Cyperus rotundus* (CR) L. belongs to *Cyperaceae* family which is well-reputed in the traditional systems of medicine. Although they grow in different agro-climatic conditions, they are often considered to be synonymous with each other. **Objective:** The present study was aimed to systematically classify both the species CS and CR through their morphological features and chemical profiling using liquid chromatography-mass spectroscopy (LC-MS), gas chromatography-mass spectroscopy (GC-MS) and thin layer chromatography patterns of the rhizome extracts. **Materials and Methods:** A method (LC-MS analysis) has been developed on Agilent LC-MSD Trap SL mass spectrometer equipped with Waters HR C18 column (3.9 mm × 300 mm, 6 μm) using isocratic elution with acetonitrile and water (70:30% v/v ratio). GC-MS analysis was performed on a Shimadzu GC-MS-QP 2010 equipped with DB-5 capillary column (30 m × 0.25 mm × 0.25 μm). **Results:** Chemoprofiling of CS and CR using LC-MS and GC-MS suggested that these two are different based on their deferential spectral pattern, however, some of the common peaks were found in both the species. In addition, we also performed the preliminary phytochemical investigation of hexane and chloroform extracts of these species, which led to the isolation of stigmasterol, β-sitosterol and lupeol as major constituents in CS. **Conclusion:** In summary, we have developed optimal chromatographic conditions (LC-MS and GC-MS) and morphological profiles to classify both the species, that is, CS and CR. Collectively, our analytical results coupled with the morphological data clearly suggested that CS and CR are morphologically different.

Key words: Chemoprofiling, *Cyperaceae*, *Cyperus rotundus*, *Cyperus scariosus*, gas chromatography-mass spectroscopy, liquid chromatography-mass spectroscopy

SUMMARY

- The huge demand for herbal medicine has put pressure on the supply of natural resources which ultimately results in use of substandard materials or substitution and adulteration. The medicinal plants, *Cyperus rotundus* L and *Cyperus scariosus* R.Br which belongs to cyperaceae family and extensively used in the traditional systems of medicine. Although these two species are grown in different soil conditions, *Cyperus scariosus* R.Br often treated as synonymous of *Cyperus rotundus*. Thus, the present study was undertaken to classify these two species systematically using the modern analytical techniques as a powerful tools. Further, we also carried out the preliminary phytochemical investigation of hexane and chloroform extracts of *cyperus scariosus* rhizomes, which resulted in the isolation of three compounds namely Sitosterol, Stigmasterol and Lupeol.



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INTRODUCTION

Medicinal plants are traditionally recognized as the primary healthcare system in many rural communities because of their effectiveness, lack of modern medicinal alternatives and cultural preferences. Overall 80% of the world's population depends primarily on traditional medicines as sources for health care.[1] Plants and plant products are reported to exhibit a wide range of biological activities, which includes nootropics, analgesics, anticonvulsants, sedatives, anti-inflammatory agents, antipyretics, neurotransmission modulators, cardio-protectives, anticoagulants, antihypertensives, anti-allergic, skin, and bone healing agents etc.[2] In recent years search for new pharmacologically active agents from plant extracts led to the discovery of clinically useful drugs that play a major role in the treatment of human diseases.[3] There is growing interest worldwide in discovering the untapped potential of medicinal plants. This increase in demand for herbal medicine has put pressure on the supply of natural resources. This ultimately results in the use of substandard materials or substitution and adulteration. To control the adulteration and maintain the quality and the efficacy of the product analytical tools play a major role. The medicinal plants, *Cyperus rotundus* (CR) L (commonly known as nut grass) and *Cyperus scariosus* (CS) R.Br (commonly known as umbrella sedge) which belongs to *Cyperaceae* family and extensively used in the traditional systems of medicine. The herbal mixtures of these plant

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species have been shown to inhibit the cellular transformation caused by ras oncogene through fighting against oxidative damage and liver carcinogenesis by up- regulating the expression of cell-adhesion protein, connexin.[4] Grasses of these species used as animal feed has been shown to enhance microbial protein synthesis in the rumen of buffaloes.[5] Along these lines, CS has an antioxidant and anti-inflammatory activities.[6] Although these two species are grown in different soil conditions, CS R.Br often treated as synonymous of CR.[7] Recently, few reports have also been appeared in the literature[8,9] where the compounds isolated from CR and its activities were reported in the name of CS. Therefore, it is desired to have proper identification and accurate analytical tools to ensure the quality and efficacy of the herb. Thus, the present study was undertaken to classify the two species systematically with morphological comparisons and chemoprofiling of different extracts using the modern analytical techniques such as liquid chromatography-mass spectroscopy (LC-MS) and gas chromatography-mass spectroscopy (GC-MS) as a powerful tools. In the present communication, we are reporting the results, analysis of rhizome extracts and comparisons of the extracts of the two species along with the preliminary phytochemical investigation study of CS. To the best of our knowledge, this is the first report on the chemical analysis of CS.

MATERIALS AND METHODS

General procedure

Different solvents hexane, chloroform, and methanol were used in extraction and isolation processes were purchased from a local distributor. Thin layer chromatography (TLC) was performed on precoated silica gel plates 60 F_{254} (E. Merck, Darmstadt, Germany). LC-MS were recorded on Agilent LC-MSD Trap SL mass spectrometer, operating in positive ion polarity. HPLC grade acetonitrile used for LC-MS analysis was obtained from Merck, India. Ultrapure water for chromatographic use was obtained from a Milli-Q system (Millipore Corp., Bedford, MA, USA). All these samples before injecting into LC-MS were filtered through the 0.45 μm membrane filter. The separation of analytes was achieved by Waters NOVAPAK H_R C18 (3.9 mm × 300 mm, 6 μm). GC-MS was performed on a Shimadzu GCMS-QP 2010 equipped with a DB-5 capillary column (30 mm × 0.25 mm × 0.25 μm). Column chromatography was carried out using silicagel 60–120 mesh (Qingdao Marine Chemical, China). Melting points were recorded on a Fisher scientific melting point apparatus. IR spectra were recorded on a Thermo Nicolet Nexus 670 FTIR spectrometer (Thermo-Fisher). Electrospray ionization mass spectrometry was measured on LC-MSD Trap SL instrument. The ^{1}H and ^{13}CNMR spectras

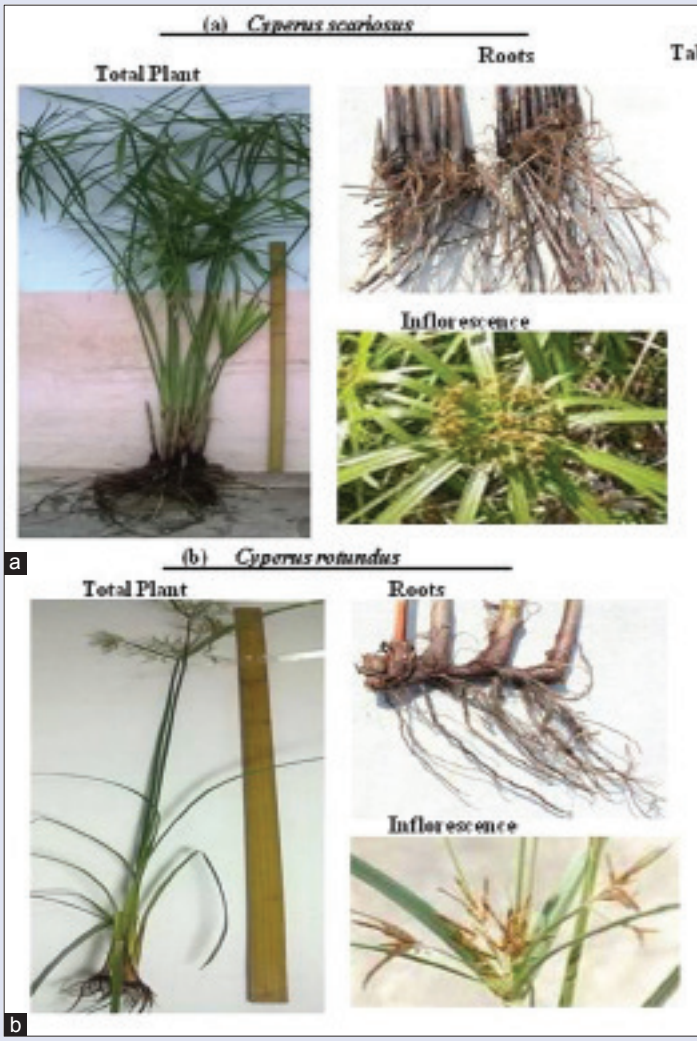

Table 1 : Morphological parameters of both the plants (CS and CR)

S. No	Morphological parameters	C.S (c.m)	C.R (c.m)
1	Plant height	180	90
2	Stem length	135	0.86
3	Root length	47	0.04
4	Rhizome thickness	0.15	0.102
5	Distance between rhizomes	0.008	0.03
6	Root thickness	0.03	0.012
7	Leaf length	30	14
8	Leaf surface area	0.03	0.01
9	Stem thickness	0.12	0.044
10	Rhizome powder	Dark brown	Light brown
11	Number of leaves at the upper end	Twelve	Three
12	Colour of flowers	light yellow to Brown	Brown
13	Flowers initiated from	Axillary buds	Axillary buds

Note: (c.m)= centimeters

Figure 1: Comparison of morphological characteristics of Total plant, Roots and inflorescence (a) *C. scariosus* (b) *C. rotundus*. Table 1: Morphological parameters of both the plants *Cyperus scariosus* and *Cyperus rotundus*

were recorded on a Bruker-300 MHz spectrometer (Bruker Scientifics) at 300 MHz for ^{1}H and 75 MHz for ^{13}C, respectively using TMS as an internal standard. The chemical shifts are expressed as δ values in part per million, and the coupling constants (j) are given in hertz (Hz).

Plant material and extraction

CS and CR plants were collected from the Botanical garden at KLEF University Campus, Vaddeswaram, Andhra Pradesh, India. These plants were taxonomically identified by Dr. A. Prasada Rao, Senior Botanist in KL University, Vijayawada, Andhra Pradesh, India. Voucher specimens have been deposited at KL University Botanical garden (voucher specimen number KLU-1250 and KLU-1251) for further use. Similar age plants were collected to discriminate phenotypic differences such as leaf, rhizome, and floral structures. Rhizomes of these herbs were shade dried and made into a fine powder used for analysis. The dried, and powdered rhizomes of these herbs were successively extracted with hexane, chloroform and methanol for 48 h. After complete extraction, the solvents were distilled off and concentrated under reduced pressure to the dryness in a rotary vaccum evaporator.

Phytochemical analysis

The concentrated different solvent extracts of CR and CS were investigated for the presence or absence of various phytoconstituents such as alkaloids, phytosterols, triterpenoids, flavonoids, phenolic compounds, tannins, carbohydrates, and proteins as per the standard methods.[10]

Thin layer chromatography

Different solvent extracts were spotted on a single silica gel G glass plate (60 F_{254}) and developed in a closed glass development tank saturated with the relevant mobile phase. The developed chromatograms were air dried at room temperature and visualized under ultraviolet (UV) light at 254 nm to detect UV-visible compounds. These were later chemically visualized by spraying with 5% H_2SO_4 in methanol solution and then charred on a hotplate to enhance color development. After visualization, the different compounds depicted by different colored spots were noted. Different solvent systems such as hexane-acetone, hexane-ethyl acetate, chloroform-ethyl acetate, chloroform-acetone, and chloroform-methanol were used as a mobile phase for the separation of the compounds.

Liquid chromatography-mass spectroscopy analytical conditions

LC-MS analysis was performed on Agilent LC-MSD Trap SL mass spectrometer (Waldron, Germany), equipped with electrospray ion interface, operating in positive ion polarity. The mobile phase consisted of acetonitrile and water in 70:30% v/v ratio, both the solvents containing

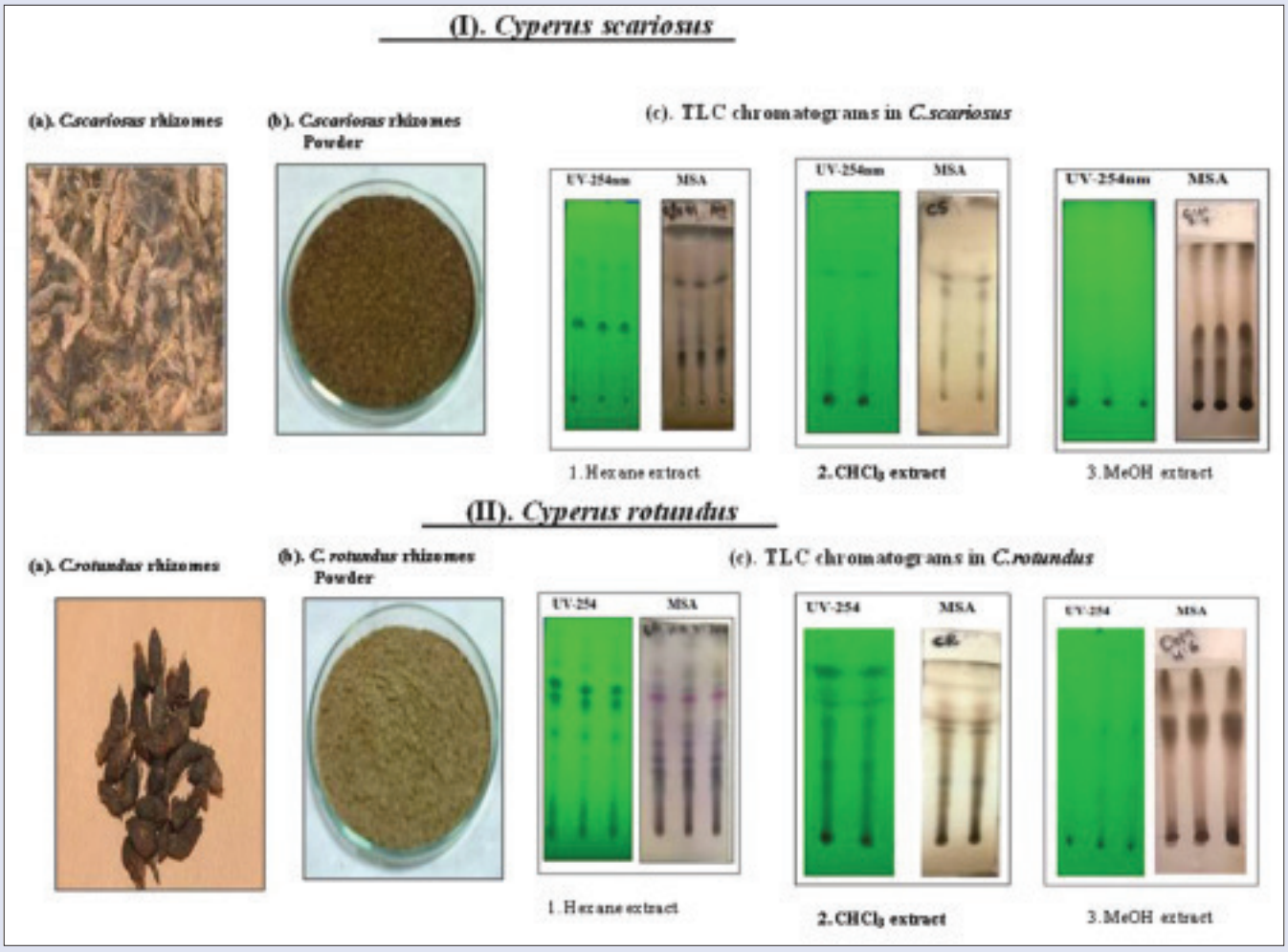

Figure 2: Comparision of morphological and thin layer chromatography banding pattern in *Cyperus scariosus* and *Cyperus rotundus*. (I. *Cyperus scariosus* and II. *Cyperus rotundus*) Morphological appearance of rhizomes (Ia and IIa) and rhizome powders (Ib and IIb) in *Cyperus scariosus* and *Cyperus rotundus*. (Ic and IIc) Thin layer chromatography banding pattern separation in hexane, chloroform and methanol extracts of *Cyperus scariosus* and *Cyperus rotundus* in ultraviolet at 254 nm and chemical treatment

Table 2: Phytochemical analysis of different extracts of *C. scariosus* and *C. rotundus rhizomes*

	C. scariosus			*C. rotundus*		
Constituents	**Hexane**	**Chloroform**	**Methanol**	**Hexane**	**Chloroform**	**Methanol**
Phytosterols	+++	+++	+	+++	+++	+
Terpenoids	+++	+++	+	+++	+++	+
Tannins	--	--	--	--	--	--
Flavanoids	--	--	--	--	--	--
Alkaloids	+	+	+	+	+	+
Saponins	--	--	--	--	--	--
Glycosides	++	++	++	++	++	++
Carbohydrates	++	++	++	++	++	++
Proteins	--	--	--	--	--	--
Phenols	--	+	+	--	+	+

-- Absent, + Present, ++ and +++ significantly present

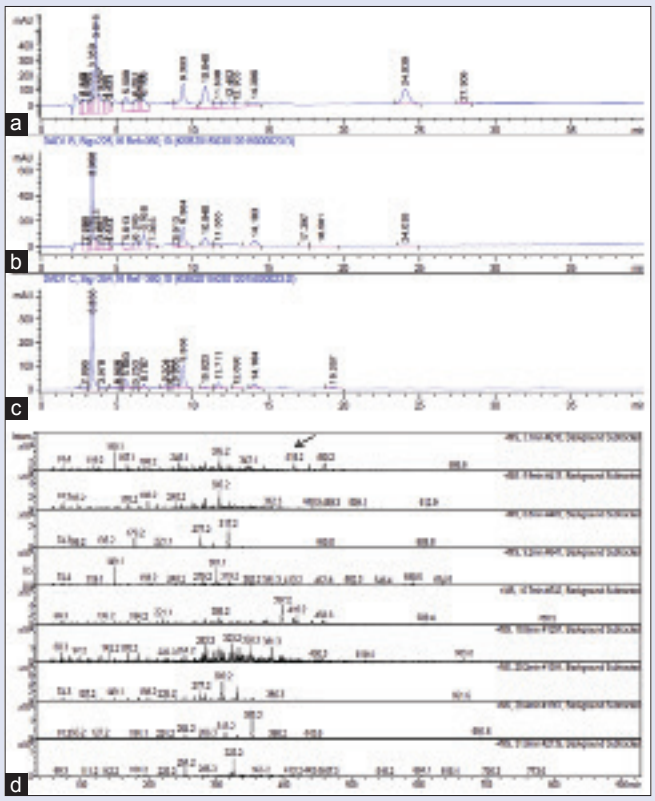

Figure 3: Chromatographic profile of *Cyperus scariosus* hexane extract by liquid chromatography-mass spectroscopy (a) at 210 nm (b) at 225 nm (c) at 254 nm. (d) Positive electron spray tandem mass spectrometry spectrum of *Cyperus scariosus* hexane extract

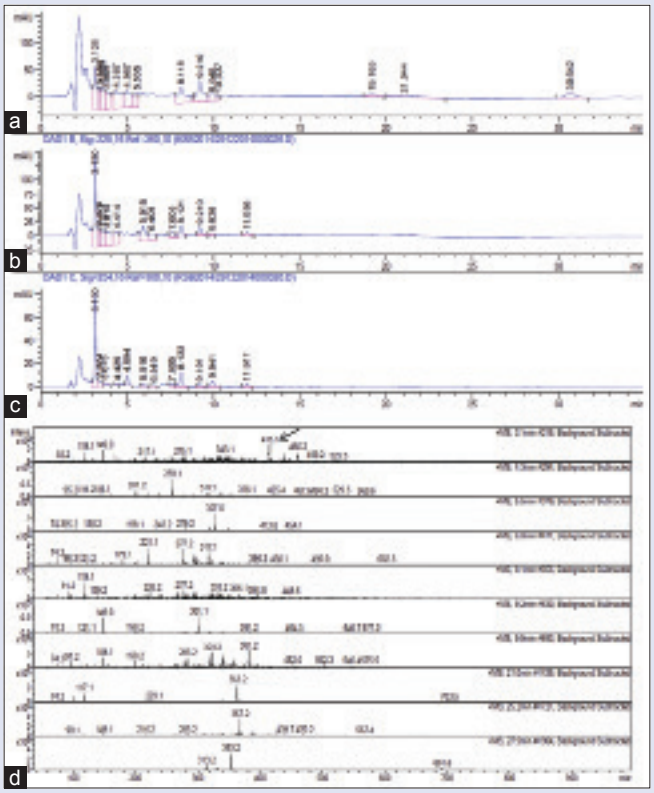

Figure 4: Chromatographic profile of *Cyperus scariosus* chloroform extract by liquid chromatography-mass spectroscopy (a) at 210 nm (b) at 225 nm (c) at 254 nm. (d) Positive electron spray tandem mass spectrometry spectrum of *Cyperus scariosus* chloroform extract

0.1% formic acid, pumped at a flow rate of 1.0 mL/min and detection wavelengths at 210, 225, and 254 nm. The injection volume was 20 µL and the total analysis time per sample was 30.0 min.

Gas chromatography-mass spectroscopy conditions

GC-MS analysis was performed on a Shimadzu GCMS-QP 2010 gas chromatograph-mass spectrometer, equipped with a DB-5 capillary column (dimensions 30 m length, 0.25 mm diameter and film thickness 0.25 µm). An autoinjector (AOC-20i) was employed for sample injection. The oven temperature was programmed from 70 to 240°C at the rate of 5 min. A split injection mode was applied for analysis. The oven temperature was held at 70°C at the start of the run for 5 min, then increased to 120°C for 5 min, before being held at 240°C for 5 min. Helium with a flow rate of 1.2 mL/min was used as a carrier gas. The ion source and interface temperatures were held at 250°C and 200°C, respectively. The retention times and characteristic ions for the samples were studied by recording the electron ionization mass spectra of analytes in scan mode (range of m/z 40–700). MS start time 2.0 min and end time 47 min. The constituents were identified after comparison with those available in the computer National Institute of Standards and Technology (NIST) library attached to the instrument.

Isolation of phytochemical compounds from *Cyperus scariosus* using column chromatography

The crude hexane extract of CS was repeatedly chromatographed on a silica gel column using gradient elution with hexane and ethyl acetate.

Table 3: Represents unique compounds in hexane and chloroform extracts of C. scariosus and C. rotundus based on GC-MS analysis

Compounds only in "CS-Hex":27	Compounds only in "CS-CHCl3":14	Compounds only in "CR-Hex":15	Compounds only in "CR-CHCl3":13
Undecane, 3,7-dimethyl-$C_{13}H_{28}$- 184	Heptane, 2,5,5-tri methyl- $C_{10}H_{22}$-142	Spiro[2.4] heptane, 1,2, 4,5- tetramethyl-6-methylene- $C_{12}H_{20}$-164	1,4-Methanocyclo octa[d] pyridazine, 1,4,4a, 5,6,9,10,10a-octa hydro-11,11-dimethyl-, (1.alpha., 4.alpha., 4a. alpha.,10a. alpha.)-$C_{14}H_{22}$-204
2-Cyclohexen-1-one, 3,5,5-tri methyl-$C_9H_{14}O$ - 138	1-Octanol-$C_8H_{18}O$-130	Tricyclo [3.3.0.0 (2, 8)]octan-3-one, 5,8-dimethyl-$C_{10}H_{14}O$-150	Cyclobutene, 4,4-dimethyl-1-(2,7-octa di enyl)-$C_{14}H_{22}$-190
1H-Pyrazole, 4,5-dihydro-5,5-dimethyl-4-isopropylidene-$C_8H_{14}N_2$,- 138	1-Heptanol-$C_7H_{16}O$-116	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methyl ethenyl)-, [1S-(1.alpha.,7. alpha, 8a.alpha.)]-$C_{15}H_{26}O$-222	2-Methyl-4-(2,6,6-tri methylcyclohex-1-enyl) but-2-en-1-ol-$C_{14}H_{24}O$-208
1-Dodecene- $C_{12}H_{24}$- 168	Cyclopropane, pentyl-C_8H_{16}-112	1-Heptatriacotanol $C_{37}H_{76}O$-536	2-Hydroxy-2,4,4-trimethyl-3-(3-methyl buta-1,3-dienyl) cyclo- hexanone – $C_{14}H_{22}O_2$- 222
3-Tetradecene, (Z)-$C_{14}H_{28}$-196	1-Tridecene-$C_{13}H_{26}$-182	Cyclo hexane, 1-methyl-2,4-bis (1-methyl ethenyl)- $C_{13}H_{22}$-178	Androstan-17-one, 3-ethyl-3-hydroxy-, (5.alpha.)-$C_{21}H_{34}O_2$-318
Tridecanol- $C_{13}H_{28}O$- 200	1-Dodecanol-$C_{12}H_{26}O$-186	Kauren-18-ol, acetate, (4. beta.)-$C_{22}H_{34}O_2$-330	Limonene diepoxide $C_{10}H_{16}O_2$-168
Hexadecane- $C_{16}H_{34}$-226	3-Hexadecene, (Z)-$C_{16}H_{32}$-224	5,9-Undecadien-1-yne, 6,10-dimethyl-$C_{13}H_{20}$-176	1H-Cyclopropa[3,4] benz[1,2-e] azulene-4a, 5,7b, 9,9a (1aH)-pentol, 3-[(acetyloxy) methyl]-1b, 4,5,7a, 8,9-hexahydro-1,1,6,8-tetramethyl-, 5,9,9a-tr- $C_{28}H_{38}O_{10}$-534
Heptadecane- $C_{17}H_{36}$-240	Heptadecane-$C_{17}H_{37}$- 240	1-Eicosanol-$C_{20}H_{42}O$-298	1H-Cyclopropa[3,4] benz[1,2-e] azulene-4a, 5, 7b, 9,9a (1aH)-pentol, 3-[(acetyloxy) methyl]-1b, 4,5,7a, 8,9-hexahydro-1,1, 6,8-tetramethyl-, 9,9a-dia-$C_{26}H_{36}O_9$-492
Octadecane- $C_{18}H_{38}$-254	1-Heptadecene-$C_{17}H_{34}$-238	1-Heptacosanol-$C_{27}H_{56}O$-396	5,8-Dimethyl-1,4,6,7-tetrahydronaphtalin dicarbonic acid, 1,4-di methyl ester-$C_{26}H_{36}O_9$-276
Phenol, 3,5-bis (1,1dimethyl ethyl)-$C_{14}H_{22}O$-206	3,4-Hexanediol, 2,5-dimethyl-$C_8H_{18}O_2$-146	Dodecanoic acid, 3-hydroxy-$C_{12}H_{24}O_3$-216	1-Tridecanol-$C_{13}H_{28}O$-200
1-Hexadecene- $C_{16}H_{32}$- 224	Nonadecane -$C_{19}H_{40}$-268	12-Methyl-E, E-2 ,13-octadeca- dien-1-ol- $C_{19}H_{36}O$-280	beta.-Stigmasterol-$C_{29}H_{48}O$-412
Cinnamyl tiglate-$C_{14}H_{16}O_2$-216	Phosphonic acid, di octa decyl ester-$C_{36}H_{75}O_3P$-586	Pregna-1,4,7,16-tetraene-3, 20-dione-$C_{21}H_{24}O_2$-308	Stigmasterol methyl ether - $C_{30}H_{50}O$-426
Propanedinitrile, dicyclohexyl- $C_{15}H_{22}N_2$-230	1-Nonadecene-$C_{19}H_{38}$-266	H-Cyclopropa [3,4]benzyl[1,2-e] azulene 4a, 5,7b, 9, 9a (1aH)-pentol, 3-[(acetyloxy) methyl]-1b, 4,5,7a, 8, 9-hexahydro-1,1,6,8 tetra methyl-, 5,9,9a-$C_{28}H_{38}O_{10}$ - 534	Tetratriacontane-$C_{34}H_{70}$-478
Cyclopropane carboxylic acid, 1- methyl-,2,6-bis (1,1-dimethyl ethyl)-4-methylphenyl ester-$C_{20}H_{30}O_2$-302	1-Pentadecanol-$C_{15}H_{32}O$-228	14. Hexacosane-C26H54-366	
Octadecanophenone-$C_{24}H_{40}O$-344		5,8-Dimethyl-1- naphtaline-dicarbonic acid, 1,4-dimethyl ester-- $C_{16}H_{20}O_4$-276	
Hexadecanophenone-$C_{22}H_{36}O$-316			
Tetradecanophenone-$C_{20}H_{32}O$-288			
Docosanoic acid-$C_{22}H_{44}O_2$-340			
1-Docosene- $C_{22}H_{44}$-308			
Isopropyl myristate-$C_{17}H_{34}O_2$-270			
1,2-Benzenedicarboxylic acid, butyl octyl ester-$C_{20}H_{30}O_4$-334			
1,2-Benzenedicarboxylic acid, butyl-8-methylnonyl ester- $C_{22}H_{34}O_4$-362			
1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester- $C_{16}H_{22}O_4$-278			
Pentadecanoic acid-$C_{15}H_{30}O_2$-242			
9-Octadecenal- $C_{18}H_{34}O$-266			
9,12-Octadecadienoicacid (Z, Z)-$C_{18}H_{32}O_2$-280			
3-Tetradecenal-$C_{14}H_{26}O$-210			

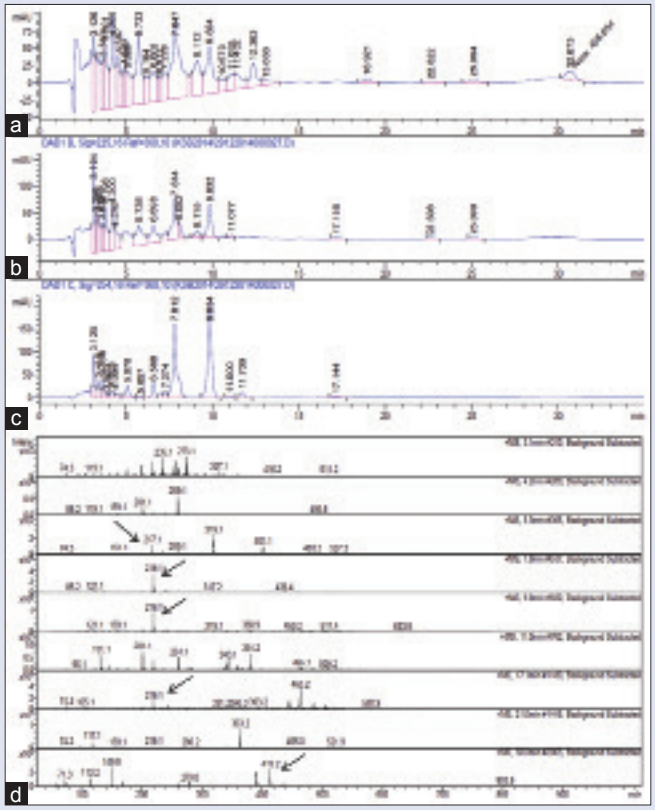

Figure 5: Chromatographic profile of *Cyperus rotundus* hexane extract by liquid chromatography-mass spectroscopy (a) at 210 nm (b) at 225 nm (c) at 254 nm. (d) Positive electron spray tandem mass spectrometry spectrum of *Cyperus rotundus* hexane extract

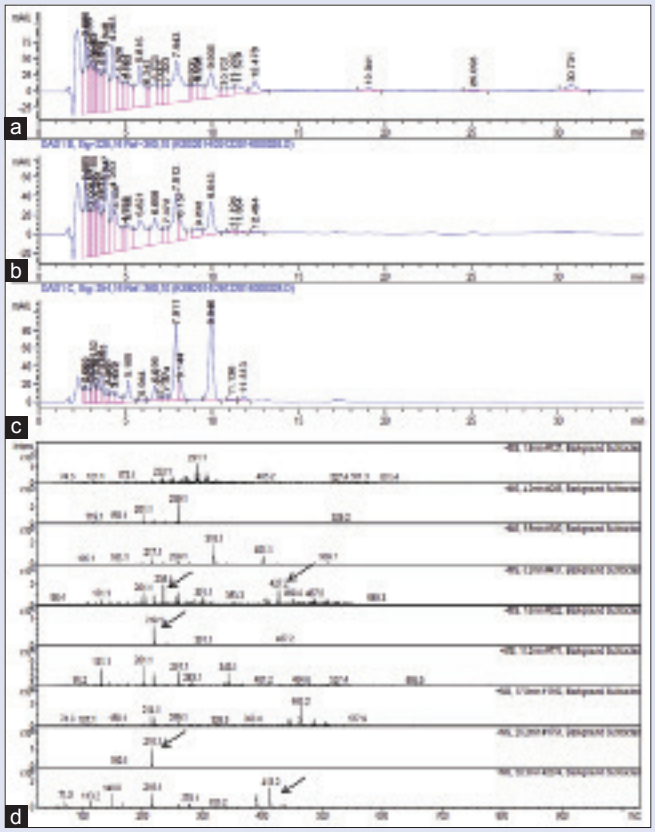

Figure 6: Chromatographic profile of *Cyperus rotundus* chloroform extract by liquid chromatography-mass spectroscopy (a) at 210 nm (b) at 225 nm (c) at 254 nm. (d) Positive electron spray mass spectrometry/mass spectrometry spectrum of *Cyperus rotundus* chloroform extract

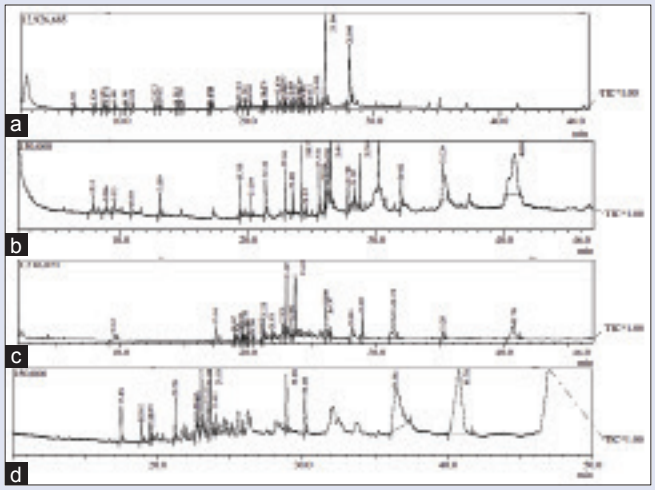

Figure 7: Representative gas chromatography-mass spectroscopy chromatograms of *Cyperus scariosus* and *Cyperus rotundus* rhizomes. (a) Hexane extracts of *Cyperus scariosus*; (b) Chloroform extract of *Cyperus scariosus*; (c) Hexane extracts of *Cyperus rotundus*; (d) Chloroform extract of *Cyperus rotundus*

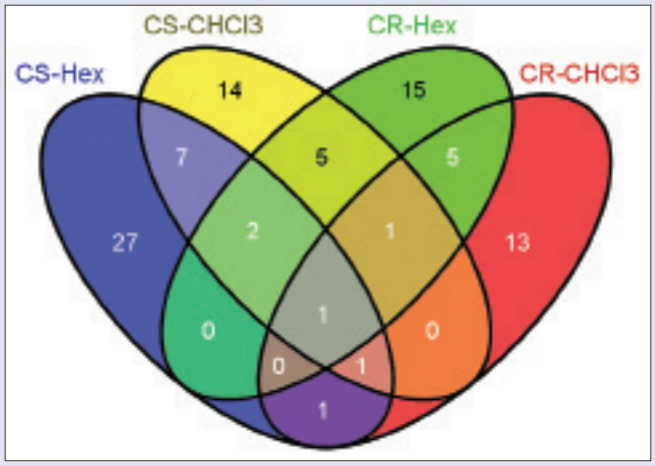

Figure 8: Venn diagram depicting the common and unique compounds in hexane and chloroform extracts of *Cyperus scariosus* and *Cyperus rotundus*

The collected fractions banding similarity were monitored by TLC. Based on TLC pattern these fractions were grouped into six major fractions (H1–H6). In H3, the fractions were combined, and the solvent was removed under vaccum to give a light brown residue. Thus obtained light brown residue has showed two major spots on TLC (solvent system, hexane: Ethyl acetate (80:20) at R_f values 0.40 and 0.50. This residue (1 g) was dissolved in hexane (20 mL) and small amount of silica gel was added (1 g) to it, the solvent was removed under vaccum and the powder

Table 4: Represents common compounds in hexane and chloroform extracts of *C. scariosus* and *C. rotundus* based on GC-MS analysis

Common Compounds in "CS-Hex" and "CS-$CHCl_3$":7	Nonane, 2,6- methyl- $C_{11}H_{24}$-156
	Undecane, 2,8-dimethyl- $C_{13}H_{28}$-184
	Dodecane, 4,6-dimethyl- $C_{14}H_{30}$-198
	Decane, 3,7-dimethyl- $C_{12}H_{26}$-170
	1-Pentadecene- $C_{15}H_{30}$-210
	Dodecane,2,6,11-tri methyl- $C_{15}H_{32}$-212
	Octadecanoic acid- $C_{18}H_{36}O_2$-284
Common Compounds in "CS-CHCl3" and "CR-Hex":5	1H-Pyrazole,4,5-dihydro-5,5-dimethyl-4-isopropylidene- $C_8H_{14}N_2$-138
	2-Cyclohexen-1-one, 3,5,5-trimethyl- $C_9H_{14}O$-138
	Eicosanoic acid- $C_{20}H_{40}O_2$-312
	1,2-Benzenedicarboxylic acid, diisooctyl ester- $C_{24}H_{38}O_4$-390
	Nonacosane- $C_{29}H_{60}$-408
Common Compounds in "CS-Hex", "CS-CHCl3" and "CR-Hex":2	n-Hexadecanoic acid- $C_{16}H_{32}O_2$-256
	1-Eicosene- $C_{20}H_{40}$-280
Common Compounds in "CR-Hex" and "CR-CHCl3":5	1H-Cyclopropa[a] naphthalene, 1a,2,3,3a,4,5,6,7b-octahydro-1,1,3a,7-tetramethyl- $C_{15}H_{24}$-204
	1,7,7-Trimethylbicyclo[2.2.1]hept-5-en-2-ol- $C_{10}H_{16}O$- 152
	Kauran-18-al, 17-(acetyloxy)-, (4.beta.)- $C_{22}H_{34}O_3$-346
	Caryophyllene oxide-$C_{15}H_{24}O$-220
	2,2,7,7-Tetramethyl tricyclo [6.2.1.0(1,6)] undec -4-en-3-one - $C_{15}H_{22}O$-218
Common Compounds in "CS-CHCl3", "CR-Hex" and "CR-CHCl3":1	Tetratetracontane- $C_{44}H_{90}$-618
Common Compounds in "CS-Hex", "CS-CHCl3", "CR-Hex" and "CR-CHCl3":1	Heneicosane- $C_{21}H_{44}$-296
Common Compounds in "CS-Hex", "CS-CHCl3" and "CR-CHCl3":1	1-Octadecene- $C_{18}H_{36}$-252
Common Compounds in "CS-Hex" and "CR-CHCl3":1	1-Hexadecanol- $C_{16}H_{34}O$-242
Common Compounds in "CS-Hex", "CR-Hex" and "CR-CHCl3":0	Zero
Common Compounds in "CS-CHCl3" and "CR-CHCl3":0	Zero
Common Compounds in "CS-Hex" and "CR-Hex":0	Zero

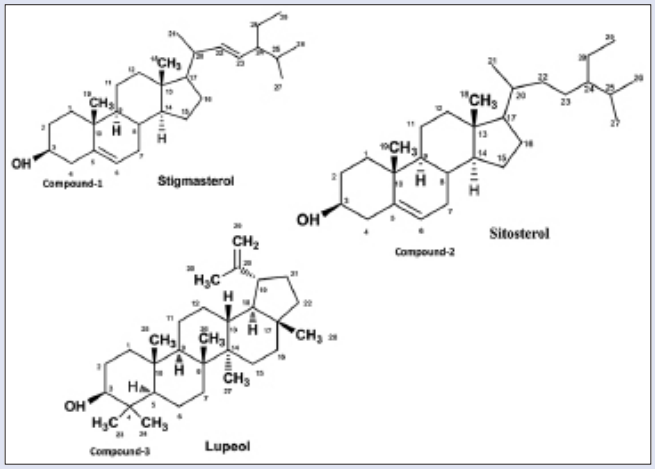

Figure 9: Isolated compounds in rhizomes of *Cyperus scariosus* R.Br

obtained was transferred onto a column of silica gel (100–200 mesh, 5 g) set in hexane. The column was successively eluted with hexane: EtOAc (1–5%).

Compound 1

A white solid (200 mg); mp 174–176°C, EIMS: (*m*/z) 412 [M^+], IR spectrum displayed absorption bands at 3424, 2936, 2867, 1640, 1464 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): 5.35 (t, *J* = 6.1 Hz, 1H), 5.14 (m, 1H), 4.98 (m, 1H), 3.52 (m, 1H), 2.52–2.08 (m, 5H), 1.98–1.92 (m, 3H), 1.03 (3H, s), 1.51 (m, 1H), 1.52 (m, 2H), 1.32–1.40 (m, 3H), 1.18 (m, 2H), 1.14 (m, 2H), 1.01 (s, 3H), 1.01 (s, 3H), 1.02 (m, 1H), 0.96 (m, 1H), 0.91 (d, *J* = 6.2 Hz, 3H), 0.83 (t, *J* = 7.2 Hz, 3H), 0.82 (d, *J* = 6.6 Hz, 3H), 0.80 (d, *J* = 6.6 Hz, 3H), 0.71 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): 140.71 (C-5), 138.2 (C-22), 72.1 (C-3), 121.7 (C-6), 56.8 (C-14), 56.2 (C-17), 51.1 (C-24), 29.6 (C-25), 42.4 (C-13), 42.6, 40.4, 39.8, 37.5, 36.4, 32.3, 32.1 (C-2), 31.8 (C-7, C-8), 20.9 (C-21), 29.3 (C-16), 18.9 (C-28), 24.4 (C-15), 20.9 (C-21), 21.5 (C-11), 19.3 (C-27), 21.7 (C-19), 12.2 (C-29), 40.6 (C-18).

Compound 2

White amorphous powder (80 mg); mp 134–135°C, EIMS: (*m*/z) 414 [M^+], IR spectrum showed absorption bands at 3424, 2930, 2852, 1724, 1463, 1378, 1271, 1059, 1023, 963 and 802 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): 5.36 (1H, d, *J* = 6.4 Hz, H-6), 3.53 (1H, m, H3), 1.01, 0.68 (3H, s, H-19 and H-18), 0.83 (3H, d, *J* = 6.4 Hz, H-21), 0.81 (3H, d, *J* = 6.4 Hz, H-29) and 0.85 (3H, t, *J* = 7.1 Hz, H-26). ^{13}C NMR ($CDCl_3$, 75 MHz): 37.5 (C-1), 31.9 (C-2), 72.0 (C-3), 42.5 (C-4), 140.9 (C-5), 121.9 (C-6), 32.1 (C-7), 32.1 (C-8), 50.3 (C-9), 36.7 (C-10), 21.3 (C-11), 39.9 (C-12), 42.6 (C-13), 56.9 (C-14), 26.3 (C-15), 28.5 (C-16), 56.3 (C-17), 36.3 (C-18), 19.2 (C-19), 34.2 (C-20), 26.3 (C-21), 36.2 (C-22), 46.1 (C-23), 23.3 (C-24), 12.2 (C-25), 29.4 (C-26), 20.1 (C-27), 19.6 (C-28), 12.0 (C-29).

Whereas, in case of chloroform extract (12 g) residue of CS elution was carried out with a stepwise gradient of chloroform: Acetone. Initially, the column was eluted with plain chloroform, further the column was eluted with chloroform: Acetone, (100:0, 90:10, 80:20, 70:30, 60:40, and 50:50) mixture in the increasing order of polarity. Fractions of 200 mL each were collected and concentrated.

Compound 3

EIMS: (*m*/z) 426 (M^+), the major mass fragments observed at 393, 330, 182, 151 and 69, mp 134–135°C. The IR spectrum displayed absorption bands at 2941, 1452, 1379, 1012 and 880 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ: 4.69 and 4.56 (each 1H, s, H-29), 3.16 (1H, dd, H-3), 2.35 and 1.49 (each 1H, m, 21A), 1.67 (1H, t, H-15A), 1.71 (3H, s, H-30), 1.61 (2H, d, H-12A, 1A), 1.67 (1H, t, H-13),1.50 (1H, q, H-2B), 1.42 (1H, m, H-22A), 1.41 (2H, m, H-7), 1.50 (1H, q, H-6B), 1.38 (1H, t, H-16A), 1.39 (1H, m, H-21B), 1.25 (1H, q, H-11B), 1.28 (1H, m, H-22B), 1.71 (3H, s, H-26),1.00 (3H, s, H-23), 0.94 (3H, s, H-27), 0.92 (1H, t, H-1B), 0.80, 0.79 and 0.73 (each 3H, H-25, 28, 29) and 0.66 (1H, d, H-5). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 150.9 (C-20), 109.3 (C-29), 79.0 (C-3), 55.3 (C-5), 50.4 (C-9), 48.0 (C-18), 48.3 (C-19), 25.1 (C-12), 42.8 (C-14), 40.8 (C-8), 40.0 (C-22), 38.8 (C-4), 38.7 (C-1), 38.0 (C-13), 37.1 (C-10), 35.6 (C-16), 34.3 (C-7), 29.1 (C-21), 28.0 (C-23), 27.4 (C-15), 27.4 (C-2), 25.1 (C-12), 28.0 (C-23), 27.4 (C-15), 20.9 (C-11), 19.3 (C-30), 18.3 (C-6), 18.0 (C-28), 15.9 (C-25), 16.1 (C-26), 15.3 (C-24), and 14.6 (C-27).

RESULTS AND DISCUSSION

Morphological differences between *Cyperus scariosus* and *Cyperus rotundus*

In CS, the stems are slender, three-sided and triangular in cross-section. An umbrella-like tuft of long narrow leaves occurs at the top. Leaves are whorled, lanceolate and green in color, with a distinct ridge. The rhizomes are initially white in color and eventually turn brown with growing age. Lateral shoots arise from the base of the stem in an immediately ascending manner. Whereas flowers are initiated from axillary buds. In contrast, CR is a grass-like weed with an extensive underground network of basal bulbs, fibrous roots, thin, wiry rhizomes and tubers born in chains of 2–6 or more on rhizomes. The leaves are mostly basal, dark green, with a prominent midrib and an abrupt taper at the top. The purplish to red-brown inflorescence is born on a stem that is triangular in cross-section and usually taller than the foliage [Figure 1].

Comparative phytochemical analysis between *Cyperus scariosus and Cyperus rotundus*

The rhizome powders showed a distinct color variation between the species [Figure 2 (I. *Cyperus Scariosus* and II. *Cyperus rotundus*)]. However, classifying the species based on color can be erratic and misleading. Based on the preliminary phytochemical analysis of various solvent extracts of CS and CR constituents of terpenoids and steroids were found in excess amount, constituents such as alkaloids, glycosides, carbohydrates, phenols, fats, and oils were also found. The compounds related to tannins; saponins and flavonoids were found to be absent [Table 2]. In TLC analysis hexane extracts of both the plant species showed better separation in hexane: EtOAC (90:10), whereas chloroform extracts showed better separation in chloroform: Acetone (70:30) as mobile phase. In case of methanolic extract in CS showed separation in $CHCl_3$: MeOH (60:40), whereas in case of CR methanolic extract (40:60) $CHCl_3$: MeOH as the mobile phase [Figure 2 (Ic and IIc)]. Based on these TLC profile pattern and their retardation factor (Rf), it is suggested that both the plant species as different.

Further, LC–tandem mass spectrometry and GC/MS techniques were used to determine the chemical profiles of CR and CS. Thus, liquid chromatogram patterns of CS hexane extract showed major peaks at their retention times 2.89, 3.359, 9.363, and 10.84 [Figure 3a-c]. Whereas in case of CS chloroform extract major peaks were observed at retention times 3.128, 4.99, 8.12 and 9.210 [Figure 4a-c]. Similarly, hexane extract of CR displayed major peaks at 3.12, 4.2, 7.81, 9.11, and 9.82 [Figure 5a-c], whereas in chloroform extract of CR major peaks observed at retention times 2.686, 3.84, 4.26, 5.82, 7.90, and 9.9, respectively [Figure 6a-c]. Furthermore, comparisons of the mass spectral patterns of hexane and chloroform extracts of CS indicated a molecular ion peaks at retention times of 3.3 and 3.1 min with m/z 415.1 $[M^+]^+$ [Figures 3d and 4d], which was correlated to the β-sitosterol from the literature study.[11] In contrary, the mass spectra of hexane and chloroform extracts of CR showed the ion peak at m/z 219.1$[M^+]^+$ with retention time 7.8 min correlated to the compounds α-cyperone and cyperotundone from the literature data.[12] In addition to this the ion peak at 26.3 min retention times with m/z 216.1$[M^+]^+$ [Figures 5d and 6d] correlated to the compounds α-cyperene and Isocyperol[13] in both the extracts of CR. Further, the ion peak with m/z 413.2 $[M^+]^+$ at retention time 30.3 min correlated to stigmasterol[14] and the ion peak at 6.3 min with m/z 427.2$[M^+]^+$ [Figure 6d] correlated to the Lupeol in chloroform extract of CR from the literature data.[15]

GC-MS chromatograms of hexane extract from the rhizomes of CS showed 30 peaks and chloroform extract showed 22 peaks. Whereas CR hexane extract showed, 23 peaks and chloroform extract showed 15 peaks. These chromatograms with retention time were shown in Figure 7. By comparing GC-MS spectra of with NIST library, we identified common and unique compounds between these extracts in the form of Venn diagram [Figure 8]. This diagram depicts the common compounds presented in CS-hexane and CS-$CHCl_3$ extracts were seven. Five compounds were common in CS-$CHCl_3$ and CR-hexane. In CS-hexane, CS-$CHCl_3$ and CR-hexane two compounds were similar. Five compounds were similar between CR-hexane and CR-$CHCl_3$. One compound is similar in CS-$CHCl_3$, CR-hexane, and CR-$CHCl_3$ between these extracts. In CS-hexane, CS-$CHCl_3$, and CR-$CHCl_3$ one compound is similar. Finally, in CS-hexane and CR-$CHCl_3$ one compound is similar. In contrast to unique compounds, 27 compounds were unique in CS-hexane, 14 compounds in CS-$CHCl_3$, 15 compounds in CS-hexane and 13 compounds in CR-$CHCl_3$. The name of the identified compounds, molecular weight, and their molecular formula were presented in Tables 3 and 4.

Isolation and structural elucidation of compounds in *Cyperus scariosus*

The concentrated hexane and chloroform extracts from the rhizomes of were chromatographed on silica gel and the resultant fractions and repeated column chromatography purification of resultant fractions led to the isolation of three compounds. The structures of isolates were established using IR, MS, 1D, and 2D NMR spectroscopic techniques. After comparing their spectral data with those reported in the literature[16] they were identified as known compounds and confirmed as stigmasterol, β-sitosterol and lupeol [Figure 9]. These compounds were found to be major constituents in both the species that is, CR[17] and CS.

CONCLUSION

In this study, we examined the morphological and chemoprofiling pattern of CS and CR *to* systematically classify these species. Based on their morphological attributes, it is found and confirmed that these two species are different. Chemoprofiling analyses revealed some of the common phytochemical compounds similar in between these herbs. Finally, we conclude that these two herbs hold some of the similar phytochemical compounds in major quantity but are morphologically different.

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Conflicts of interest

There are no conflicts of interest.

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