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: Chemoprophiling, 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. 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 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.

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][2] 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.[5][6] 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. This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

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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 F254 (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 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 NOVAPA K H 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 'H and 13CNMR spectra

![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](image-url)
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 vacuum 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 F254) 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% $\text{H}_2\text{SO}_4$ 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...
Table 2: Phytochemical analysis of different extracts of C. scariosus and C. rotundus rhizomes

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

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

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_{11}H_{20} | Heptane, 2,5,5-tri methyl- C_{8}H_{12} | Spiro[2.4] heptane, 1,2, 4,5- tetramethyl- 6-methylene- C_{8}H_{19} | 1,4-Methanocyclo octa[d] pyridazine, 1,4,4a, 5,6,9,10a-octa hydro-11,11-dimethyl- (1.alpha., 4.alpha., 4a. alpha,10a. alpha.)-C_{16}H_{33} |
| 2-Cyclohexen-1-one, 3,5,5-tri methyl-C_{9}H_{14}O | 1-Octanol- C_{9}H_{18}O | Tricyclo hexane, 1, methyl-2,4-bis (1-methyl ethenyl)- C_{21}H_{44} | 2-Hydroxy-2,4,4-trimethyl-3 (3-methyl buta-1,3-dienyl) cyclo- hexane – C_{12}H_{26}O-222 |
| 1H-Pyrazole, 4,5-dihydro-5,5-dimethyl-4-isopropylidene- C_{8}H_{12}N_{2} | 1-Heptanol- C_{7}H_{16}O | Pyran-1-ol- C_{9}H_{18}O | Androstan-17-one, 3-ethyl-3-hydroxy-, (1H.-C_{21}H_{42}O)-318 |
| Hexadecane- C_{16}H_{34} | Hexadecane- C_{17}H_{38} | Heptadecane- C_{20}H_{42}O-396 | 5,8-Dimethyl-1,4,6,7-tetradicarboxylic acid, 1,4-di methyl ester- C_{25}H_{50}O-276 |
| 3-Hexadecene, (Z)- C_{16}H_{32} | 5,9-Undecadien-1-yn, 6,10-dimethyl-C_{15}H_{28} | 1-Eicosanol- C_{20}H_{42}O-298 | 1-Tridecanol- C_{13}H_{28}O-200 |
| 1-Heptadecene- C_{17}H_{34} | 1-Heptadecane- C_{17}H_{36} | Heptacosanol- C_{27}H_{56}O-396 | 5,8-Dimethyl-1,4,6,7-tetradicarboxylic acid, 1,4-di methyl ester- C_{28}H_{58}O-276 |
| 2-Uncyclonene- C_{16}H_{30} | 1-Hexadecene- C_{16}H_{32} | Docosanoic acid- C_{22}H_{44}O_{2}-340 | 1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester- C_{26}H_{48}O_{2}-340 |
| 1-Hexadecene- C_{16}H_{32} | Nonadecane - C_{19}H_{38} | 12-Methyl-E. E-2,13-octadeca-dien-1-octyl-C_{20}H_{42}O-280 | Stigmasterol methyl ether - C_{27}H_{42}O-426 |
| Cyclonitrenitrile, dicyclohexyl- C_{36}H_{64}N_{2} | H-Cyclopentene[3,4]benz[1,2-e] azulene-4a, 5,7b, 9,9a (1A)-pentol, 3-[acetoxy methyl] ester- C_{32}H_{50}O-492 | 14. Hexacosane-C_{26}H_{54}O_{3}-366 | Tetratriacontane-C_{34}H_{72}-478 |
| Docosane- C_{22}H_{44} | 3-Hexadecene- (Z)- C_{16}H_{32} | 1-Tridecasenoic acid- C_{26}H_{50}O-376 | 5,8-Dimethyl-1-naphthalene dicarboxylic acid, 1,4-dimethyl ester- C_{27}H_{54}O_{2}-276 |
| 1-Heptadecene- C_{17}H_{36} | 1-Nonadecene- C_{19}H_{38} | 1,2-Benzenedicarboxylic acid, butyl-8-methylphenyl ester- C_{22}H_{48}O_{3}-362 | 1,2-Benzenedicarboxylic acid, butyl-8-methylene- C_{18}H_{32}O_{3}-362 |
| Octadecanophenone-C_{20}H_{34}O_{3}-344 | 1,2-Benzenedicarboxylic acid, butyl-8-methylphenyl ester- C_{18}H_{28}O_{3}-302 | 1,2-Benzenedicarboxylic acid, butyl-8-methylene- C_{18}H_{32}O_{3}-362 | 1,2-Benzenedicarboxylic acid, butyl-8-methylene- C_{18}H_{32}O_{3}-362 |
| Hexadecanophenone-C_{20}H_{30}O_{3}-341 | 2-Heptadecenyl- C_{18}H_{34}O_{3}-302 | 1,2-Benzenedicarboxylic acid, butyl-8-methylene- C_{18}H_{32}O_{3}-362 | 1,2-Benzenedicarboxylic acid, butyl-8-methylene- C_{18}H_{32}O_{3}-362 |
| Tetradecanophenone-C_{20}H_{30}O_{3}-341 | Docosanoic acid-C_{22}H_{44}O_{2}-340 | 1,2-Benzenedicarboxylic acid, butyl-8-methylene- C_{18}H_{32}O_{3}-362 | 1,2-Benzenedicarboxylic acid, butyl-8-methylene- C_{18}H_{32}O_{3}-362 |
| 1-Docosene- C_{22}H_{44} | 1,2-Benzenedicarboxylic acid, butyl-8-methylene- C_{18}H_{32}O_{3}-362 | 1,2-Benzenedicarboxylic acid, butyl-8-methylene- C_{18}H_{32}O_{3}-362 | 1,2-Benzenedicarboxylic acid, butyl-8-methylene- C_{18}H_{32}O_{3}-362 |
| 3-Octadecenyl- C_{26}H_{42}O_{3}-302 | 5,8-Dimethyl-1-naphthalene dicarboxylic acid, 1,4-dimethyl ester- C_{27}H_{54}O_{2}-276 | 1,2-Benzenedicarboxylic acid, butyl-8-methylene- C_{18}H_{32}O_{3}-362 | 1,2-Benzenedicarboxylic acid, butyl-8-methylene- C_{18}H_{32}O_{3}-362 |
| 3-Octadecenal- C_{26}H_{42}O_{3}-302 | 1,2-Benzenedicarboxylic acid, butyl-8-methylene- C_{18}H_{32}O_{3}-362 | 1,2-Benzenedicarboxylic acid, butyl-8-methylene- C_{18}H_{32}O_{3}-362 | 1,2-Benzenedicarboxylic acid, butyl-8-methylene- C_{18}H_{32}O_{3}-362 |
| 9-Octadecenal-C_{26}H_{42}O_{3}-302 | 1,2-Benzenedicarboxylic acid, butyl-8-methylene- C_{18}H_{32}O_{3}-362 | 1,2-Benzenedicarboxylic acid, butyl-8-methylene- C_{18}H_{32}O_{3}-362 | 1,2-Benzenedicarboxylic acid, butyl-8-methylene- C_{18}H_{32}O_{3}-362 |
light brown residue has showed two major spots on TLC (solvent system, hexane: Ethyl acetate (80:20) at Rf 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

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

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 spectrometry 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
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 (CDCl₃, 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).

**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 (CDCl₃, 300 MHz): 5.36 (1H, d, J = 6.4 Hz, H-6), 3.53 (1H, m, H-1), 1.01, 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, d, J = 7.1 Hz, H-26). ¹³C NMR (CDCl₃, 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).

**Table 4**

| Common Compounds in “CS‑Hex” and “CS‑CHCl₃” | 7/7 |
|---------------------------------------------|----|
| Nonane, 2,6- methyl C₂₅H₅₄, C₃₀H₆₄, C₃₀H₆₀, C₃₀H₆₀ | 156 |
| Undecane, 2,8-dimethyl C₂₅H₅₂ | 184 |
| Dodecane, 4,6-dimethyl C₂₅H₅₂ | 198 |
| Decane, 3,7-dimethyl C₂₀H₄₂ | 170 |
| 1-Octadecane C₂₀H₄₂ | 210 |
| 2-Methylpentane C₁₂H₂₆ | 212 |
| Octadecanoic acid C₁₈H₃₇O₂ | 284 |
| 1H-Pyrazole, 4,5-dihydro-5,5-dimethyl-4-isopropylidine C₁₂H₁₆N₂ | 138 |
| 1,2-Benzene dicarboxylic acid, diisooctyl ester C₂₀H₃₇O₂ | 312 |
| Nonacosane C₂₉H₅₈ | 408 |
| n-Hexadecanoic acid C₂₀H₄₂O₂ | 256 |
| 1-Octacosane C₂₈H₅₆ | 280 |

**Common Compounds in “CR‑Hex” and “CR‑CHCl₃”**: 5

| Common Compounds in “CR‑Hex” and “CR‑CHCl₃” | 5 |
|----------------------------------------------|----|
| 1-Octadecane C₂₀H₄₂ | 252 |
| 1-Hexadecanol C₂₀H₄₂O₂ | 242 |
| Tetratetracontane C₄₄H₉₂ | 618 |

**Common Compounds in “CS‑CHCl₃”**: 4

| Common Compounds in “CS‑CHCl₃” | 4 |
|--------------------------------|----|
| Heneicosane C₂₁H₄₄ | 296 |

**Common Compounds in “CS‑CHCl₃”**: 1

| Common Compounds in “CS‑CHCl₃” | 1 |
|--------------------------------|----|
| 1-Octadecene C₂₀H₄₂ | 252 |

| Common Compounds in “CR‑Hex” and “CR‑CHCl₃” | 0 |
|----------------------------------------------|----|
| Zero | 0 |

**Common Compounds in “CR‑Hex” and “CR‑CHCl₃”**: 0

| Common Compounds in “CR‑Hex” and “CR‑CHCl₃” | 0 |
|----------------------------------------------|----|
| Zero | 0 |

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

Morphological differences between *Cyperus scariosus* and *C. 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, wavy 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 *C. 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 CHCl₃: MeOH (60:40), whereas in case of CR methanolic extract (40:60) CHCl₃: MeOH as the mobile phase [Figure 2 (lc and lIc)]. 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 2.68, 3.84, 4.26, 5.82, 7.90, and 9.9, respectively [Figure 6a-c]. In addition to the above, 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 isocyperone from the literature data.[29] In contrast, 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-cyperene and cyperotundone from the literature data.[30] 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 isocyperone[29] 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[31] 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.[32]

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-CHCl₃ extracts were seven. Five compounds were common in CS-CHCl₃ and CR-hexane. In CS-hexane, CS-CHCl₃ and CR-hexane two compounds were similar. Five compounds were similar between CR-hexane and CR-CHCl₃. One compound is similar in CS-CHCl₃, CR-hexane, and CR-CHCl₃ between these extracts. In CS-hexane, CS-CHCl₃, and CR-CHCl₃ one compound is similar. Finally, in CS-hexane and CR-CHCl₃ one compound is similar. In contrast to unique compounds, 27 compounds were unique in CS-hexane, 14 compounds in CS-CHCl₃, 15 compounds in CS-hexane and 13 compounds in CR-CHCl₃. 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,[33] 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[34] and CS.

CONCLUSION

In this study, we examined the morphological and chemoprophiling 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. Chemoprophiling 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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