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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