BIODIVERSITAS
Volume 23, Number 5, May 2022
Pages: 2329-2341

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d230511

RP-HPLC based analysis of different polyphenols in seven species of *Carex* L. (Cyperaceae Juss.) from West Bengal, India

POOJA RAJAK, ASOK GHOSH♥
Taxonomy and Biosystematics Laboratory, UGC-CAS Department of Botany (DST-FIST sponsored), University of Burdwan. Golapbag 713104, West Bengal, India. Tel.: +91-342-2656427, ♥email: asokcarex@gmail.com; asokcarex@rediffmail.com

Manuscript received: 22 February 2022. Revision accepted: 20 April 2022.

Abstract. *Rajak P, Ghosh A. 2022. RP-HPLC based analysis of different polyphenols in seven species of* Carex *L. (Cyperaceae Juss.) from West Bengal, India. Biodiversitas 23: 2329-2341. Carex* L. is one of the important genera of the family Cyperaceae having immense ecological significance and probable therapeutic potentiality due to the presence of bioactive phytochemicals i.e. polyphenols. RP-HPLC (reversed-phase high-performance liquid chromatography) based evaluation of polyphenols concerned with the chemotaxonomy of this genus, was a fundament of reported research but the evaluation of its therapeutic possibilities are still an open field of research. In concerned with the above aim, phytochemical investigation was done by analyzing the seven species of Indian *Carex* to evaluate polyphenols employing RP-HPLC. The above-mentioned analysis reveals differences in the presence as well as the amount of these marker phytochemical components compositions of each and every studied species of *Carex*. Total of nine polyphenols was used as standard among which, except coumaric acid, all of the polyphenols were detected in considerable amount in the studied species. Among these seven species, *Carex stramentitia* Boott ex Boeckeler and *C. alopecuroides* D. Don were found to contain a higher number of polyphenols. Gallic acid was quantified in the highest amount in *C. baccans* Nees. A higher amount of phloroglucinol was also detected in *C. insignis* Boott and *C. baccans*. Remarkably, biochanin A, a highly significant therapeutic phytochemical, was only observed in higher amounts in *C. alopecuroides*.

Keywords: Bioactive phytochemicals, *Carex*, Cyperaceae, RP-HPLC, therapeutic potentiality

INTRODUCTION

Cyperaceae Juss., the third largest family in Monocots, under the order Poales, has a large number of species (ca. 5570) under 89 genera worldwide (POWO 2022). In India, the family is the sixth largest, comprised of 545 species under 38 genera as reported by Arisdason and Lakshminarasimhan (2017). The megadiverse genus *Carex* L. is consisting of ca. 1997 species around the world (POWO 2022) and ca.163 species in India (Karthikeyan et al. 1989). The genus *Carex* has the delimitation based on its complexity and similarity in reproductive morphology, which makes this genus taxonomically challenging (Reznicek 1990).

Phytochemical investigation of plants had been carried out a long time ago to report the presence of bioactive components which in turn helped the field of pharmacology in a good way. Apart from Cyperaceae, in previous studies of different angiosperms families like Asteraceae, Vitaceae, Poaceae phytochemical screening along with chemotaxonomy has been successfully used as a promising method (Emerenciano et al. 2001; Mika et al. 2005; Rivière et al. 2012). Among all the phytochemicals, polyphenols are one of the most important groups of secondary metabolites, widely distributed in plants with numerous promising bioactivities such as antioxidant and anti-inflammatory activities (Mojzer et al. 2016; Zhang et al. 2016). Phytochemical screening of Cyperaceae other than *Carex*, especially based on polyphenols, had been done worldwide by several authors. The two-dimensional chromatographic analysis by Harborne (1971) revealed the presence of five pharmacologically important flavonoids i.e. kaempferol, quercetin, glycoflavone, luteolin and tricin in the leaves of different members of the tribe *Scirpae*, *Rhynchosporae* and Cypereae. The research found similar distribution pattern of foliar flavonoids suggesting the close relationship of Cyperaceae with Poaceae as well as Palmae. Nakajima et al. (1978) had identified two new hydroxystilebene named scirpusin A and B along with two known hydroxystilbene, resveratrol and 3,3',4,5'- tertrahydroxystilbene from the rhizome of *Scirpus fluviatilis* (Torr.) A. Gray. Methoxycyperotundol, cyperotundol, salicylic acid, caffeic acid, protocatechuic acid, *p*-coumaric acid, pongamone A and biochanin A, cassigarol E, scirpusin A and B were isolated from the rhizomes of *Cyperus rotundus* L. (Zhou et al. 2012, 2013; Tran et al. 2013). Abdel-Mogib et al. (2001) had found four stilbenes along with a new acetophenone derivative from the tubers of *Scirpus holoschoenus* L. Arraki et al. (2017) have extracted scirpusin B and cyperusphenol B from the seeds of *Cyperus eragrostis* Lam. Similarly, Zaki et al. (2018) have revealed the presence of three new flavan derivatives; two new stilbene derivatives along with four known stilebene derivatives compounds from the aerial parts of *Cyperus conglomeratus* Rottb. Recently, Datta et al. (2018) have reported vanillic acid, ferulic acid, rutin, myrcetin, quercetin and apigenin from *Cyperus compressus* L.

Carex, the largest genus of the family Cyperaceae, had gained the attention of many authors due to the availability of a large number of bioactive polyphenols in it. Several investigations have been previously carried out in this genus throughout the world. Harborne (1971) had performed two-dimensional chromatography and found different distribution patterns of kaempferol, quercetin, glycoflavone, luteolin and tricin in leaf extracts of different species under the tribe Cariceae. They have concluded that the presence of flavonols in seven of all the studied species of *Carex* suggests their primitive nature. Manhart (1986) had studied *Carex* section *Laxiflorae* and found some foliar flavonoids viz. quercetin glycoside, luteolin, tricin and apigenin 7-o- glycoside. The broad-scale comparisons of flavonoid aglycone distributions among the various numbers of sedges were critically examined to incorporate some members of *Carex*. These comparisons were proved to be restricted to explain the phylogenetic relationships among the species within the genus *Carex*. The effectiveness of this investigation in *Carex* at the infra-sectional levels and possibly at the inter-sectional levels was also explained by detailed flavonoid analyses in the sections *Laxiflorae* and *Acrocystis* (Manhart 1990).

A tetrastilbene (cis-miyabenol A) and two known oligostilbenes (kobophenol B and cis-miyabenol C) were found to be present in the seeds of *Carex pendula* Huds. (Meng et al. 2001). The flavonoid, tricin was identified in the leaves of *Carex arenaria* L. (Van de Staaij et al. 2002). Prenyl stilbenoid derivatives namely carexane A-L along with distachyasin, a novel antioxidant metabolite were isolated from the leaves and roots of *Carex distachya* Desf and this species was proved to be a potent source of polyphenolic compounds by D’Abrosca et al. (2005) and Fiorentino et al. (2006). Two resveratrol oligomers i.e. pallidol and kobophenol A along with five flavonoids i.e, isoorientin, luteolin, quercetin, 3-O-methylquercetin and rutin were isolated from seeds of *Carex folliculata* L. from the northern United States by Li et al. (2009). Resveratrol oligomers viz. pallidol, α-viniferin, trans- miyabenol, kobophenol A and B have been isolated from *Carex folliculata* L. and *C. gynandra* Schwein. by González-Sarrias et al. (2011).

Similarly, Bogucka-kocka et al. (2011) have estimated eight phenolic acids viz. caffeic, ferulic, *p*-coumaric, *p*-hydroxybenzoic, protocatechuic, sinapic, syringic, vanillic acid in different quantities from the aerial parts of 18 species of *Carex* from Central Europe. In their extensive study, some stilbenes viz. carexinol A, resveratrol-diglucoside, miyabenol A & C, kobophenol A and α-viniferin have been isolated from five species of *Carex* from France by Arraki et al. (2013). In the phytochemical screening on aerial parts and root tubers of *Carex baccans* Nees by Dávid et al. (2021); Kumar et al. (2013) and Giri et al. (2015), the species was found to be the potent source of bioactive compound α-viniferin, smiglasid A, B and trans-resveratrol respectively. Two new phenolics named vulpinoideol A and B along with ten known polyphenolic compounds were isolated from the seeds of *Carex vulpinoidea* Michx. by Niesen et al. (2015). Some bioactive flavonoids were isolated from the roots of three Iranian *Carex* species (Noori et al. 2015). α-viniferin was isolated from the roots of *C. humilis* Leyss. by Seo et al. (2017). Virgatanol, resveratrol diglucoside, piceatannol and ε-viniferin were isolated from the seeds of *C. appressa* var. *virgata*; kobophenol A and carexinol A were isolated from the whole plant of *Carex cuprina* in their recent study by Arraki et al. (2017).

Due to very limited investigation, there is a lack of information on phytochemical screening on Indian Cyperaceae in general and the genus *Carex* in particular, which was the basis to initiate the present study. In this present investigation, altogether seven species of *Carex* representing three sub-genera namely *Vigneastra*, *Carex* and *Vignea* have been collected and extracted for RP-HPLC analysis. The comparison was done with nine standards (gallic acid, phloroglucinol, catechin, caffeic acid, coumaric acid, ferulic acid, rosmarinic acid, quercetin and biochanin A). The aim of the present study is the qualitative and quantitative assessment of these polyphenolics (phenolic acids and flavonoids) in the studied species of the sedge genus *Carex*.

MATERIALS AND METHODS

Materials

Field collection and sample preparation

Fresh live samples of seven species of *Carex* were collected in the field at their fruiting stage. Details of the collected information are presented in Table 1. After collection, the samples were washed thoroughly and air-dried at room temperature. The dried samples were ground into coarse powder with the help of a mixer grinder and stored in airtight sterile containers for further extraction procedures.

Plant material authentication

The plant materials for the present study were collected from their natural habitats in Darjeeling Himalayas, West Bengal, India. The details of the collection information along with systematic position are presented in Table 1. The plant materials were identified by consulting CAL herbarium physically, the protologues and original materials (type materials) are now available in different online databases like JSTOR (https://plants.jstor.org/), BHL (https://www.biodiversitylibrary.org/), HathiTrust (https://www.hathitrust.org/), internet archive (https://archive.org/) and digital herbaria like B, LE, LINN, P, S, SBT, BM, BR, E, K, NY (http://www.bgbm.org, http://www.binran.ru, http://www.linnean-online.org/, http://science.mnhn.fr/institution/mnhn/collection/p/item/search/form, https://www.nrm.se/en/forskningochsamlingar/botanik.8999708.html, http://www.bergianska.se/english/collections/the-bergius-herbarium/the-bergius-herbarium-1.88853, https://www.nhm.ac.uk/, https://www.botanicalcollections.be/#/en/home, https://data.rbge.org.uk/search/herbarium/, http://apps.kew.org/herbcat/navigator.do, https://www.nybg.org/; herbaria acronyms were according to Theirs 2022). Literature like, Boott (1858) and subsequent publications like *Flora of British India* (Clarke

1894), and *Flora of China* (Lunkai et al. 2010) were also consulted. The voucher specimens of the studied species were deposited at BURD (Thiers 2022), Herbarium of the Department of Botany, The University of Burdwan.

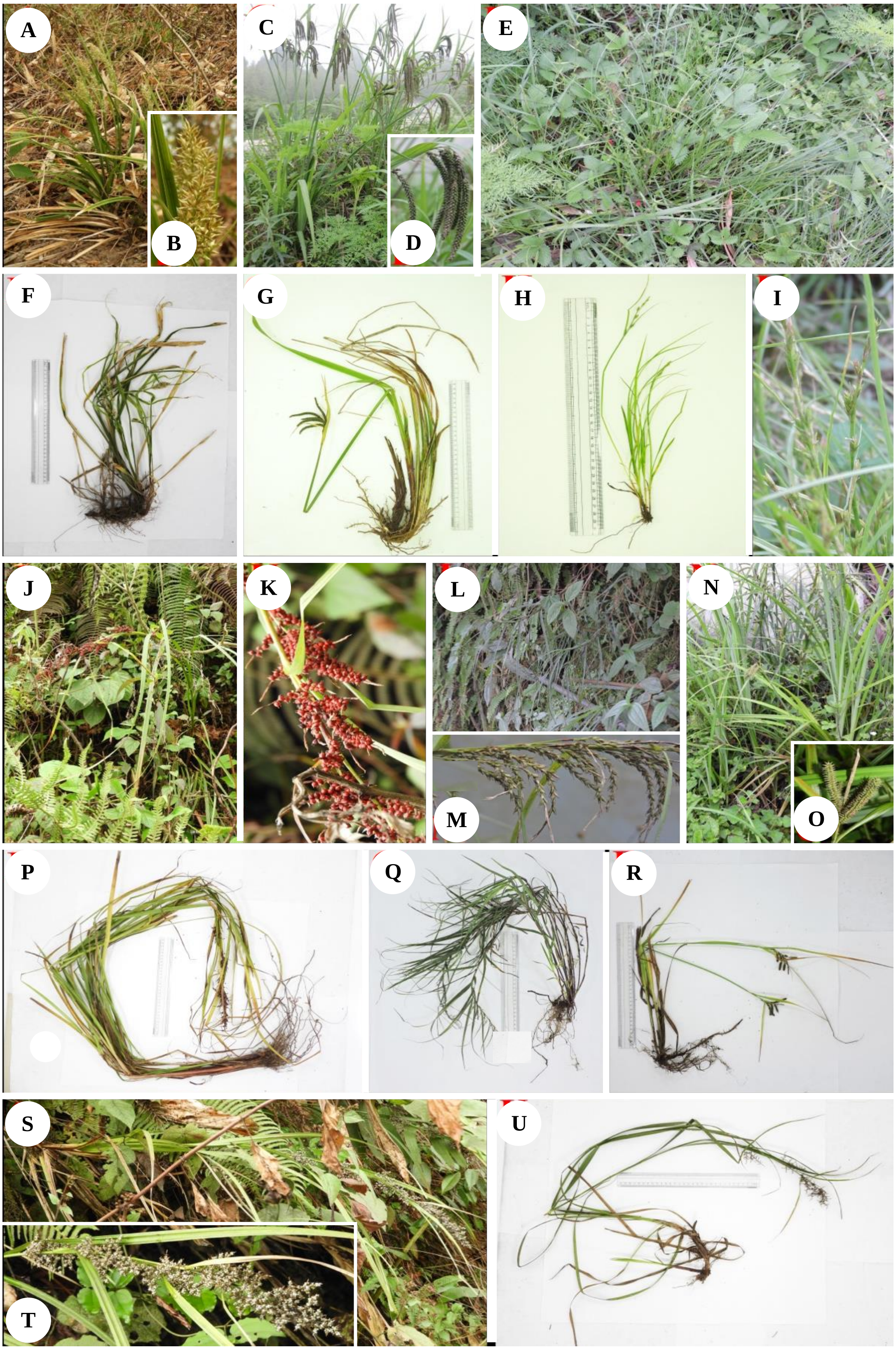

Figure 1. Habitat, inflorescence and habit of the studied seven species of *Carex* L. respectively. A, B, F: *C. stramentitia* Boott ex Boeckeler. C, D, G: *C. teres* Boott. E, I, H: *C. remota* L. J, K, P: *C. baccans* Nees. L, M, Q: *C. insignis* Boott. N, O, R: *C. alopecuroides* D. Don. S, T, U: *C. cruciata* Wahlenb.

Table 1. Collection detail, systematic position and accession number of the voucher specimen of the studied seven species of *Carex* L.

Subgenus	Section	Species	Location	Date of collection	Voucher number
Carex subgenus *Vigneastra*	*Carex* sect. *Polystachyae*	*Carex baccans* Nees	Lebong, Darjeeling 27º03’07” N, 88º16’05” E	27.10.2019	BURD 12161
	Carex sect. *Indicae*	*Carex cruciata* Wahlenb.	Lebong, Darjeeling 27º03’10” N, 88º16’07” E	31.03.2019	BURD 12156
		Carex stramentitia Boott ex Boeckeler	Darjeeling 26º56’14’’ N, 88º20’28’’ E	29.03.2019	BURD 12155
Carex subgenus *Carex*	*Carex* sect. *Decorae*	*Carex insignis* Boott	Lebong, Darjeeling 27º02’35” N, 88º16’01” E	27.10.2019	BURD 12160
	Carex sect. *Molliculae*	*Carex alopecuroides* D. Don	Lopchu, Darjeeling 27º03’31” N, 88º22’26” E	29.05.2019	BURD 12157
	Carex sect. *Forficulae*	*Carex teres* Boott	Tiger Hill, Darjeeling 26º59’44” N, 88º16’44” E	01.06.2019	BURD 12159
Carex subgenus *Vignea*	*Carex* sect. *Remotae*	*Carex remota* L.	Tiger Hill, Darjeeling 26º59’42” N, 88º16’49” E	01.06.2019	BURD 12158

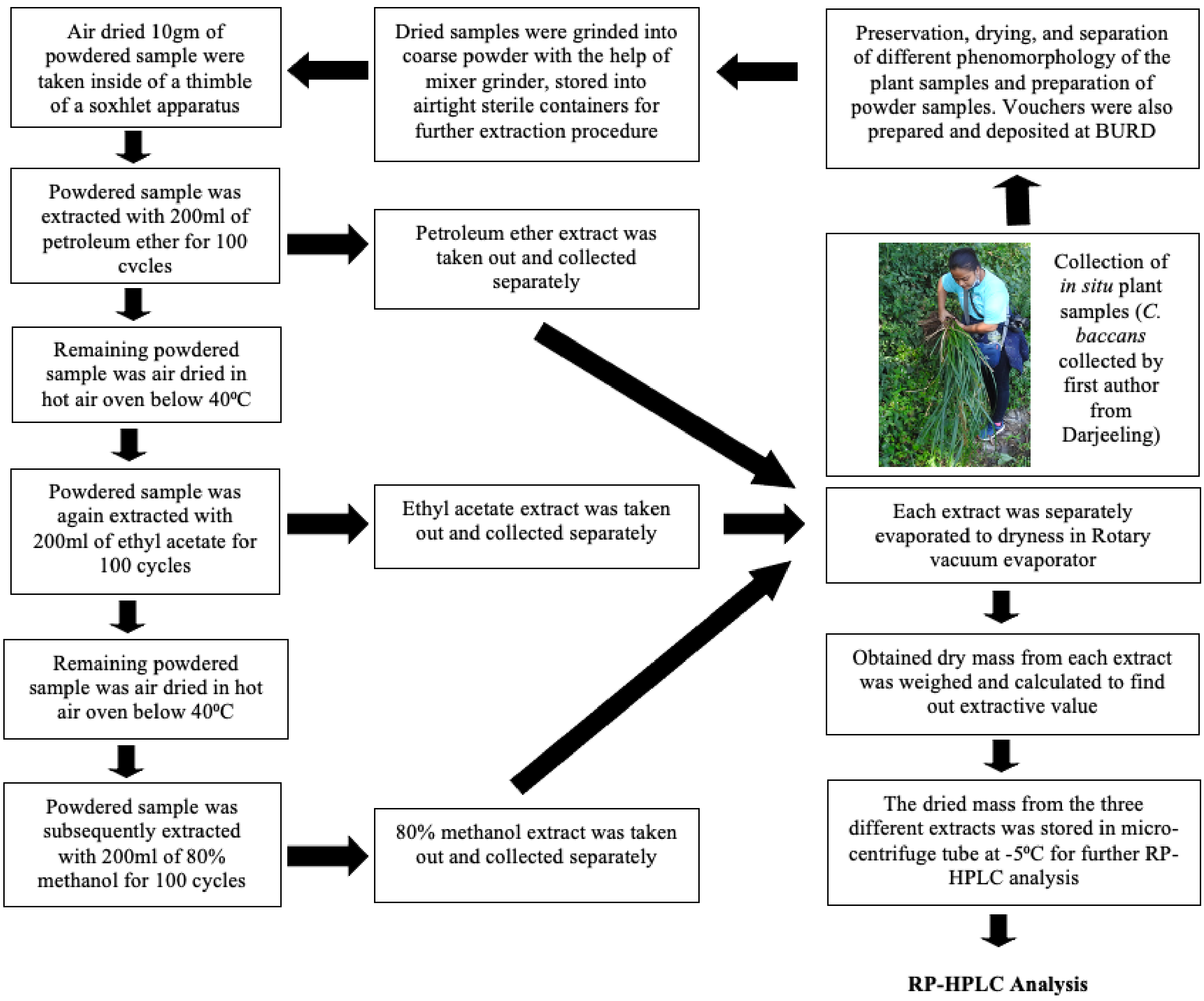

Figure 2. The extraction process of plant samples

Chemicals and reagents

Petroleum ether, ethyl acetate, methanol, acetonitrile, acetic acid used in the present study were from Merck, Germany. Nine standards viz. gallic acid, phloroglucinol, catechin, caffeic acid, coumaric acid, ferulic acid, rosmarinic acid, quercetin and biochanin A were used for the detection of phytochemicals procured from Sigma Aldrich.

Extraction of plant samples

Phytochemical extraction was done following Kumar et al. (2016) with some modifications. Details of the extraction process are presented in Figure 2.

Extract yield

Here, extractive capacity signifies how much dry extract is yielded from 10 g powdered sample. Three different solvents of different polarity viz. petroleum ether, ethyl acetate, 80% methanol were used as extracting solvents. The process of extraction solubilizes the polyphenolic compounds of the plant sample studied and thus it is an essential part of polyphenol extraction and estimation. The obtained dry mass was weighed and calculated to find out the Extractive value (% extractive yield) with the help of the following formula. Results are shown in Table 2.

$$\text{Extractive value} = \frac{\text{Amount of crude extract from 10gms of dried plant material} \times 100}{10}$$

RP-HPLC analysis of phenolic acids and flavonoids

Sample preparation

For RP-HPLC analysis, 4 mg/mL concentration of 80% methanolic extracts of all the studied species was prepared and filtered through Puradisc Nylon syringe filter of 0.2 µm pore size (Brand Agilent) and 10 µL sample was loaded into HPLC column C18.

Standard preparation

Standard stock solution of gallic acid (G), phloroglucinol (P), catechin (C), caffeic acid (CA), coumaric acid (CM), ferulic acid (FR), rosmarinic acid (RS), quercetin (Q) and biochanin A (B) were prepared at 1 mg/ml concentration in HPLC grade methanol and 10 µL of the sample was loaded into HPLC column C18 separately. The mixture of nine standards run into HPLC column C18 for 65 minutes and the HPLC chromatograms were detected at six different wavelengths (254 nm, 270 nm, 280 nm, 310 nm, 320 nm and 350 nm) using a photodiode array UV detector.

Chromatographic condition

Shimadzu CTO-10AS, DGU-20A, SIL-20A, LC-20AT, RF-10Axl, SPD-M20A, RID-20A, CMB-20A, FRC-10A with gradient flow was used for HPLC analysis. The mobile phase contains solvent A (0.1% acetic acid water) and solvent B (0.1% acetic acid acetonitrile). The column was thermostatically controlled at 24.4ºC oven temperature. Subsequently, gradient elution was performed by changing the proportion of solvent A to solvent B. The HPLC chromatograms were detected at six different wavelengths (254 nm, 270 nm, 280 nm, 310 nm, 320 nm and 350 nm) using a photodiode array UV detector [followed Seal (2016) with some modifications].

Qualitative estimation of phenolic acids and flavonoids

Each polyphenolic compound was identified by considering its Retention time (RT) and by spiking with standards under the same condition. The quantification of polyphenols was carried out by the measurement of the integrated peak area. Initially, the qualitative results were taken for the presence or absence of specific group of polyphenols for 65 minutes program.

Quantitative estimation of phenolic acids and flavonoids

The amount of individual polyphenols (five phenolic acids and four flavonoids) in 80% methanol extracts of all the seven species of *Carex* was calculated using the following formula by considering the peak area of the respective compound present in the sample chromatogram with the peak area of respective standard along with the concentration of sample extract and standard along with standard purity. The quantity of the phytochemicals was expressed in mg/g dry weight (followed Mohan et al. 2017).

$$\frac{\text{Peak area of sample}}{\text{Peak area of standard}} \times \frac{\text{Concentration of standard}}{\text{Concentration of sample}} \times \text{Purity of standard}$$

RESULTS AND DISCUSSION

Extractive capacity

Table 2 shows the percentage yield of the three different solvent extracts of studied seven species of *Carex* in successive soxhlet extraction method in increasing polarity order. The extractive yield of all 21 extracts of these seven species of *Carex* varied from 0.25% to 10.7%. For all of the studied species, 80% methanol was proved to be a potent solvent for the extraction of phytochemicals as it showed the highest extractive value. For *Carex stramentitia*, *C. cruciata*, *C. alopecuroides*, *C. remota*, *C. teres*, *C. insignis* and *C. baccans* extractive values varied from 0.4% to 4.78%, 0.78% to 1.54%, 0.3% to 4.46%, 0.56% to 2.64, 0.42% to 3.87%, 0.34% to 4.59% and 0.52% to 10.7% respectively.

Analytical RP-HPLC study

The HPLC chromatograms of 80% methanol extract of *Carex teres* in 4mg/ml concentration (Figure 3) and the mixture of nine standards in 1mg/ml are presented in figure (Figure 4).

Qualitative analysis

By comparing retention times of all the chromatograms of 80% methanol extracts with the chromatogram of standards, it can be said that successive soxhlet extraction of *Carex stramentitia* showed the presence of only gallic acid, catechin, rosmarinic acid and quercetin; whereas *C.*

cruciata showed the presence of only caffeic acid and rosmarinic acid. Similarly, *C. alopecuroides* showed the presence of catechin, caffeic acid, ferulic acid and biochanin A. *C. remota* showed the presence of only ferulic acid and rosmarinic acid. *C. teres* showed the presence of gallic acid, phloroglucinol and quercetin. *C. insignis* showed the presence of only phloroglucinol and quercetin. *C. baccans* showed the presence of phloroglucinol, caffeic acid and ferulic acid (Table 3).

Quantitative analysis

The amount of phenolic acids and flavonoids were calculated using the abovementioned formula (given in materials and methods) and presented in Table 4. The amount of gallic acid varied from 0.036-0.481 mg/g dry weight, whereas the amount of phloroglucinol varied from 0.406-0.583 mg/g dry weight. Catechin was present in a range of 0.111-0.375 mg/g dry weight; whereas caffeic acid was present in a range of 0.003-0.017 mg/g dry weight. Coumaric acid was absent in all the studied species. Ferulic acid was present in a range of 0.013-0.067 mg/g dry weight; whereas rosmarinic acid varied from 0.018-0.042 mg/g dry weight. Quercetin present in a range of 0.007-0.146 mg/g dry weight; whereas biochanin A present in 0.303 mg/g dry weight.

Table 2. Extractive capacities (% extractive yield) of studied seven species of *Carex* L.

Species name	Extractive value (% extractive yield) of different solvent extracts in successive soxhlet extraction method		
	Petroleum ether extract	Ethyl acetate extract	80% Methanol extract
Carex stramentitia	0.4	0.55	4.78
Carex cruciata	1.48	0.78	1.54
Carex alopecuroides	0.3	0.36	4.46
Carex remota	0.58	0.56	2.64
Carex teres	0.47	0.42	3.87
Carex insignis	0.35	0.34	4.59
Carex baccans	0.52	0.8	10.7

Table 3. Qualitative analysis of 5 phenolic acids and 4 flavonoids in 80% methanol extracts of all seven species of *Carex* L.

Phytochemicals	*Carex stramentitia*	*Carex cruciata*	*Carex alopecuroides*	*Carex remota*	*Carex teres*	*Carex insignis*	*Carex baccans*
Gallic acid (GA)	+	ND	ND	ND	+	ND	ND
Phloroglucinol (PHL)	ND	ND	ND	ND	+	+	+
Catechin (CT)	+	ND	+	ND	ND	ND	ND
Caffeic acid (CA)	ND	+	+	ND	ND	ND	+
Coumaric acid (CM)	ND	ND	ND	ND	ND	ND	ND
Ferulic acid (FR)	ND	ND	+	+	ND	ND	+
Rosmarinic acid (RS)	+	+	ND	+	ND	ND	ND
Quercetin (QT)	+	ND	ND	ND	+	+	ND
Biochanin A (BA)	ND	ND	+	ND	ND	ND	ND

Note: ND: Not detected

Table 4. Quantification of 5 phenolic acids and 4 flavonoids in 80% methanol extracts (unit: mg/g dry weight) of all seven species of *Carex* L.

Phytochemicals	*Carex stramentitia*	*Carex cruciata*	*Carex alopecuroides*	*Carex remota*	*Carex teres*	*Carex insignis*	*Carex baccans*
Gallic acid (GA)	0.481±0.346	0	0	0	0.036±0.027	0	0
Phloroglucinol (PHL)	0	0	0	0	0.406±0.230	0.583±0.441	0.491±0.278
Catechin (CT)	0.111±0.046	0	0.375±0.449	0	0	0	0
Caffeic acid (CA)	0	0.017±0.013	0.009±0.006	0	0	0	0.003±0.003
Coumaric acid (CM)	0	0	0	0	0	0	0
Ferulic acid (FR)	0	0	0.067±0.004	0.042±0.006	0	0	0.013±0.005
Rosmarinic acid (RS)	0.018±0.005	0.042±0.033	0	0.032±0.007	0	0	0
Quercetin (QT)	0.146±0.023	0	0	0	0.007±0.001	0.05±0.01	0
Biochanin A (BA)	0	0	0.303±0.145	0	0	0	0

Note: Value: Average of three readings±SD

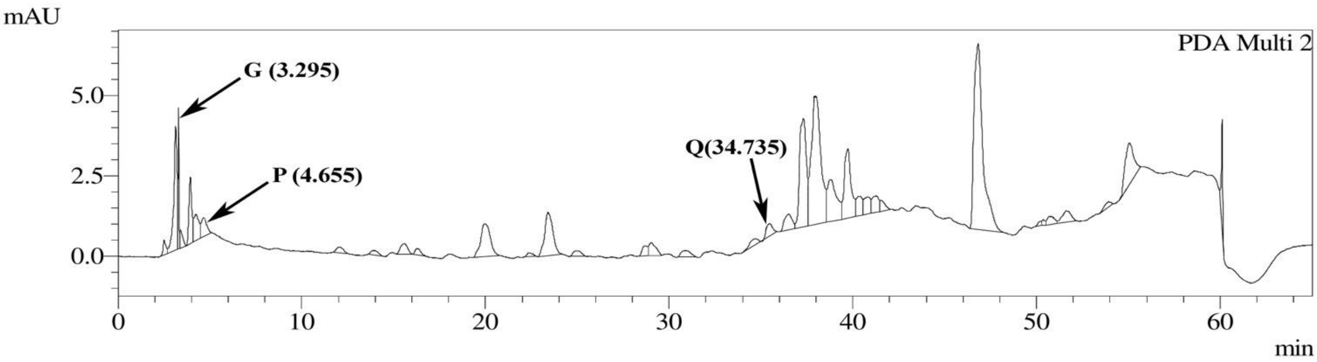

Figure 3. HPLC chromatogram of 80% methanol extract (successive soxhlet extraction) of *Carex teres* at 270nm. G: gallic acid, P: phloroglucinol, Q: quercetin

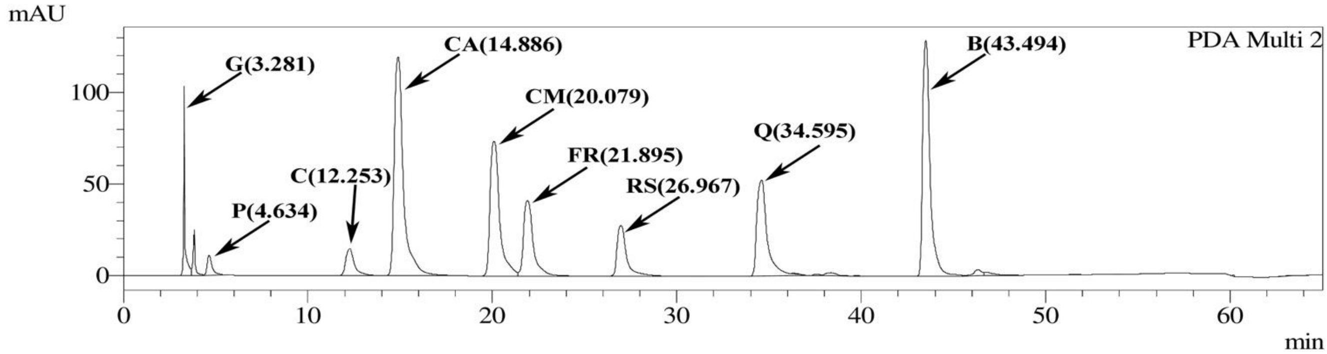

Figure 4. HPLC chromatogram of mixture of nine standards (gallic acid, phloroglucinol, catechin, caffeic acid, coumaric acid, ferulic acid, rosmarinic acid, quercetin and biochanin A) at 270nm. G: gallic acid, P: phloroglucinol, C: catechin, CA: caffeic acid, CM: coumaric acid, FR: ferulic acid, RS: rosmarinic acid, Q: quercetin, B: biochanin A

Discussion

Traditionally, the members of the family Cyperaceae are being treated as non-potent, thus remain underutilized except in very few specified cases like *Cyperus rotundus*, *C. exaltatus*, C. *compressus* (Banerjee et al. 2012; Peerzada et al. 2015; Kumar 2016; Datta et al. 2018; Kamala et al. 2018; Chung and Kang 2019; Bezerra et al. 2022; Hosseini et al. 2022). Phytochemical screenings of the different taxa of the genus *Carex* L., Cyperaceae have been done only by very few authors (Manhart 1986; Giri et al. 2015; Noori et al. 2015; Arraki et al. 2017; Dávid 2021). Recently Giri et al. (2015) have reported that *Carex baccans* is one of the most potent sources of some very important bioactive compounds and the root tuber of the species produces trans-resveratrol. Though, most of the Indian species under the genus *Carex* are still not evaluated for bioactive components as well as their therapeutic potentiality and thus remain neglected. The genus *Carex* has several infra-generic taxa like sub-genera and sections, mostly identified based on morphological characters and genetic characters (Hendrichs et al. 2004) and very rarely on the basis of phytochemicals. Thus, chemotaxonomic aspects are rare in Cyperaceae (Harborne 1971; Bogucka-Kocka et al. 2011). The therapeutic evaluation of the genus is also very rare (González-Sarrias et al. 2011). Even, those species which are native and confined to the studied phytogeographical region like *Carex insignis, C. stramentitia, C. teres, C. polycephala* Boott, *C. burttii* Noltie, *C. daltoni* Boott etc. have not yet even been screened for their phytochemical constituents including polyphenolics, which could be a good source of the bioactive components and also be important species in therapeutics.

Plants having different types of polyphenolic compounds in the form of simple phenolics, flavonoids, phenolic acids, tannins, etc. have been used as potential therapeutics due to the presence of antioxidative, anticancerous, anti-inflammatory, free radical scavenging properties and others (Gil et al. 2000). These properties of polyphenols can play an important role in human health by preventing certain chronic diseases such as insulin sensitivity, obesity, type 2 diabetes, neurodegenerative diseases including Alzheimer’s disease (Del Rio et al. 2012). Polyphenols can act as anti-carcinogenic by arresting cellular growth via cell apoptosis. Among them, phenolic acids and flavonoids showed a protective mechanism against biotic and abiotic stresses in plants. Also, many dietary polyphenols can be found in fruits, vegetables, grains, spices and herbs. Consumption of these foods can lead to a lowered risk of most common degenerative and chronic diseases mostly caused by oxidative stress (Zhang et al. 2016). This interaction between polyphenolic compounds and gut microbes may improve insulin sensitivity (Bagarolli et al. 2017). There is substantial evidence that specific polyphenols can contribute to health benefits by showing atheroprotective and hepatoprotective effects (Zanotti et al. 2014; Porras et al. 2017; Cory et al. 2018; Santhakumar et al. 2018). Being therapeutically important, these groups of polyphenols viz. gallic acid, phloroglucinol, catechin, caffeic acid, coumaric acid, ferulic acid, rosmarinic acid, quercetin and biochanin A are considered as bioactive compounds (Durazzo et al. 2019; Câmara et al. 2021).

Results of the present investigation suggest that different species of *Carex* contain a considerable amount of different polyphenolic compounds viz. gallic acid, phloroglucinol, catechin, caffeic acid, ferulic acid, rosmarinic acid, quecetin, biochanin A, except coumaric acid. These species may be a good source of free radicals (Kilani-Jaziri 2011). The results of the present study showed similar findings as analyzed by Bogucka-Kocka et al. (2011) that the content of the studied polyphenolic compounds varied in the investigated species (Table 4). This is probably due to the non-specificity of these polyphenolic compounds among the species of *Carex*, Cyperaceae. Gallic acid was proven to protect kidney dysfunction (Kotb et al. 2021), human stem cells (Shan et al. 2021), can also prevent breast cancer by inducing apoptosis in breast cancer cells (Aborehab et al. 2021; Ashrafizadeh et al. 2021). In the present study, this phenolic acid was present in *Carex stramentitia* and *C. teres*, while absent in other species confirming that these species could be a good source of the component. Phloroglucinol, an important polyphenolic compound, showed an inhibitory effect on oxidative stress, inflammation (Hong et al. 2021; Marinho et al. 2021) and breast cancer (Pádua et al. 2015). This compound also revealed various therapeutic properties such as anti-bacterial, anti-tubercular, anti-fungal activities *etc*. (Wei et al. 2021; Iqbal et al. 2022; Ivanov et al. 2022). Phloroglucinol also showed therapeutic effects in cellular and animal models of Parkinson’s disease (Ryu et al. 2013), anti-cancerous effect on colon cancer (Lopes-Costa et al. 2017) and anti-diabetic effect (Yoon et al. 2017).

In the present study, phloroglucinol was present only in *C. teres*, *C. insignis* and *C. baccans.* In addition, Kumar et al. (2013) reported that the aerial part of *C. baccans* is a very good source of bioactive compounds like α-viniferin, smiglasid A and B. Very recently, the species was also evaluated by Giri et al. (2015) for its bioactive components and underground part (root tuber) of *C. baccans* was found to be a very good source of bioactive trans-resveratrol. Studies prove that one of the gigantic, easily available Darjeeling Himalayan species under the genus *Carex*, *C. baccans* could be a very good species for future commercial purposes. Catechin was proven to improve several medical conditions like obesity (Suzuki et al. 2016), type-2 diabetes (Alipour et al. 2018). It was also mentioned in many previous studies that catechin has antioxidant properties, which can protect several diseases like cancer, cardiovascular and neurodegenerative diseases, associated with reactive oxygen species (Bae et al. 2020; Nallasamy et al. 2021). This medicinally important polyphenolic compound can also act as an anti-infective agent (Shimamura et al. 2007). Among the studied species, catechin was found to present in *Carex stramentitia* and *C. alopecuroides,* while absent in other species in our present investigation suggesting the bioactive potentiality of these native species of *Carex*. Similarly, caffeic acid, one of the potent polyphenolic acids also has many therapeutic potentialities such as antimetastatic, antitumor (Klein et al. 2021; Sari et al. 2022), anti-hyperglycemic (Oršolić et al. 2021), antimutagenic effect and radical scavenging property (Bogacz et al. 2021; Temviriyanukul et al. 2021). Several lines of evidence showed that this phenolic acid has some physiological effects like antioxidative property (Genaro-Mattos et al. 2015; Bajomo et al. 2022), antiviral activity (Sinha et al. 2022). Apart from all these, caffeic acid also showed some other pharmacological activities related to clinical biology such as anti-inflammatory, antibacterial, anti-atherosclerotic, immuno-stimulatory, cardioprotective, antiproliferative, hepatoprotective and anti-hepatocellular carcinoma activities (Espíndola et al. 2019).

In addition, Bogucka-Kocka et al. (2011) have reported caffeic acid in almost all the studied species of *Carex* except *C. remota*. The present study is also in accordance with the observation of Bogucka-Kocka et al. (2011) and reports the absence of caffeic acid in *C. remota*, but presence in *C. cruciata*, *C. alopecuroides* and *C. baccans*. Ferulic acid is one of the most important, naturally occurring phenolic acids in plants with innumerable health benefits against various clinical disorders namely cardiovascular and neurodegenerative disease, cancer, diabetes, hypertension, hypercholesterolemia (de Paiva et al. 2013; Mancuso et al. 2014; Panwar et al. 2018; Alam 2019; Zheng et al. 2019; Zhang et al. 2021). This phenolic acid can contribute efficiently to the treatment of one of the most common neurodegenerative disorders, Alzheimer’s disease (Sgarbossa et al. 2015). Ferulic acid also possesses strong antioxidative properties (Zduńska et al. 2018). Bogucka-Kocka et al. (2011) have evaluated 18 species of *Carex* from the Central European Lowland region and the presence of ferulic acid was confirmed in the studied species of *Carex* including *C. remota,* except *C. elata*. In accordance with this, present screening on *Carex* spp. from Darjeeling Himalayas also revealed the presence of this phenolic acid in *C. alopecuroides*, *C. remota* and *C. baccans*. Rosmarinic acid showed a very important therapeutic application in the prevention of neurodegeneration (Fachel et al. 2019). Some previous studies revealed its nephroprotective activity by preventing apoptosis in the kidney (Domitrović et al. 2014). Several evidences showed innumerable bioactivity of this phenolic acid like photoprotective, antioxidant, anti-inflammatory, cardioprotective, anti-depressive, anti-hemorrhage, antiviral, etc. Rosmarinic acid also plays an important role in the treatment of several diseases such as diabetic neuropathy, colon cancer, lung cancer, diabetic cerebral injury, acute lung injury, skin cancer, tumor metastasis, rheumatoid arthritis (Al-Dhabi et al. 2014;; Hossan et al. 2014; Kim et al. 2015; Adomako-Bonsu et al. 2017; Nadeem et al. 2019; Anwar et al. 2020; Luo et al. 2020; Li et al. 2021; Zhou et al. 2021). This medicinally important phenolic acid was detected in considerable amount in *C. stramentitia*, *C. cruciata* and *C. remota* in the present study. Quercetin, another important flavonoid is proven to have many therapeutic properties such as anti-cancerous, anti-aging, antiviral, anti-inflammatory, cardioprotective activities. It is a potential polyphenolic compound that can act against cancer proliferation, chronic inflammation and also negatively regulates various crucial signaling pathways associated with life-threatening diseases (Sharma et al. 2018; Batiha et al. 2020; Yin et al. 2021). This polyphenol is also known as phytoestrogen and it can improve rheumatoid arthritis (Kim et al. 2018; Yuan et al. 2020; Chakraborty et al. 2021) and viral pneumonia (Schettig et al. 2020; Brito et al. 2021). It is proven to be used in the treatment of ophthalmic diseases (Zhao et al. 2021).

Combined administration of quercetin along with vitamin C can show antiviral activity by blocking several steps into the virus such as entry of the virus, its replication, protein assembly etc. for both the prophylaxis and the early treatment of respiratory tract infections, especially in COVID-19 patients (Colunga Biancatelli et al. 2020). A recent study revealed that this polyphenolic compound may have the potential to treat the symptoms of COVID-19, which is an emerging disease in the present world (Bastaminejad et al. 2021). It can work in the early stage of COVID-19 as it has anti-coagulant, anti-inflammatory and antioxidant properties (Di Pierro et al. 2021; Manjunath et al. 2021). Quercetin also showed its chemotherapeutic efficacy in the treatment of prostate cancer (Lu et al. 2020; Ghafouri-Fard et al. 2021; Hussain et al. 2021). Few authors have previously reported quercetin in different species of *Carex*. Harborne (1971) had found Quercetin in six species of *Carex* from the British Isles. Manhart (1986) had also reported this flavonoid in the section *Laxiflorae* of *Carex*. Recently, Noori et al. (2015) have also reported quercetin in *C. stenophylla* Wahlenb., one of the temperate species from Iran. Li et al. (2009) had a similar observation on the occurrence of quercetin in the seeds of *C. folliculate* L. from Pennsylvania. But none of the species of *Carex* from Darjeeling Himalayas, India were included in the earlier studies. Even the species reported to have quercetin are not even common in this phytogeographical region. The present study for the first time reports the presence of quercetin in three studied species of *Carex* from Darjeeling Himalayas, namely *C. stramentitia, C. teres* and *C. insignis.* Though, this flavonoid was also previously reported in other genera of the tribe *Scirpae* viz. *Eriophorum latifolium* Hoppe and *Fuirena pubescens* (Poir.) Kunth. by Harborne (1971) and in *Cyperus compressus* by Dutta et al. (2018).

Biochanin A is one the most renowned bioactive polyphenolic compound, also known as phytoestrogen, exhibits several therapeutic properties like antioxidative, neuroprotective, anti-inflammatory, anticancerous, anti-hyperglycemic, osteogenic (Raheja et al. 2018; Chakraborty et al. 2021), antibacterial (Hanski et al. 2014). This polyphenol is proved to be protective against neurotoxicity (Wu et al. 2021) and also aids in oxidative stress reduction (Rathinasamy 2021; Singh et al. 2021). It also improves hepatic steatosis and insulin resistance (Park et al. 2016) and rheumatoid arthritis (Chakraborty et al. 2021). In the present study, this phytoestrogen was detected only in *C. alopecuroides* with the amount of 0.409 mg/g dry weight. Among the nine polyphenolic compounds, only coumaric acid was found to be absent in all the studied species.

The quantity of these therapeutically important polyphenolic compounds varied among the studied species of *Carex* (Table 4). The hydro-methanolic (4:1) extract of the seeds of *Saraca asoca* (Roxb.) De Wilde showed 0.09 mg/g dry weight (Dhanani et al. 2016), while in the present study the highest concentration of gallic acid was found in 80% methanolic extract of whole plant body of *Carex stramentitia* i.e. 0.481 mg/g dry weight. *C. insignis* showed the highest amount of phloroglucinol i.e. 0.583 mg/g dry weight. These two species of *Carex*, confined to Darjeeling Himalaya, can be a potent source of these polyphenolic compounds based on their notable concentration. Catechin was known to be an important tea flavonoid generally found in many plants. The amount of this flavonoid ranges from 0.028 to 0.616 mg/g dry weight in different parts of *Fagopyrum esculentum* Moench (Kalinova et al. 2009).

In the present study, catechin was present in the highest amount i.e. 0.375 mg/g dry weight in *C. alopecuroides*, which may suggest that different parts of this species can reveal further variation in concentration of this specific flavonoid. Caffeic acid was detected in aerial parts of some central European members of *Carex* at an average amount i.e. 0.13 mg/g dry weight (Bogucka-Kocka et al. 2011); whereas, the amount of caffeic acid was highest in whole plant body extract of *C. cruciata* i.e. 0.017 mg/g dry weight. This type of variation in the amount of phenolic acid may be due to variation in geographical regions or selection of different sub-generic members or following different extraction methods. In the present investigation, ferulic acid was present in the highest concentration in *C. alopecuroides* i.e. 0.067 mg/g dry weight; whereas, rosmarinic acid was present in a maximum concentration in *C. cruciata* with 0.042 mg/g dry weight. The presence of these phenolic acids (caffeic acid, ferulic acid, rosmarinic acid) in the studied members of Cyperaceae was also previously detected in Poaceae (Mika et al. 2005). This type of same phytochemical composition of these two families signifies the placement of these families in same order under Monocotyledons. The previous study revealed that quercetin isolated in the amount of 10mg/64g dry weight i.e. 0.156 mg/g from the methanolic extract of *Carex folliculata* seeds (Li et al. 2009), whereas, in the present study, the highest amount of quercetin i.e. 0.146 mg/g dry weight was estimated from 80% methanolic extract of whole plant body of *C. stramentitia.* The extracted amount of quercetin varied among the studies species. This is probably due to the whole plant being used for extraction in the present investigation and the previous authors extracted quercetin from mature seeds only. Thus, quercetin may be localized in occurrence. Biochanin A was earlier detected from the root of *Gynerium sagittatum*

(Aubl.) P. Beauv. (Poaceae) in an amount of 0.373 mg/g dry weight (Benavides et al. 2007). Whereas, this phytoestrogen was the less abundant component and was present only in *C. alopecuroides* with the amount of 0.303 mg/g dry weight which may be due to restricted occurrence. The similar abundance of this isoflavonoid in the members of both Poaceae and Cyperaceae may be due to their phytochemical composition similarity.

To conclude, in order to the isolation of the mentioned polyphenolic compounds, the present investigation revealed that *Carex* can be one of the most important genera in Cyperaceae as different species under the genus contain a significant amount of pharmacologically potent and bioactive polyphenolic compounds. Thus, it can be said that these species may be a good resource for bioactive compounds, can be used to treat various clinical disorders and these bioactive components can also be extracted from the species of *Carex* in a controlled manner which are prevalent in Darjeeling Himalayas, India. As well, these species could be a potent source of novel phytochemicals and bioactive components, particularly those species which are confined to this region (eg. *Carex insignis*, *C. burttii*, *C. daltoni*, *C. polycephala* etc.). Thus, extensive phytochemical screening of other species of *Carex* present in this region is needed. Besides the pharmacological approach, the obtained phytochemical data can also be used in the identification and separation of closely related Indian species of *Carex*. So these data can be useful in the future chemotaxonomic study which is one of the main objectives of the present investigation.

ACKNOWLEDGEMENTS

The authors thankfully acknowledge DHESTBT (W.B.D.S.T.), Government of West Bengal, India, for sanctioned Research Grant (Memo no. 284 (Sanc.)/ST/P/S&T/1G-61/2017 Dt. 28. 03. 2018). The authors are also grateful to Prof. Sujoy Kumar Das Gupta (C.I.F. In-Charge), Mr. Swaroop Biswas of C.I.F. Department of Bose Institute, Kolkata for giving the permission to analyze samples in RP-HPLC. The authors are also thankful to the Director, Botanical Survey of India and Scientist E, Head of the office, Central National Herbarium, Howrah, for permitting herbarium consultation. The authors express their thanks to the herbarium authorities of B, E, K, LE, LINN, NY, P, S and SBT for making the digital images of the original material available on the internet. The authors are also thankful to Prof. Soumen Bhattacharjee and Dr. Sujit Roy of the Department of Botany, the University of Burdwan for providing laboratory facility and other technical support. The present work is financially supported by DHESTBT (W.B.D.S.T.), Government of West Bengal, India, with the sanctioned grant (Memo no. 284 (Sanc.)/ST/P/S&T/1G-61/2017 Dt. 28. 03. 2018).

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