AQ2 Original Article

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Pharmacognostic and chromatographic evaluation of male and female flowers of *Coscinium fenestratum* for Berberine content and its effect on antioxidant activity

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ABSTRACT

Coscinium fenestratum (CF) (Gaertn.) Colebr., [Family: Menispermaceae] is an endangered species of dioecious liana found in Western Ghats of India. The present investigation aimed with systematic analytical studies on the macroscopic, microscopic examination of male and female CF flowers, various solvent extractions, accompanied by phytochemical screening through chemical tests, identification of Berberine alkaloids, antioxidant activity, and Berberine quantification by high-performance thin-layer chromatography (HPTLC) process. Two different amounts of solvents (10 ml and 20 ml) with two different time intervals, that is, 15 min and 25 min, respectively, were used for extraction. Results revealed that both flowers had their own unique characters. Chemical tests revealed the group of phytoconstituents present, especially Berberine alkaloids, in both the CF extracts. The yield and antioxidant activity for female flowers resulted higher in methanol extract than other extracts. Validated HPTLC method with good linearity of standard Berberine was established, and the estimated amount of Berberine resulted higher in methanol extract of the female flower (low detectable in male flower). Finally, it concluded that the methanol extract was better for extraction of Berberine from the female CF flower (10 ml volume with 15 min time period) which also showed higher antioxidant activity using the DPPH method.

Keywords: Antioxidant activity, berberine, *Coscinium fenestratum*, flowers, high-performance thin-layer chromatography, microscopy

INTRODUCTION

Western Ghats Mountains are the World's second largest sanctuary for rare and endangered species. Due to their lesser knowledge and rarity some plants are of greater significance, and others are due to their use in traditional medicine systems. Notwithstanding this, due to scarcity and over-exploitation, some plants are listed in the endangered plant species.[1] Therefore, a minimum information and empirical research is recorded about these species. One such species is that of *Coscinium fenestratum* (CF), belongs to the family Menispermaceae, and is a woody floral climber. It is usually referred to as tree turmeric or yellow vine. A lot of research literature has been clarified regarding CF leaf, stem, and seed related pharmacological activities. Some of the significant recent activities are among them, namely, antioxidant and antibacterial function of the CF leaf and stem extracts;[2,3] anticytotoxic activity of stem extract,[4] cytotoxic effect of crude water extract of CF stem on human HN31 cell line,[5] antioxidant and anthelmintic activity of CF Fruit Pulp,[6], antihypertension effect of CF stem crude extract,[7] Dual COX/LOX inhibitory activity of aqueous CF stem extract,[8] and identified new phytoconstituents found in stem and leaf of CF through HPLC-MS[9]. Not only that, tissue culture of the leaf through callus and suspension-grown on vermicompost extract media along with coelomic fluid,[10] stem callus culture and early development of stem rooting by the use of zinc sulfate in MS media were also studied.[11]

The plant is used in more than 62 Ayurvedic preparations, but no attempts have been made to generate analytical data regarding the active constituents present in the species due to its non-availability/rarity.[12] Very few or scanty research on flowers available since the flowering season is once a year from November to December[13] and it is very difficult to collect flowers, because the number of flowers is very limited. It has been stated that floral component evaluation would provide more details on its successful use.[14,15] No studies have yet been carried out on the microscopic evaluation of flowers and their behavior[16] and it is therefore worth learning about the pharmacognostic identification of male and female CF flowers by morphology and microscopy, which further will contribute to pollination, successful pollen collection, various therapeutic effectiveness, and even newer drug discovery. In this present research, microscopical examination, pharmacognostic screening, percentage yield and quantitative analysis on the male and female flowers are assessed with this purpose. High-performance thin-layer chromatography (HPTLC) is a valuable method for investing and evaluating active ingredients in the flower extracts that will assist both the creation of biomarkers and the discovery of drugs by future researchers to create scientific evidence for various traditional uses.

MATERIALS AND METHODS

Collection of Plant Materials

Flowers were collected from the Western Ghats region of Tamil Nadu (Coimbatore) in the month December (2nd week) by Dr. Gokul Sivaraman, Post Doctorate, CIMAP, Bangalore. The male and female flowers were identified and authenticated by Dr. P.E. Rajashekaran, Principal Scientist, Indian Institute of Horticultural Research, Bengaluru. The specimen flowers are kept for herbarium (Herbarium no: KCP/CF-Male flower-KD/2018-19; KCP/CF-Female flower-KD/2018-19) in the Department of Pharmacognosy, Krupanidhi College of Pharmacy, Bengaluru.

Morphology and Microscopic Evaluation

The morphological characteristics of CF male and female flowers such as color, odor, taste, and size have been investigated and the findings are shown in Figure 1. More dried male and female CF flowers are powdered, mounted on slide separately with glycerine by staining with phloroglucinol and HCl in 1:1 ratio and observed anatomical characters under the microscope. The results are shown in Figures 2 and 3.

Figure 1: *Coscinium fenestratum* male and female flower

Extraction of Flower Samples

The male and female CF flowers are dried for 7 days in shade and coarsely powdered by hand for the extraction process. 20 g of dried, powdered drugs are divided into 10 g each and then extracted separately by microwave extractor with petroleum-ether, chloroform, ethyl acetate, methanol, and water successively at two different volumes and two different time durations. The temperature was adjusted at 70°C with a power of 400 W throughout extraction. The volume of solvents is fixed in 10 ml and 15 min and for 20 ml and 25 min. After extraction, solvents are filtered with ash-free filter paper and vacuum evaporated to get the residue of extract. In each case, the yield is measured and calculated the percentage yield [Figure 4]. AQ5

HPTLC Estimation

All the samples are prepared at a concentration of 5 mg/ml using methanol and further TLC is performed using the mobile phase n-propanol:formic acid:water (90:1:9 v/v) for the detection of phytoconstituents (Berberine).[17,18] Various concentrations of standards are prepared for a standard calibration curve within the range of 80–400 ng/spot.

Chromatographic condition

Samples are applied to precoated silica gel 60 F_{254} TLC plates (20 × 10 cm) by Linomat V sample applicator. The sample volume is applied in 4.0 µl each as a 5 mm band length in 3 × 10 silica gel. After sample application, the plates are developed up to 80 mm in CAMAG twin trough chamber previously saturated with the solvent system, n-propanol:formic acid:water (90:1:9 v/v). Then, the plate is scanned at 254 nm and 366 nm followed by spectral analysis. Reprostar Chromatography Documentation Apparatus is used for photographs of the HPTLC plates. The software used is winCATS 1.3.4 version.

Method validation

The analytical approach has been validated according to the guidelines of the International Council for Harmonization (ICH, 2005) for linearity, precision, accuracy, specificity, the limit of detection (LOD), and limit of quantitation (LOQ). The linearity is done by applying various standard berberine hydrochloride concentrations. LOD and LOQ are determined on the basis of standard deviation (SD) and slope (S) of the calibration curve at levels approaching to the LOD according to formula {LOD = 3.3 (SD/S) and LOQ = 10 (SD/S)}. Precision studies include repeatability and system precision. Accuracy by recovery studies is achieved by spiking a known concentration of standard to pre-analyzed samples. The robustness is achieved by a small variation in optimized system parameters such as variation in mobile phase composition, and chamber saturation period.

Isolation of Berberine

Based on the identification of the chromatography, berberine was isolated from the CF flower extract by column chromatography method using silica gel (60/120 mesh size) as a stationary bed and based on the polarity of the solvent used, namely, petroleum ether, chloroform, diethyl ether, ethyl acetate, acetone, and methanol. Finally, methanol fraction was collected (yellow fluorescent fraction no. 60, volume 300 ml) and further

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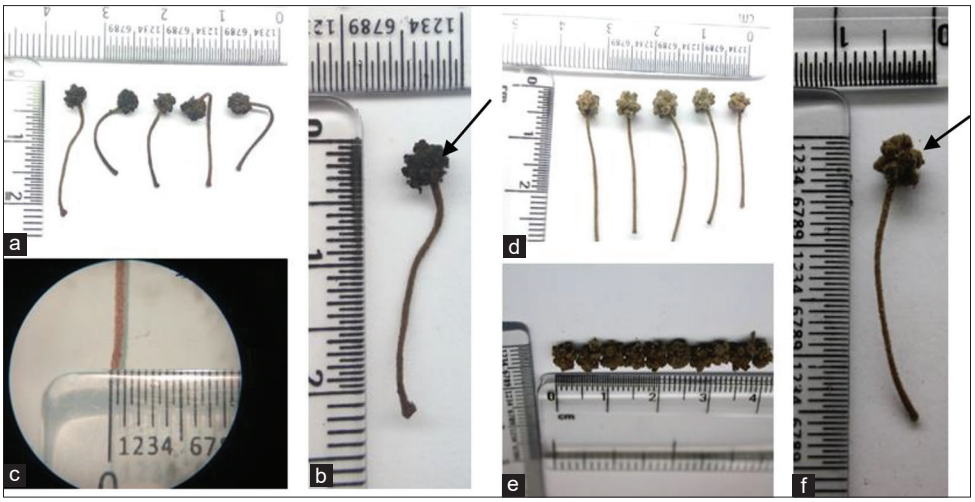

Figure 2: Photograph of morphological evaluation of *Coscinium fenestratum* (CF) male and female flowers, respectively. (a) Length of multiple flowers, (b) length of single male flower, and (c) width of male flower stalk of CF Male flower, and (d) length of multiple female flowers, (e) length of single female flower, and (f) length of multiple female flower heads of CF female flower

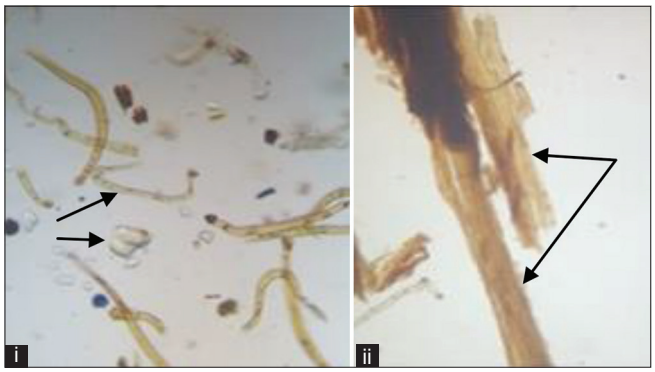

Figure 3a: Photograph of microscopic evaluation of *Coscinium fenestratum* (CF) female flowers (×10). (i) Trichomes with prismatic crystals, (ii) vascular bundles (All the figures are identified by arrows)

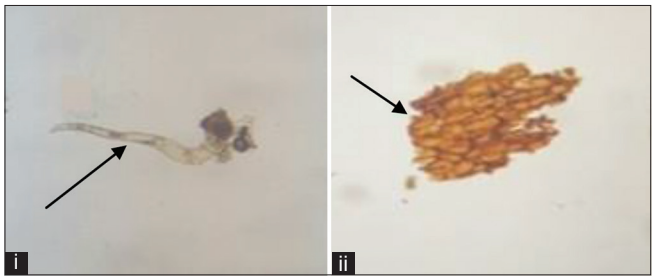

Figure 3b: Photograph microscopic evaluation of *Coscinium fenestratum* (CF) male flowers. (i) Trichomes, (ii) hexagonal shaped cork cells of CF male flower (All the figures are identified by arrows)

evaporated to dryness and procured yellow colored powder which was reconfirmed with TLC, HPTLC, and NMR study.

Antioxidant Activity

Ferric reducing antioxidant power (FRAP) assay

In 0.9 ml of extract, 2.7 ml of FRAP reagent and 270 µl of water are added and then the mixture is incubated at 37°C for 30 min. This is cooled at room temperature and then

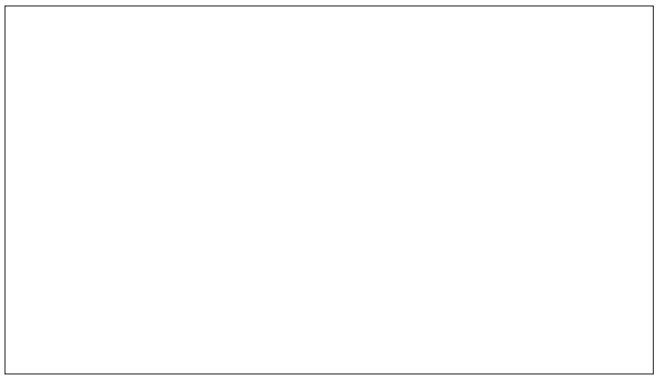

Figure 4: ???

absorbance is measured at 595 nm. Varying concentrations of the sample (25, 50, 100, 200, and 400 µg/ml) in triplicates are prepared. Reduction of a ferric tripyridyltriazine complex to ferrous-(2,4,6-tripyridyl-s-triazine)-2, that is, ferric (III) [colorless] to ferrous (II) [blue] is detected by measuring absorbance at 595 nm where Gallic acid is used as standard.[19] The sample concentration providing 0.5 of absorbance, that is, 50% of inhibition (IC_{50}) was calculated by plotting absorbance against the corresponding sample concentration.

DPPH radical scavenging assay

The sample extracts are prepared by dissolving it in methanol at different concentrations. 2.0 ml of varying concentrations of the sample (25, 50, 100, 200, and 400 µg/ml) in triplicates is prepared. 0.5 ml of DPPH reagent is added to all test tubes and mixed well. The test tubes are incubate in the dark for 30 min and then measured in a spectrophotometer at 517 nm.[20] 2.0 ml of methanol and 0.5 ml of DPPH reagent are used as a control. Methanol served as blank and gallic acid is used as standard. The IC_{50} value (µg/ml) of the sample, that is, the concentration of the sample required to inhibit 50% of the DPPH free radical, is calculated by linear regression. The percentage inhibition of DPPH radical by the antioxidants in the extracts is calculated by the following formula:

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% Activity: = $[(A_{control}–A_{sample}/A_{control}) \times 100]$

Statistical Analysis

Data are expressed as mean ± SEM from three replications. For antioxidant assays, a one-way ANOVA test followed by a post-Tukey's test ($P < 0.05$) and the differences among IC_{50} of various extracts for different antioxidant assays are applied. The IC_{50} values are determined using the GraphPad Prism 5 software. $P < 0.05$ is considered to be statistically significant.

Further 2 × 2 factorial designs with two center points are applied for the determination of significant percentage yield and antioxidant activity with respect to solvent volume and extraction time. Replicated experiments are conducted with varying the volume and time using JMP version 11, software. Using this design, the magnitude of the effect of each parameter on the resulted response of percentage yield and antioxidant activity is calculated. Two levels are used for tests, that is, the volume of solvent (10 ml as low and 20 ml as high) and extraction time (15 min as low and 25 min as high).

RESULTS

Morphology and Microscopic Characters

The observed morphological characters of the CF male and female flower are depicted in Table 1 and Figure 2. Thereafter, powder microscopy of both the flowers is carried out and revealed that the presence of plenty of unicellular and multicellular covering and glandular Trichomes, Prismatic crystals, Pigment cells (Golden and Violet colored cells), hexagonal shaped cells, and vascular bundles [Figure 3a] in CF female flower and further CF male flower characterized with few unicellular trichomes, Prismatic crystals, Pigment cells (Golden colored cells), and hexagonal shaped cells and pollen grains [Figure 3b], etc.

Extraction and Yield

Extraction of CF male and female flowers is performed separately using different solvents with the conditions above stated. Table 2 shows the percentage yield of the extracts, where methanol extract yielded higher (25.6%) with 10 ml of solvent microwave extracted for 15 min.

Phytochemical screening of flower extracts is carried out by chemical tests. Based on higher yield in particular volume and temperature, methanol and aqueous extracts of CF male and female flowers are selected. Various chemical tests resulted from female flower methanol and aqueous extracts showed the presence of different phytoconstituents such as alkaloids, glycosides, phytosterols, flavonoids, carbohydrates, and proteins while male flower extracts showed the presence of alkaloids, glycosides, flavonoids, and carbohydrates [Table 3].

HPTLC Study

Based on the chemical test, the presence of alkaloid, that is, berberine is calculated through the HPTLC process using standard berberine. The HPTLC approach is validated in terms of linearity, robustness, LOD, LOQ, and accuracy for both the extracts, but male flowers do not show proper results hence data represented in this manuscript for female flower [Table 4]. The R_f value of berberine for CF female flower is found to be 0.33 [Graph 1 and Figure 5] whereas berberine is not detected in a male flower prominently. The calibration

Table 1: Organoleptic characters and size of CF male and female flower

Macroscopic characters	CF male flower	CF female flower
Color	Reddish black	Grey
Odor	Characteristic	Characteristic
Taste	Bland	Bland
Size	1.92 cm in length and 3.9 mm in diameter	2.52 cm in length and 4.67 mm in diameter
Length of flower head	3.75 mm	4.4 mm
Thickness of flower stalk	0.5 mm	0.5 mm
Subglobular parts	12 Nos.	12 Nos.

CF: *Coscinium fenestratum*

Table 2: Yield of the extract in various solvents

Solvent used	Amount of powdered drug (g)	% yield (Female flower)	% yield (Male flower)
Pet ether			
10 ml for 25 min	10	4.2	3.6
20 ml for 15 min	10	4.6	3.4
Chloroform			
10 ml for 25 min	10	3.3	3.1
20 ml for 15 min	10	3.5	3.0
Ethyl acetate			
10 ml for 25 min	10	6.1	4.7
20 ml for 15 min	10	6.4	4.4
Methanol			
10 ml for 25 min	10	25.6	13.4
20 ml for 15 min	10	21.4	12.7
Water			
10 ml for 25 min	10	16.8	12.2
20 ml for 15 min	10	14.6	11.4

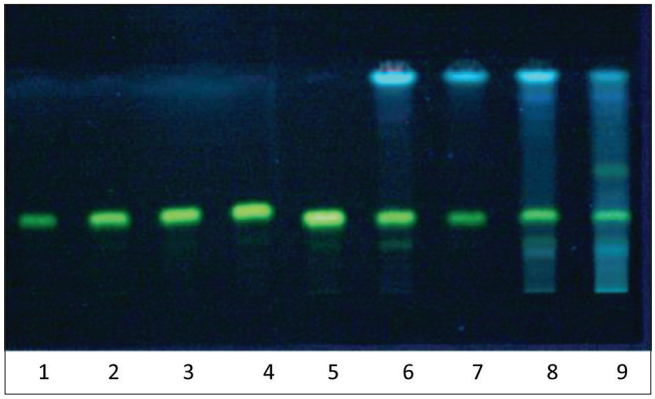

Figure 5: (For female flower) - Track 1–5: Standard berberine hydrochloride (5–25 µg/ml); track 6: Aqueous (10 ml) extract track 7: Aqueous (20 ml) extract; Track 8: Methanol (10 ml) extract; Track 9: Methanol (20 ml) extract

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Table 3: Phytochemical analysis through chemical tests

Plant constituents	Tests	CF male flower extract		CF female flower extract	
		Methanol extract	Aqueous extract	Methanol extract	Aqueous extract
Lipids	Sudan III reagent	--	--	--	--
Proteins	Biuret test	--	--	++	+
Carbohydrates	Benedict's test	+	+	++	+
Glycosides	Keller-Kiliani test	+	+	++	+
Alkaloids	Dragendroff's test	+	+	++	+
	Wagner's test	+	+	++	+
Flavonoids	$FeCl_3$ test	+	--	++	+
	Shinoda test	+	--	++	+
Tannins	$FeCl_3$ test	--	--	--	--
Steroids	Liebermann–Burchard test	--	--	++	--
Saponins	Foam test	--	--	--	--
Terpenoids	Salkowski test	--	--	--	--
Phenols	FC reagent test	+	+	++	+
Resins	Acetic anhydride+ H_2SO_4 test	--	--	--	--

++: Positive; (--): Negative; +: Less present

Table 4: LOD and LOQ of CF female flower

Berberine	Female flower		Male flower	
	Limit of detection (ng)	Limit of quantification (ng)	Limit of detection (ng)	Limit of quantification (ng)
With respect to height	379.17	1149.01	513.13	1420.11
With respect to area	32.97	99.93	58.10	134.02

LOD: Limit of detection, LOQ: Limit of quantitation, CF: *Coscinium fenestratum*

Table 5: Content of berberine in CF female and male flower extracts

Extract (volume)	Berberine content Female flower (mg/g)	Berberine content male flower (mg/g)
Methanol (10 ml)	0.019	0.005
Methanol (20 ml)	0.023	0.009
Aqueous (10 ml)	0.011	0.003
Aqueous (20 ml)	0.017	0.004

CF: *Coscinium fenestratum*

curve is linear in the range of 80–400 ng/spot for berberine (r = 0.99832 and 0.99041 with respect to peak height and area, respectively). The regression equation is found to be y = 0.03107x + 0.8195 and y = 0.8826X +79.42 with respect to height and area. The content of berberine in female flower extracts is given in Table 5. Quantification of berberine in male flower is very negligible for the mentioned concentration.

Specificity

The specificity of the method was determined by analyzing standard drug and sample. The presence of berberine in flowers was confirmed by comparing R_f and ultraviolet–visible spectra of the sample with the standard. The purity of the berberine sample spot in the sample and standard was analyzed by superimposing the spectrum of standard and sample peaks at 350 nm [Graph 2].

Accuracy

Accuracy of the method was studied by performing recovery studies at three levels of berberine. The pre-analyzed samples were spiked with 80%, 100%, and 120% of the standard berberine and analyzed by the proposed HPTLC method. The actual yield of berberine was calculated as 0.011 mg (with 80% spike), 0.019 mg (with 100% spike), and 0.023 mg (with 120% spike). The experiment was conducted 6 times the percentage recovery at three different levels of berberine was found to be 98.57 (% RSD = 0.87), 98.54 (% RSD = 0.81), and 98.58% (% RSD = 0.78), respectively.

Robustness

The robustness at 300 ng/b and was tested in triplicate by making a slight variation in optimized system parameters such as variation in the composition of the mobile phase, and chamber saturation period. The findings were analyzed in terms of the relative standard deviation (% RSD) and standard error of the peak area. Mobile phase prepared by solvent system n-propanol:formic acid:water with three different ratios such as 89.9: 0.5: 8.9 v/v, 90:1:9 v/v, and 90.5: 1.5: 9.5 v/v and the duration of saturation time change during chromatograph

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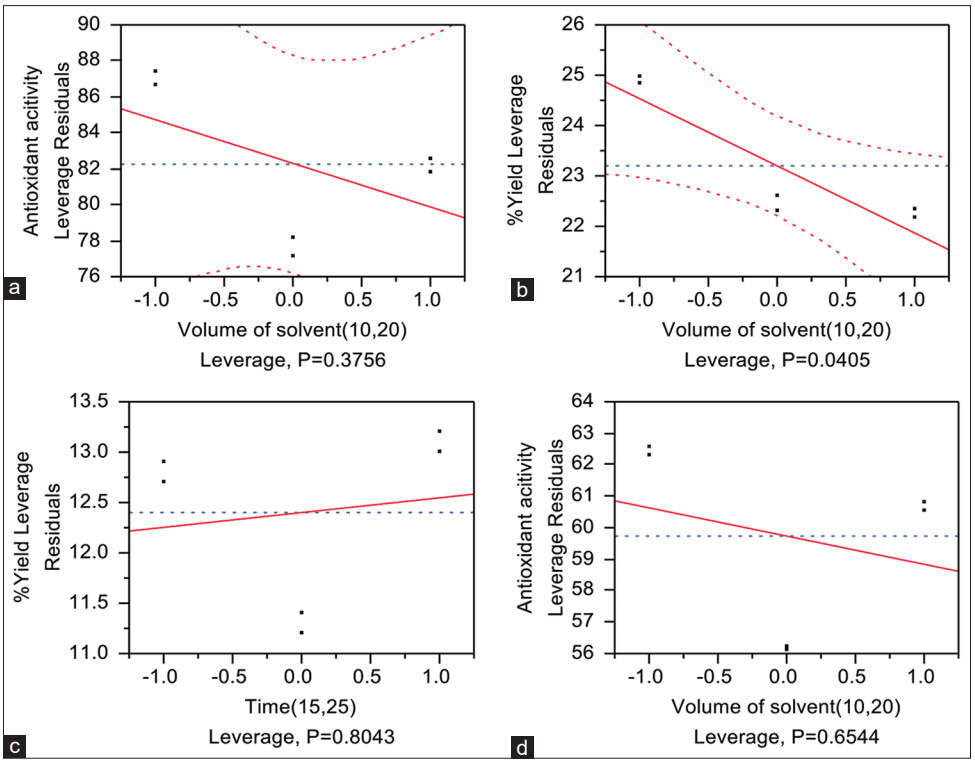
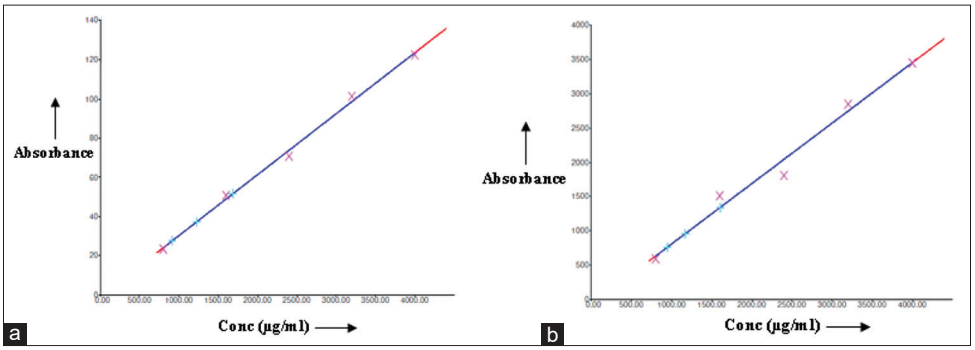
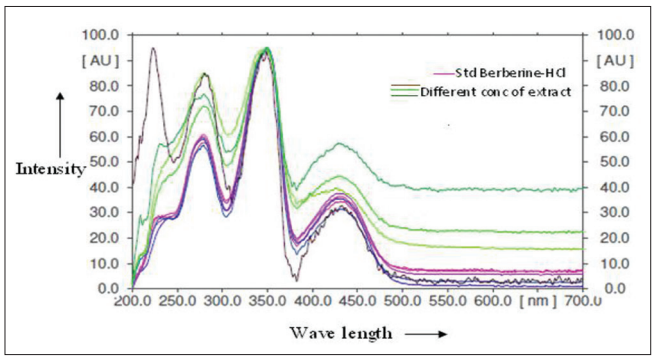


Figure 6: Leverage plot for percentage yield and antioxidant study of *Coscinium fenestratum* female (a and b) and male (c and d) methanolic (10 ml) extract



Graph 1: Calibration curve of berberine with respect to height and area samples of *Coscinium fenestratum* (CF) female flower. Graphs showing linearity [with respect to (a) height and (b) area] obtained by the CF female flower methanol extract with the standard berberine hydrochloride



Graph 2: UV spectrum of *Coscinium fenestratum* female flower extracts with standard berberine hydrochloride from 199 nm to 800 nm

development (18, 20 and 22 min), respectively. The plate was activated at 110°C for 20 min and analyzed at 350 nm. By introducing small changes into the TLC method, % RSD was obtained <2% proved the robustness of the proposed method.

Isolation of Berberine

Isolated berberine was yellow colored powder (yield of isolated berberine was 8.6%) which was reconfirmed with same HPTLC method and proton NMR study.

1H-NMR

Isolated berberine further reconfirmed with Proton NMR study and correlated with the standard berberine hydrochloride. The signals at 9.91, 8.96, 8.20, 8.02, 7.80, and 7.09 PPM represent the presence of H atom in the structure of berberine which was nearly similar to the H–atom present in standard berberine hydrochloride (9.90, 8.95, 8.21, 8.01, 7.80, and 7.09 PPM). Thereafter, three CH_2 groups are showing at the signal of 6.17,

4.94, and 3.35 PPM which were also correlated with the three CH_2 groups present in the standard berberine hydrochloride (6.17, 4.95, and 3.35, respectively). Furthermore, two CH_3 groups were also identified and confirmed at the signals of 4.09 and 4.07 PPM by coinciding with 4.09 and 4.07 PPM of standard drug (Figures not showed).

Antioxidant Activity

All the extracts of CF female and male flowers are assayed by DPPH and FRAP method. The female flower methanol extract (10 ml for 15 min) showed higher antioxidant activity using DPPH and FRAP assay methods, while the male flower extract (methanol) showed very less antioxidant activity using the DPPH method but did not display any results with FRAP assay methods. Further IC_{50} values are determined for the potentiality of antioxidant study. Gallic acid showed IC_{50} values of 19.12 µg/ml and 28.12 µg/ml by DPPH and FRAP methods, respectively, whereas the methanolic female flower extract showed IC_{50} value at a concentration of 26.01 µg/ml (10 ml) and 29.14 µg/ml (10 ml) by DPPH and FRAP methods respectively which are nearby standard gallic acid [Table 6] but no activity showed by the male flower extracts.

Correlation Study

Percentage yield is correlated with an antioxidant study carried out by the DPPH method. It is observed that a significant correlation among female flower extract (10 ml) with the antioxidant study with the same extract of DPPH method ($P < 0.05$) [Table 7].

Analysis of the design yielded the leverage plots and surface response plot for both the female and male flower extract. It is found that in both cases the volume of solvent showed a significant effect of yield and antioxidant activity [Figure 6]. The response profile showed the linear relationship between the volume of solvent with % yield and antioxidant activity for both the extract [Figure 7].

DISCUSSION

All the plant sections are of considerable medicinal value and have been demanding on the drug market. The flower is the most vulnerable part of the plant because it blooms once a year and also plants are dioecious in nature as well. With a slow growth rate, this species requires 15 years to reach its reproductive stage.[1] Therefore, the study of usage of flowers would, therefore, be of greater value of understanding the therapeutic potential and in maintaining the plant population. CF flowers and fruits are one such unusual plant materials as the plant is a dioecious climber and bares flowers once in a year in a particular season with specific weather conditions for a limited period of time. Hence, such an exotic, rare plant material is not explored in any of its medicinal benefits. Although there is awareness of the importance of this species to the human population, there is still a lack of research work on the conservation methods, pharmacological potentials, and therapeutic applications of the CF plant flowers.

Documentation for pharmacognostic descriptions of male and female flowers for clear identification and distinction between them is defined in this report. Therefore, morphological and microscopical characters are initially performed and histological characters are discovered which are rare and very unique. Such specifics are of great use to classify flowers.

Therefore, sophisticated microwave extraction is carried out with different solvents for two separate volumes (10 ml and 20 ml) with two constant periods (15 min and 25 min)

Table 6: IC_{50} values of methanolic and aqueous CF flower extracts

Extracts	IC_{50} value (µg/ml)			
	Female flower		Male flower	
	DPPH method	FRAP method	DPPH method	FRAP method
Methanol (10 ml)	26.01±0.02***	29.14±0.02***	---	---
Methanol (20 ml)	28.11±0.13***	33.30±0.11***	---	---
Aqueous (10 ml)	47.23±0.11***	52.11±0.03***	---	---
Aqueous (20 ml)	49.23±0.20***	54.04±0.02***	---	---
Isolated berberine	23.11±0.22***	24.06±0.10***		
Gallic acid	19.12±0.03	28.12±0.12	19.12±0.03	28.17±0.12

Mean±SEM (n=); One-way ANOVA study followed by Tukey's post-test was carried out at significant level when compared with standard as control, ***P<0.01=Significant; (---)=No activity. CF: *Coscinium fenestratum*, FRAP: Ferric reducing antioxidant power, DPPH: 2,2-Diphenyl-1-picryl-hydrazyl-hydrate

Table 7: Correlation study of methanolic extract of yield (for 10 ml) and antioxidant activity (by DPPH method)

	Male flower yield	Male flower antioxidant study	Female flower yield	Female flower antioxidant study
Male flower yield	1			
Male flower antioxidant study	0.158	1		
Female flower yield	−0.109	0.923	1	
Female flower antioxidant study	−0.237	0.828	0.980*	1

*P<0.05=Significant

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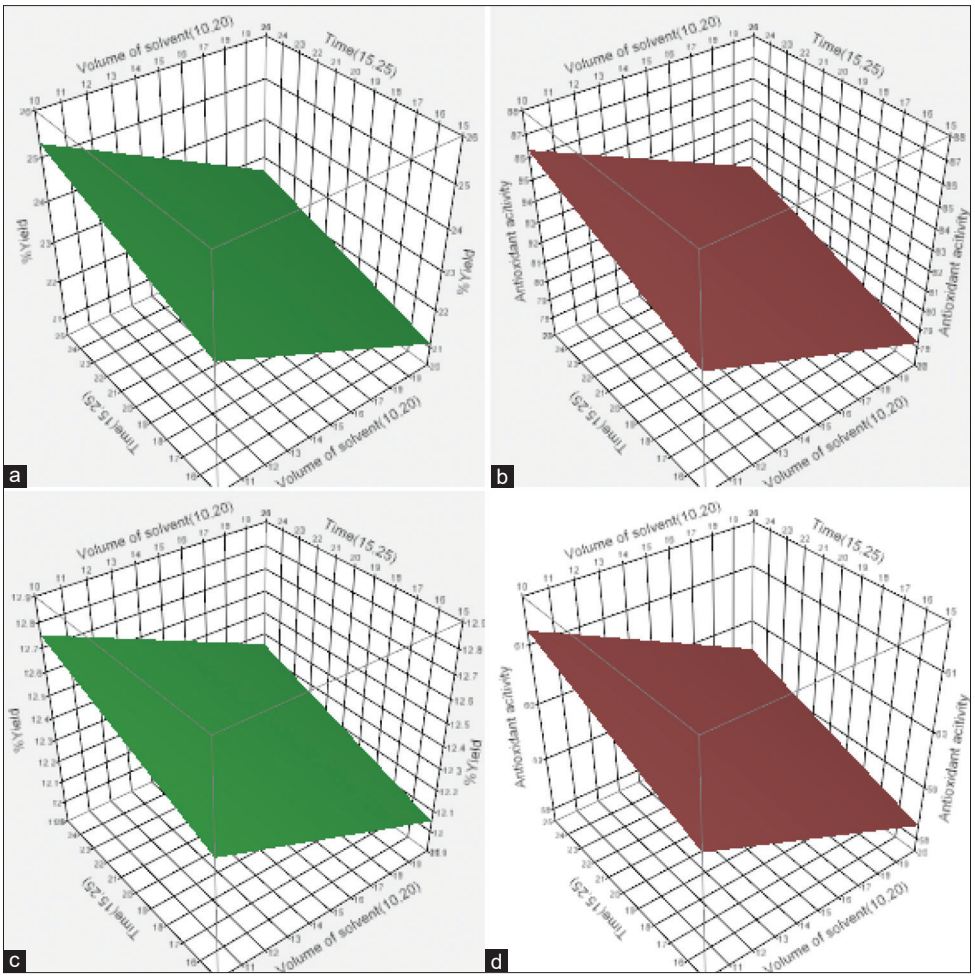

Figure 7: Response curve for percentage yield and antioxidant study of CF female (a and b) and male (c and d) methanolic (10 ml) extract with respect to time and volume of solvent

to standardize the efficient extraction process as well as the quantity of the percentage yield. Earlier many literature works report the same results where saponins content is reduced with longer extraction time.[21-23] The test subsequently showed that the methanol extract yielded a higher percentage yield than other extracts which is also a similar result to the previous report.[24,25] Further phytochemical natures of male and female flowers are screened through chemical tests separately. Different groups of phytoconstituents such as alkaloids, glycosides, phytosterols, flavonoids, carbohydrates, and protein are found in both flower extracts which have potential therapeutic efficacy and also indicated the potential for specific pharmacological activity. Based on the group of constituents present, and alkaloid is identified as the presence of berberine by HPTLC. Further, it is worthwhile to quantify the content of phytoconstituents especially alkaloid (Berberine) in both the flowers because of its present in larger quantity in the stem and other parts. Hence, the amount of berberine is quantified in flower extracts by a validated HPTLC technique for the first time. Many literature works revealed that various solvents are used for the detection of berberine as mobile phase such as ethyl acetate:butanol:formic acid:water (50:30:12:10 v/v) for CF stem extract,[26] butanol:glacial acetic acid:water (14:3:4 v/v) for CF stem extract,[27] n-butanol, acetic acid, and water (8:1:1v/v) for CF stem callus[11] but in this present investigation n-propanol:formic acid:water (90:1:9 v/v) is used which resulted in more clear separation and identification of the constituent. Finally, result revealed female flowers showed higher amounts of berberine content as compared to male flowers, though the TLC of the extracts from different flowers showed a similar pattern. This may be due to the presence of unicellular and multicellular covering and glandular Trichomes which are more quantities of female flowers than the male flowers. Trichomes are known to play an important role in the storage of secondary metabolites and research literature is also evident that it is thus confirmed that the female flowers stored more secondary metabolites (especially berberine) than the male flower.[28-30] The same type of result reported earlier when the percentage content of berberine is estimated in stems, leaves, roots, and fruits of CF plants with respect to sex of the individual, female plant resulted in more berberine content than male plant.[31] Thereafter, berberine was isolated and confirmed with analysis of HPTLC and 1H NMR.

Based on the presence of berberine in flowers, further antioxidant activity is attempted using DPPH and FRAP

assay methods. Isolated berberine was also shown good antioxidant activity which was also correlated with the earlier report.[32] The study found that berberine is the actual active constituent of the extract. Since the antioxidant action is a fundamental and essential function of a plant for its use in the treatment, diagnosis, and prevention of various diseases, the female flower extracts also result in antioxidant activity in this report (no results for male flower). This result is directly proportional to the active constituent, that is, content of berberine in the flower that was also reported earlier.[33] Hence, due to the presence of higher berberine content than the male flowers, female flowers resulted in better performance. Female flowers, therefore, exhibited potential antioxidant activity which is an indication for further therapeutic activity exploration.

CONCLUSION

CF female flowers have great potentials for further investigation of their pharmacological and therapeutic uses because to the presence of high berberine content when quantified using the HPTLC system. This berberine content often depends on the existence of abundant trichomes in female flowers relative to male flower but it is worthwhile to perform experiments on male and female flowers with regard to morphology and microscopy are worthwhile, followed by pharmacognostic screening to evaluate their individual characteristics through chemical testing, which helped to establishment of potential antioxidant activity of the isolated berberine.

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CONFLICTS OF INTEREST

The authors declare that no conflicts of interest exist in the course of conducting this research.

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