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Research article

Unveiling the bioactive potential of indigenous Caralluma fimbriata extract: Integrated nutritional, phytochemical and structural profiling

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Abstract: Medicinal plants have long been a cornerstone of traditional and modern medicines. Their extracts are recognized for their significant safety and therapeutic potential. *Caralluma fimbriata*, a member of the *Apocynaceae* family, has a historical application in the treatment of diabetes, oxidative stress, and metabolic disorders. We focused on the nutritional and mineral analysis of *C. fimbriata* and assessed four extraction techniques, Maceration (MC), Soxhlet extraction (SOX), microwave-assisted extraction (MAE), and ultrasonic-assisted extraction (UAE), to evaluate their impact on extraction yield and antioxidant potential. The study revealed that the UAE exhibited a significantly higher extraction efficiency (21.99%) compared to MAE (15.29%), SOX (14.01%), and MC (11.72%). The UAE extraction method revealed the highest total phenolic content (37.72 ± 1.75 mg/g) and flavonoid content (18.47 ± 1.59 mg/g) compared to all other methods. The enhanced antioxidant activity was corroborated by the findings, as demonstrated through the 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay (91.29%) and the ferric-reducing antioxidant power (788.32 µmol FeSO4/g). FTIR spectroscopy confirmed that the core chemical composition of extracts was unchanged across all extraction techniques. The findings indicated that the UAE is an effective and sustainable method for optimizing phytochemical extraction from *C. fimbriata*, highlighting its potential as a source of natural antioxidants for the nutraceutical sector and functional foods.

Keywords: pregnane glycosides; *Caralluma fimbriata*; ultrasound-assisted extraction; LC-MS; FTIR

1. Introduction

Over the past few decades, there has been a growing global trend toward the utilization of herbal medicines as primary therapeutic agents, driven by concerns over the adverse effects associated with synthetic pharmaceutical drugs [1]. As a result, wild edible plants have re-emerged as valuable therapeutic agents, particularly in traditional healthcare systems like Ayurveda and Traditional Chinese medicine [2]. Among these, *Caralluma fimbriata*, a succulent plant belonging to the *Asclepiadaceae* family, has gained attention for its ethnopharmacological and nutritional properties [3]. It is distributed in Asia, Southeast Europe, Egypt, and Africa, specifically in the arid areas of Pakistan, locally known as "choonga" or "kali mulian". Historically, it has been consumed as a vegetable and is widely recognized for quenching thirst and managing obesity among ethnic populations [4]. These traditional uses have sparked scientific interest in its bioactive profile and structural analysis. The pharmacological properties of *C. fimbriata* are attributed to its diverse secondary metabolites, which include steroidal glycosides, alkaloids, terpenoids, flavonoids, and tannins [5]. Among these, pregnane glycosides are the major bioactive compounds that have drawn considerable interest in the pharmaceutical and functional food industries [6]. In addition, it contains flavone glycosides, saponins, triterpenoids, and other flavonoids that have been extensively investigated for their efficacy against various pathological conditions and metabolic disorders [7,8].

The recovery of these bioactive compounds from plant matrices is a critical step that directly influences the extraction yield and the biological potency of the final extract [9,10]. The phytochemicals recovery is strongly influenced by several interrelated factors, i.e., type of solvent, extraction method, temperature, and duration [11,12]. Traditional extraction techniques, such as MC, decoction, and SOX, are widely used but have significant limitations. These include long processing times, high energy consumption, excessive solvent usage, and thermal degradation that can lead to hydrolysis and oxidation of phenolic compounds [13,14]. These conventional methods rely primarily on swelling and diffusion processes during extended extraction periods, which makes them less effective in completely disrupting cell structures and may restrict the release of bioactive substances found deep within the cells [15]. These limitations have led to the exploration of innovative green extraction technologies that enhance yield, reduce solvent usage, minimize processing time, and preserve bioactivity. Among these, ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) are considered as promising eco-friendly alternatives [16]. UAE utilizes acoustic cavitation generated by ultrasonic waves to disrupt plant cell walls, improving solvent penetration and facilitating the release of intracellular phytochemicals [17]. The major parameters influencing UAE efficiency include ultrasonic power, extraction time, temperature, solvent type, and solid to liquid ratio[18]. Optimal adjustments of these parameters enhance the extraction yield while minimizing thermal degradation and preserving the structural integrity of heat-sensitive compounds. The cavitation phenomenon creates micro-jets and shock waves that mechanically break cell walls, thereby increasing mass transfer rates and reducing extraction time from hours to minutes [13]. Similarly, MAE relies on dielectric heating to induce rapid internal heating of plant matrices, leading to efficient cell rupture and accelerated release of bioactive compounds. Key factors influencing MAE performance include microwave power, extraction, duration, solvent polarity, and temperature control . The volumetric heating mechanism of MAE enables rapid and uniform heat distribution, significantly reducing extraction time while maintaining high yields of thermolabile compounds. Moreover, MAE requires less solvent and energy compared to conventional methods, making it a sustainable alternative for

industrial applications [19]. UAE and MAE offer significant advantages over conventional methods, including higher extraction efficiency, reduced processing time (from hours to minutes), lower solvent consumption (up to 70% reduction), better preservation of thermolabile compounds, improved repeatability, and greater scalability for industrial applications [20,21]. Various species of *Caralluma* have been used in traditional practices for both dietary purposes and the management of diverse health ailments. However, research comparing different extraction techniques and evaluating the antioxidant potential of *C. fimbriata* remains limited [22]. Therefore, this investigation was designed to comprehensively compare the efficiency of four extraction methods, like MC, SOX, MAE, and UAE, for recovering bioactive compounds from *C. fimbriata*. Our objectives were to: Analyze the nutritional and mineral composition of *C. fimbriata*; evaluate and compare extraction efficiency among the four extraction techniques based on extraction yield; determine total phenolic content (TPC) and total flavonoid content (TFC); assess antioxidant activities using DPPH and FRAP assays to identify the optimal extraction approach; and characterize the phytochemical profile using FTIR and LC-MS/MS analysis. The findings provide valuable insights into the application of green extraction strategies for producing high-value, bioactive-rich extracts with potential applications in functional foods and nutraceuticals.

2. Materials and methods

2.1. Procurement and preparation of raw materials

C. fimbriata was purchased from the local market in Kamra, Attock, Pakistan. After being thoroughly cleaned with distilled water to remove dust, dirt, and surface impurities, it was chopped into smaller pieces and dried in the dehydrator (Harvest Saver, R 5A, 1-800369-4283/USA). For subsequent analysis, the dried materials were crushed into powder, sieved to obtain a uniform particle size of approximately 60 mesh (≈250 μm), packed in polythene bags, and kept at −20°C. The analytical-grade chemicals and reagents used in the study. Ethanol (70% v/v), methanol (LC-MS grade, purity ≥ 99.9%), and acetonitrile (LC-MS grade, purity ≥ 99.9%) were obtained from Merck KGaA (Darmstadt, Germany). Formic acid (≥ 98%) was obtained from Sigma-Aldrich (Tokyo- Japan). The 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, 2,4,6-tris(2-pyridyl)-S-triazine (TPTZ), aluminum chloride ($AlCl_3$), sodium acetate (CH_3CO_2Na), and all other reagents for phenolic, flavonoid, and antioxidant assays were purchased from Sigma-Aldrich or Merck with ≥98% purity. Analytical standards for pregnane glycosides, saponins, and flavonoids, used for LC-MS/MS calibration and quantification, were procured from Cayman Chemical (Ann Arbor, MI, USA). Calibration solutions were prepared in ethanol at concentrations ranging from 0.08 to 5 μg/mL using Class A volumetric flasks and calibrated pipettes.

2.2. Nutritional analysis of C. fimbriata

The proximate analysis of *C. fimbriata* was assessed following standard methods of Association of Official Analytical Chemist (AOAC) [23]. Purposely, fresh *C. fimbriata* was dried at 105 ± 5°C to determine its moisture content (Method No. 925.08), whereas dried samples were incinerated at 600°C for 6 hours to measure the ash content (Method No. 923.03). The Kjeldahl method (Method No. 979.09) was used to determine the crude protein, while Soxhlet's ether extraction protocol (Method No. 920.39) was used to extract the crude fat as ether extract. Moreover, these fat-free samples were analyzed for crude

fiber (Method No. 962.09). Mineral analyses were carried out by adopting the protocols of AOAC [23]. In the end, non-nitrogenous extract (NNE) was calculated using the following expression:

$$NNE(\%) = [100 - moisture\% - crude\ protein\% - ether\ extract\% - ash\% - crude\ fiber\%] \quad (1)$$

2.3. Extraction methods

2.3.1. Maceration (MC)

The *C. fimbriata* extract was prepared by adopting the protocol of Priya [24], with minor adjustments. Powdered plant material (20g) was mixed with 300 mL of 70% ethanol (70:30, v/v) in a 500 mL glass beaker and subjected to continuous magnetic stirring for 3 hours at 25 ± 2°C. Thereafter, the extract was filtered through Whatman filter paper no. 2, and the residue extraction was repeated once more. The samples were then centrifuged for 15 minutes at 8000 rpm (Centrifuge 5804 R, Germany). The supernatant was filtered and concentrated to a minimal volume through a rotary evaporator (EYELA Rotary Vacuum Evaporator N-N Series), freeze-dried and maintained at −20 °C until subsequent analysis.

The following formula (equation 2) was used to calculate the yield:

$$Yield\ \left(\frac{w}{w}\right) = \frac{(weight\ of\ C.fimbriata\ extract)(g)}{(weight\ of\ raw\ C.fimbriata\ powder)(20g)} \times 100 \quad (2)$$

2.3.2. Soxhlet extraction (SOX)

This extraction was done by following the method of Ugwah-Oguejiofor [25], with a few modifications. Purposely, 20 g of sample was enclosed within filter paper and placed in the extraction assembly of the Soxhlet apparatus (Lab Tech, LEX-3030F, Korea). The extraction was carried out using 300 mL of 70% ethanol (1:15, w/v) for 6 hours. Subsequently, the solvent containing the extract was subjected to rotary evaporation (EYELA Rotary Vacuum Evaporator N–N Series), freeze-dried, and kept at −20 °C for further assay.

2.3.3. Microwave-assisted extraction (MAE)

A slight modification in the procedure demonstrated by Verma et al. carried out the *C. fimbriata* extraction through microwave [26]. Thr sample (20 g) was combined with 70% ethanol and positioned in a microwave (Dawlance-Microwave Oven, Japan) for 3 minutes at 60 ± 5 °C. Thereafter, the mixture was subjected to a centrifugation machine (Centrifuge-5804 R, Germany) at 5000 rpm for 15 minutes. Finally, the microwave-extracted sample of *C. fimbriata* freeze-dried in (Christ/LCG Layo Chamber Guard 1-215-550 PMMA) was collected and preserved at −20°C for further assessment.

2.3.4. Ultrasound-assisted extraction (UAE)

Ultrasound-assisted extraction (UAE) was done utilizing a formerly optimized technique with some minor adjustments [27]. A quantity of 20 g of *C. fimbriata* was submerged in 300 mL of 70%

ethanol employing an Ultrasonic Processor (UP) (6.5 KW, 2400Hz, Scientz, China). The parameters for time and temperature were set at 30 minutes and 45 ± 5°C, respectively. Following the extraction process, the extract was centrifuged (Centrifuge-5804 R, Germany) at 8000 rpm for 10 minutes, supernatant was filtered through Whatman filter paper to eliminate any insoluble residues. Subsequently, the *C. fimbriata* extract obtained through ultrasound extraction was freeze-dried and preserved at −20°C until additional experiments.

2.4. Physiochemical screening and antioxidant capacity assessment

2.4.1. Total phenol content (TPC) determination

The total phenolic content (TPC) were determined using the Folin-Ciocalteu method, as outlined by Maheshu et al. [28], with slight changes. A 0.1 mL extract was combined with 3 mL of H_2O, then adding 0.5 mL of Folin-Ciocalteu reagent. Subsequently, 2 mL of 10% sodium carbonate was added after 3 minutes and mixed thoroughly. The resultant mixture was incubated for 1 minute in a water bath set at 100 °C. Post-incubation, the solution was allowed to cool for 2 minutes before measuring the absorbance using a UV-visible spectrophotometer (Perkin Elmer Lambda 40 UV/VIS Spectrophotometer) at 650 nm. Gallic acid was used as the calibration standard, with a concentration range of 10–100 μg/mL. All measurements were conducted in triplicate using a reagent blank as reference, and results are reported as milligrams of Gallic acid equivalents per gram of dry weight (mg, GAE/g dw).

2.4.2. Total flavonoid content (TFC) determination

TFC determination in the samples were conducted following the modified version of the procedure described by Kumaran [29].A total of 2.0 mL of the sample or a standard was added to 2.0 mL of a 2% $AlCl_3$ solution, followed by adding 3.0 mL of 0.6 M sodium acetate (CH_3CO_2Na). Subsequently, the mixture was incubated at 20°C for 30 minutes. The absorbance was measured in triplicate utilizing a UV-Vis spectrophotometer (Perkin Elmer Lambda 40 UV/VIS Spectrophotometer) at 440 nm. The calibration curve of catechin was used as the standard, and results were denoted as catechin equivalent (CTE)/g of extracts.

2.5. Antioxidant assays

2.5.1. 2,2-diphenyl-1-picrylhydrazyl (DPPH)

The DPPH of the samples was evaluated following the protocol of Medina et al. [30], with certain adjustments. A total of 0.1 mL of extract (1 mg/mL) was mixed with 3.9 mL of DPPH solution and vortexed for 30 seconds.

Subsequently, the mixture was left to incubate for 30 minutes at room temperature in dark, and the absorbance was measured using a UV-Vis spectrophotometer (Perkin Elmer Lambda 40 UV/VIS Spectrophotometer) at 515 nm while methanol was used as a blank. The outcomes were described as percent inhibition of DPPH radical by extract using the following equation 3:

$$DPPH\ radical\ scavenging\ activity\ (\%) = \frac{(Absorbance\ control - sample\ absorbance)}{Absorbance\ of\ control} \times 100 \quad (3)$$

2.5.2. The ferric reducing antioxidant power (FRAP) method

The FRAP was evaluated following a modified procedure outlined by Maheshu et al. [28]. A volume of (500 µl) of a sample was introduced to 3 mL of FRAP reagent (comprising 10 parts of a 300 mM sodium acetate buffer at pH 3.6, 1 part of 10 mM 2,4,6-tris(2-pyridyl)-S-triazine (TPTZ) solution, and 1 part of 20 mM $FeCl_3$. $6H_2O$ solution). Subsequently, the mixture was incubated in a water bath at 37°C. The absorbance was measured at 593 nm after 30 minutes. The $FeSO_4$ calibration curve was obtained, and the readings were denoted µmol $FeSO_4$/gram of plant material on a dry weight basis.

2.6. Fourier-transformed infrared (FTIR) spectrometry of C. fimbriata *extract*

To determine chemical spectra and examine the structure of *C. fimbriata extract* derived from various extraction methods, FTIR spectroscopy was performed. The *C. fimbriata* extract spectra were measured using an FTIR spectrophotometer (Perkin Elmer, Inc., Waltham, MA, USA), in accordance with the protocol of Ahmed et al. [31]. The sample 1.0 mg of *C. fimbriata* extract powder was positioned on the universal diamond ATR top plate and subjected to a force of 120 N. The spectral data were collected in the 400 to 4000 $cm^{-}1$ range with a resolution of 4 $cm^{-}1$ and an average of four scans. Subsequently, the universal diamond ATR top plate was cleaned using alcohol after each scan.

2.7. Liquid chromatography-mass spectrometry (LC-MS/MS) of C. fimbriata *extract*

Liquid chromatography fitted with a mass spectrometer detector (LC-MS/MS) was used to evaluate the phytochemical compositions using the modified method of Nichitoi et al. [32] in the Mass Spectrometry & Metabolomics Core Facility, Michigan State University, USA. The analysis by LC-MS/MS was conducted utilizing Qaudrupole-Exactive, which was manufactured by Thermo and equipped with Electrospray Ionization/Turbo Ion Spray mode. During the chromatographical examination, a Synergi, C_{21} column (Fusion/RP 80 A, 4-µm particle size; Phenomenex-Inc., Torance, USA) injected a volume of 5 µL. The solvents were formic acid (0.5%) and methanol. The gradient elution encompassed a range from 2% to 98% B at 32°C, with a flow of elution rate at 900 µL/min and an elution duration of 15 minutes. With nitrogen acting as the collision gas, the mass spectra were recorded in the positive ion mode between 50 m/z and 5000 m/z. The temperature of the MS instrument's ionization source was kept at 500°C. The nebulizer's gas flow pressure was set to 1000 psi. By appropriately diluting individual stocks, a mixed working standard with a concentration of 10 µg/mL in ethanol was obtained. Ethanol calibration solutions were prepared in the concentration range from 0.08 to 5 µg/mL. Class A volumetric glass flasks and automated pipettes were used in the experimental setup. The peak area list was generated using the progenesis QI software concerning pregnane glycosides, saponins, and flavonoids based on accurate mass and fragmentation.

2.8. Statistical analysis

Statistical analysis was performed using Statistix software (version 8.1) [34]. All experiments were conducted in triplicate and results are expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was applied to determine significant differences among extraction methods, followed by Tukey's honestly significant difference (HSD) test for post-hoc multiple

comparisons. Graphical representations were generated using GraphPad Prism software.

3. Results

3.1. Nutritional analysis of C. fimbriata

The mean percentage findings and standard deviation for the proximate and mineral composition of *C. fimbriata* (dry weight basis) are summarized in Table 1. The analysis indicated that *C. fimbriata* contained 11.42% ash, 17.81% ether extract, 13.54% crude protein, 56.41% crude fiber, and 4.62% NNE on a dry basis. Being a cactus species, *C. fimbriata* possesses a significant amount of moisture (74.00 ± 3.65%) and can resist water stress. The results of proximate composition agreed with prior investigations [33–35]. Fiber content results aligned with previous research by Maheshu [28], who reported 15.2% crude fiber in *C. fimbriata* on a fresh weight basis, which corresponds to approximately 58% on a dry weight basis, comparable to our observation.

The mineral profile of *C. fimbriata* depicted the amounts as mg/100 g of iron (Fe) (21.26 ± 1.05), zinc (Zn) (23.9 ± 1.18), calcium (114.86 ± 5.71), copper (7.66 ± 0.35), manganese (Mn) (6.45 ± 0.31), magnesium (Mg) (19.67 ± 0.88), sodium (Na) (175.78 ± 7.51), potassium (K) (26.73 ± 1.23), and phosphorus (38.65 ± 1.84).

Table 1. Proximate (%) composition of *C. fimbriata* on a dry weight basis.

Proximate components (%)	Content
Ash	11.42 ± 0.50
Ether extract	17.81 ± 1.11
Crude protein	13.54 ± 0.16
Crude fiber	56.41 ± 2.78
NNE	4.62 ± 0.19
Mineral composition (mg/100g) of *C. fimbriata* on dry weight basis	
Minerals (mg/100g)	Content
Iron	21.26 ± 1.05
Zinc	23.9 ± 1.18
Calcium	114.86 ± 5.71
Copper	7.66 ± 0.35
Manganese	6.45 ± 0.31
Magnesium	19.67 ± 0.88
Potassium	26.73 ± 1.23
Sodium	175.78 ± 7.51
Phosphorus	38.65 ± 1.84

3.2. Extraction yield

A comparison was conducted to evaluate the impacts of different extraction methodologies on the yield of *C. fimbriata extract*. The outcomes are delineated in Figure 1. As per the results, the yield of *C. fimbriata* extract exhibited a declining pattern: UAE (23.99%) > MAE (21.29%) > SOX (18.8%) > MC (16.81%). The UAE extraction approach demonstrated the highest yield, while MC extraction

displayed the lowest yield. Conversely, microwave extraction resulted in a higher yield of 21.29% than SOX extraction.

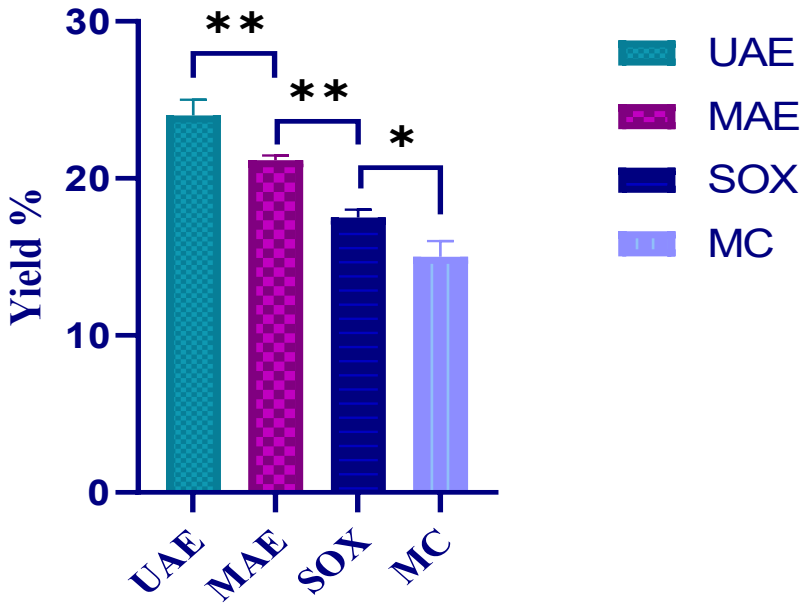

Figure 1. The extraction efficiency of *C. fimbriata* by employing UAE, MAE, SOX, and MC techniques. Results are expressed as Mean ± SD. Statistical significance is denoted at a threshold of *$P<0.05$, ** $P<0.01$.

3.3. TPC

The outcomes of the TPC of *C. fimbriata* for all extraction techniques are presented in Figure 2. The statistical analysis revealed that TPC differed significantly among all UAE (37.72 ± 1.75 mg GAE/g dw) > MAE (32.47 ± 2.23 mg GAE/g dw) > SOX (21.66 ± 2.15 mg GAE/g dw) > MC (19.90 ± 1.16 mg GAE/g dw). Among these, the UAE yielded the highest TPC concentration (37.72 ± 1.75 mg/g dw), demonstrating that the extraction technique markedly influences the recovery of phenolic compounds.

3.4. TFC

The flavonoid concentration of the *C. fimbriata* samples in UAE 18.47 ± 1.59 mg CE/g dw > MAE 13.33 ± 0.58 mg CE/g dw > SOX 12.05 ± 1.51 mg CE/ g dw > MC 11.89 ± 0.58 mg CE/ g dw, with the highest in the UAE extract of *C. fimbriata* and the lowest in the MC.

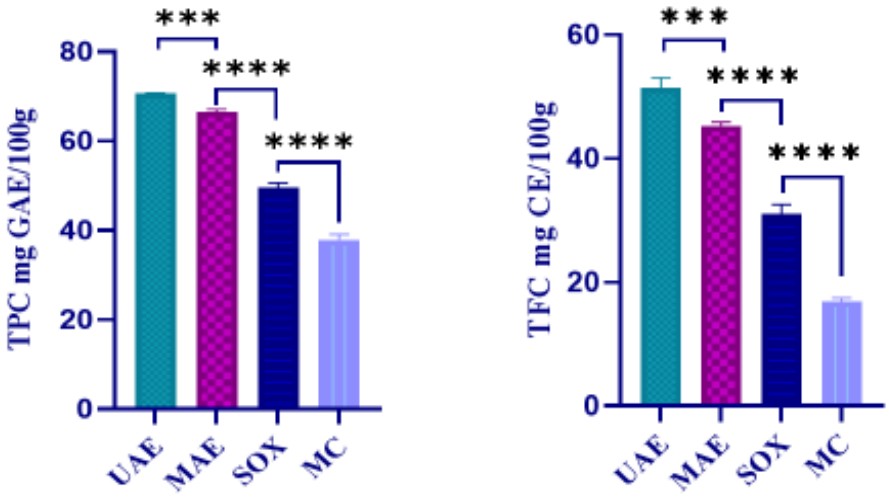

Figure 2. Effect of UAE, MAE, SOX, and MC techniques on TPC and TFC of *C. fimbriata* extract. Results are expressed as Mean ± SD. Statistical significance is denoted at a threshold of *$P<0.05$, ** $P<0.01$, *** $P<0.001$, and **** $P<0.0001$.

3.5. Antioxidant assays

3.5.1. DPPH

The stable free radical DPPH is recognized for its ability to undergo electron or hydrogen radical acceptance, resulting in the formation of a stable molecule. These molecules are widely accredited for their utility in assessing the efficacy of antioxidants in scavenging free radicals. Experimental evaluations involving MC, SOX, MAE, and UAE were conducted to quantify their impact on the DPPH radical, as illustrated in Figure 3. Mean values elucidated that UAE of *C. fimbriata extract* exhibited the highest scavenging activity (91.29 ± 3.23%), while MC displayed the lowest activity (68.88 ± 2.06%). The SOX (78.32 ± 2.15%) had lesser scavenging activity compared to MAE (89.58 ± 1.15%).

3.5.2. FRAP

FRAP is an additional parameter to assess antioxidant activity by correlating with the presence of reductones, which are molecules that contribute electrons. These reductones play a crucial role in providing electrons (hydrogen ions) to disrupt the chain reaction of free radicals, thereby impeding lipid peroxidation through the reduction of oxidized intermediates generated during the process. The data presented in Figure 3 illustrates the FRAP assay values as follows: UAE 788.32 ± 31.18 µM $FeSO_4$/g DW, MAE 689.81 ± 24.47 µM $FeSO_4$/g DW, SOX 691.33 ± 34.56 µM $FeSO_4$/g DW, and MC 643.55 ± 31.06 µM $FeSO_4$/g DW.

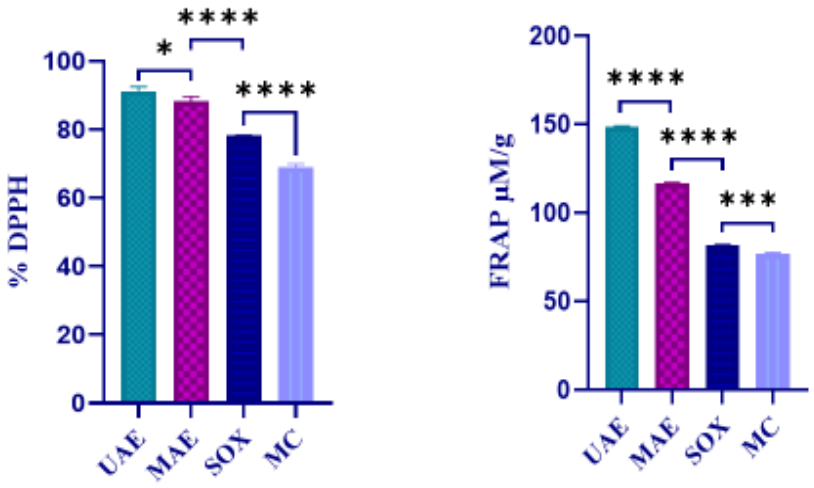

Figure 3. Effect of UAE, MAE, SOX, and MC techniques on %DPPH, and FRAP of *C. fimbriata* extract. Results are expressed as Mean ± SD. Statistical significance is denoted at a threshold of *$P<0.05$, ** $P<0.01$, *** $P<0.001$, and **** $P<0.0001$.

3.6. The FTIR spectrum

The principal constituents of the *C. fimbriata* extract consist of pregnane glycosides, saponins, and flavones. The utilization of FTIR spectroscopy within the frequency range of 400-4000cm^{-}1 facilitated the identification of the primary functional groups and chemical bonds within the *C. fimbriata* extract samples. Table 2 and Figure 4 display the similarity in the FTIR spectra of MC, SOX, MAE, and UAE, suggesting that the structural features of *C. fimbriata* extracts remained unchanged despite using various extraction techniques. Although minor variations were observed in the 1660–1000 cm^{-1} region, particularly in the MAE spectrum, these differences were attributed to changes in the intensity of C–O, C=C, and C–N vibrations caused by variations in extraction conditions rather than any alteration in the chemical structure. The major characteristic peaks corresponding to hydroxyl (–OH), aliphatic (C–H), carbonyl (C=O), and aromatic (C=C) groups remained consistent across all methods, confirming that the fundamental structural features of *C. fimbriata* extracts were preserved despite the use of different extraction techniques.

Table 2. FTIR of MC, SOX, MAE, and UAE extraction techniques of *C. fimbriata* extract.

Extraction	Functional group name	Bond	Frequency (cm^{-1})
MC (a)	Alcohol	O-H stretching	3317
	Alkanes	C-H stretch	2936
	Alkanes	C-H stretching	2827
	Carbon dioxide	O=C=O stretching	2331
	Aromatics	C-C stretching	1449
	Alkanes	C-H Stretching	1409
	Alcohol	C-O stretching	1024
SOX (b)	Easters	C-O stretching	1024
	Amides	N-H Stretching	3317
	Alkanes	C-H Stretch	2913
	Alkanes	C-H Stretching	2845
	Carboxylic acid	C-O stretching	1728
	Amides	N-H Stretching	1681
	Alkanes	C-H Stretching	1415
	Aliphatic amines	C-N Strech	1021
MAE (c)	Alcohol	O-H stretching	3467
	Alcohol	O-H stretching	3317
	Phenols	O-H stretching	3216
	Alkanes	C-H Stretch	2934
	Alkanes	C-H Stretching	2849
	Carbon dioxide	O=C=O stretching	2368
	Aromatics	C-C stretching	1587
	Alkanes	C-H stretching	1409
UAE (d)	Alcohol	O-H stretching	3467
	Phenols	H-bonded	3451
	Phenols	O-H stretching	3216
	Aliphatic primary amine	N-H stretching	3320
	Alkanes	C-H stretch	2916
	Alkanes	C-H stretching	2849
	Carbon dioxide	O=C=O stretching	2368
	Aromatics	C-C stretching	1587
	Alkanes	C-H stretching	1415
	Amides	C-N stretch	1268

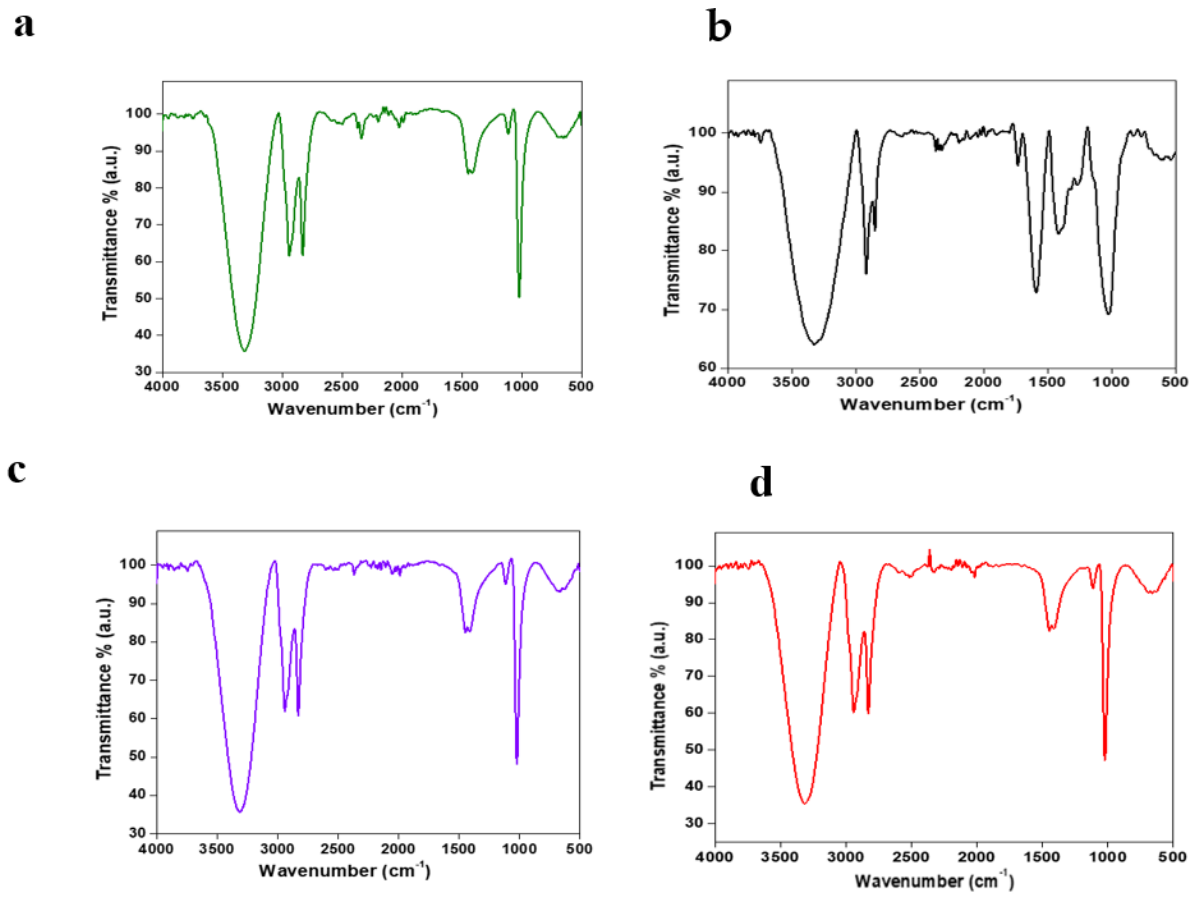

Figure 4. (a) FT-IR Spectra of MC (b), SOX (c), MAE, and (d) UAE techniques.

3.7. LCMS

In this section, the LC–MS/MS analysis was carried out for the UAE of *C. fimbriata,* which exhibited the highest extraction yield and optimal phytochemical composition among all extraction techniques. *The C. fimbriata* extract was examined for additional phytochemical screening through LC-MS in this investigation. Several phenolic components were found in the *C. fimbriata* extract according to the LC-MS/MS study pregnane glycosides, steroids, saponins, Kaempferol, sucrose, and kaempferol-3-O-hexose deoxyhexose, which are the principal flavonoids that have been identified, and trans-ferulic acid and syringic acid are the principal phenolic acids. The presentation of the m/z and relative mass defect (RMD) values for the identified phyto-compounds are shown in Table 3 and Figure 5.

Table 3. Phytochemical profiling of UAE of *C. fimbriata* through LC-MS/MS.

Serial No.	Type of molecule	Neutral mass (Da)	m/z	RMD	Retention time (min)	Sample peak area
1	Steroids		1124.6	534	11.87	1137715918
2	Pregnane glycoside	944.512644	962.546	568	13.29	897686300.6
3	Pregnane glycoside	1168.581209	1186.62	518	13.49	680048116.4
4	Pregnane glycoside		1024.56	549	14.94	556586459.2
5	Pregnane glycoside		964.56	581	13.40	485370528.1
6	Kaempferol-3-O-hexosedeoxyhexose	592.2679361	593.275	464	16.68	349680652.4
7	Pregnane glycoside	837.4689948	838.476	568	8.30	344089561.9
8	Kaempferol-3-O-hexosedeoxyhexose	592.2679361	593.275	464	16.68	349680652.4
9	Pregnane glycoside	656.3765269	674.41	608	9.16	343753572.6
10	Pregnane glycoside		920.536	582	12.46	305426551.1
11	Luteolin7-O-glucoside		609.27	444	16.40	197862068.1
12	Pregnane glycoside	675.4164605	676.424	626	9.13	287028811.6
13	Pregnane glycoside		836.463	554	8.37	265527830.2
14	Pregnane glycoside		834.448	537	8.23	225173431.3
15	Pregnane glycoside		836.461	552	8.20	211529297.7
16	Pregnane glycoside		672.395	587	8.99	198674054.9
17	Pregnane glycoside		674.408	605	8.98	184611206.3
18	Pregnane glycoside		814.494	606	9.95	112083800.1
19	Pregnane glycoside		978.541	553	12.19	99741499.11
20	Pregnane glycoside		962.546	567	13.06	97106907.99
21	Pregnane glycoside		946.515	544	11.55	81244443.52
22	Pregnane glycoside	1008.541994	1026.58	561	15.24	74528368.37
23		608.2627197	609.27	443	16.07	74405643.99
24	Pregnane glycoside		816.508	623	10.13	63922196.37
25	Pregnane glycoside		948.53	559	11.67	63897500.37
26	Pregnane glycoside	884.4906012	885.498	562	12.59	62858056.84
27		581.2849186	582.292	502	10.82	62466889.69
28		744.3682087	745.375	504	8.58	59394217.39
29	Pregnane glycoside	798.4752468	816.509	623	9.28	54994601.53
30	Quercetin		309.241	781	15.46	9388118.142
31	Sucrose	342.1157789	360.15	415	1.02	51107287.46
32	Luteolin	284.2192117	295.226	767	13.77	23474119.39
33	Pregnane glycoside	962.4716396	980.505	516	7.15	45443297.61
34	kaempferol glycoside	594.1582936	595.166	278	6.86	45332124.52
35	Amentoflavone	532.2520113	533.259	486	6.28	10147298.31

Continued on the next page

Serial No.	Type of molecule	Neutral mass (Da)	m/z	RMD	Retention time (min)	Sample peak area
36	Quercetin-3-O-hexosedeoxyhexose	606.2469654	607.254	419	15.41	10137587.83
37	Pregnane glycoside	656.3760167	674.41	608	7.82	41652026.64
38	Pregnane glycoside	675.4186386	676.426	630	7.98	41234610.01
39	Pregnane glycoside		918.52	566	13.46	37115002.51
40	Pregnane glycoside	800.4896682	818.523	640	9.48	33398559.06
41	Syringicacid		198.097	490	0.95	15658310.63
42	Ferulicacid	193.0741253	194.081	419	5.20	15525549
43		790.5217076	808.556	687	17.29	32971450.12
44	Pregnane glycoside	884.491014	885.498	563	12.47	28819119.48
45	Pregnane glycoside	904.5164982	887.513	578	12.68	27123016.31
46			1174.61	521	13.15	26873506.06
47	Pregnane glycoside	780.4650128	798.499	625	10.35	26864021.99
48		987.5572574	1010.55	541	13.70	26599932.5
49	Pregnane glycoside		920.535	581	13.61	26556665.23
50	Pregnane glycoside		996.5	501	7.89	23352608.7
51	Pregnane glycoside	656.3404946	674.374	555	7.64	23091673.4
52	Pregnane glycoside		838.478	570	7.27	21115795.03
53	Pregnane glycoside		816.509	624	9.52	20877142.44
54	Pregnane glycoside		998.514	515	7.98	20595662.79
55	Pregnane glycoside	654.360944	672.395	587	8.08	20043922.7

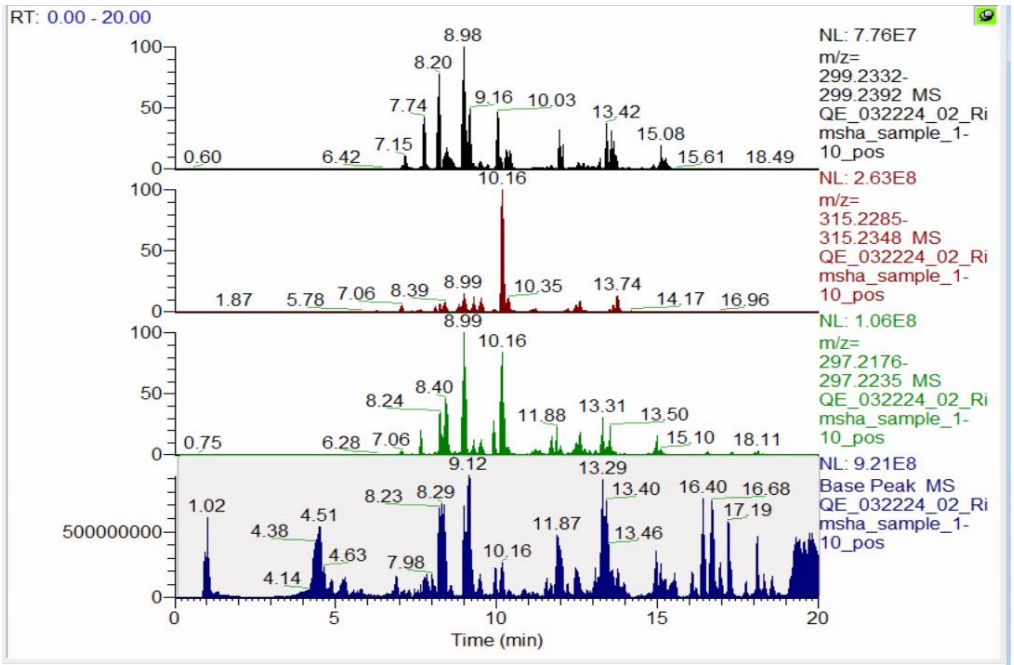

Figure 5. LCMS/MS extracted ion chromatograms of UAE of *C. fimbriata* obtained in positive ion mode, showing peaks that fragment to release the (C_{21}) steroidal core. The bottom trace is the base peak intensity chromatogram for the sample.

4. Discussion

Minerals are necessary for crucial physiological activities, including acid-base and water balance. Our mineral composition results are similar to those reported by Kumar et al. [36]. Fe is an important element for the production of haemoglobin in red blood cells [37]. Likewise, Mohan investigated mineral content in different unconventional wild edible plants [33]. Zn assumes a crucial function in the stabilization of macromolecular synthesis and structure [38]. Mn is an essential element required for various biochemical and enzymatic processes in the body. Mg is critical in maintaining normal nerve and muscle function in human physiology [39]. The mineral composition of vegetables may differ due to several factors, including variety, geographic origin, cultivation, and soil type [40].

The outcomes of this research of yield extraction are favored by a previous study [41], which reported the maximum yield of *Caralluma tuberculate* through UAE (17.08%) compared to MAE (14.26%) and orbital shaker extraction (11.74%). Romes conducted another investigation to evaluate the impact of novel extraction techniques on the obtained yield from *Elaeis guineensis* leaves [42]. The results indicated that the UAE provided a higher yield (14.38%) compared to Soxhlet (6.86%) and maceration (3.73%). Another study, explored by Hosseini [43], reported the highest pectin yield obtained from sour oranges (28.07%) in the UAE, in contrast to traditional techniques. Likewise, the UAE technique has favorable potential and improved efficacy in extracting grapefruit peel.

Consequently, there has been a rise of approximately 16.34% in yield compared to conventional heating extraction [44]. This improvement is attributed to enhanced cell disruption through dielectric heating. However, the extraction parameters must be carefully optimized, as extending microwave exposure beyond the optimal point can lead to a plateau in yield. This plateau occurs because most of the easily extractable compounds are released, and prolonged microwave treatment may cause the degradation of heat-sensitive bioactive compounds, reducing further recovery [45].

The UAE approach maintained higher efficiency by minimizing heat degradation and enhancing solvent penetration, which allign with our observations of superior yield under ultrasound treatment. The increased yield obtained through the utilization of the UAE is likely attributed to the application of ultrasonic waves, which leads to the fracturing of plant tissue and thereby enhances the liberation of the compounds of interest. Therefore, an extended interface exists between the target compounds and the solvent employed for extraction due to the disturbance of the cellular structure [46]. Ultrasound improves the efficiency of the extraction process by enhancing mass transfer rates and potentially disrupting the cell wall through the formation of microcavities. This leads to elevated product yields while decreasing processing time, solvent usage, thermal degradation, and water and energy consumption [47]. Hence, research indicates that ultrasound-assisted extraction results in an increased yield of *C. fimbriata* extract, reflecting the indicative of the dependence of the extraction yield on the employed extraction technique.

Our findings agree with Maheshu, who reported that solvent polarity and the extraction method significantly affect the phenolic yield of *C. fimbriata* when extracted through Soxhlet extraction [28]. In a research, Vats found that the UAE produced the highest phenolic yield (54.4 mg GAE/g) from lime peel waste, outperforming MAE (41.8 mg GAE/g) and Soxhlet extraction (33.2 mg GAE/g) with a 33% shorter extraction time [48]. Comparable results were reported for *Cynara scolymus* L. extracts; UAE showed greater TPC (38.12 mg GAE/g) than SOX (29.65 mg GAE/g) and MC (24.37 mg GAE/g) [13]. These results support the idea that improved mass transfer and cavitation effects during ultra-sonication facilitate the release of bound phenolic, which enhances the antioxidant potential and extract quality.

The decrease in total phenolic content in the MC and SOX extraction extracts can be attributed to the breakdown of phenolic compounds during prolonged heating, as observed in *Carica papaya* leaf extract, where extended extraction time leads to a proportional reduction in overall phenolic content [49]. A similar trend has been documented for *Caralluma arabica,* where phenolic content is directly associated with its antioxidant potential [50]. The extraction method is crucial in determining the content of phytoconstituents and their antioxidant potential [51]. Thus, our findings confirm that UAE provides a non-thermal, effective method for recovering phenolic compounds from *C. fimbriata*, yielding higher TPC than alternative methods. This improvement can be attributed to ultrasonic cavitation's mechanical disruption of plant cell walls, which improves solvent penetration and intracellular phenolic release.

Similarly, *C. tuberculate* has been reported to contain promising secondary metabolites [52]. Another study revealed a significant presence of polyphenolic compounds in *C. fimbriata,* including saponins and flavonoids [53]. Flavonoids, phenolic compounds, and natural antioxidants found in *C. fimbriata* are implicated in plant physiological processes. The ability of flavonoids to modulate the body's reactions to allergens, viruses, and carcinogens has earned them the label of natural biological response modifiers. Their documented effects include anti-inflammatory, anti-allergic, and anti-cancer properties [54]. The concentration of total phenolic compounds in the *Caralluma flava* extract is 10.14 mg GAE/g, while the total flavonoid content is 4.13 mg QE/g [55]. Additionally, *C. tuberculata* demonstrated a higher TFC value of approximately 37.14 mg CE/g dw, confirming that members of the *Caralluma* genus have a significant flavonoid content that enhances their bioactivity [56]. Hence, these results supported that *C. fimbriata* extracts obtained by UAE can yield relatively high flavonoid concentrations because of improved extraction efficiency. The *C. fimbriata extract* exhibited significant antioxidant activities. Our results agree with work for *C. tuberculate* extract in which maximum DPPH inhibition was noted for UAE in comparison to MAE [41]. Our results are consistent with the previous study, where the *C. quadrangular* exhibited higher antioxidant activity; inhibition over 85% of the extract was observed at 1000 μg/ml [57]. Another investigation supported our findings, there is a strong scavenging activity in *Caralluma edulis* extract [58].

The results of another study also depicted the highest inhibition activity by the UAE of *Rosa rugosa* as opposed to the conventional methods [59]. Likewise, researchers have discussed the effect of UAE on peaches and pumpkins in which maximum DPPH was observed. The MC method is commonly employed and regarded as the simplest technique for *Caralluma* extraction. Nevertheless, this method is characterized by inefficiency, time consumption, and excessive solvent usage. The structural integrity of phenols and glycosides is compromised by MC extraction, leading to a reduction in phenolic content as well [60].

We assessed the flavone glycosides derived from orange peel and observed the superior scavenging efficacy in the UAE in contrast to the traditional method [61]. The mechanical impacts of ultrasound were predominantly observed on cellular membranes, as demonstrated by cell disruption, thereby facilitating the liberation of intracellular contents. This stands in opposition to traditional methods i.e., MC, where plant extract diffusion through glandular walls leads to cell rupture occurring over an extended period [62]. Ultrasonic extraction of *C. fimbriata* enhances the efficacy of DPPH radical scavenging by disrupting the plant cell wall, enhancing mass transfer rates, and optimizing parameters such as temperature and power settings.

These results agree with the findings reported by Noreen [41], who assessed extraction techniques for *C. tuberculata* and found that UAE had greater FRAP activity and a higher total phenolic content

than MAE and orbital shaker extracts.

Their study also revealed a significant correlation between phenolic content and antioxidant capacity, further supporting that UAE enhances the efficiency extraction of bioactive components in *Caralluma* species. Our findings are supported by Poorhashemi [63], who found the antioxidant and FRAP (99.38 mmol/g) assays of *Myristica fragrans* seeds through the optimized conditions of UAE. The observed variations in FRAP activity can also be influenced by factors such as growth conditions, soil composition, seasonal variation, sunlight exposure, and geographical location. Research was done on *Caralluma nilagiriana* in which FTIR confirmed the presence of phenol, alcohol, and terpene group compounds [64]. Another study was performed to evaluate the functional groups. FTIR identified the presence of aliphatic primary amines, carboxylic acids, alcohols, aromatics, aliphatic amines, and alkanes in *Caralluma bicolor* [65]. The different techniques did not affect the functional groups of *Abelmoschus esculentus,* as reported in the literature [66], and these results are comparable to our findings. The existence of O-H groups signifies the hydrophilic characteristic of the pregnane glycosides. Our findings agree with a prior study [67], which elaborated high peaks at 3425.45 (O-H tensile bond), 1575.19 (the CC aromatic rings or alkenes), and 1400.57 (C-H bonds) in *C. tuberculate.* FTIR analysis of *C. fimbriata* confirmed the presence of various functional groups such as phenols, aliphatic primary amines, alkanes, carboxylic acids, nitriles, aromatics, alcohols, aliphatic amines, alkyl halides, and alkynes [65].

Another investigation validated our findings and demonstrated the LC-MS profiles of *C. tuberculate* and *Caralluma europaea* [1,68]. The identification of isomers sharing identical molecular weights was validated by examining their distinctive fragmentation and the principal products ions detected in the MS-MS spectrum, as documented in another study [69]. LC-MS is a robust method for qualitative and quantitative analyses, enabling precise determination of analyte molecular weights, as identification of analytes based on molecular weight exhibits high selectivity. Moreover, the fragmentation profiles detected in the mass spectrum proved to be valuable for characterizing the compounds. Compounds displayed specifically to pregnane glycosides distinct fragment patterns at m/z 962.546, 1186.62, and 1024.56, while copmounds 6,7, and 8 displayed fragment ions at m/z 838.476, 593.275, and 674.41. These ions were ascribed to the loss of hydroxyl groups and hydrogen atoms from the pregnane backbone under positive ionization mode [70]. Additionally, 609.1 was employed for kaempferol-3-O-glucose, and for quercetin-3-O-hexose deoxyhexose [71]. However, there are many unknown compounds that remain undetected. Although LC-MS/MS provided comprehensive qualitative profiling of pregnane glycosides in *C. fimbriata*, the absence of pure reference standards limited quantitative estimation. Preganane glycosides are a diverse group of secondary metabloites characterized by structural heterogeneity and multiple glycosidic substitutions, which hinder standardization and quantitative calibration. Hence, further explorations are required regarding the comprehensive phytochemical profiling of *C. fimbriata*, with a focus on the isolation and purification of individual pregnane glycosides to facilitate quantitative validation and enhance analytical robustness.

5. Conclusions

In this study, we have demonstrated that the UAE approach is the most effective for extracting bioactive components from *C. fimbriata*. According to the DPPH (91.29%) and FRAP (788.32 μmol $FeSO_4$/g) data, the UAE also had higher levels of phenolic and flavonoids, as well as exceptional

antioxidant activity. However, LC-MS/MS identified the presence of significant bioactive substances, including pregnane glycosides and kaempferol. FTIR analysis revealed alignment in the chemical profiles across all extraction techniques. According to morphological research, the UAE preserved cellular integrity, enabling the extraction of high-quality phytochemicals. The UAE has strong industrial process capabilities for functional foods and nutraceutical products, thanks to its high efficiency, cost-effectiveness, and scalability. In the future, researchers should focus on the quantitative analysis, bioavailability, and in vivo effectiveness of *C. fimbriata* extracts derived from UAE, particularly in clinical models of metabolic diseases. Furthermore, incorporating these extracts into advanced delivery systems, such as microencapsulation or nanoemulsions, might enhance stability, targeted delivery, and therapeutic efficacy, thereby supporting their application in functional food and nutraceutical compositions.

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Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

Conflict of interest

The authors have declared no conflicts of interest for this article.

Author contributions

Rimsha Anwar: Conceptualization, methodology, software, validation, formal analysis, investigation, writing-original draft preparation, visualization. Allah Rakha: Conceptualization, resources, supervision, project administration. Imran Pasha: writing- review, data curation, editing, visualization. Muhammad Asghar: writing-review and editing.

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