

A Comparative Study on Wet and Dry extracts of *Caralluma fimbriata* for Phytochemicals and Evaluation of Therapeutic activity

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ABSTRACT: To compare the phytochemicals and evaluate the antioxidant, antidiabetic and antimicrobial activity of different extracts (petroleum ether, ethanol and aqueous) of dry and wet samples of *Caralluma fimbriata* (*C. fimbriata*) is proposed. Phytochemicals are screened in all the extracts by standard methods and total alkaloids, flavonoids, phenols and tannins are quantified by High-Performance Liquid Chromatography (HPLC). The elemental profiling and proximate analysis are carried out to determine the nutritive value of *C. fimbriata*. Further the evaluation of antioxidant activities by 2, 2-diphenyl-picrylhydrazyl (DPPH), 2, 2-azino-bis-3-ethylenebenzothiazoline-6-sulfonic acid (ABTS) radical scavenging activity, Ferric Reducing Ability of Plasma (FRAP), Super Oxide Dismutase (SOD) assay and antidiabetic activity by α -amylase and α glucosidase inhibition are performed. The antimicrobial activity of the extracts is determined *in vitro* by MIC (minimum inhibition Concentration). Results revealed that the phytochemical components tested are present in varying concentrations in all the extracts corresponding to differences in their antioxidant, free radical scavenging activities, antidiabetic and antimicrobial activities with high nutritive value. Therefore, one can conclude that there is a significant correlation between the phytochemicals and activities exhibited by *C. fimbriata*.

The comparative study on wet and dry extracts of *Caralluma fimbriata* for phytochemicals and therapeutic activity evaluation face challenges related to maintaining consistent extraction conditions between wet and dry methods, ensuring accurate quantification of phytochemical compounds, and establishing relevant and reliable therapeutic activity assays. Additionally, variability in the composition of plant material due to environmental factors and batch-to-batch variations could introduce complexity to the study.

The challenges of the study include controlling for variability in plant material, optimizing extraction methods for both wet and dry extracts, and establishing reliable methods for phytochemical analysis and therapeutic activity evaluation.

Keywords: Nutritive value, *Caralluma fimbriata*, Antioxidants, Antimicrobial, α -amylase.

INTRODUCTION

Plant-based medicines and their herbal formulations are resourceful for therapeutic applications that are widespread and used in traditional medicine, as they are easily accessible and non-toxic (Grover *et al.*, 2002). Natural products are the main sources of essential macro elements and micronutrients, and pharmacologically active compounds that are necessary for the healthy development of the body and the

maintenance of the well-being of human health (Alzahrani, 2016). The best alternative therapies for treating ailments are always thought to be medicinal plants and their extracts as they have high antioxidant content to forage free radicals & restore damaged cells thereby slowing down oxidative stress in the body.

In this context, *Caralluma fimbriata* (*C. fimbriata*) an edible, sturdy, succulent shrub with cactus- alike leaves used by Indian tribes for centuries as an appetite suppressant and thirst quencher and called as 'Famine

food of India' is used (Bouarab-Chibane *et al.*, 2019; Jayawardena *et al.*, 2021; Gujjala *et al.*, 2017; Asmi *et al.*, 2017; Lakshmi *et al.*, 2014). The present study is performed to screen for the effective phytochemicals present in different extracts (petroleum ether, ethanol and aqueous) of dry and wet samples of *C. fimbriata* in terms of quantitatively and qualitatively as shown in Fig. 1, where one see both dry and wet *C. fimbriata*. The main objective is to determine its nutritive value and potentiality towards antioxidant, antimicrobial and anti-diabetic activities.



Fig. 1. Dry and wet samples of *C. fimbriata*.

PROPOSED METHOD

A. Plant Material and Preparation of Extract

We collect fresh plant from Chitradurga district, washed with plain water and shade dried, and powdered using a blender. The initial weight of the plant is 3.7 kg which yielded 230 g of powder which is used for further estimations. The powdered and filtered plant sample of 100 g are extracted successively with petroleum ether, ethanol and aqueous using Soxhlet apparatus at 55-85 °C for 8-10 h in order to extract the polar and non-polar compounds. The solvents of the respective extracts were reduced under room temperature and stored at 4 °C for further use. The dried plant extracts are then redissolved in dimethyl sulfoxide and to get the solution of 10 mg/10 mL for each extract which is subjected to analysis of *in vitro* activities.

B. Qualitative and quantitative phytochemical screening

Preliminary qualitative phytochemical analysis was performed to identify the nature of phytoconstituents in a dry and wet extract (Pet ether, Ethanol, and aqueous extraction) of *C. fimbriata* as described by (Harborne, 1998; Sazada *et al.*, 2009).

Quantitative analysis of phytochemical compounds was made on the C18 column in isocratic mode with the mobile phase methanol and water in the ratio 7:3 at a flow rate of 1 mL/min. The standard solution with the concentration of 0.4 mg/mL and sample (10 mg/mL) were dissolved in the mobile phase and 20 µL was injected and the elution was monitored at 230 nm. The amounts of alkaloids, tannins, flavonoids, and phenols present in the sample were estimated using the formula (Lone Shabir *et al.*, 2015). As depicted in Fig. 2.

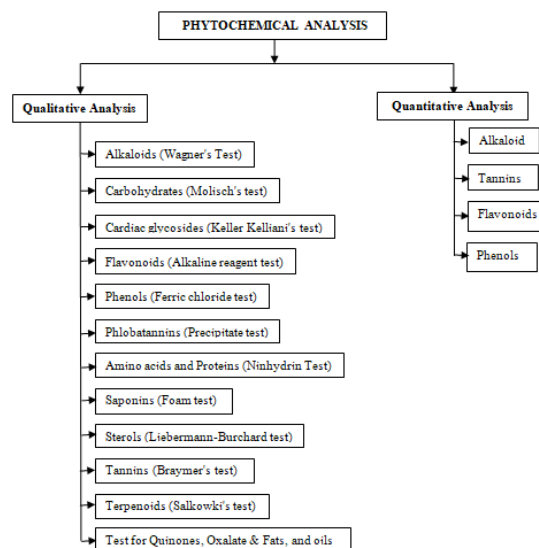


Fig. 2. Qualitative and Quantitative analysis of Phytochemical compounds Phytochemical screening.

C. Elemental (ICP-OES analysis) and Proximate Analysis

Dry and wet extracts from *C. fimbriata* are analyzed for elemental detection using ICP- OES technique. This is because the system is fast, and it has the ability to detect elements in extreme minute concentrations as shown in Fig. 3. Elements such as Calcium (Ca), Potassium (K), Magnesium (Mg), Phosphorus (P), Copper (Cu), Zinc (Zn), and Sodium (Na), Iron (Fe), Manganese (Mg), Chromium (Cr) are estimated. The required amount of quantity of the sample is collected in the vessel and the samples are expose to 15 minutes with 10ml of Concentrated HNO₃ before sealing vessels. The vessel is microwaved and cooled, and the final volume is made up to 25ml with water. The above solution is aspirated into ICP-OES and quantified. The calibration standards are prepared by diluting the stock multi-elemental standard solution (1000mg/l) in nitric acid. The proximate analysis (moisture, fiber, ash, crude fats, proteins and carbohydrates) and energy content of the samples are determined, using standard methods (Nanda *et al.*, 2003). The quantitative evaluation of nutritional value and organic content is performed to measure the actual percentage proximate composition. The examiner parameters are total protein, fat total carbohydrate crude fiber acid digest fiber and ash value (Shukla *et al.*, 2012).

D. Therapeutic Activities of *C. fimbriata*

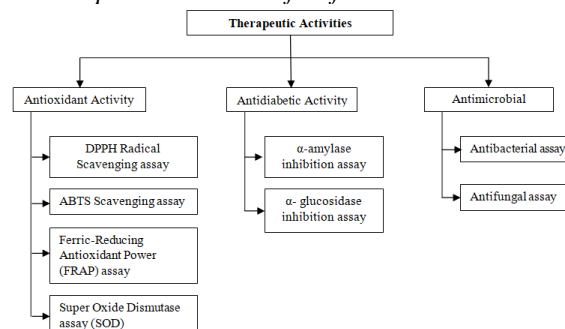


Fig. 3. Block diagram of Therapeutic Activities.

(i) Determination of Antioxidant Activity

DPPH Radical Scavenging Activity Assay: The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is performed to evaluate the free radical scavenging activity of the extract. DPPH solution (0.004%) is prepared in 100 mL of methanol. 200 μ L of the sample and the standard are mixed at different concentration and incubated in dark for 15 minutes at room temperature (Blois 1958). All the measurements are performed in triplicate.

ABTS Scavenging Assay: The 2,2'-azino-bis-3-ethylbenzotiazolin-6-sulfonic acid (ABTS) scavenging assay is used based on radical cation decolorization method. Reduction of ABTS and radicals depend on plant extracts antioxidant capacity (electron donating capacity) which leads to decolorization of the radical. ABTS and Potassium per sulphate is prepared in deionized water and are incubated in dark for 24–48 h. The absorbance is read at 745 nm (Arnao *et al.*, 2001). The percentage inhibition of ABTS is estimated. All the measurements are performed in triplicate. **Ferric-Reducing Antioxidant Power (FRAP) Assay:** The antioxidant activity of the extract is determined through standard Fe³⁺ reducing power assay (Benzie and Strain 1999). Both extracts and standard (ascorbic acid) (0.5 mL) are mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 1% 2.5 mL of potassium ferricyanide. The incubation of reaction mixture is done at 50°C for 30 minutes. Then, 2.5 mL (10%) of trichloroacetic acid is added, centrifuged at 13,000 rpm for 10 minutes. Finally, 2.5 mL supernatant is separated and mixed with 2.5 mL distilled water and 0.5 mL (0.1%) of Ferric chloride. The absorbance is read at 700 nm. The reducing power of the extract is expressed as optical density of tested extract.

SOD Activity: The procedure adopted is that of Beauchamp and Fridovich (1971). The reaction mixture composed of 2.6 μ M riboflavin, 13 mM methionine, 75 μ M nitrobluetetrazolium (NBT), 0.1 mM ethylene diamine tetra acetic acid (EDTA), phosphate buffer saline (PBS- pH 7.4) and various concentrations of test samples. The sample is randomly placed in a light storage box and replaced, randomly, every 5 minutes for 15-minutes. During the light illumination, NBT is reduced to blue formazan formation that is measured by the absorbance at 560 nm. The inhibition of blue formazan formation is taken as a superoxide quenching activity.

(ii) Determination of Anti-Diabetic Activity

Invitro α -amylase inhibition assay: The α -amylase inhibitory effect of different extracts and its solvent fractions is measured by employing the chromogenic 3, 5-Dinitrosalicylic acid method. The total reaction mixture containing 1.4 mL of 0.05M sodium phosphate buffer (pH 6.9), 50 μ L of α -amylase and acarbose (used as a positive control) or test samples (the crude extract and its solvent partitions) at concentrations of 100, 200, 300, 400 and 500 μ g/mL are at 37°C for 10 minutes. After pre-incubation, 500 μ L of 1% (w/v) starch solution dissolved in the above mentioned buffer is added to each tube and incubated for 15 minutes at

37°C. One milliliter of DNS reagent is added to the reaction tube and it is boiled in water bath for 5 minutes to stop the reaction. It is cooled and absorbance is measured at 540 nm. The inhibitory activity of the samples and the standard drug (acarbose) is calculated in comparison with the negative control (100% enzyme activity).

Invitro α -glycosidase inhibition assay: The inhibitory activity is determined by incubating 1 mL of starch solution (2% w/v maltose) with 0.2 M tris buffer (pH 8) and various concentration of sample (100-500 mg/mL). The reaction mixture is incubated at 37°C for 10 minutes. The reaction is initiated by adding 1 mL of α -glucosidase enzyme (1 U/ml) to it and incubation at 35°C for 40 minutes. Then the reaction is terminated by the addition of 2 mL of 6 N HCl. The intensity of the color is measured at 540 nm and IC₅₀ values are determined.

(iii) Determination of Antimicrobial Activity by Well Diffusion Method

The wet and dry sample extracts (Petroleum ether, Ethanol, Aqueous,) are tested in triplicates for their MIC property against selected organisms (*Aspergillus niger*, *Candida albicans*, *Bacillus cereus*, *Staphylococcus aureus*, *Streptococcus mutans*, *E. coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*). Potato Dextrose Broth and Luria Bertani broth are prepared for culturing fungal and bacterial species respectively. 10 mg of samples are dissolved in 1 mL of dimethyl sulfoxide and different aliquots are prepared by pipetting 10 μ L (100 μ g), 20 μ L (200 μ g), 30 μ L (300 μ g), 40 μ L (400 μ g) and the final volume is made upto 50 μ L by adding DMSO. Media is prepared and inoculated with 200 μ L inoculum of fungal and bacterial strains. Five wells measuring 0.6 cm was is in each plate using the borer and 50 μ L of prepared sample containing 100 μ g, 200 μ g, 300 μ g and 400 μ g are loaded into the respective wells and 50 μ L of DMSO is loaded in the middle well as control blank. The bacterial plates incubated at 37°C for 24 h and Fungal plates incubated at 25°C for 72 h. Later, zone of inhibition is recorded in mm (Millimeter).

E. Statistical Analysis

The mean of the replicate values is calculated after each experiment and runs in triplicate on at least three separate occasions. Values are expressed in terms of mean \pm SD. Students t-test is used for the statistical analysis of the data, and comparisons between the control and treated groups.

RESULTS AND DISCUSSION

The phytochemical screening of wet and dry extract of *C. fimbriata* (petroleum ether, ethanol, and aqueous) shows the presence of phytochemicals like alkaloids, flavonoids, phenols, tannins, terpenoids, oxalates, saponins, sterols and amino acids. But Phlobatannins, sterols, quinones and oxalates are absent in all extracts as shown in the Table 1.

Table 1: Qualitative phytochemical screening of dry and wet extracts of *C. fimbriata*.

Sr. No.	Components	Dry extract/wet extract		
		Pet. ether	Ethanol	Aqueous
1.	Alkaloids	+/+	+/+	-/+
2.	Carbohydrates	+/-	+/+	-/+
3.	Cardiac glycosides	+/+	+/+	-/-
4.	Flavonoids	+/-	+/-	+/+
5.	Phenols	+/+	+/+	+/-
6.	Phlobatannins	-/-	-/-	-/-
7.	Amino acids/proteins	+/-	+/+	+/+
8.	Saponins	+/+	+/-	-/+
9.	Sterols	-/-	-/-	-/-
10.	Tannins	+/-	+/+	+/-
11.	Terpenoids	+/+	+/+	-/-
12.	Quinones	-/-	-/-	-/-
13.	Oxalates	-/-	-/-	-/-
14.	Oils and fats	+/+	+/+	+/+

+: Presence; -: Absence

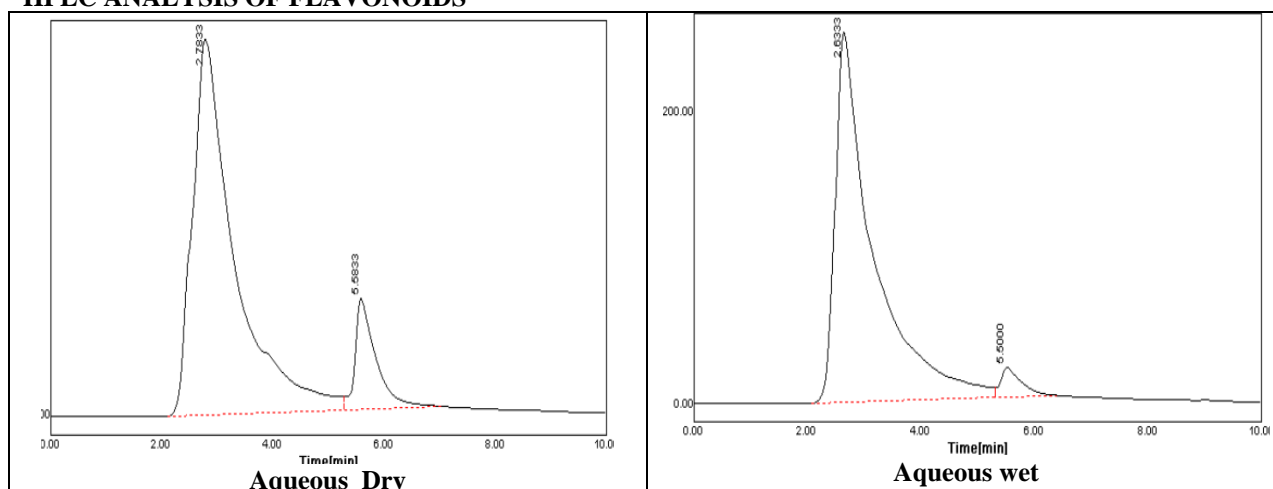
Alkaloids, flavonoids, phenols, and tannins are discovered to be the most potent bioactive chemicals, and their quantities are thus quantified by HPLC. The results are shown in Table 2. The dry extracts shows the

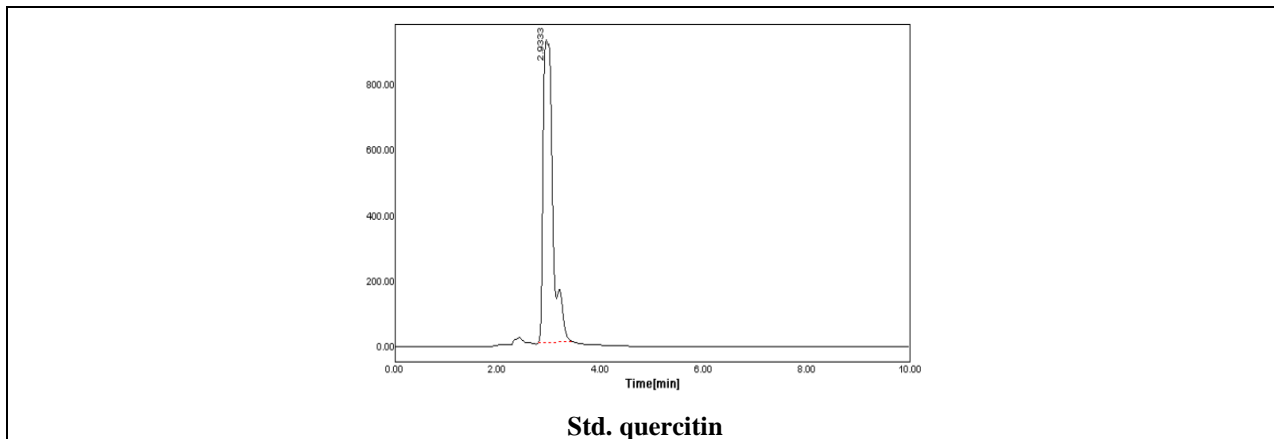
presence of higher amounts of phytochemicals as compared to the wet extracts. It may be hypothesized that these phytochemicals are the main source for antimicrobial and antioxidant property.

Table 2: Quantitative phytochemical screening of dry and wet extracts of *C. fimbriata*- HPLC analysis of flavonoids, alkaloids, phenols and tannins.

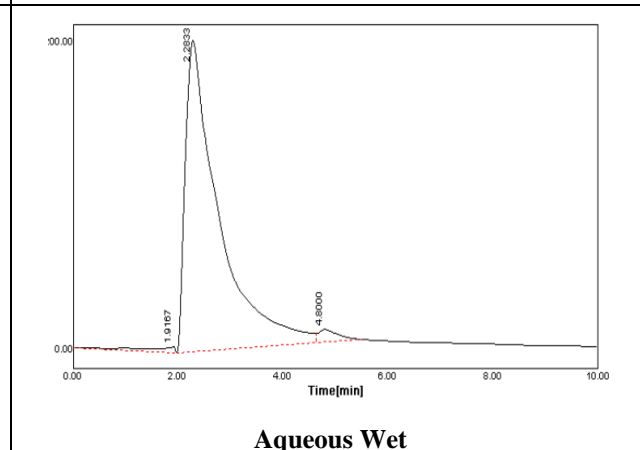
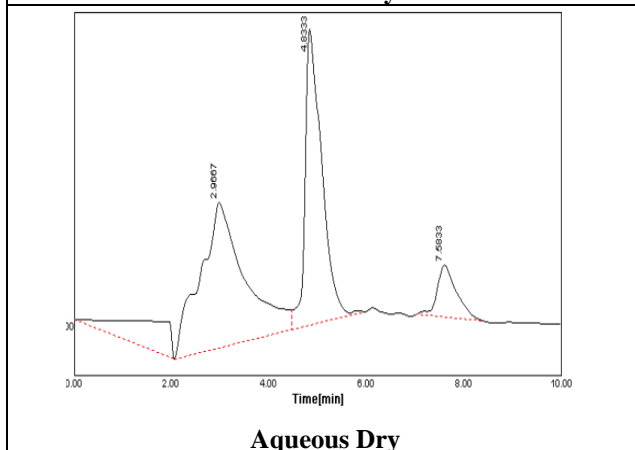
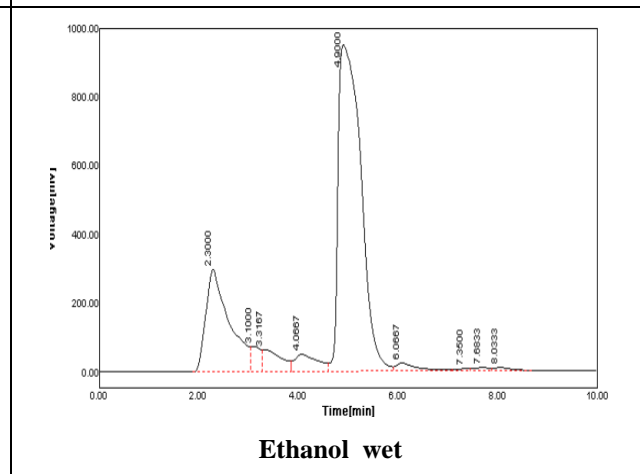
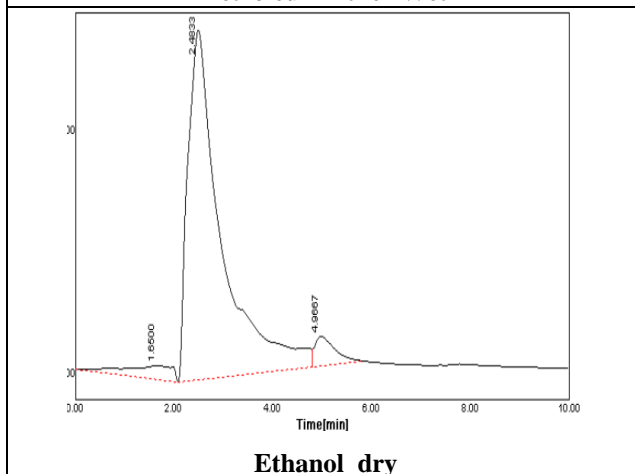
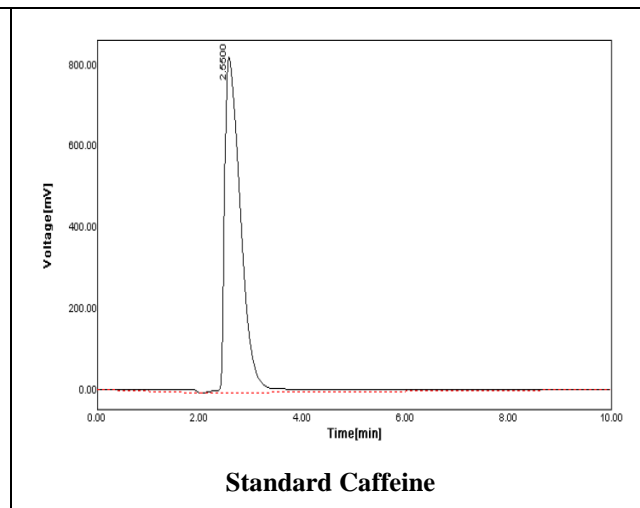
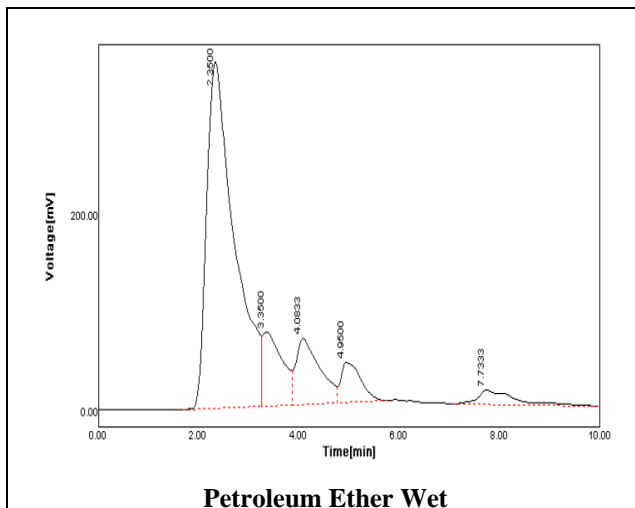
Compounds	Solvents	Wet sample Amount in mg/g	Dry sample Amount in mg/g
1. Flavonoids	Petroleum Ether	-	0.464
	Ethanol	-	18.92
	Aqueous	38.544	24.106
2. Alkaloids	Petroleum Ether	28.938	-
	Ethanol	20.84	21.15
	Aqueous	19.35	28.84
3. Phenols	Petroleum Ether	19.601	1.192
	Ethanol	35.34	37.69
	Aqueous	-	33.31
4. Tannins	Petroleum Ether	-	13.562
	Ethanol	68.468	74.54
	Aqueous	-	146.31

HPLC ANALYSIS OF FLAVONOIDS

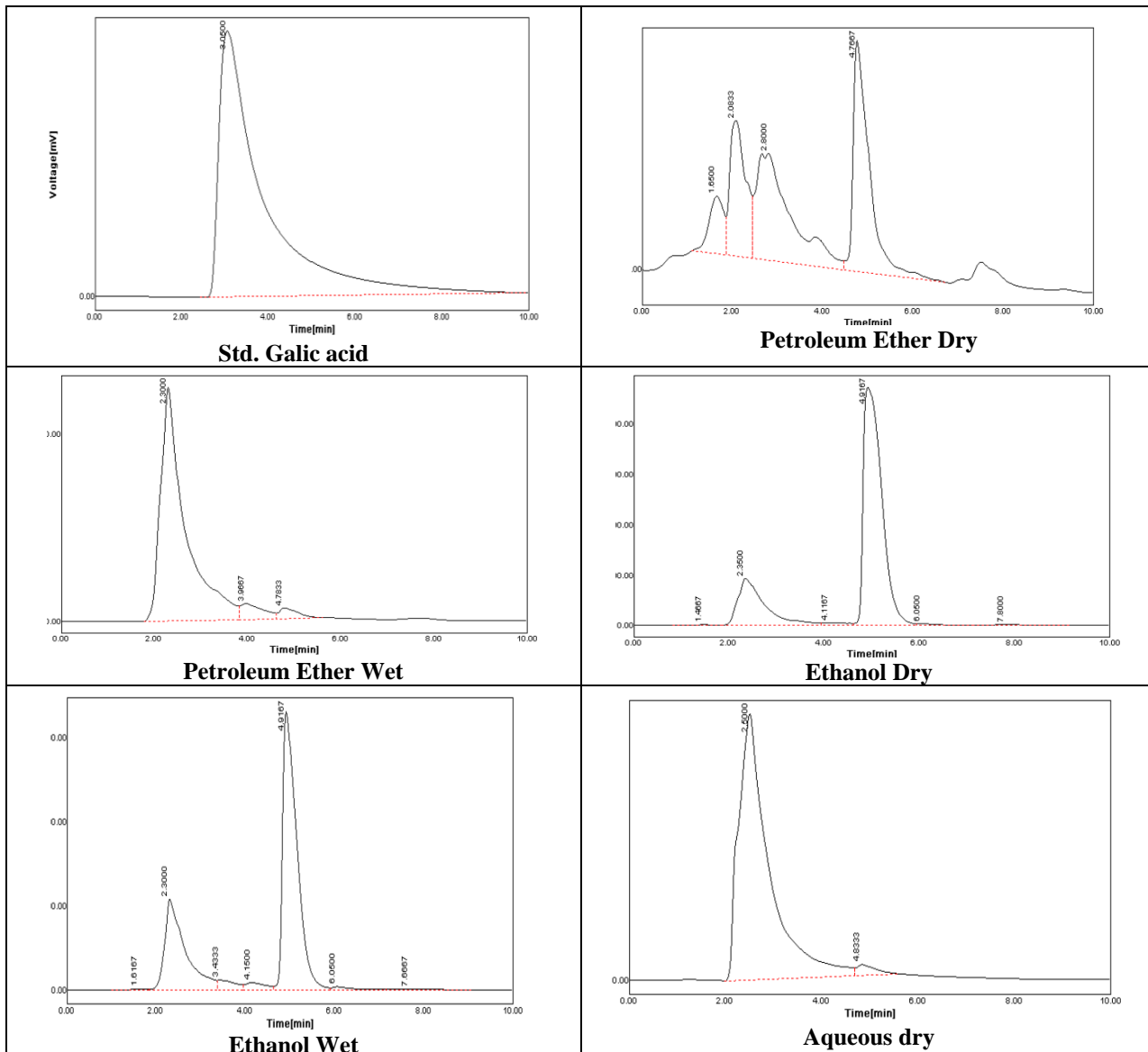




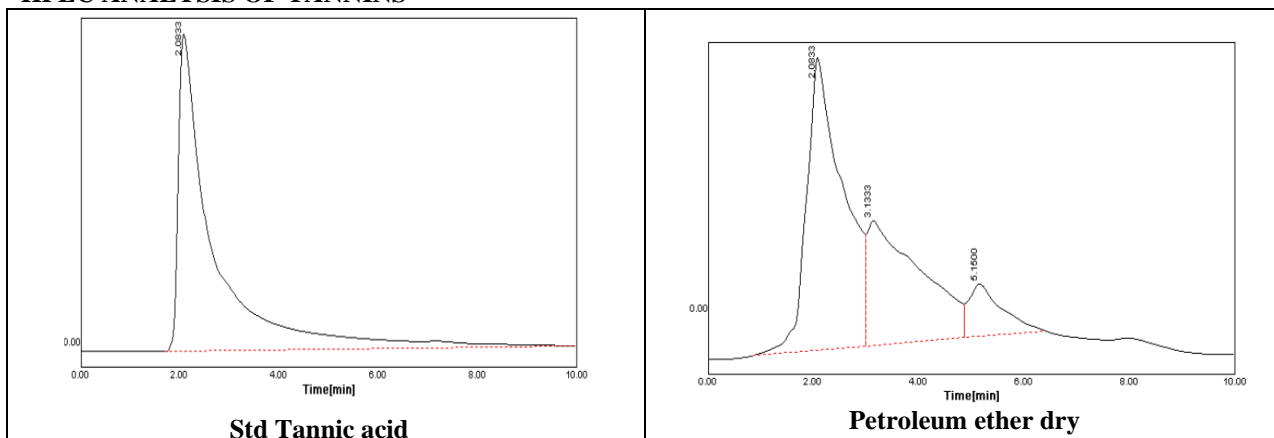
HPLC ANALYSIS OF ALKALOIDS



HPLC ANALYSIS OF PHENOLS



HPLC ANALYSIS OF TANNINS



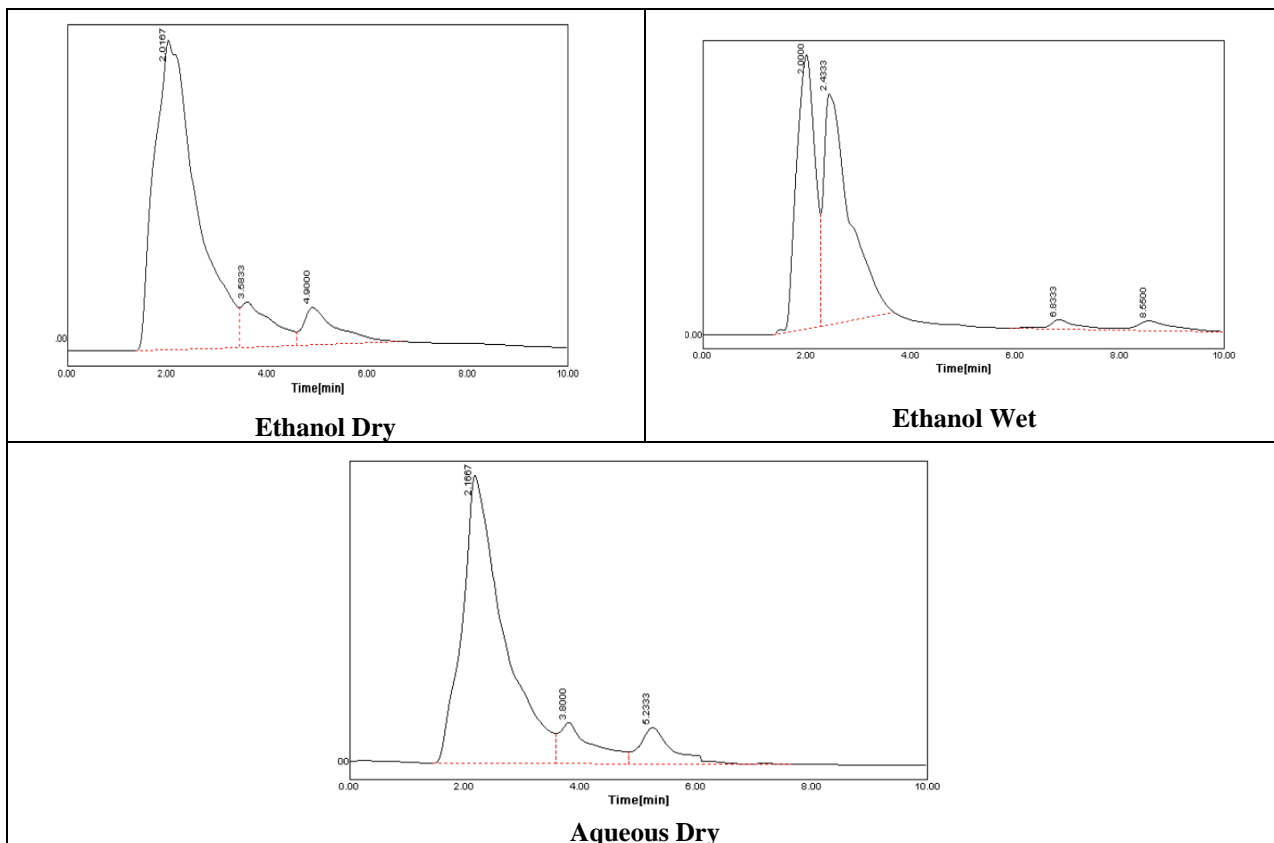


Fig. 5. HPLC Chromatogram of dry and wet extracts of *C. fimbriata*- Quantitative analysis of flavonoids, alkaloids, phenols and tannins.

The results of proximate analysis are depicted in Table 3 and moisture content is in the range of 50-70%, *C. fimbriata* appeared to be a good supplement for protein(37.4%), carbohydrate(89.6%) and fat(15.9%), which are major building blocks indicative of higher nutritive value as calculated. Nutritive value is expressed in kilo calories per 100 g of dry weight of *C. fimbriata*, which is calculated using the formula and shows high nutritive value of *C. fimbriata* (651.1k.cal/100g) with high amount of proteins, carbohydrates, cardiac glycosides, oils and fats. Nutritive Value (NV) = (4x% of protein+9x% of crude fat+4x% of total Carbohydrate)= (4x37.4) + (9x 15.9) + (4x89.6)= 149.6 + 143.1 + 358.4= 651.1 kcal /100 g.

Mineral content is a measure of amount of specific inorganic components such as calcium, sodium, phosphorous, magnesium, zinc and copper present within the sample. Elemental analysis of *C. fimbriata* by ICP-OES technique confirmed the presence of pharmaceutically active, major, minor and trace elements. The results of elemental analysis are illustrated in Table 4, which reveal the presence of Calcium (452ppm), Potassium (10.7ppm), Magnesium (686ppm), Phosphorus (7.6ppm), Copper (3.1ppm), Zinc (174ppm) and Sodium (22.6ppm), Iron (17ppm), Manganese (23ppm), Chromium (0.03ppm) as compared to the standards. The results implicate those increasing concentrations of biologically essential elements as against the credible biologically essential elements.

Table 3: Elemental analysis of dry and wet extracts of *C. fimbriata*.

Sr. No.	Metal ions	Standard Concentration in ppm	Concentration in ppm
1.	Iron	0.5-50	17
2.	Calcium	360-800	452
3.	Magnesium	400-600	686
4.	Sodium	4-5	22.6
5.	Potassium	0.10-1	10.7
6.	Phosphorus	4-5	7.6
7.	Zinc	15-20	174
8.	Copper	1-3	3.1
9.	Manganese	10-20	23
10.	Chromium	-	0.03

Table 4: Proximate analysis of dry and wet extracts of *C. fimbriata*.

Sr. No.	Parameters	Percentage
1.	Moisture content	50 – 70
2.	Ash content	1.96
3.	Crude fiber	5.6
4.	Crude fat	15.9
5.	Acid Digest fiber	11.56
6.	Protein	37.4
7.	Carbohydrates	89.6

Nutritive value is calculated in kcal/100 g of *C. fimbriata*.

The extracts antioxidant activity is evaluated using DPPH which is affected by antioxidants due to its ability to donate electrons. In order to prevent the damaging effects of free radicals, radical scavenging is crucial. The DPPH assay also shows that extracts include phenolic and flavonoid chemicals. Our findings shows that all extracts have significant antioxidant activity, along with positive control and value results. Table 5a and 5b display DPPH activity values with the IC₅₀ values of 1.471±0.34, 3.998±0.42 and 1.302±0.22 mg respectively in Petroleum ether, ethanolic and aqueous samples of wet extracts. However, the percentage inhibition of positive control (ascorbic acid) is higher than that of extracts with percentage inhibition of 97.27 % at 300 µg/mL and IC₅₀ value of 74.16 ± 0.23 µg. The reaction between ABTS and potassium persulphate generate ABTS cations scavenging activity, which indicate the peculiar donating or reducing competences. Extracts showed an inhibition at the concentration of samples used in a dose dependent manner as depicted in Table 6a and 6b. Lowest IC₅₀ value is found in aqueous sample of the dry extract and is found to be 325.41±0.23 mg. The higher scavenging power of extracts correlate with the higher amounts of flavonoids and phenols. This further proved the *C. fimbriata* potential to scavenge the free radicals

therefore, *C. fimbriata* could be a better choice in preventing chain reaction in lipid peroxidation and could also serve as potential nutraceuticals. Flavonoids and phenols in the extracts may be associated to antioxidant action. On the other hand, the antioxidant activity is also evaluated by its reducing power competence. Existence of reductants in extracts leads to terminates free radicals chain reaction by donating Hydrogen and hence, extract reduce Fe³⁺ to Fe²⁺. The formation of blue color indicates the reducing activity of extracts and absorbance is directly proportional to reducing capability of the extracts. In current study, all the extracts show a significant electron-donating activity by reducing Fe³⁺ to Fe²⁺ in a concentration-dependent manner as depicted in the Table 7a and b. Results also prove that the extracts have the potential to react with free radicals and terminate them into non-reactive stable form as depicted and the lowest IC₅₀(71.408±0.26 mg) is observed in aqueous fraction of dry sample. Table 8a and 8b displays the various levels of SOD activity that the extracts exhibited. The results shows that when compared to other extracts, *C. fimbriata* wet extract have a substantially higher antioxidant capacity with the lowest IC₅₀ of 71.71±0.57 and 74.55±0.22 µg in Petroleum ether and ethanolic fractions respectively as compared to other.

Table 5a: Shows the absorbance and percentage inhibition of wet and dry extracts of *C. fimbriata* as determined by SOD activity.

Concentration of sample in µg	Percentage inhibition		
	Pet. ether	Ethanol	Aqueous
100	52.33/10.28	48.59/42.05	22.42/32.71
200	59.81/19.62	66.35/45.79	30.84/44.85
300	71.02/40.18	73.83/64.48	50.46/55.14
400	74.76/54.20	83.17/74.76	58.87/67.28
500	85.98/73.83	89.71/85.98	78.50/80.37

Two line graphs for Absorbance at 560 nm, Percentage inhibition. Two separate graphs.

Table 5b: Shows the IC₅₀ values of wet and dry extracts of *C. fimbriata* as determined by SOD activity.

Sample	IC ₅₀ value (µg) of Wet/dry sample
Petroleum ether extract	71.71±0.57***/364.14±2.51***
Ethanol extract	74.55±0.22***/192.097±0.31**
Aqueous extract	312.69±0.45***/248.34±0.12***

Values are expressed as mean ±SD, Students 't' test - Comparisons are made with the control
***P< 0.001; **P< 0.01; *P< 0.05; NS: Non significant.

Table 6a: Shows the results of FRAP assay of wet and dry extracts of *C. fimbriata*.

Concentration of sample (µg)	Standard	Wet/dry extract		
	Gallic acid	Pet. Ether	Ethanol	Aqueous
100	0.09	0.322/0.387	0.168/0.353	0.305/0.353
200	0.119	0.381/0.430	0.264/0.384	0.338/0.344
300	0.224	0.411/0.439	0.268/0.424	0.361/0.394
400	0.244	0.421/0.444	0.273/0.475	0.375/0.420
500	0.313	0.455/0.462	0.293/0.489	0.389/0.433

Table 6b: Shows the IC₅₀ values of FRAP assay of wet and dry extracts of *C. fimbriata*.

Samples	IC ₅₀ value (mg) of Wet/ dry sample
Gallic acid	83.288
Petroleum ether	165.646±0.22***/248.084±0.19***
Ethanol	166.081±0.62**/124.209±0.45**
Aqueous	248.539±0.22***/71.408±0.26***

Values are expressed as mean ±SD, Students 't' test - Comparisons are made with the control
 ***P< 0.001; **P< 0.01; *P< 0.05; NS: Non significant

Table 7a: Shows the absorbance and percentage inhibition of wet and dry extracts of *C. fimbriata* as determined by DPPH assay.

Concentration of sample(µg)	Standard	Percentage inhibition		
	Gallic acid	Pet. ether	Ethanol	Aqueous
100	0.857	0.7/0.4	11.5/5.1	7.9/6.2
200	0.753	3.3/2.0	12.4/7.5	11.4/8.0
300	0.657	4.5/2.3	13.3/9.1	15.2/9.3
400	0.544	10.3/3.4	14.1/10.8	18.9/12.8
500	0.429	15.6/6.6	15.6/13.8	21.6/16.6

Table 7b: Shows the IC₅₀ values of wet and dry extracts of *C. fimbriata* as determined by DPPH assay.

Sample	IC ₅₀ value (mg)
Wet sample/ dry sample	
Petroleum ether extract	1.471±0.34**/3.710±0.11***
Ethanol extract	3.998±0.42**/2.268±0.24***
Aqueous extract	1.302±0.22***/1.839±0.28***

Values are expressed as mean ±SD, Students 't' test - Comparisons are made with the control
 ***P< 0.001; **P< 0.01; *P< 0.05; NS: Non significant.

Table 8a: Shows the absorbance and percentage inhibition of wet and dry extracts of *C. fimbriata* as determined by ABTS assay.

Concentration of sample(µg)	Standard	Percentage inhibition		
	Gallic acid	Pet. ether	Ethanol	Aqueous
100	0.710	18.1	13.2	25.1
200	0.516	29.1	19.7	39.7
300	0.387	34.9	20.9	47.5
400	0.234	47.4	25.1	56.2
500	0.123	60.4	29.1	68.4

Table 8b: Shows the IC₅₀ values of wet and dry extracts of *C. fimbriata* as determined by ABTS assay.

Sample	IC ₅₀ value (µg)
Wet sample/ Dry sample	
Petroleum ether extract	416.812±0.63*/949.89±0.20***
Ethanol extract	579.31±0.64*/1063.44±0.43**
Aqueous extract	292.94±0.22**/325.41±0.23***

Values are expressed as mean ±SD, Students 't' test - Comparisons are made with the control
 ***P< 0.001; **P< 0.01; *P< 0.05; NS: Non significant.

As reported in Table 9a and b, the extracts of different solvent fractions show concentration-dependent *in vitro* α-amylase inhibitory activities with the highest percentage inhibition exhibited by the ethanolic fractions (dry and wet). Similarly, the standard drug, acarbose, at the medium and highest doses show roughly 100% enzyme inhibition. The ethanolic fraction (dry and wet) exhibited the highest α-amylase inhibitory potential with the lowest IC₅₀ value of

333.48±1.03 µg and 391±1.42 µg. As shown in the Table 10a and Table 10b, the extracts of different solvent fractions show concentration-dependent *in vitro* α-glucosidase inhibitory activities with the highest percentage inhibition exhibited by the ethanolic fractions. The wet ethanolic fraction is found to be more effective with an IC₅₀ value of 137.37±1.27 µg as compared to acarbose which is used as a positive control.

Table 9a: Shows the absorbance and the percentage inhibition of wet and dry extracts of *C. fimbriata* at concentrations from 100-500 µg as obtained by α-amylase inhibition assay.

Concentration of sample(µg)	Percentage inhibition		
	Pet. ether	Ethanol	Aqueous
100	34.22/27.83	35.87/41.85	16.49/23.09
200	37.52/30.10	37.93/46.18	37.11/40.0
300	37.73/40.58	38.35/47.21	39.58/40.41
400	42.06/42.26	52.16/50.92	40.41/41.03
500	43.50/49.07	58.55/57.73	45.97/43.09

Table 9b: Shows the IC₅₀ values of wet and dry extracts of *C. fimbriata* as determined by α-amylase inhibition assay.

Samples	IC ₅₀ value (µg)
Wet/dry sample	
Petroleum ether	775.93±1.32***/520.58±1.83***
Ethanol	391.023±1.42***/333.48±1.03**
Aqueous	525.939±2.02**/604.512±1.22***

Values are expressed as mean ±SD, Students 't' test - Comparisons are made with the control

***P< 0.001; **P< 0.01; *P< 0.05; NS: Non significant.

Table 10a: Shows the absorbance and the percentage inhibition of wet and dry extracts of *C. fimbriata* concentrations from 100-500 µg as obtained by α-glucosidase inhibition assay.

Concentration of sample(µg)	Percentage inhibition		
	Pet. ether	Ethanol	Aqueous
100	33.23/5.58	47.20/39.26	1.76/31.03
200	45.0/31.47	55/53.67	13.38/55.44
300	48.97/55.14	58.82/65.29	35/59.11
400	55.0/61.61	67.35/69.26	53.08/59.85
500	64.11/67.2	72.05/70	63.38/65.88

Table 10b: Shows the IC₅₀ values of wet and dry extracts of *C. fimbriata* as determined by α-glycosidase inhibition assay.

Samples	IC ₅₀ value (µg)
Wet /dry sample	
Petroleum ether	310.11±1.42***/337.77±2.33**
Ethanol	137.37±1.27**/176.71±1.2***
Aqueous	402.46±1.21**/242.52±1.39***

Values are expressed as mean ±SD, Students 't' test - Comparisons are made with the control

***P< 0.001; **P< 0.01; *P< 0.05; NS: Non significant.

The data obtained, through the determination of MIC, from the association of antibiotics with extracts to observe any synergistic effects are presented in Table 11a. The zone of inhibition is calculated, and the results revealed variability in the inhibitory concentrations of each extract for given bacteria and fungi. The extracts show activities in the range (concentrations) above 100 µg/mL. Wet aqueous extract show antimicrobial activity against *Candida albicans*, *Salmonella mutants* and *Salmonella typhimurium* at a concentration ranging above 200 µg/mL also wet ethanolic extract show

antimicrobial activity only against *Salmonella typhimurium* above 100 µg/mL. Dry ethanolic extract antimicrobial activity is observed against *Salmonella typhimurium* and *Salmonella mutants* at concentration 100 and 200 µg/mL respectively. The results obtained are compared to the standards used as depicted in the Table 11b. The activity exhibited against the *Salmonella* species in particular may be correlated with the non-mutagenic potential of *C. fimbriata* which needs to be studied in detail.

Table 11a: Table depicts the Zones of Inhibition (mm) of wet and dry extracts of *C. fimbriata* against the microorganism.

Organism	Aqueous Extract				Ethanol Extract				Petroleum ether Extract			
	Dry/ Wet				Dry/ Wet				Dry/ Wet			
	100µg	200µg	300µg	400µg	100µg	200µg	300µg	400µg	100µg	200µg	300µg	400µg
<i>A. niger</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>C. albicans</i>	-	-/10	-/11	-/12	-	-	-	-	-	-	-	-
<i>B. cereus</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>S. aureus</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>S. mutans</i>	-	-/10	-/11	-/12	-	10/-	13/-	14/-	-	-	-	-
<i>E. coli</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>P. aeruginosa</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>S. typhi</i>	-	-/10	13/11	14/13	11/10	12/12	14/14	16/15	10/-	11/-	12/11	13/12

Table 11b: Table depicts the Zones of Inhibition (mm) of standards used against the microorganism.

Organism	Zone of Inhibition(mm) of standards(tetracyclin)			
	100µg	200µg	300µg	400µg
<i>A. niger</i>	15	18	20	24
<i>C. albicans</i>	25	30	30	32
<i>B. cereus</i>	27	29	30	31
<i>S. aureus</i>	20	22	24	26
<i>S. mutans</i>	28	30	31	34
<i>E. coli</i>	29	31	32	34
<i>P. aeruginosa</i>	30	32	34	36
<i>S. typhi</i>	30	31	32	34

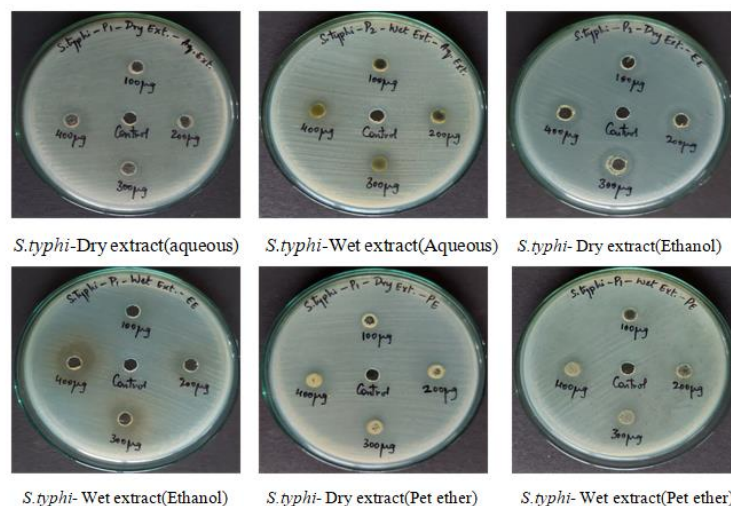


Fig. 4. Zone of inhibition.

The Results obtained from the qualitative phytochemical screening show the presence of Alkaloids, Flavonoids, Phenols, Tannins, Quinones, Terpenoids, Oxcaletes, Saponins, Sterols and Amino acids which further paved the way for quantitative phytochemical analysis for selected phytochemicals. Studies confirmed the identified phytochemicals that is alkaloids, flavonoids, tannins and phenols to be the bioactive compounds and presence of these phytochemicals contribute to medicinal properties to the *C. fimbriata*. Flavonoids and phenolic chemicals, which are naturally occurring antioxidants in *C. fimbriata*, play a significant role and are related to all of the plant's functions. Due to their capacity to alter how the body responds to allergens, viruses, and cancer-causing agents, flavonoids have been referred to as nature's biological response modifiers. They have been shown to have anti-inflammatory, anti-allergic, and anti-cancer properties (Yamamoto and Gaynor 2001). The amounts of flavonoids that were analysed are associated with biological processes such defence against tumours, free radicals, platelet aggregation, bacteria, ulcers, hepatotoxins, and viruses. They are strong, water-soluble antioxidants and free radical scavengers that guard against several stages of carcinogenesis, prevent oxidative cell damage, and fight cancer (Okwu and Morah 2004).

Phenolic compounds are reported to serve as antioxidants, and exhibit a wide range spectrum of medicinal properties such as anti-cancer, anti-inflammatory and diabetes (Nagavani *et al.*, 2010). Phenolic contents in extracts indicate that *C. fimbriata*

is a good source of phenols and can be used for reducing blood pressure, lowering of cancer and cardiovascular diseases, for level free radical adsorption and neutralization and anticancer agents. These natural phenolics in *C. fimbriata* have the potential for application in food systems to maintain food quality. Consumption of approximately 1000 mg GAE/day of total phenolic compounds is recommended (Scalbert and Williamson 2000). According to reports, phenolic compounds have anti-inflammatory, anti-cancer, and diabetes-fighting capabilities in addition to acting as antioxidants (Ghasemzadeh and Ghasemzadeh 2011). The presence of phenols in extracts suggests that *C. fimbriata* is a good source of phenols, which can be utilised as anticancer agents, to lessen the risk of cancer and cardiovascular illnesses, as well as to lower blood pressure. These natural phenolics found in *C. fimbriata* may be used in food systems to preserve food quality. It is advocated for a daily intake of total phenolic compounds of about 1000 mg GAE (Scalbert and Williamson 2000). Therefore, consuming 100g of *C. fimbriata* a day can help achieve the normal daily intake of total phenols. Proximate test results also indicated the presence of carbohydrate and fats in the extract. The principal nutritional role of carbohydrate is the production of energy and hence *C. fimbriata* could be recommended as nutraceutical.

In addition to providing a larger portion of energy, carbohydrates are essential for the immune system, fertility, pathogenesis, blood coagulation, and development (Madziga *et al.*, 2010). *C. fimbriata* with its increased proportions of fats, carbs, and proteins, has

the potential to be a food supplement, energy beverage, and nutraceutical. According to the research, *C. fimbriata* has the potential to be a nutraceutical, energy drink, and food supplement. Food's source of amino acids is protein. The figure found in this study is more ($p > 0.05$) than the Food and Agriculture Organization's suggested range of 12–15%. The high protein content proved efficiency of *C. fimbriata* as a complete source of protein in the meal.

Mineral elements not only serve as nutritional sources for both plant and animals. The heavy elements and essential trace elements are known to influence various body functions based on their concentrations. Elements such as K, Na, Ca, Mg, Mn and Fe play important roles and are critical in the regulation of a number of cell membrane, permeability, muscles contraction, heart function, blood clotting, protein and red blood synthesis (Hendricks, 2002). The presence of considerable amount of magnesium, calcium, phosphorus is an indication that it can supply some essential minerals needed for healthy life. Calcium, magnesium further adds on to the control of hyperglycaemic cases through the enhancement of insulin secretion. Magnesium is vital in human nutrition due to its function as a cofactor for more than 300 essential enzyme systems, its requirement for increased DNA and RNA synthesis, energy generation as well as glycolysis and has also been shown to be essential for mitochondria to carry out oxidative phosphorylation (Elinge *et al.*, 2012). The current study indicates that *C. fimbriata* contains about 68.6 mg/100 g of Mg which could significantly increase this mineral's availability in the human body if they are consumed. Phosphorus is needed for healthy bones and teeth, energy metabolism, and acid base balance in the body. It maintains blood sugar level and normal heart contraction and also important for normal cell growth, bone growth, kidney function and cell growth. *C. fimbriata* contains about 0.67mg/100 g of phosphorus. Therefore studies were performed to evaluate the antidiabetic, antioxidant and antimicrobial activity of *C. fimbriata*.

For plants to effectively scavenge free radicals, phenol must exist (Singh *et al.*, 2009). Lipid peroxidase or hydroperoxidase free radicals, which are important promoters of the autoxidation of lipid chains, can be given hydrogen by plant extracts that include antioxidants with free radical scavenging properties (Prakash *et al.*, 2011). Studies on antioxidants *in vitro* revealed that the extracts not only have antioxidant properties but also have strong free radical scavenger properties. One of the therapeutic approaches for controlling postprandial hyperglycemia in diabetic patient is to prevent or decreasing absorption of carbohydrate after food intake. Complex starches, oligosaccharides, and disaccharides must be broken down into monosaccharides by α -amylase and α -glucosidases before they are absorbed in the duodenum and upper jejunum. Recent advances in understanding the activity of intestinal enzymes helped in the development of newer pharmacological agents. α -glucosidase inhibitors reduce intestinal absorption of starch, dextrin, and disaccharides by inhibiting the action of α -glucosidase in the intestinal brush border.

Vyshali *et al.*,

Inhibition of this enzyme slows the absorption of carbohydrates from the GI tract and decreases the rate of rise of postprandial glucose (PP hyperglycemia). This delay digestion and breakdown of starch may have beneficial effects on insulin resistance and glycemic index control in people with diabetes (Mohamed *et al.*, 2012; Narkhede *et al.*, 2011; Uddin *et al.*, 2014; Abd Elkader *et al.*, 2022). Acarbose is α -glucosidase inhibitor and was used as a standard. Experimental results showed that the extracts significantly inhibited the α -glucosidase and α -amylase enzymes. Ethanolic extract showed better α -amylase and α -glucosidase inhibitory activity than aqueous and petroleum ether extract. These results indicate that *C. fimbriata* exhibits hypoglycemic activity possibly by inhibition of pancreatic α -amylase. Thus, the potential antidiabetic activity of *C. fimbriata* could be attributed to the bioactive phytochemicals implicated for their potential antihyperglycemic activity which might exert their effects individually or in synergy with each other. Further, the search for substances with highly antimicrobial activity has been one of the most intensive field of research to minimize the risk of infectious diseases that caused by bacteria, fungi, viruses, and parasites, which are pathogenic to humans. Plants extracts are still the major sources of many therapeutic agents including antimicrobial agents for the treatment of infectious diseases and plants rich in secondary metabolites such as tannins, alkaloids and flavonoids, have been found *in vitro* to have antimicrobial properties (Lewis and Ausubel 2006; de Boer *et al.*, 2005) and henceforth the study was carried to determine the antimicrobial activity of *C. fimbriata* and it was startling to know that *C. fimbriata* did not exhibit any significant antimicrobial activity. To conclude *C. fimbriata* acts as a necessary nutrient and can also be included in other foods as nutraceuticals for efficient and proper metabolism as well as the maintenance of a healthy physiological condition and also has the full potential to be employed as an ingredient in food on an individual basis. The study's findings suggested that *C. fimbriata* can potentially play a substantial, advantageous role in providing a healthy and balanced diet because they are nutritionally dense and abundant in phytochemicals, particularly antioxidants. Additionally, *C. fimbriata* has strong anti-inflammatory and anti-diabetic properties. But *C. fimbriata* had the least antibacterial activity of the studied species due to its high phenolic content. Need of the hour is to conduct more scientific research in the areas of agronomy, breeding, postharvest handling, value addition, and connecting farmers to markets. Although the study uncovers a wealth of information on *C. fimbriata*, additional research is required to speculate on its potential for treating diabetes, cancer, and inflammation. More research is needed to establish the *in vivo* activities supporting the so far conducted *in vitro* studies.

CONCLUSIONS

The utilization of natural compounds and their structural equivalents has a significant impact on the

field of pharmacology, particularly in the treatment of cancer and infectious diseases. However, the discovery and development of natural products as drugs have been hindered by various challenges, such as the technical difficulties involved in screening, isolating, characterizing, and optimization. These challenges prompted the pharmaceutical industry to largely abandon the search for natural products after the 1990s. Fortunately, recent advancements in technology and scientific understanding have provided new avenues to overcome these obstacles. As a result, there is a renewed focus on studying *C. fimbriata*, which holds promise as both a potential drug lead and a functional food. These advancements in technology and science have opened up new possibilities for harnessing the therapeutic potential of natural compounds, revitalizing the search for novel drug candidates derived from natural sources. The study's findings suggested that both the wet and dry extracts of *C. fimbriata* can potentially play a substantial, advantageous role in providing a healthy and balanced diet as they are abundant in phytochemicals with antioxidants and antidiabetic properties and with respect to variances in their antidiabetic, antimicrobial, antioxidant, and free radical scavenging capabilities. These natural bioactive phytochemicals work in tandem with diet to protect against a number of diseases.

Another important prospect regarding *C. fimbriata* is that more scientific study is needed in the fields of agronomy, breeding, postharvest handling, value addition, and connecting farmers to markets.

The study's contributions include a comprehensive analysis of phytochemical profiles in both wet and dry *Caralluma fimbriata* extracts, along with an evaluation of their therapeutic potential, shedding light on the optimal extraction method and potential applications of the plant for various health benefits.

The study provides valuable insights into the differences in phytochemical composition and therapeutic potential between wet and dry extracts of *Caralluma fimbriata*, contributing to a better understanding of the optimal extraction method for harnessing its bioactive compounds and guiding potential applications in healthcare and pharmaceutical industries.

FUTURE SCOPE

Conducting animal and human trials to evaluate the effects of *C. fimbriata* consumption on specific health conditions, such as diabetes, cancer, and inflammation, will provide valuable insights into its therapeutic potential.

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Conflict of Interest. None.

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