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ORIGINAL ARTICLE

Utilizing Chromatographic Techniques, Quantitative Estimation of n-Hexadecanoic Acid, a Potential Bioactive Compound Found in *Caralluma fimbriata* Wall

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Abstract

Background and Objectives: It is well known that *Caralluma fimbriata* has a wide range of therapeutic applications in traditional medical practices. The n-hexadecanoic acid present in *Caralluma* species has wide range of therapeutic potential. The present study was designed for quantitative estimation of n-hexadecanoic acid in chloroform and hydro-methanolic extracts of *C. fimbriata* Wall. **Methods:** *C. fimbriata* extracts were obtained by cold maceration methods and preliminary phytochemical screening was performed. Further, the extracts were subjected for chromatographic purity analysis such as high-performance liquid chromatography, thin-layer chromatography, column chromatography, gas chromatography-high-resolution mass spectrometry (GC-HRMS), and *in vivo* anti-emetic activity. **Results:** The quantity of n-hexadecanoic acid in *C. fimbriata* methanolic extract was found to be higher, that is, 160.06 ppm then the chloroform extract, that is, 118.38 ppm. Furthermore, GC-HRMS analysis resulted that hydro-methanolic extract has n-hexadecanoic acid with % peak area of 78.23% which is higher than the chloroform extract, that is, 60.44%. The *in vivo* study found that the 100 mg/kg *C. fimbriata* extract showed 41% inhibition. **Conclusion:** Even though n-hexadecanoic acid is more soluble in chloroform, it is isolated in greater quantity in methanolic extract.

Key words: *Caralluma fimbriata*, chloroform extract, chromatographic purity analysis, hydro-methanolic extract, n-hexadecanoic acid, etc

INTRODUCTION

Caralluma fimbriata is a perennial succulent herb widely distributed throughout the peninsular regions of India. The plant typically grows to a height of 20–30 cm and is characterized by erect, branched, fleshy, four-angled green stems that taper toward the apex. In recent decades, *C. fimbriata* has attracted considerable scientific attention due to its rich phytochemical profile and diverse pharmacological activities. Various studies have reported the presence of glycosides, flavonoids, saponins, alkaloids, sterols, triterpenoids, fatty acids, and phenolic compounds. These phytoconstituents contribute to the plant's medicinal properties, including anti-obesity, antidiabetic, antioxidant, anti-inflammatory, antimicrobial, and anticancer activities.[1,2]

Among the numerous bioactive constituents identified in *C. fimbriata*, n-hexadecanoic acid has been reported as one of the significant fatty acid components. Chemically, it is a saturated long-chain fatty acid. N-hexadecanoic acid has been detected in various medicinal plants through chromatographic techniques such as gas chromatography–mass spectrometry, high-performance liquid chromatography (HPLC), and thin-layer chromatography (TLC).[3,4] Several biological activities have been attributed

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to this compound. Studies have demonstrated that N-hexadecanoic and its derivatives may influence cellular signaling pathways involved in apoptosis and cell-cycle regulation, thereby exhibiting potential anticancer effects against selected cancer cell lines. Certain investigations suggest that fatty acids may modulate neurotransmitter activity and neuronal membrane function, contributing to antidepressant-like effects. It has shown inhibitory effects against several Gram-positive and Gram-negative bacterial strains by disrupting microbial membrane integrity. N-hexadecanoic acid containing plant extracts have demonstrated free radical scavenging properties, which may help protect biological systems against oxidative stress. Derivatives of it shown the ability to modulate inflammatory mediators and cytokines, thereby contributing to anti-inflammatory effects.[5,6]

Although *C. fimbriata* contains numerous phytochemicals, the isolation and quantification of specific marker compounds are essential for quality control, standardization, and pharmacological evaluation. Determining the concentration of n-hexadecanoic acid can establish phytochemical standards for herbal formulations and support pharmacological investigations.

The primary objective of the study is to quantitative estimation of n-hexadecanoic acid, in a *C. fimbriata* wall by chromatographic techniques. Chromatographic purity testing will be employed to evaluate the effectiveness of the isolation process and to establish the concentration of n-hexadecanoic acid present in the plant extract.

MATERIALS AND METHODS

Plant collection and authentication

The whole plants of *C. fimbriata* wall were collected from the mountain ranges of Sangamner village, District Ahmednagar, Maharashtra in the month of August. The plant was authenticated from the Department of Botany and Research Center, PVP College of Arts, Science and Commerce, Pravaranagar, Maharashtra.

Drying and grinding

The collected plant parts of plant (stem) were sun-dried for 4–5 weeks. The plant parts were ground into a coarse powder with the help of a suitable grinder as well as mortar and pestle. The powder was stored in an airtight container and kept in a cool, dark, and dry place until analysis commenced.

Extraction of active constituents

Different methods used for extraction of active constituents present in *C. fimbriata* wall, detail procedure of two methods are given below:

Chloroform extract using cold maceration method

20 g powder of *C. fimbriata* wall was weighed and then soaked in 100 mL chloroform for 48 h in room temperature. Then, the same was filtered with Whatmans filter paper. The obtained extract is stored at 4°C for further study.[7]

Methanolic extract using cold maceration method

20 g powder of *C. fimbriata* wall was weighed and then soaked in 200 mL solvent mixture of methanol and water (80:20) using magnetic stirrer and stirred for 3 h and was kept aside for 48 h in room temperature. Then, the same was filtered with Whatman filter paper. Again, the residue was dissolved with 200 mL solvent and stirred for 2 hrs. The solvent containing the extract is dried under room temperature. The obtained extract is stored in 4°C for further study.[8]

Determination of extractive yield

The percentage yield or extractive yield of the *C. fimbriata* wall extract was obtained using below formula,[9]

$$Extract\ Yield = \frac{(W2 - W1)}{W0} \times 100$$

Where, W_2 is weight of the extract and container; W_1 is weight of empty container, and W_0 weight of dried sample.

Preliminary phytochemical screening

Phytochemical screening was carried out on the crude chloroform and hydro-methanolic extract to identify the chemical constituents of *C. fimbriata* so as to determine the presence of secondary metabolites such as alkaloids, carbohydrate, glycosides, saponins, phenol, flavonoids, protein, terpenoids, and tannins. Different tests are used to test the active constituents present in extract and these are given below.[10,11]

Alkaloids

Dragendorff"s test is used for finding presence of alkaloids in extract, in which Dragendorff's reagent is added into 2–3 mL of filtrate. After addition of this reagent if orange brown precipitate is observed, it indicates presence of alkaloids.

Carbohydrates

Molisch's and Fehling's test are used for finding presence of carbohydrates in extract. In Molisch's test, take 2–3 mL of extract and add few drops of alpha-naphthol solution in alcohol, shake well, and add concentrated sulfuric acid from sides of the tube. After completion of this all steps if violet ring is form at the junction of two liquids indicate the presence of carbohydrates. In Fehling's test, take mixture of 1 mL of Fehling's solution-A, 1 mL of Fehling's solution-B and boil for 1 min. Add equal volume of test solution and

heat on boiling water bath for 5-10 min. After completion of all the steps if initially yellow precipitates are form and then formation of brick red precipitates indicate the presence of carbohydrate.

Glycosides

Keller-Killiani test is used for finding presence of glycosides in extract, in which glacial acetic acid, one drop of 5% ferric chloride, and sulfuric acid is added into 2–3 mL of filtrate. After addition of this reagent if reddish-brown color appears at junction of the two liquid layers and upper layer appears bluish green indicates presence of glycosides.

Saponins

Shake the drug extract vigorously with water and persistent stable foam is obtained; it indicates presence of saponins.

Phenols

Add few drops of 5% ferric chloride ($FeCl_3$) solution in 2–3 mL of extract. If deep blue-black color is form, it indicates the presence of phenol.

Flavonoids

Sodium hydroxide test is used for finding presence of flavonoids in extract, in which add few drops of 1% ammonia solution is added into 2–3 mL of filtrate. After addition of this reagent if yellow color appears, it indicates presence of flavonoids.

Proteins

Biuret and Million's test are used for finding presence of proteins in extract. In Biuret test, take 2–3 mL of extract and add few drops of 4% sodium hydroxide solution and 1% copper sulfate solution. After completion of this all steps if violet or pink color appears, it indicates the presence of proteins. In Million's test, 5 mL of million's reagent is added into 3 mL of test solution. After completion of this all steps if white precipitate is obtained, Warm the precipitate turns brick red or the precipitate dissolves giving red colored solution indicates presence of protein.

Steroids

Salkowski test is used for finding presence of steroids in extract. In this test, take 2 mL chloroform and 2 mL concentrated sulfuric acid is added to 2 mL extract. After completion of this all steps if appearance of red color in chloroform layer and greenish yellow fluorescence in acid layer indicates the presence of steroids.

Tannins

Add few drops of 5% $FeCl_3$ solution into 2–3 mL of extract. After addition of this reagent if deep blue-black color appears, it indicates presence of tannins.

Fats

Press powder of crude drug between two filter paper. If filter paper gets permanently stained due to oil, it indicates the presence of fats.

HPLC quantification of extracts

Analytical wavelength of pure n-hexadecanoic acid is selected by multiple steps. 1000 ppm solution was prepared by dissolving 100 mg pure palmitic acid in 100 mL methanol then 10 mL of the solution was diluted in 100 mL methanol to make 100 ppm solution. Further serial dilutions of the solution were then made to obtain several concentrations. The solutions were scanned to obtain the maximum wavelength of absorption using a ultraviolet visible (UV-Vis) spectrophotometer. Chromatographic conditions for HPLC are mentioned in Table 1.

The quantity of n-hexadecenoic acid (Palmitic acid) in given extract is calculated using below formula.[12]

$$Conc.of\ Sample = \frac{\text{Area of Sample}}{Area\ of\ Standard} \times Conc.of\ Standard$$

Fractionation of extracts by column chromatography

Crude chloroform extract of *C. fimbriata* was purified by silica gel column chromatography (Silica 60–120). The column was prepared using silica gel slurry in 100% petroleum ether. Petroleum ether: Acetone solvent at ratio 6:4 was used as a solvent system. Fractions were collected at a flow rate of 1 mL/min 10 mL each.[7]

Crude hydro-methanolic extract of *C. fimbriata* was purified by silica gel column chromatography (Silica 60–120). The column was prepared using silica gel slurry in 100% hexane. Hexane: Ethyl acetate solvent at ratio 7:3 was used as a solvent system. Fractions were collected at a flow rate of 1 mL/min 10 mL each.[13]

TLC

TLC analysis was carried out according to the method described by Harborne.[14] The TLC plates were prepared with stationary phase as silica gel G and then the plates were air dried at room temperature and kept for activation in hot air oven for 10 min at 110 °C. Sample spots of fractions from both the extracts column were given and then mobile phase Petroleum ether: Acetone (6:4) and Hexane: Ethyl acetate (7:3) was allowed to run, respectively, over the stationary phase and then was air dried. Detection of retention factor (R_f) values was done in UV light at 366 nm and was compared with standard R_f value of palmitic acid. The movement of

Table 1: Chromatographic conditions for HPLC

Sample	Standard (Palmitic acid)	Chloroform extract	Methanolic extract
Mobile phase	Methanol: Water (80:20)	Methanol: Water (80:20)	Methanol: Water (80:20)
Flowrate	0.8 mL/min	0.8 mL/min	0.8 mL/min
Wavelength	220 nm	220 nm	220 nm
Sample volume	20 µL	20 µL	20 µL
Pressure	10–11 MPa	10–11 MPa	10–11 MPa
Run time	12.29 min	12.64 min	12.60 min

HPLC: High-performance liquid chromatography

the bioactive compound was expressed by its R_f values, calculated as,

$$Rf = \frac{Distance\,travelled\,by\,Solute}{Distance\,travelled\,by\,solvent\ front}$$

Gas chromatography-high-resolution mass spectrometry (GC-HRMS) analysis fractions

The active fractions from both the extracts of *C. fimbriata* were subjected for GC-HRMS analysis for identification of bioactive compounds present in the extracts. The active fractions obtain from the column chromatography were sent for GC-HRMS to SAIF, IIT Bombay, Powai, Mumbai, Maharashtra.

In vivo anti-emetic activity

Poultry chicks (25–35 g) were obtained from the P.I.R.E.N.S. and placed in controlled temperature (25 ± 2°C) with light-dark cycle in the animal house of the Institute. All the Chicks were kept on fasting 16 h before the start of experiment with the free access to water.[15]

Young 4 days chicks having weight 25–35 g selected for study. Initially, all chicks stabilize for 10 min. Animals were divided into five groups for comparative study such as A, B, C, D, and E. To first group 0.9% saline solution containing 5% dimethyl sulfoxide and 1% Tween 80 (10 mL/kg), to second group chlorpromazine (150 mg/kg), to third group crude extract (100 mg/kg), to fourth group crude extract (200 mg/kg), and to fifth group crude extract (300 mg/kg) were administered. Wait for 10 min after solution/extract administration and copper sulfate is administered orally (50 mg/kg). Count the number of retches for 10 min and calculate the mean number of retches as well as percentage inhibition by following formula.

$$\%\,Inhibition = \frac{(A-B)}{A} \times 100$$

Where, A is frequency of retching in control groups, B is frequency of retching in test groups.

Table 2: Percentage yield of plant extracts in different solvents

S. No.	Extract	Extract value (%w/w)
1.	Chloroform extract	16.72
2.	Hydro-methanolic extract	23.42

Table 3: Preliminary phytochemical screening of *Caralluma fimbriata* extracts

S. No.	Test	Chloroform extract	Methanolic extract
1.	Alkaloids	+	+
2.	Carbohydrates	+	-
3.	Glycosides	-	+
4.	Saponins	+	+
5.	Phenol	-	+
6.	Flavonoids	+	+
7.	Proteins	-	-
8.	Steroids	+	+
9.	Tannin	-	+
10.	Fats	+	+

RESULTS AND DISCUSSION

Determination of % yield of extract

The extraction yield is a metric for how effectively a solvent extracts particular component from the starting substance. It will provide information on the plant's capacity to be extracted under various circumstances that the results were reported in Table 2.

Phytochemical analysis

Phytochemical test was performed for the two different extracts of *C. fimbriata.* The chloroform extract shows presence of alkaloids, carbohydrates, saponins, flavonoids, proteins, steroids, tannin, and fats, as shown in Figure 1 and Table 3.

While methanolic extract shows presence of alkaloids, saponins, phenols, flavonoids, steroids, and fats, as shown in Figure 2a and b.



Figure 1: *In vivo* antiemetic activity

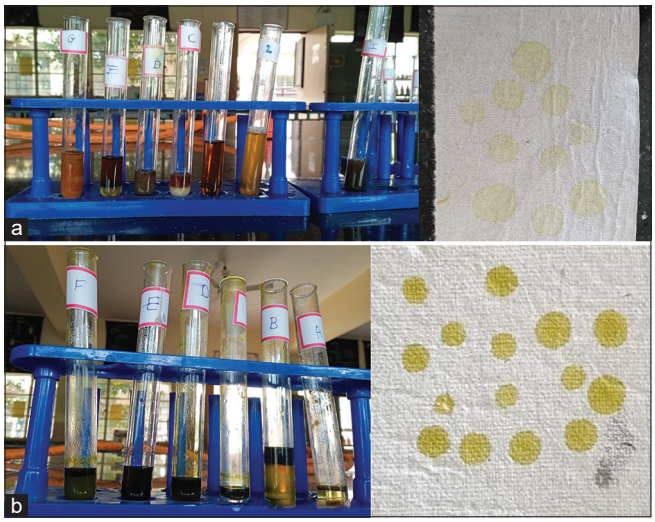


Figure 2: Phytochemical screening of *Caralluma fimbriata*, (a) chloroform extract and (b) methanolic extract

Quantification of extracts

UV spectrum and HPLC chromatogram of n-hexadecanoic acid

The UV spectrum shows a prominent absorption peak in the deep UV region (approximately 220–230 nm), attributed to electronic transitions within the sample. Maximum absorbance of n-hexadecanoic acid (Palmitic acid) was observed at wavelength 220 nm. The absorbance rapidly decreases with increasing wavelength and remains negligible above 250 nm, indicating the absence of significant chromophores absorbing in the near-UV-Vis regions and same is presented in Figure 3a. The obtain wavelength 220 nm is used for further HPLC analysis.

In Figure 3b, chromatographic profile of the sample showing several minor peaks between 3 and 7 min and a dominant, well-resolved peak at approximately 11 min. (10.798 min.) retention time and area of standard is 49405. The major peak indicates the presence of the principal component, while the smaller peaks correspond to minor constituents or impurities. The stable baseline and symmetrical peak shape suggest satisfactory chromatographic performance.

HPLC chromatogram for C. fimbriata extracts

Figure 4a is a HPLC chromatogram of chloroform extract in which a major peak appears at approximately 3.0 min and another minor peak appears around 11.0 min (11.019 min) which is close to the standard peak. While Figure 4b is a HPLC chromatogram of methanolic extract, in which a very large peak appears at approximately 3.2–3.5 min. Another minor peak appears around 11.0 min (11.023 min) which is close to the standard peak.

The quantity of palmitic acid in *C. fimbriata* chloroform extract is 118.38 ppm and the quantity of palmitic acid in *C. fimbriata* methanolic extract is 160.06 ppm.

Silica gel column chromatography

The fractionation by the column was done and the fractions of chloroform and hydro-methanolic extracts were collected and concentrated as shown in Figure 5 and were store at room temperature for further experimental works. The TLC of fractions was conducted to identify active fractions. The active fractions were concentrated and subjected to spectroscopic studies to identify the individual phytochemicals present.

TLC

TLC was performed to evaluate the separation of phytochemical constituents present in *C. fimbriata* extracts using suitable solvent systems. The chloroform extract exhibited good separation when petroleum ether and acetone were used in a ratio of 6:4, resulting in an Rf value of 0.73. This value suggests that the compound migrated a considerable distance on the TLC plate, indicating a balanced interaction between the mobile and stationary phases. In contrast, the hydro-methanolic extract showed optimal separation with a solvent system comprising hexane and ethyl acetate (7:3), producing an Rf value of 0.67. The variation in Rf values between the two extracts reflects differences in the polarity and chemical nature of the compounds extracted by each solvent. Overall,

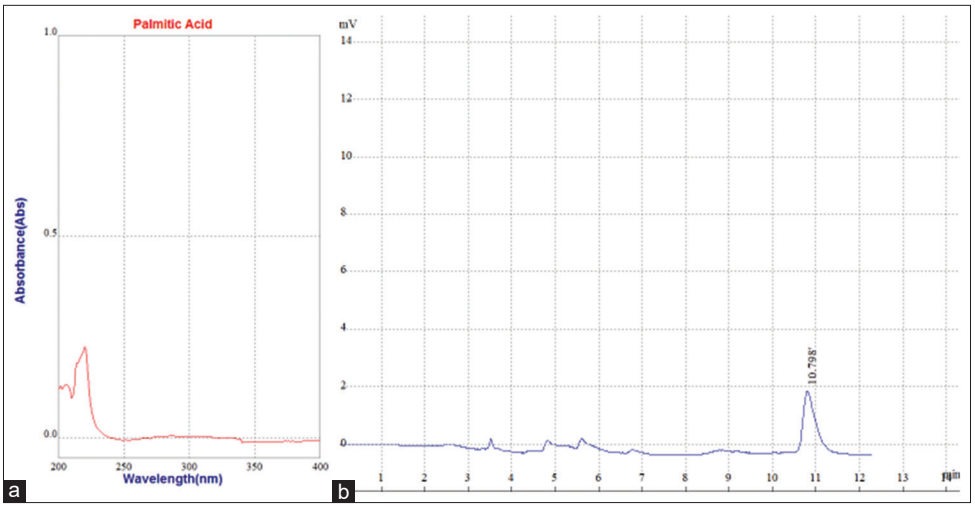


Figure 3: (a) Ultraviolet spectrum of n-hexadecanoic acid (palmitic acid) and (b) high-performance liquid chromatography chromatogram of n-hexadecanoic acid (palmitic acid)

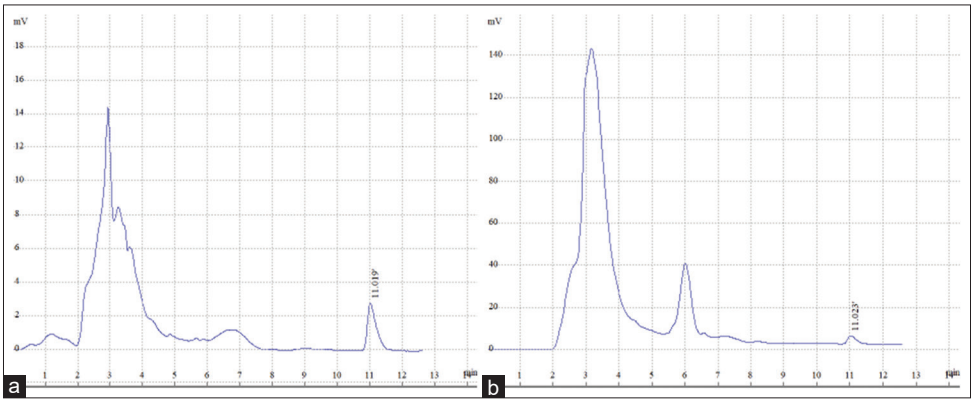


Figure 4: (a) High-performance liquid chromatography (HPLC) chromatogram of chloroform extract and (b) HPLC chromatogram of methanolic extract

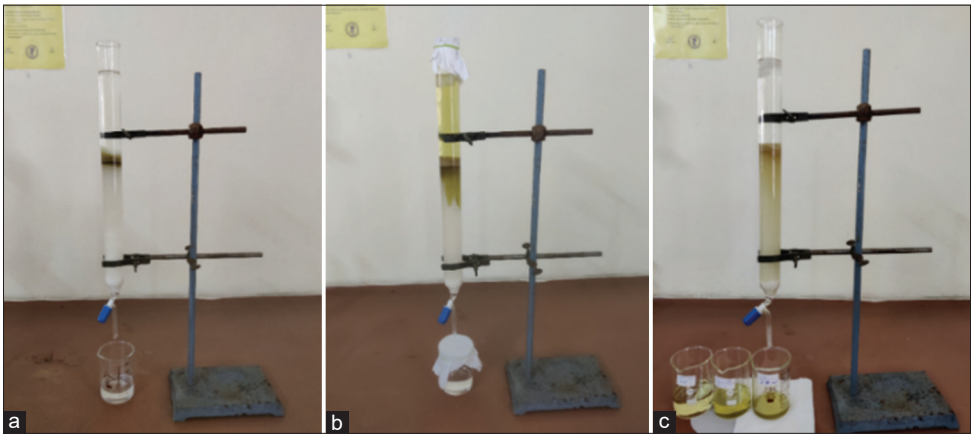


Figure 5: Column chromatography for both the extracts of *Caralluma fimbriata* wall. (a) Sample loading into the column, (b) packing column by introducing the mobile phase, and (c) collection of fractions

the selected solvent systems were effective in separating the major constituents of both extracts, providing a reliable basis for subsequent phytochemical analysis and compound identification. The results of same are shown in Figure 6.

GC-HRMS analysis

GC-HRMS analysis of the chloroform and hydro-methanolic extracts of *C. fimbriata* revealed the presence of several phytochemical constituents, as indicated by multiple

Table 4: Screening test of *Caralluma fimbriata* plant extract for anti-emetic effect against copper sulfate induced emesis in young chicks

S. No.	Groups	No. of chicks	Average weight	Mean No. of retches	% inhibition
1.	Negative control	6	32.17±0.31	117.67±5.48	-
2.	Positive control	6	30.5±0.22	66.00±1.51	43.91
3.	100 mg crude extract	6	35.33±0.33	67.33±1.38	41.36
4.	200 mg crude extract	6	26.17±0.40	56.5±1.65	51.98
5.	300 mg crude extract	6	27.33±0.21	44.33±1.36	62.32

n=6, SEM: Standard error of mean; **P*<0.0001 versus control shows significant values using unpaired student *t*-test

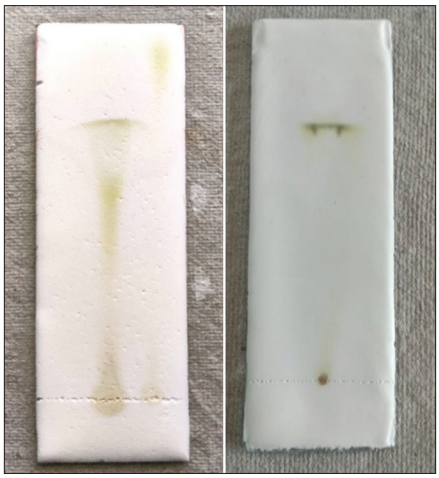


Figure 6: Thin-layer chromatography of active fractions

well-defined peaks in the chromatograms. Both extracts exhibited similar retention times, suggesting the presence of common compounds, although differences in peak intensities reflected variations in their concentrations due to the extraction solvent. Mass spectral analysis identified hexadecanoic acid, 2-oxo-, methyl ester and n-Hexadecanoic acid (palmitic acid) as the major constituents based on their characteristic fragmentation patterns and molecular ion peaks, same are shown in Figure 7. The detection of these fatty acid derivatives indicates that they are important metabolites in *C. fimbriata*. Overall, the GC-HRMS results provide valuable information on the chemical composition of *C. fimbriata* and support its potential as a source of bioactive compounds.

In vivo anti-emetic activity

For *in vivo* antiemetic activity, the Chicks were divided into 5 groups each group containing six chicks (n = 6). Emesis was induced using copper sulfate and the number of retches was recorded of each group. In this study, the negative control Group I showed the maximum number of retches in chicks (117.67 ± 5.48). Group II treated with chlorpromazine exhibited the percentage inhibition of retches (43.91%), while the treatment Group III, IV, and V orally administered with crude extract 100, 200, and 300 mg/kg showed % inhibition of the number of retches in chicks 41.36%, 51.98%, and 62.32%, respectively. All the groups were compared with the Group I (negative control group) and the results of same are shown in Table 4 and Figure 8.

DISCUSSION

The present study was performed to find out the amount of potent bioactive compound n-hexadecanoic acid that can be extracted using different solvents from *C. fimbriata*. Two solvents, that is, chloroform and hydro-methanol were used for the extraction process. The preliminary phytochemical screening of both the extracts was done to find out various phytochemicals present in the *C. fimbriata* extracts of which main focus was on steroids or fatty acids and the results showed presence of fatty acid in both the extracts. The chromatographic purity testing was done using TLC, column chromatography, GC-HRMS, and HPLC. For the quantitative estimation of n-hexadecanoic acid, HPLC Quantification of both extracts was performed and the hydro-methanolic extract yields high concentration, that is, 160.06 ppm of n-hexadecanoic acid then the chloroform extract.

The column chromatography was used for fractionation of the extracts so as to find out active fractions containing n-hexadecanoic acid. The fractions were collected separately and TLC was performed for identification of active fraction. From TLC, the R_f value of the active fraction from hydro-methanolic extract, that is, 0.67 was observed very close to the standard R_f value, that is, 0.60[14] which showed presence of palmitic acid in hydro-methanolic extract. Furthermore, the R_f value of the active fractions from chloroform extract was 0.73 which was observed not that close to the standard R_f value of palmitic acid. The active fractions of each extract were then concentrated and were processed for GC-HRMS analysis. The hydro-methanolic extract showed presence of n-hexadecanoic acid with % peak area of 78.23% which is higher than the chloroform extract i.e., 60.44%.

The *in vivo* study was performed and found that the 100 mg/kg *C. fimbriata* extract showed 41% inhibition as compared with standard chlorpromazine which showed 43.91% inhibition.

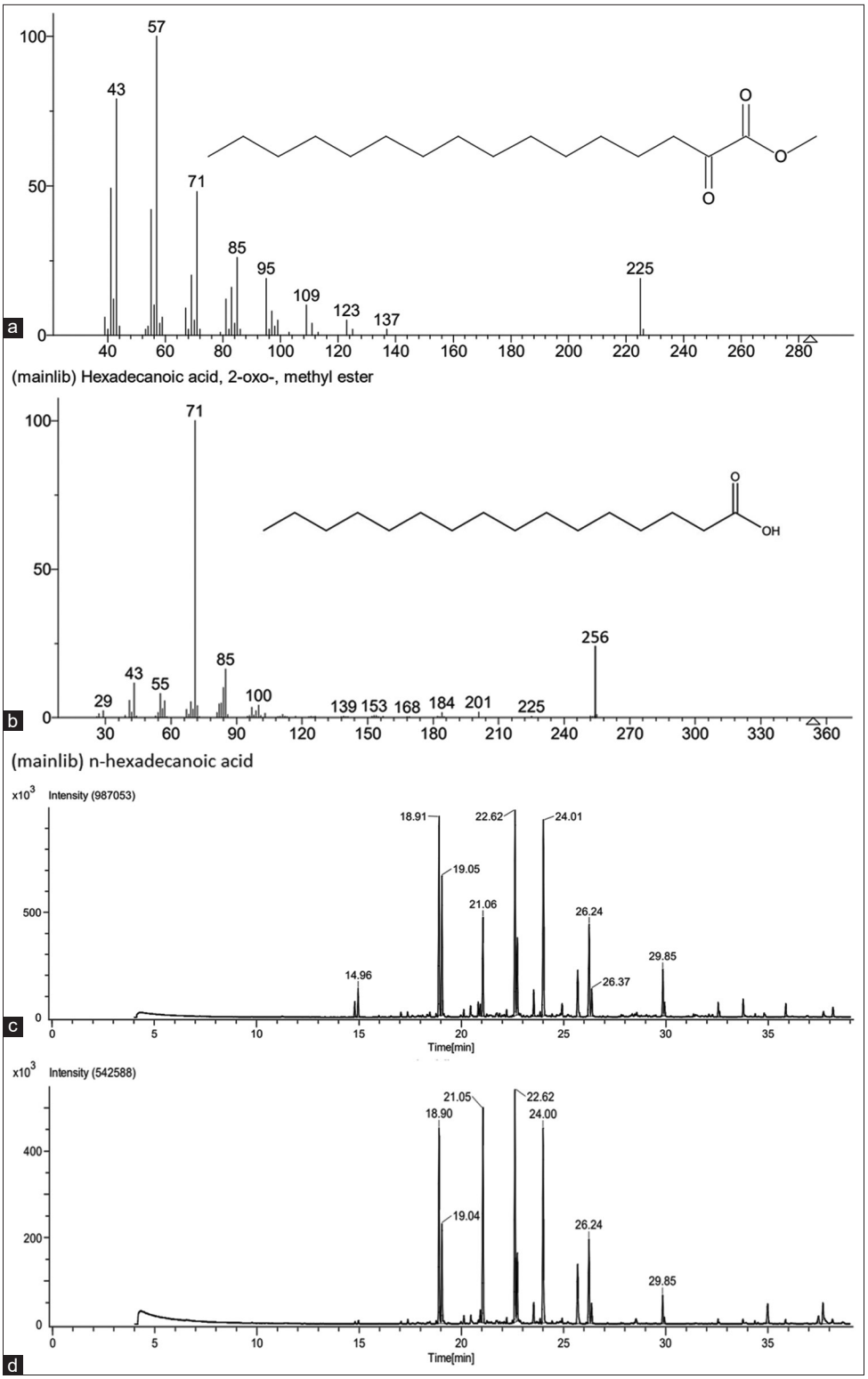


Figure 7: (a) Hexadecanoic acid, 2-oxo-, methyl ester structure obtain from gas chromatography-high-resolution mass spectrometry (GC-HRMS), (b) n-Hexadecanoic acid structure obtain from GC-HRMS, (c) GC-HRMS spectrum of chloroform extract of *Caralluma fimbriata*, and (d) GC-HRMS spectrum of hydro-methanolic extract of *C. fimbriata*

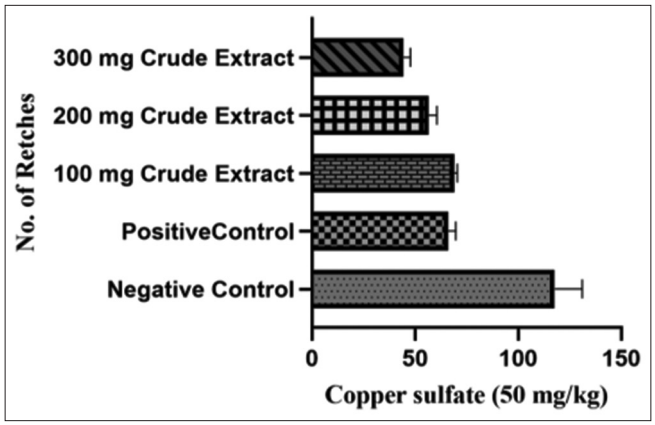


Figure 8: In comparison to standard chlorpromazine, crude chloroform extract of *Caralluma fimbriata* wall inhibited copper sulfate-induced retches in chicks

CONCLUSION

As the study conclude that even with a higher solubility of n-hexadecanoic acid in chloroform, it gets isolated in more concentration in methanolic extract. Furthermore, the hydro-methanolic extract can be used for commercial purpose for extraction as it yields was higher than chloroform extract.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

REFERENCES

1. Anwar R, Rabail R, Rakha A, Bryla M, Roszko M, Aadil RM, *et al*. Delving the Role of Caralluma fimbriata: An Edible Wild Plant to Mitigate the Biomarkers of Metabolic Syndrome. Oxid Med Cell Longev 2022;2022:5720372.
2. Harborne AJ. Phytochemical methods a guide to modern techniques of plant analysis. Berlin: Springer Science and Business Media; 1998.
3. Snyder LR, Kirkland JJ, Dolan JW. Practical HPLC Method Development. United States: Wiley.
4. Snyder LR, Kirkland JJ, Dolan JW. Introduction to Modern Liquid Chromatography. United States: Wiley Publications.
5. Akpuaka A, Ekwenchi MM, Dashak DA, Dildar A. Biological activities of characterized isolates of n-hexadecanoic acid methyl ester and n-hexadecanoic acid. Sci Res Essays 2013;8:1067-74.
6. Krishnamoorthy K, Subramaniam P. Phytochemical profiling of leaf, stem, and tuber parts of *Solena amplexicaulis* (Lam.) Gandhi using GC-MS. Int Sch Res Notices 2014;2014:567409.
7. Ravi L, Krishnan K. Cytotoxic potential of n-hexadecanoic acid extracted from *Kigelia pinnata* leaves. Asian J Cell Biol 2016;12:20-7.
8. Priya D, Rajaram K, Suresh Kumar P. Phytochemical studies and GC-MS analysis of Caralluma fimbriata wall. Int J Pharm Res Dev 2011;3:105-10.
9. Ngamkhae N, Monthakantirat O, Chulikhit Y, Boonyarat C, Maneenet J, Khamphukdee C, *et al*. Optimization of extraction method for Kleeb Bua Daeng formula and comparison between ultrasound-assisted and microwave-assisted extraction. J Appl Res Med Aromat Plants 2022;28:100369.
10. Veshalini K, Arapoc DJ, Adam Z, Razali R, Suliman NA, Bakar NA. Phytochemical Screening, *in vitro* antioxidant activities and Zebrafish embryotoxicity of *Abelmoschus esculentus* extracts. Pharmacogn J 2022;14:690-701.
11. Patel M, Mishra RP, Patel G. Phytochemical screening of aqueous extracts of *Abelmoschus esculantus* leaf and root. J EmergTechnol Innov Res 2019;6:772-5.
12. Kupiec T. Quality-control analytical methods: High-performance liquid chromatography. Int J Pharm Compd 2004;8:223-7.
13. Othman AR, Abdullah N, Ahmad S, Ismail IS, Zakaria MP. Elucidation of *in-vitro* anti-inflammatory bioactive compounds isolated from *Jatropha curcas* L. Plant root. BMC Complement Altern Med 2015;15:11.
14. Harborne JB. Phytochemical Methods A Guide to Modern Techniques of Plant Analysis. 3rd ed. London: Chapman and Hall; 1998.
15. Kirkwood JK, Hubrecht R, Universities Federation for Animal Welfare. The UFAW Handbook on the Care and Management of Laboratory and Other Research Animals. New Jersey: Wiley-Blackwell; 2010.

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