



OPEN Phytochemical profiling of *Moltkiopsis ciliata* using DART-ToF-MS and GC-MS

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The environment of Saudi Arabia is particularly rich in plants utilized in folk medicine to cure various diseases. Therefore, the quality assessment of these plants and the study of their primary constituents are crucial for human health. Chromatographic techniques and mass spectrometry are effective methods for separating, profiling, and analyzing natural products. The primary objective of this work is to extract and profile the natural constituents present in the *Moltkiopsis ciliata* plant and identify their major bioactive compounds. More than 30 compounds were rapidly identified from the fresh parts of the plant—flowers, leaves, roots, and stems—without any prior sample preparation by direct analysis in real-time of flight-mass spectrometry (DART-ToF-MS), highlighting the novelty of this method in profiling the phytochemical composition of the plant. The total extracts of the plant were fractionated according to their polarity by liquid-liquid extraction and analyzed by gas chromatography-mass spectrometry (GC-MS). In addition, the hexane extracts of flowers, leaves, stems, and roots were analyzed using GC-MS and showed the presence of many volatile constituents, in which more than 60 compounds were successfully identified. Many of the detected compounds highlight the potential biological significance of the plant, such as indolizine, echinatine, heliotrine, vitamin E, campesterol, stigmaterol, γ -sitosterol, and phytol, which are known to have anti-inflammatory, antimicrobial, and antioxidant properties. Whereas the abrine compound was identified in this species for the first time. In a comparison between the two MS techniques used in this work, the results indicate that the proposed methods are complementary and can be used together for the comprehensive screening and regular analysis of bioactive compounds in plants.

Keywords Natural product analysis, High-resolution mass spectrometry, Direct analysis in real time, Gas chromatography-mass spectrometry, Maceration.

Natural products, including lipids, proteins, carbohydrates, and nucleic acids, are chemicals produced by a living organism. They play a significant role in metabolic reactions¹. Natural products have been employed as active ingredients in herbal treatments and other traditional therapies, having developed over centuries and gained unrivalled chemical diversity, which results in a diverse range of biological activities². Nowadays, some traditional medicine practices, such as Indian, Chinese, and African herbal medicines, are still the main treatment options for many people worldwide³. Thus, natural products have been the focus of the scientific community's attention for the past few decades, and interest in them is still rising.

The Kingdom of Saudi Arabia's traditional medicine is based on medicinal plants and exhibits connections to healthy living, diet, and folk healing practices recognized by a particular community⁴. Since 1990, the Saudi people's perception of traditional medicine has evolved. Several ethnobotanical surveys conducted in Saudi Arabia revealed that many Saudi citizens depend on classical medicine, either by itself or in conjunction with modern medicine⁵. The flora of Saudi Arabia has around 2250 species, including approximately 1,200 medicinal plants⁶. Additionally, many significant studies should be done to identify the essential chemical components and bioactive substances.

Moltkiopsis ciliata (Forssk.) (*M. ciliata*) I.M. Johns, also known as *Lithospermum callosum* Vahl., is one of the medicinal plants that grow in the north, east, and central regions of Saudi Arabia. It belongs to the family Boraginaceae, which has about 100 genera and more than 2000 species distributed all over the world, and about 66 species are distributed over 20 genera in Saudi Arabia⁷. *M. ciliata* is the only species of the *Moltkiopsis* genus. Locally, this species in the southern tribes is known as "Halam", while in the north of the kingdom, it is called

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“Hamat”, also called “Crapsi” (Al-Dahna). It grows in the sandy and stony deserts of the Kingdom of Saudi Arabia⁸.

The entire plant is used as a medication, while the fresh plant pulp has hemostatic characteristics. It has anti-tumor, antithrombotic⁹, antimicrobial¹⁰, anticancer, and wound-healing activities. The preliminary phytochemical analysis of *M. ciliata* showed the presence of alkaloids, sterols, saponins, flavonoids, triterpenes, tannins, and essential oils¹¹. Three aryl dihydronaphthalene lignan compounds were isolated and identified from *M. ciliata*, which are Moltkiopsin A, Trigonotin A, and Trigonotin C¹². In the present research, spectral techniques such as DART-ToF-MS and GC-MS were used, as they offered accurate details on the structure and properties of organic bioactive compounds¹³. The advantages of using spectral methods are that they provide data faster, need little material, are accurate, and enable continuous analysis at various processing steps of the compound extracted without altering the composition of the bioactive compound¹⁴.

The first and most important step in herb analysis is sample preparation, since it is vital to extract the desired chemical compound from the herbal plants¹⁵. The initial crude extracts using these methods contain complex mixtures of numerous plant metabolites, such as alkaloids, phenolics, terpenoids, and flavonoids¹⁶. A number of methods have been confirmed to extract natural products from diverse matrices. These methods include percolation, maceration, soxhlet extraction, ultrasound-assisted or sonication extraction, and microwave-assisted extraction¹⁷. One of these methods, maceration, is considered the suitable way for thermolabile plant material¹⁸.

Given that the plant samples are complex and the total active constituents cannot be achieved with a single maceration, more than one extraction cycle has been repeated using the same solvents or with a different one¹⁹. This procedure is known as double or triple maceration. The advantages of conventional maceration extraction are that it does not require equipment or specific skills to do the maceration process, and the soaking procedure is rapid. The main disadvantages of this method are that the extraction time is long and the efficacy is low; these will limit its use, but they can be avoided by repeating the soaking process²⁰.

Gas chromatography (GC) is one of the most widely used techniques in plant analysis to separate secondary metabolites. It has the inherent ability to quantify compounds present at low concentrations by using electron capture detectors, which have very high sensitivities²¹. Gas chromatography-mass spectrometry (GC-MS) is mostly considered a versatile analytical technique since it has high separation ability, selectivity, robustness, sensitivity, and reproducibility, as well as a great number of well-established libraries of both trade and ‘in-house’ metabolite databases available²². In this device, the end of the GC system is coupled with a single quadrupole MS detector. Electron ionization and chemical ionization are the two primary ionization types utilized in GC-MS. Many analysts choose the first procedure because there are numerous mass spectral databases and libraries that correspond to electron ionization-based GC-MS²³.

Direct analysis in real-time of flight-mass spectrometry (DART-ToF-MS) is an established technique for rapid mass spectral analysis of a wide range of samples. It can analyse samples at atmospheric pressure, basically in an open laboratory environment²⁴. DART-MS differs from conventional mass spectrometry in the ionization of low-molecular-mass components, which are found on the surfaces of liquids or solids in a gas stream without the need for sample preparation or chromatographic separation of the sample's components. For this reason, DART-MS is suitable for plant material component analysis²⁵. High-throughput fingerprinting study of natural samples is possible since vacuum ionization or sample preparation steps can be omitted in DART-MS analysis. This feature is considered one of the most advantageous characteristics of DART ion sources²⁶.

Current studies have emphasized the value of DART-MS for rapid profiling, authentication, and quality control of medicinal plants²⁷ and others have highlighted its wider applications in natural products chemistry²⁸. More recently, studies have shown that a combination of DART-MS and chromatographic techniques such as GC-MS gives a more complete picture of plant metabolites²⁹. Regardless of these advances, *M. ciliata* has not yet been analyzed by DART-MS, leaving a gap in the literature about its phytochemical characterization using this new technique. Thus, the target of this research is to use a combination of DART-ToF-MS and GC-MS to address this gap by providing the first complete description of the *M. ciliata* plant from Saudi Arabia. On the other hand, a qualitative analysis of the phytochemicals in the hexane extract of each plant part and the total extract in the different fractions was accomplished by GC-MS. Accordingly, this study not only aims to detect new bioactive constituents not previously reported in *M. ciliata* but also explains the practical significance of combining GC-MS and DART-MS in the phytochemical analysis of medicinal plants.

Materials and methods

Plant materials and reagents

HPLC-grade solvents (methanol, hexane, chloroform, ethyl acetate, and n-butanol) were purchased from Aldrich (Steinheim, Germany) and Fisher Scientific (Leicestershire, UK). Sigma-Aldrich (Steinheim, Germany) supplied polyethylene glycol with an average molecular weight of 600, which was used as an internal standard. Helium, used as a carrier gas for DART-ToF-MS and GC-MS, was provided by the Saudi Industrial Gas Company (Riyadh, Saudi Arabia). A saturated *n*-alkane standard mixture used for the determination of retention indices, specifically the C7–C30 series, was purchased from Merck (Darmstadt, Germany).

The whole plants of *M. ciliata* (Fig. 1) were collected in March 2022 from plants grown in a nature reserve over a sandy area (N: 25.967516, E: 44.040977), Al-Da'atha, Unaiza, Al-Qassim province, Saudi Arabia. The botanical identification and confirmation were achieved by Dr. Jacob Thomas, Professor of Taxonomy, Botany and Microbiology Department, College of Science, King Saud University. A voucher specimen of *M. ciliata* has been deposited in the Advanced Materials Research Chair Laboratories, Department of Chemistry, King Saud University, under the deposition number AMRC-PL-03. All plant samples were collected in accordance with the relevant institutional, national, and international regulations and guidelines.



Fig. 1. Photograph of the plant under investigation (*M. ciliata*).

Preparation of plant samples

The fresh plant was thoroughly washed two to three times with running tap water. The plant parts—flowers, leaves, roots, stems, and the whole plant of *M. ciliata*—were separated; a sample of each fresh plant part was analyzed directly by the DART-ToF-MS instrument, and the rest of the plant was dissected into small pieces and dried under shades at room temperature for 15 days, then ground by using a commercial grinder (Moulinex, Electric Grinder 180 W AR110010). The amount of powder obtained after grinding is 84 g of flowers, 63 g of leaves, 76 g of stems, and 100 g of roots. They were transferred to polyethylene plastic bottles until further use.

Extraction and fractionation

30 g of dried powder of each part (flowers, leaves, stems, and roots) was taken and soaked in closed flasks using 300 mL of methanol (solid-to-solvent ratios 1:10) for each. Also, 80 g of the whole plant powder was soaked in 800 mL of the same solvent. Methanol was selected based on previous studies where methanolic extracts showed the best result and higher activity compared to ethanol and chloroform extracts^{11,30}.

To ensure most of the bioactive compounds are extracted, maceration was carried out three times for the whole plant and each part of the plant separately using a shaker (Unimax 1010, Heidolph, Germany), with agitation fixed at 200 rpm, at ambient temperature for 24 h, followed by filtration of each part separately.

Filtrates of the whole plant and each part were evaporated under reduced pressure (277 mbar) using a rotary evaporator (BUCHI Rotavapor, Switzerland) at a heating bath's temperature not more than 40 °C and a chiller's temperature (5 °C). The dry residue of the whole plant and each part was collected and weighed.

For both the whole plant and parts, 0.5 g from each extract was dissolved in 25 mL of hexane. The solutions were placed on the shaker with agitation fixed at 150 rpm for 1.30 h to ensure that all of the compounds were dissolved. After that, they were filtered through a membrane filter (fine pore, 0.45 µm). Finally, the filtrates were collected and stored at refrigeration temperature for further use.

On the other hand, the total extract of the whole plant was fractionated according to its polarity using each of the following solvents, respectively: hexane, chloroform, ethyl acetate, and *n*-butanol. First, the total extract was dissolved in 50 mL of distilled water, and an equal volume of hexane was added. The solutions were shaken for 30 min. The bottom layer (hexane layer) drains into an airtight container after the layers are fully separated. The fraction was stored in the refrigerator to avoid hexane evaporation. The same procedure was repeated for the remaining aqueous layer with chloroform, ethyl acetate, and *n*-butanol, respectively. The separated solvent layers were kept in the refrigerator till used for analysis later.

Instrumentation and analysis conditions

DART-ToF-MS system

All the parts of the fresh plant (flowers, leaves, stems, and roots) were analyzed by DART-ToF-MS without any sample preparation. The DART-ToF-MS system includes a DART ion source (IonSense, Saugus, MA, USA), atmospheric pressure ionization ToF-MS (Jeol, Tokyo, Japan), and the mass spectrometer, a Jeol, the accurate mass time-of-flight (AccuToF) LC-plus JMS-T100 LP. The mass spectrometer was operated in positive (+ve-ion) mode. It should be noted that as the DART-MS experiments were carried out under soft ionization conditions (i.e., orifice 1 = 5 V), there was little to no fragmentation, and therefore, each peak in the mass spectrum points to one constituent in the protonated form.

To implement mass drift compensation for accurate mass values. A 200 µg/mL solution of polyethylene glycol (PEG 600) in methanol was applied just before each sample. Data acquisition was observed in the mass range

of 100–1000 amu at an acquisition rate of 10 spectra/min. Elemental compositions of chemical compounds detected were compared with accurate mass spectral data with a well-developed library of plant components isolated from the National Institute of Standards and Technology (NIST) standard reference database. All the operating conditions of the DART-ToF-MS are present in Table S1. Additional details regarding the DART-ToF-MS data processing workflow are available in Supplementary Text: Data processing workflow – DART-ToF-MS.

Gas chromatography-mass spectrometry system

In this instrument, the volatile constituents of *M. ciliata* extracts are separated and identified. The hexane extracts of flowers, leaves, stems, roots, and the whole plant were transferred to the GC vial and became ready for GC-MS analysis. The analysis was performed using a GC-MS system consisting of a Thermo Trace GC Ultra gas chromatograph equipped with an AI 3000 autoinjector and linked to a Thermo TSQ Quantum MS (Thermo Scientific, Sunnyvale, CA, USA). Major peaks were scanned in the m/z range from 50 to 1000. Monitoring all apparatus parameters, in addition to data storage and processing, was achieved using Xcalibur™ software from Thermo Fisher Scientific. The NIST and Wiley MS libraries were used to identify the unknown components, and a comparison with the literature was reported. The operating conditions are presented in Table S2. Further details of the GC-MS data processing and post-acquisition analysis are provided in the Supplementary Text: Data processing workflow – GC-MS.

1 Results and discussion

Extraction and fractionation procedure

Extraction of plants is a process of separating active plant constituents (secondary metabolites), such as flavonoids, glycosides, saponins, alkaloids, terpenes, and steroids, from inactive material using a suitable solvent and extraction procedure¹⁸. The solvent used for the extraction process affects the isolated biologically active compounds from plants. In general, polar solvents such as water, methanol, and ethanol are used in the extraction of polar compounds, whereas nonpolar solvents such as hexane and dichloromethane are used in the extraction of nonpolar compounds^{31,32}.

On the other hand, the choice of a suitable extraction method depends on various factors, including the solvent used, temperature, the nature of the plant material, and the solvent-to-sample ratio³³. As the crude plant extracts contain a complex mixture of many metabolites, which could be affected by temperature, the maceration extraction technique is convenient and very suitable for thermolabile plant material, so it was used in this step to avoid degradation of the major components³⁴ and a fractionation procedure, which refers to the separation process where components are separated into different fractions, is used to enable the screening of natural products in the plant extracts more efficiently¹⁸. This process of separating components in plant extracts using liquid-liquid extraction according to polarity, from hexane, which is the least polar, to *n*-butanol, which is the most polar.

In this work, methanol was used as a solvent in the maceration extraction technique, while the fractionation procedure for the whole plant extract was performed using different solvents according to their polarity. The weights of extracts obtained from the whole plant and each part of the plant are represented in Table 1.

Fast profiling of plant using DART-ToF-MS

DART-ToF-MS is a technique that introduces highly definitive screening data leading to the identity of substances present in a sample³⁵. It can provide soft ionization and rapid analysis of samples without the need for sample preparation. With the assistance of DART, exact mass measurements can be done rapidly with high-resolution mass spectrometers.

In this work, all the plant parts were subjected to DART-ToF-MS analysis under the same conditions as demonstrated in Table S1, and the spectra of the fresh plant parts were obtained. More than 30 organic chemical components were determined from different parts of *M. ciliata* (flowers, leaves, stems, and roots) using the DART-ToF-MS technique.

DART-ToF-MS analysis of flowers

The fresh flowers were analyzed by DART-ToF-MS within the mass range of 0–650 Da, and fourteen compounds were tentatively identified based on their exact mass, molecular formula, and literature reports⁹. These compounds are shown in Table 2 and S3. In the mass spectrum, abrine ($m/z=219.12$) was found to be the major alkaloid, with the highest peak intensity among the observed alkaloids, and hence its relative abundance was assigned to be 100%, as shown in Figs. 2a and S1. Although abrine is an alkaloid previously isolated from the *Abrus precatorius* plant³⁶ this study reports its identification in *M. ciliata* for the first time. Therefore, the detection of abrine in this species is considered novel. A higher resolution of the closely related peaks displayed

Part of plant	Sample weight (g)	Extract weight (g)	Extraction yield%
Flowers	30	2.14	7.13
Leaves	30	2.67	8.90
Stems	30	1.70	5.67
Roots	30	3.15	10.5
Whole plant	80	7.12	8.90

Table 1. Weights of methanol extracts for whole plant and each part of the plant.

	No.	Experimental mass	Calculated mass	Mass difference (mmu)	Possible formula	Unsaturation degree	Expected compound
Flowers	1	409.23883	409.23788	0.95	C ₂₆ H ₃₃ O ₄	10.5	Nimbocinol
	2	300.18484	300.18110	3.75	C ₁₅ H ₂₆ NO ₅	3.5	Echinatine
	3	314.19774	314.19675	-0.09	C ₁₆ H ₂₈ NO ₅	3.5	Heliotrine
	4	240.14942	240.15007	-0.65	C ₁₅ H ₁₈ N ₃	8.5	Anatalline
	5	219.11532	219.11335	1.97	C ₁₂ H ₁₅ N ₂ O ₂	6.5	Abrine (N-methyltryptophan)
Leaves	1	300.18484	300.18110	3.75	C ₁₅ H ₂₆ NO ₅	3.5	Echinatine
	2	314.19974	314.19675	-2.18	C ₁₆ H ₂₇ NO ₅	3.5	Heliotrine
	3	268.17827	268.18137	-3.10	C ₁₇ H ₂₂ N ₃	8.5	Elaeocarpidine
	4	430.38369	430.38108	2.59	C ₂₉ H ₅₀ O ₂	5	Vitamin-E
	5	96.04630	96.04494	1.36	C ₅ H ₆ NO	3.5	3-Hydroxypyridine
Stems	1	409.23529	409.23788	-2.60	C ₂₆ H ₃₃ O ₄	10.5	Nimbocinol
	2	261.16163	261.16030	1.33	C ₁₅ H ₂₁ N ₂ O ₂	6.5	Baptifoline
	3	96.04394	96.04494	-1.01	C ₅ H ₆ NO	3.5	3-Hydroxypyridine
	4	299.17242	299.17327	-0.85	C ₁₅ H ₂₅ NO ₅	4	Echinatine
	5	453.24848	453.24750	0.37	C ₂₄ H ₃₇ O ₈	6.5	Colroforoskolin
Roots	1	328.21286	328.21240	0.47	C ₁₇ H ₃₀ NO ₅	3.5	3'-Acetyltrachelanthamine
	2	286.20292	286.20183	1.09	C ₁₅ H ₂₈ NO ₄	2.5	Viridiflorine
	3	437.27090	437.26918	1.72	C ₂₈ H ₃₇ O ₄	10.5	Azadirone
	4	299.17606	299.17327	2.79	C ₁₅ H ₂₆ NO ₅	4	Echinatine
	5	314.19732	314.19675	0.57	C ₁₆ H ₂₈ NO ₅	3.5	Heliotrine

Table 2. Exact mass data for some constituents of *M. ciliata* plant fresh parts by DART-ToF-MS spectra. Table 3 Phytochemicals detected in hexane extract of all parts of *M. ciliata* plant analyzed by GC-MS.

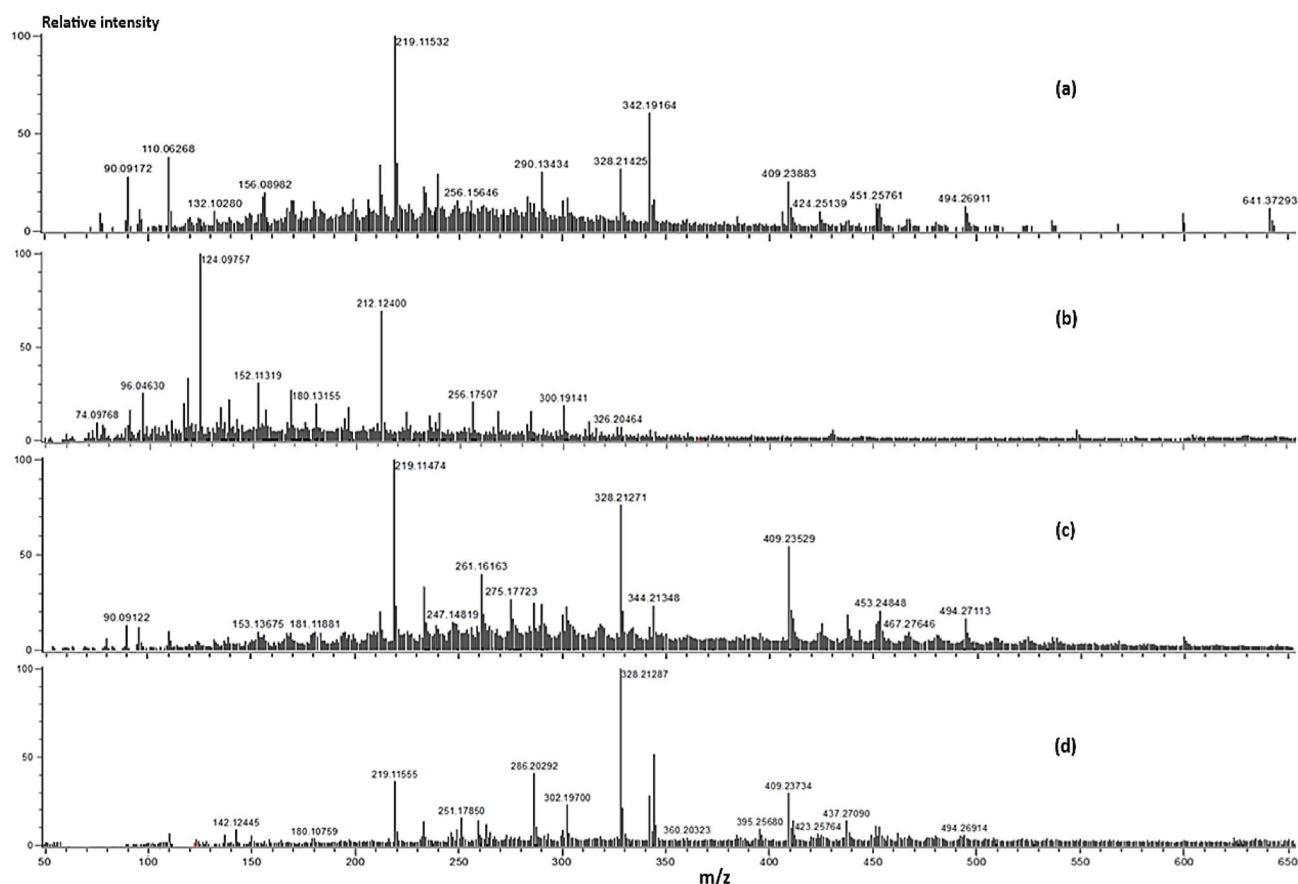


Fig. 2. Full range DART-ToF-MS spectrum from fresh *M. ciliata* plant of (a) flowers, (b) leaves, (c) stems, and (d) roots.

other major alkaloids such as echinatine and heliotrine; these compounds are pyrrolizidine alkaloids, which have been reported as the isolated component from *M. ciliata* growing in Qatar³⁰. Echinatine has a protective effect against ischemia-induced myocardial injury in hearts; this effect may be due to the antioxidant and anti-inflammatory activities of this compound³⁷. Furthermore, two compounds, vitamin B5 and nimboicinol, have been observed in flowers; nimboicinol is a tetracyclic triterpenoid that has inhibitory activity against insects³⁸. The exact mass of constituents identified from the flower sample is listed in Table 2 and S3.

DART-ToF-MS analysis of leaves

Chemical fingerprint analysis of the leaves by DART-ToF-MS is shown in Figs. 2b and S2. The results showed that it contains about fifteen major peaks; the highest peak is not identified, but various compounds have been observed in both leaves and flowers, such as 9-ethyl-9 H-pyrido[2,3-b] indol-2-amine, echinatine, and heliotrine. On the other hand, compounds such as ethanamine, *n*-ethyl-*n*-nitro-, 3-hydroxypyridine, *n*-methylololine, and elaeocarpidine were detected only in the leaves. The compounds ethanamine and *n*-ethyl-*n*-nitro- have been reported in the *Cynoglossum zeylanicum*, which belongs to the same Boraginaceae family³⁹. Data of all compounds detected in the leaves are shown in Table 2 and S4.

DART-ToF-MS analysis of stems

The results of the DART-ToF-MS analysis of stems demonstrate the presence of fifteen phytochemical compounds. The main observation from the spectrum, shown in Figs. 2c and S3, is that the peaks corresponding to the molecular species of echinatine, heliotrine, abrine, 3'-acetyltrachelanthamine, nimboicinol, and 2-amino-6-[(2-amino-2-carboxyethyl) amino] hexanoic acid were observed in the DART-ToF-MS spectra for both stems and flowers.

The stems also contain 3-hydroxypyridine, which is present in the leaves. Furthermore, some compounds, like baptifoline and huperzine U, have been observed in the stems but not detected in leaves and flowers. The spectrum presents three peaks corresponding to the ions of phthalic acid, butyl hept-4-yl ester, 6-beta-hydroxytestosterone, and phthalic acid, butyl hex-3-yl ester, which were detected only in the stems. It is noticeable from Table 2 and S5 that the difference between the detected and calculated mass was less than ± 4.61 mDa in all cases, which means a high accuracy.

DART-MS analysis of roots

Fourteen compounds were tentatively identified from the fresh roots by DART-ToF-MS, as shown in Table 2 and S6. The main result from the spectrum, shown in Figs. 2d and S4, is the six peaks corresponding to pyrrolizidine alkaloids such as 3'-acetyltrachelanthamine, viridiflorine, 3'-acetylcopsamine, megalanthoin, echinatine, and heliotrine. Pyrrolizidine alkaloids are common secondary metabolites in the Boraginaceae family and serve as chemical defense compounds mainly against herbivores and microbes⁴⁰. The same phytochemicals present in both the flowers and the stems mentioned above are also present in the roots. The detected and calculated masses are mentioned in Table 2 and S6.

The analysis of volatile constituents of *M. ciliata* plant parts by GC-MS

Optimization of the GC-MS conditions

GC-MS is one of the most accurate methods to identify several secondary metabolites present in plant extract⁴¹. Optimization of GC-MS parameters in the analysis of the constituents of the non-polar parts of *M. ciliata* plant extract was achieved by investigating the influence of the carrier gas flow speed, temperature program, and detection conditions, where the best possible separation of the compounds was considered. The GC-MS operating conditions and the detection conditions in the mass spectrometer are presented in Table S2.

To mark the identity of peaks, each spectrum was compared to the spectra found in the main MS spectral libraries, NIST and Wiley. Also, all of the identified compounds were confirmed by specialized natural products online electronic databases such as COCONUT (<https://coconut.naturalproducts.net/>) and LOTUS (<https://lotus.naturalproducts.net/>).

GC-MS analysis of hexane extracts of *M. ciliata* plant parts

The GC-MS analysis of hexane extracts of each part of *M. ciliata* revealed the presence of twenty-one, twenty-four, twenty-four, and eighteen volatile constituents from flowers, leaves, stems, and roots, respectively. Among those, fourteen compounds are present in all parts of the plant. A steroid compound (γ -sitosterol) with a retention time of 57.5 min was identified by GC-MS analysis as the main constituent of the hexane extract.

The GC-MS chromatograms for all four extracts are presented in Fig. 3. The active principles found in all the parts, with their retention time (t_R), molecular weight (MW), and main fragments, are presented in Table 3.

The GC-MS chromatograms obtained revealed that *M. ciliata* is rich in bioactive compounds in all parts of the plant. For example, γ -Sitosterol shows anticancerous⁴² anti-hyperglycemic, and hepatoprotective activity and may be used as a potential antidiabetic⁴³. Campesterol showed anticancer and antioxidant activities^{44,45}. Likewise, stigmasterol has antiviral, cancer-preventive, anti-inflammatory, and anti-osteoarthritic activity⁴⁶. Vitamin E showed antiaging, antitumor, antidiabetic, and anticancer activity⁴⁷. Hexadecanoic acid methyl ester has been reported as an antibacterial, antioxidant, antitumor, immunostimulant, chemopreventive, and lipoxigenase inhibitor⁴⁸.

The graph in Fig. 4 shows that the plant is rich in steroids, and the γ -sitosterol is the highest phytochemical in all parts of the plant, followed by campesterol and stigmasterol in both the stems and the roots. As for pyrrolizidine alkaloid compounds, echinatine and heliotrine are present more in the flowers than in the rest of the parts, and the echinatine percentage is higher than heliotrine in all of the parts, which is the same observation in the DART

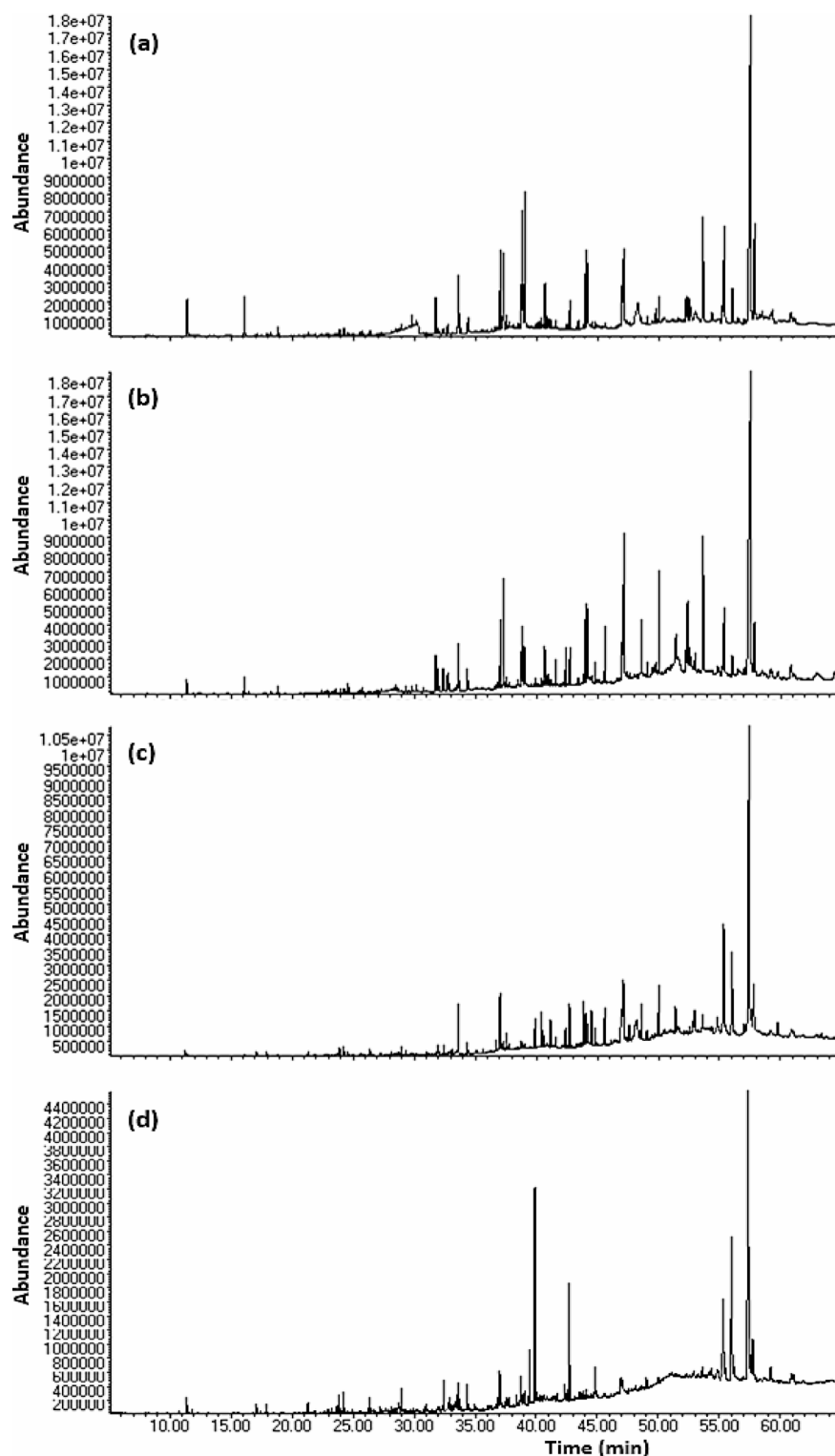


Fig. 3. The GC-MS chromatograms of hexane extract for *M. ciliata* parts showing relative abundance and retention time of phytochemicals. Where: (a) flowers, (b) leaves, (c) stems, and (d) roots.

results. Fig. S5 shows experimental and standard mass spectra for some of the identified compounds in the hexane extract.

The phytochemical compound that is detected in all aerial parts (flowers, leaves, and stems) is pentacosane (Table 4). It is noted that both flowers and leaves contain five major constituents; they are benzofuran, 2,3-dihydro-, methyl ferulate, neophytadiene, phytol, and squalene. Abdel-Hak and his co-workers reported that squalene has antitumor and antioxidant properties¹¹. A previous study reported that phytol is present in

No	t _R (min)	Expected compound	Formula	MW	Main fragments
1	11.38	Indolizine	C ₈ H ₇ N	117	117,89,90,63,116
2	33.60	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270	74,87,55,143,75,227
3	34.35	Dibutyl phthalate	C ₁₆ H ₂₂ O ₄	278	149,150,223,104,205
4	36.95	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (linoleic acid, methyl ester)	C ₁₉ H ₃₄ O ₂	294	67,81,55,95,82,79
5	37.04	9-Octadecenoic acid (Z)-, methyl ester (oleic acid, methyl ester)	C ₁₉ H ₃₆ O ₂	296	202,55,79,57,67
6	38.73	Echinatine	C ₁₅ H ₂₅ NO ₅	299	138,93,94,139,80,95
7	39.09	Heliotrine	C ₁₆ H ₂₇ NO ₅	313	138,93,94,139,80
8	46.17	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (palmitin, 2-mono-)	C ₁₉ H ₃₈ O ₄	330	98,57,55,74,239
9	46.96	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester (Linolein, 2-mono-)	C ₂₁ H ₃₈ O ₄	354	67,81,55,95,79
10	53.66	Vitamin-E	C ₁₉ H ₃₈ O ₄	330	165,430,164,431,166
11	55.33	Campesterol	C ₂₈ H ₄₈ O	400	400,105,107,145,55
12	56.03	Stigmasterol	C ₂₉ H ₄₈ O	412	414,107,105,145,55
13	57.43	γ-Sitosterol	C ₂₉ H ₅₀ O	414	414,55,107,57,145
14	57.79	Stigmasta-5,24(28)-dien-3-ol, (3β,24Z)-	C ₂₉ H ₄₈ O	412	314,55,207,281,69

Table 3. Phytochemicals detected in hexane extract of all parts of *M. ciliata* plant analyzed by GC-MS.

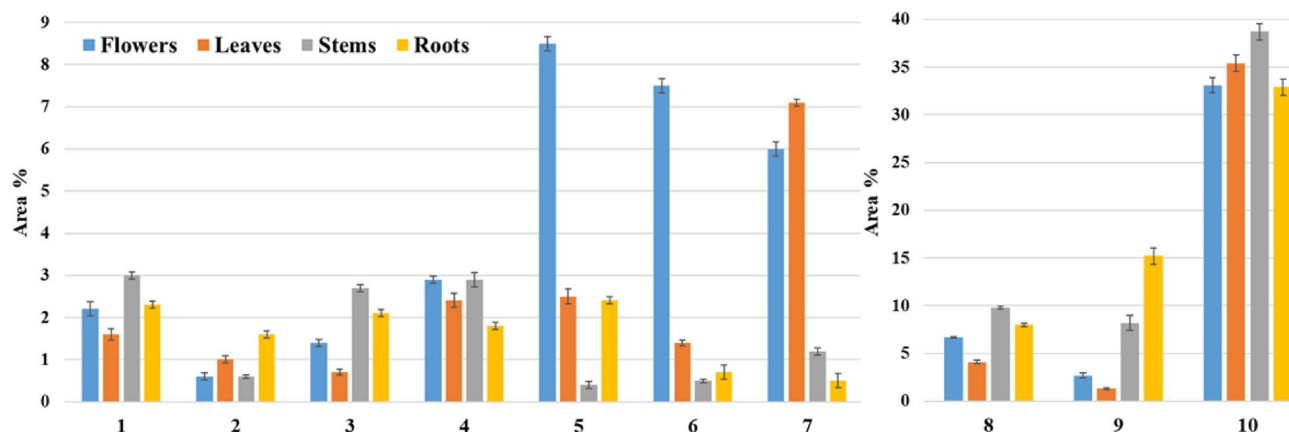


Fig. 4. Some of the phytochemicals detected in all parts of the plant. Where: (1) Hexadecanoic acid, methyl ester, (2) Dibutyl phthalate, (3) Linoleic acid, methyl ester, (4) Oleic acid, methyl ester, (5) Echinatine, (6) Heliotrine, (7) Vitamin-E, (8) Campesterol, (9) Stigmasterol, and (10) γ-Sitosterol.

“*Cynoglossum zeylanicum*,” which belongs to the same family as *M. ciliata* and has biological activity as an anticancer, antioxidant, anti-inflammatory, and diuretic⁴². Also, neophytadiene has been reported as an antipyretic, analgesic, anti-inflammatory, anti-microbial, and antioxidant⁴⁹. Moreover, leaves and stems share two significant phytochemicals; they are behenic alcohol and hexacosane. While it was observed that there were three compounds common in the stems and roots, they are benzene, 1,3-bis (1,1 dimethylethyl), 6β-Hydroxytestosterone, and diisooctyl phthalate. In addition, there are three constituents that were detected in the leaves: 4,8,12,16-tetramethylheptadecan-4-olide, octadecane (3-ethyl-5-(2-ethylbutyl)) and tetracosane. Moreover, four constituents were observed in the stems: eicosanoic acid, methyl ester, docosanoic acid, methyl ester, tetratetracontane, and stigmasta-3,5-dien-7-one. Further, androstan-9-thiocyanato-3,11,17-trione was detected in roots. All of the phytochemicals detected from hexane extracts of *M. ciliata* plant parts are listed in Table 4.

To facilitate identification of the detected compounds, the retention indices of the identified compounds were calculated in parallel with the mass spectral library matching. For this purpose, a linear alkane series mixture has been injected at the same chromatographic conditions as the plant part extracts. The calculated retention indices for the detected compounds were in good agreement with those reported for authentic standards injected on the most related stationary phase used in this work (HP-5MS). For example, indole showed a calculated retention index of 1341, which closely matched the experimental value of 1340. Similarly, myristic acid exhibited a calculated retention index of 1772, in agreement with the reported value of 1771, and 9,12-octadecadienoic acid (Z, Z)- showed a calculated retention index of 2106, consistent with the reference retention index of 2104. The details of the retention index calculations are provided in Tables S7 and S8, Fig. S6, and Supplementary Text: Retention index calculations.

	No	t _R (min)	Expected compound	Formula	MW	Area%	Main fragments
Flowers	1	16.08	Benzofuran, 2,3-dihydro-	C ₈ H ₈ O	120	1.7	120,91,119,65,121
	2	31.76	Methyl ferulate	C ₁₁ H ₁₂ O ₄	208	1.2	208,177,145,117,89
	3	31.81	Neophytadiene	C ₂₀ H ₃₈	278	0.8	68,95,82,57,69,123
	4	37.30	Phytol	C ₂₀ H ₄₀ O	296	3.4	71,123,57,81,55
	5	40.26	Docosane	C ₂₂ H ₄₆	310	1.7	57,43,71,85,99
	6	44.02	Tetracosane	C ₂₄ H ₅₀	338	3.2	57,71,85,55,99
	7	52.40	Squalene	C ₃₀ H ₅₀	410	1.2	69,81,95,55,93
Leaves	1	16.08	Benzofuran, 2,3-dihydro-	C ₈ H ₈ O	120	0.7	120,91,119,65,121
	2	31.76	Methyl ferulate	C ₁₁ H ₁₂ O ₄	208	1.2	208,177,145,117,89
	3	31.81	Neophytadiene	C ₂₀ H ₃₈	278	1.0	68,95,82,57,69,123
	4	37.30	Phytol	C ₂₀ H ₄₀ O	296	3.9	71,123,57,81,55,69
	5	40.60	Behenic alcohol (docosanol)	C ₂₂ H ₄₆ O	326	1.2	83,57,55,97,69
	6	41.59	4,8,12,16-Tetramethylheptadecan-4-olide	C ₂₁ H ₄₀ O ₂	324	0.9	99,55,57,69,71,83
	7	42.40	Tricosane	C ₂₃ H ₄₈	324	1.3	57,71,85,55,99
	8	44.04	Tetracosane	C ₂₄ H ₅₀	338	3.2	57,71,85,55,99
	9	45.60	Pentacosane	C ₂₅ H ₅₂	352	2.0	57,71,85,55,99
	10	47.15	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	C ₂₆ H ₅₄	366	9.5	57,71,85,55,69
	11	52.40	Squalene	C ₃₀ H ₅₀	410	2.6	69,81,95,55,93
Stems	1	17.11	Benzene, 1,3-bis(1,1 dimethylethyl)-	C ₁₄ H ₂₂	190	0.2	175,57,190,176,91
	2	39.90	6β-Hydroxytestosterone	C ₁₉ H ₂₈ O ₃	304	1.7	274,137,87,304,191
	3	40.40	Behenic alcohol (docosanol)	C ₂₂ H ₄₆ O	326	2.1	83,57,55,97,69
	4	41.18	Eicosanoic acid, methyl ester	C ₂₁ H ₄₂ O ₂	326	1.5	74,87,75,55,143
	5	44.02	Tetracosane	C ₂₄ H ₅₀	338	1.1	57,71,85,55,99
	6	44.52	Docosanoic acid, methyl ester	C ₂₃ H ₄₆ O ₂	354	2.0	78,87,75,55,57
	7	44.82	Diisooctyl phthalate	C ₂₄ H ₃₈ O ₄	390	0.9	149,167,57,71,70
	8	45.61	Pentacosane	C ₂₅ H ₅₂	352	1.9	57,71,85,55,99
	9	50.01	Nonacosane	C ₂₉ H ₆₀	408	2.9	57,71,85,43,99
	10	59.78	Stigmasta-3,5-dien-7-one	C ₂₉ H ₄₆ O	410	1.9	174,410,57,161,71
Roots	1	17.11	Benzene, 1,3-bis(1,1 dimethylethyl)-	C ₁₄ H ₂₂	190	0.5	175,57,190,176,91
	2	39.45	Androstan-9-thiocyanato-3,11,17-trione	C ₂₀ H ₂₅ NO ₃ S	359	3.3	287,304,91,203,219
	3	39.89	6β-Hydroxytestosterone	C ₁₉ H ₂₈ O ₃	304	13.4	274,137,191,175,55
	4	44.82	Diisooctyl phthalate	C ₂₄ H ₃₈ O ₄	390	2.1	149,167,57,71,70

Table 4. Phytochemicals detected from hexane extract of *M. ciliata* plant parts analyzed by GC-MS.

Method	Compounds
DART-ToF-MS	Acetylintermediate Cornucervine Ethanamine, <i>n</i> -ethyl- <i>n</i> -nitro- 3'-Acetyltrachelanthamin
GC-MS	γ-Sitosterol Stigmasterol Campesterol Hexadecanoic acid Methyl ester Phytol Squalene
Both Methods	Echinatin Heliotrin Vitamin-E Phthalic acid Butyl hept-4-ester ester Butyl hex-3-yl ester 6β-Hydroxytestosterone

Table 5. Comparison of phytochemicals detected from *M. ciliata* by DART-ToF-MS and GC-MS methods.

Correlation between MS techniques

The results of both DART-ToF-MS and GC-MS for the different parts of the plant showed the presence of the same phytochemicals, for example, echinatin, heliotrin, vitamin E, phthalic acid, butyl hept-4-ester, 6β-hydroxytestosterone, and phthalic acid, butyl hex-3-yl ester. Table 5 presents some of the phytochemicals

detected by both DART-ToF-MS and GC-MS in correlation with the compounds that have been detected only by one technique. On the other hand, some compounds were present in more than one part of the plant. However, only echinatin was detected in all parts of the plant (flowers, leaves, stems, and roots) based on the DART-ToF-MS method. Tables S9 and S10 demonstrate the distribution of the common compounds detected across different plant parts using DART-ToF-MS and GC-MS techniques, respectively.

While both techniques use mass spectrometry for the detection of the sample constituents, DART-ToF-MS and GC-MS differ significantly in their ionization mechanisms, resolution, and the way analytical information is interpreted. DART-ToF-MS employs ambient ionization, where metastable species such as excited-state helium transfer energy to analyte molecules in open air, producing ions with minimal sample preparation. This process represents a soft ionization technique that generally generates intact molecular ions, facilitating rapid identification but often providing limited structural fragmentation for mechanistic interpretation. The coupling of DART with ToF analyzers enables high-resolution mass measurements, allowing accurate determination of elemental compositions and improved molecular formula elucidation^{27–29}. In contrast, GC-MS typically employs electron ionization within a controlled vacuum environment, where analytes are first separated by gas chromatography before undergoing high-energy ionization. As a hard ionization technique, electron ionization induces extensive fragmentation, producing reproducible fragmentation patterns that act as molecular fingerprints for compound identification and enable detailed mechanistic insight into bond cleavage and molecular structure. Based on the results shown in Table 5, S9, and S10, we conclude that DART-ToF-MS and GC-MS are complementary analytical techniques, and together they provide a powerful approach for the comprehensive identification and efficient analysis of the bioactive compounds in plants.

Fractionation and GC analysis of the plant extracts

GC-MS analysis of the hexane fraction

Total extract in a hexane fraction of *M. ciliata* revealed the presence of 31 chemical compounds. The whole plant the hexane extract shows higher phytoconstituent peaks than other plant parts. The GC-MS chromatogram of the total extract in hexane is presented in Fig. 5a. The compounds identified in this fraction (Table 6, and S11) are the same as those identified in the different parts of the plant, except for three compounds that did not show clearly in the other different parts; they are *n*-hexadecanoic acid, myristic acid, and 9,12-octadecadienoic acid (Z, Z). A previous study reported that these three phytochemicals are present in *Cynoglossum zeylanicum*, which belongs to the same family. *n*-Hexadecanoic acid showed some activity as an antioxidant and antibacterial⁵⁰. Myristic acid is used in cosmetic and topical medicinal preparations where good absorption through the skin is desired⁵¹. 9,12-Octadecadienoic acid has been reported to show some properties as anti-inflammatory, cancer preventive, hepatoprotective, nematocide, insectifuge, antihistaminic, antieczemic, antiacne, and anticoronary⁴². Fig. S7 reveals experimental and standard mass spectra for *n*-hexadecanoic acid, methyl ester, and vitamin E in the hexane fraction.

GC-MS analysis of the chloroform fraction

The chloroform fraction of *M. ciliata* was processed for GC-MS analysis to identify its phytochemical content. GC-MS analysis of the chloroform fraction showed the presence of forty chemical compounds (Table 6, and S12, Fig. 5b). In our GC-MS analysis, major bioactive components of the chloroform fraction are γ -sitosterol, stigmasterol, campesterol, 6 β -hydroxytestosterone, vitamin E, indolizine, and diisooctyl phthalate. Diisooctyl phthalate is one of the derivatives of phthalate ester, which has been reported to possess strong inhibitory effects against the growth of different bacterial, fungal, and algal species⁵². The chromatogram of the chloroform fraction was marked by new peaks. For example, 7-dehydrodiosgenin, tetratetracontane, and 9,12-octadecadienoic acid (Z, Z)-2-hydroxy-1-(hydroxymethyl)ethyl ester. 7-dehydrodiosgenin is a steroid compound that has been reported as an anticancer⁵³. Tetratetracontane showed some activity as an antioxidant and cytoprotective⁵⁴. Fig. S8 shows the experimental and standard mass spectra for 6 β -hydroxytestosterone and phytol compounds in the chloroform fraction.

GC-MS analysis of the Ethyl acetate fraction

The extract of *M. ciliata* in the ethyl acetate fraction revealed the presence of twenty-four chemical constituents. The GC-MS chromatogram of the total extract in the ethyl acetate fraction is presented in Fig. 5c. Some of the identified compounds in the ethyl acetate fraction (Table 6, and S13) are benzofuran, 2,3-dihydro-, 2-methoxy-4-vinylphenol (vinylguaiaicol), γ -sitosterol, benzaldehyde, 2-hydroxy-6-methyl-, phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-, indole, indolizine, 3,5'-dimethoxyacetophenone, and diisooctyl phthalate. The highest peak is benzofuran, 2,3-dihydro-, known as coumaran; it is reported to have antihelminthic, anti-inflammatory, and antiarrhythmic activities⁵⁵. Results showed the presence of some phenolic compounds in the ethyl acetate fraction. For example, benzenethanol, 4-hydroxy- (tyrosol), which shows an antioxidant property⁵⁶ 2-methyl-4-vinylphenol (vinylguaiaicol), benzaldehyde, 2-hydroxy-6-methyl-, trans-coniferyl alcohol, and trans-sinapyl alcohol. This might be due to the increase in the polarity of this solvent compared to hexane and chloroform. Experimental and standard mass spectra for some of the identified compounds of the total extract in the ethyl acetate fraction are shown in Fig. S9.

GC-MS analysis of the n-butanol fraction

The *n*-butanol fraction of *M. ciliata* was processed for GC-MS analysis to identify its phytochemical content. GC-MS analysis of the *n*-butanol fraction showed the presence of eleven chemical compounds (Fig. 5d; Table 6, and S14). In our GC-MS analysis, major bioactive components of the *n*-butanol fraction are heliotrine, echinatin, propane, 2,2'-[methylenebis(oxy)]bis[2-methyl-, benzofuran, 2,3-dihydro-(coumaran), butanoic acid, butyl ester, butane, 1,1-dibutoxy, and orthoformic acid, tri-isobutyl ester. Experimental and standard mass spectra

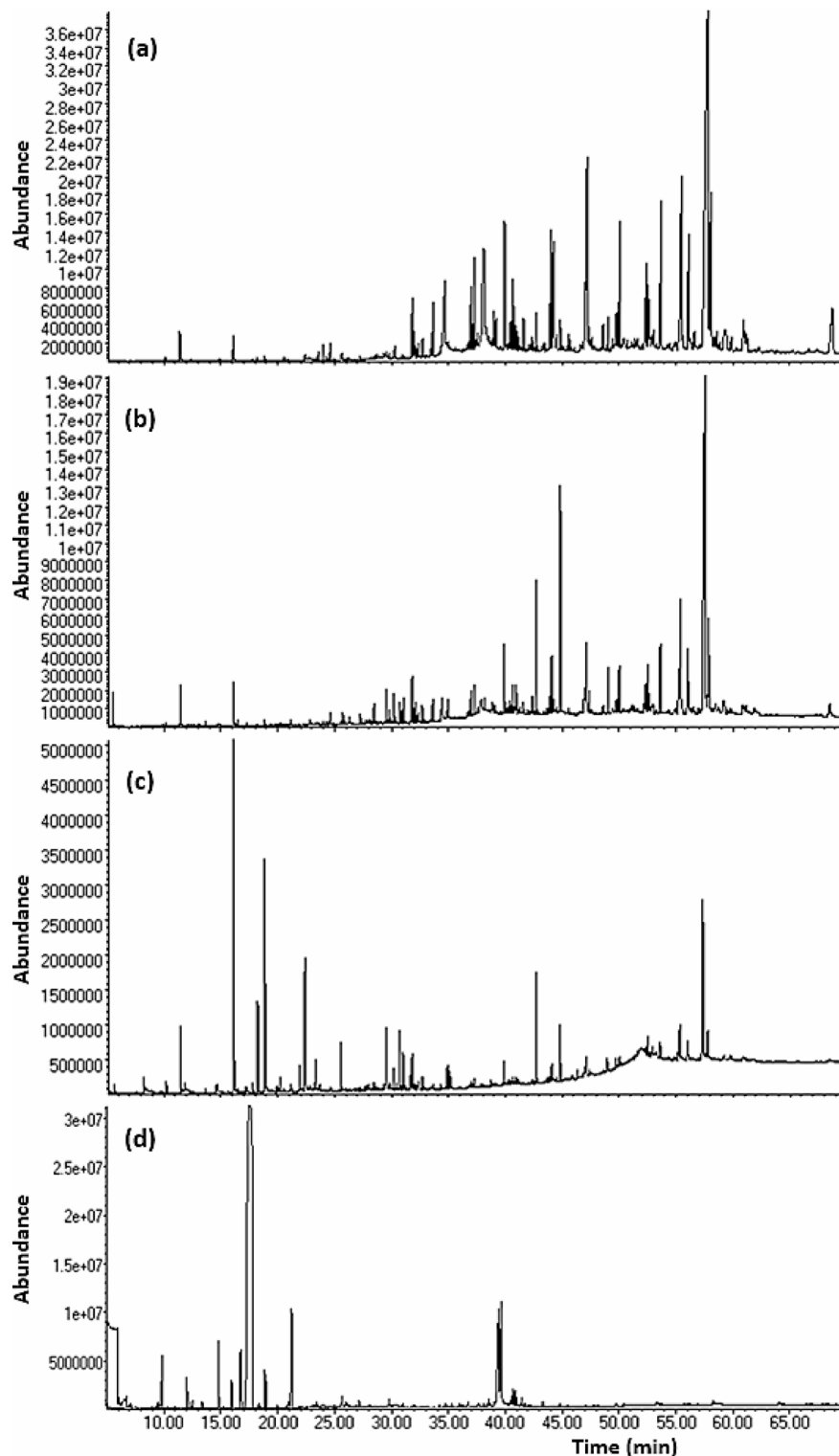


Fig. 5. The GC-MS chromatogram of the total extract for *M. ciliata* in (a) hexane fraction, (b) chloroform fraction, (c) ethyl acetate fraction, and (d) *n*-butanol fraction.

for the last two compounds in the *n*-butanol fraction are revealed in Fig. S10. The chromatogram shows that the highest peak is butane,1,1-dibutoxy. Kawuri and his co-workers reported that it has antimicrobial activity⁵⁴. Finally, no peak of steroids in the butanol fraction appeared in the range of 50–60 min, which is already the steroid's peak range under the same conditions of analysis.

Again, the results show the presence of some compounds in more than one fraction. Table S15 displays the distribution of the common compounds detected across different solvent fractions using the GC-MS technique.

	No	t _R (min)	Expected compound	Formula	MW	Area%	Main fragments
Hexane	1	11.38	Indolizine	C ₈ H ₇ N	117	0.7	117,89,90,63,116
	2	30.26	Tetradecanoic acid (Myristic acid)	C ₁₄ H ₂₈ O ₂	228	0.3	73,60,129,55,57
	3	34.69	<i>n</i> -Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	3.1	73,60,55,57,129
	4	38.10	9,12-Octadecadienoic acid (Z,Z)-	C ₁₈ H ₃₂ O ₂	280	6.3	67,81,79,95,55
	5	53.66	Vitamin-E	C ₂₉ H ₅₀ O ₂	430	4.8	165,430,164,431,166
	6	55.33	Campesterol	C ₂₈ H ₄₈ O	400	8.5	400,145,107,105,382
	7	57.78	γ-Sitosterol	C ₂₉ H ₅₀ O	414	32.1	414,55,107,57,145
Chloroform	1	11.44	Indolizine	C ₈ H ₇ N	117	1.4	117,89,90,63,116
	2	39.90	6β-Hydroxytestosterone	C ₁₉ H ₂₈ O ₃	304	2.6	274,137,87,304,191
	3	53.64	Vitamin-E	C ₂₉ H ₅₀ O ₂	430	3.4	165,430,164,431,166
	4	55.37	Campesterol	C ₂₈ H ₄₈ O	400	6.5	400,145,107,105,382
	5	44.85	Diisooctyl phthalate	C ₂₄ H ₃₈ O ₄	390	8.1	149167,57,71,70
	6	56.06	Stigmasterol	C ₂₉ H ₄₈ O	412	4	55,83,412,81,255
	7	57.55	γ-Sitosterol	C ₂₉ H ₅₀ O	414	32.5	414,55,107,57,145
Ethyl acetate	1	11.46	Indolizine	C ₈ H ₇ N	117	3.1	117,89,90,63,116
	2	18.24	Indole	C ₈ H ₇ N	117	4.4	117,90,89,63,118
	3	55.32	Campesterol	C ₂₈ H ₄₈ O	400	2.6	400,145,107,105,382
	4	16.27	Benzofuran, 2,3-dihydro-(Coumaran)	C ₈ H ₈ O	120	18.1	120,91,119,65,121,94
	5	57.36	γ-Sitosterol	C ₂₉ H ₅₀ O	414	16.1	414,55,107,57,145
	6	18.86	2-Methoxy-4-vinylphenol (Vinylguaicol)	C ₉ H ₁₀ O ₂	150	14.3	150,135,107,77,151
	7	21.94	Benzeneethanol, 4-hydroxy- (Tyrosol)	C ₈ H ₁₀ O ₂	138	1.6	107,138,77,108,78
<i>n</i> -Butanol	1	6.10	Cyclobutane	C ₄ H ₈	56	1.2	56,55,57,53,73
	2	10.10	Butanoic acid, butyl ester	C ₈ H ₁₆ O ₂	144	0.8	71,89,56,57
	3	12.25	Indolizine	C ₈ H ₇ N	117	1.6	117,89,90,63,116
	4	38.73	Heliotrine	C ₁₆ H ₂₇ NO ₅	313	7.2	138,93,94,139,80,95
	5	17.66	Butane, 1,1-dibutoxy-	C ₁₂ H ₂₆ O ₂	202	0.8	57,73,129,55,159
	6	39.09	Echinatine	C ₁₅ H ₂₅ NO ₅	299	5.3	138,93,94,139,80,95

Table 6. Phytochemicals detected of the total methanol extract of *M. ciliata* in different fractions by GC-MS. .

As an example, campesterol and γ-sitosterol were registered in three fractions, i.e., hexane, chloroform, and ethyl acetate, while only indolizine was detected in all fractions based on the optimized GC-MS method.

Comparison study

The aim of this section is to compare the characteristics of the proposed extraction and analysis methods with other reported works that studied species that belong to the Boraginaceae family. All of the comparison data are given in Table 7. In a recent study, Abdel-Haq and his co-workers analyzed the chemical composition of *M. ciliata* from Egypt¹¹ and reported some compounds that agree with our results in this work. For example, *n*-hexadecanoic acid, hexadecanoic acid methyl ester, tetradecanoic acid, 9,12-octadecadienoic acid methyl ester, eicosanoic acid methyl ester, and 4,8,12,16-tetramethylheptadecan-4-olide are all present in a hexane fraction. Also, there are other constituents presented in other fractions, like 2-methoxy-4-vinylphenol, squalene, and 6-hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one.

Conclusion

Natural products analysis is particularly challenging due to the complexity of its chemical components. Therefore, the separation and analysis of plant extracts are critical in developing accurate and reliable analytical methods. In this work, the main natural constituents and bioactive compounds of the *M. ciliata* plant were extracted and investigated. The triple maceration extraction technique has been used to obtain the total plant extracts, followed by liquid-liquid extraction to reduce the matrix effect. For comprehensive screening, the plant's extracts and fresh parts were analyzed using two complementary mass spectrometric methods, DART-ToF-MS and GC-MS. DART-ToF-MS detected more than 30 compounds and over 60 compounds using the GC-MS method, with many of the identified compounds known to possess different biological activities. Future researches should focus on applying quantitative analytical methods to determine the precise concentration levels of key compounds and to confirm their biological activities. However, Table S16 summarizes the most important compounds successfully identified in the *M. ciliata* plant using both mass spectrometric methods, along with their chemical classes and reported pharmacological properties. The results indicate that the developed methods are complementary and can be used together to expand the scope of investigation and analysis of active compounds present in plants, including guiding bioactivity assays, supporting quality control in herbal drug formulations, and ensuring the safety of plant-based products. Thus, the results of this study enhance the potential use of *M. ciliata* in pharmacological and biological contexts and also advance analytical procedures.

Plant name		<i>M. ciliata</i>	<i>M. ciliata</i>	<i>Cynoglossum zeylanicum</i>	<i>Cordia retusa</i>	<i>Cordia dichotoma</i>
Country		Saudi Arabia	Egypt	Tamil Nadu	India	India
Studied part		Whole plant	Aerial parts	Whole plant	Aerial parts	Leaves
Extraction metho		Triple maceration	Maceration	Maceration	Maceration	Maceration
Detection technique		GC-MS	GC-MS	GC-MS	GC-MS	GC-MS
Extraction solvent		Methanol	Methanol	Ethanol	Ethanol	Methanol
Common compounds	<i>n</i> -Hexadecanoic acid	✓	✓	✓	-	✓
	Hexadecenoic acid methyl ester	✓	✓	-	-	✓
	Tetradecanoic acid	✓	✓	✓	-	-
	9,12-Octadecadienoic acid methyl ester	✓	✓	-	-	-
	2-Methoxy-4- vinylphenol	✓	✓	-	-	-
	Squalene	✓	✓	✓	✓	✓
	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	✓	✓	-	-	-
	4-((1E)-3-hydroxy-1- propyl)-2- methoxyphenol	✓	✓	-	-	-
	Vitamin-E	✓	-	-	✓	-
	Phytol	✓	-	✓	✓	✓
	Stigmasterol	✓	-	✓	-	-
	γ-Sitosterol	✓	-	✓	-	-
References		This work	11	39	57	58

Table 7. Comparison study for some species belonging to the Boraginaceae family.

Data availability

All data generated or analyzed during this study are included in this article and supplementary materials. Further inquiries can be directed to the corresponding author.

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N.S. Formal analysis, investigation, data curation, methods validation, software. A.M. Methodology, formal analysis, investigation, supervision. A.R. Research methodology, software, writing—original draft. A.B. Conceptualization, research methodology, software, writing—review and editing. Z.A. Conceptualization, research methodology, resources, supervision, writing—review and editing. A.A. Conceptualization, methodology, formal analysis, investigation, data curation, methods validation, supervision, writing—original draft. All authors reviewed the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

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