RESEARCH PAPER

INDIGENOUS MICROALGA (CHLORELLA SOROKINIANA SM1) ISOLATION, CARBON CAPTURE POTENTIAL AND PHARMACEUTICAL METABOLITE PROFILING

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Received: 13th Feb, 2026; Revised: 8th April 2026; Accepted: 09th May, 2026; Available Online: 10th May, 2026

Abstract

Background: Microalgae are becoming widely recognized as efficient agents for sustainable carbon capture and bioactive metabolite production with potent pharmaceutical applications**.** At present there is an argent need for biobased pharmaceutical bio compounds.

Objectives: To isolate new microalgal species for carbon capture and utilization as biopharma products and confirm the presence of bioactive compounds.

Methods: Traditional methods of serial dilutions from soil, monoculture establishment, morphological analysis, axenic culture development, molecular identification were carried out for the identification of indigenous microalgal species. Microalga *Chlorella sorokiniana* SM1 growth analysis, pigment profile and theoretical carbon capture potential was carried out. The LCMS analysis was performed using *Chlorella sorokiniana* SM1 extract for the identification of potential pharmaceutical compounds.

Results: The indigenous microalga Chlorella sorokiniana SM1, a novel strain, was isolated, made axenic and identified using molecular techniques. The cells were spherical in size and were around 2.68 µm in diameter. The biomass of isolated Chlorella sorokiniana SM1 strain was 0.74 g/L as compared to 0.63 g/L of already known Chlorella sorokiniana species used for comparison purposes. The theoretical C capture potential of the isolated Chlorella sorokiniana SM1 and Chlorella sorokiniana were calculated to be 1.33 and 1.13g/g biomass respectively, which was 37.5% higher. Chlorophyll a, b and carotenoids of the isolated strain were found to be 14%, 16& and 33% greater than that of known Chlorella sorokiniana. Also, 23 unique bioactive substances, including metabolites with possible antibacterial, antiviral, anticancer, antidiabetic, antioxidant, and anti-inflammatory qualities, were found by LC-MS analysis.

Conclusion: Overall, the findings highlight the dual utility of *Chlorella sorokiniana* SM1 in climate change mitigation and pharmaceutical applications, positioning *C. sorokiniana* SM1 as a promising candidate for integrated environmental and pharmaceutical industry sustainability.

KEYWORDS: *Chlorella sorokiniana*, Molecular studies, LCMS Analysis, Microalgal metabolites, Carbon sequestration

How to cite this article: Mulani M, Kothari V, Pawar S, Nikule H, Bhagat S, Jadhav P, Rathod JP. Indigenous Microalga (Chlorella sorokiniana SM1) Isolation, Carbon Capture Potential and Pharmaceutical Metabolite Profiling. Int J Drug Deliv Technol. 2026;16(48s): 826-834. DOI: 10.25258/ijddt.16.48s.79

Introduction

Carbon capture, its storage, or utilization is gaining significant importance worldwide due to the catastrophic effects of climate change. It includes global warming, caused by excess greenhouse gas emissions such as methane and carbon dioxide, which also leads to a decrease in food productivity and has adverse effects in various sectors, including agriculture and health [1]. In recent years, growing concerns over nutrient and fuel energy shortages—driven by rapid population growth, climate change, and environmental degradation—have highlighted the urgent need for sustainable resources to restore balance between humans and the planet [2]. Microalgae are unicellular organisms capable of photosynthesis, harnessing carbon dioxide and sunlight to produce a diverse array of valuable metabolites [3-5]. Through CO_2 assimilation, they generate compounds such as carbohydrates, proteins, lipids, astaxanthin, omega-3 fatty acids, β-carotene, lutein, chlorophylls a and b, and phycocyanin. These substances serve as antioxidants and bioactive agents, and are widely applied in industries including dyes, cosmetics, food, and as animal feed [6-10]. Estimates suggest that algae encompass between 70,000 and 1 million distinct species [11]. The use of molecular markers such as 18S and ITS

genes have significantly advanced the classification of microalgal taxa. Furthermore, both morphological and genetic analyses have been instrumental in identifying specific microalgal species [12]. Accurate identification of microalgae in various environments is essential for exploring their potential applications [13,14]. Advanced molecular approaches, such as DNA barcoding, have significantly contributed to building phylogenetic relationships and resolving taxonomically challenging groups, thereby reducing the risk of misidentification due to morphological similarities [15,16]. Continued exploration and analysis of specific and previously unexamined environments may lead to the discovery of new strains and species possessing superior or yet-to-be-explored biotechnological capabilities [17]. This study focused on isolating a microalga from native agricultural soils for assessment of its potential in carbon capture and the production of bioactive compounds. The strain, initially named BMT (SM1), was identified morphologically and confirmed as Chlorella sorokiniana SM1 through molecular techniques. Examination of its growth, pigment profile, and ability to absorb CO_2 was undertaken. LC-MS analysis revealed 23 bioactive compounds with properties such as antibacterial, antiviral, anticancer, antidiabetic, antioxidant, and anti-inflammatory effects. Axenic cultures were developed to study their growth and metabolite production, highlighting their promise for sustainable biotechnological and pharmaceutical applications .

2. MATERIALS AND METHODS

2.1 Sampling site

Soil samples were collected from an open field in Shardanagar, Malegaon, Baramati, India (Latitude 18.139062°, Longitude 74.534432°). Using a sterile scalpel, samples were collected from the open field in July and August 2022 and transported to the lab in a sterile zip-lock pouch (Fig. 1A, B, C).

2.2 Isolation and preparation of axenic culture

The soil sample was inoculated into sterile BG11 medium and incubated for one month until a green coloration indicated microalgal growth. During initial isolation, antibiotic treatments (streptomycin, kanamycin, and ampicillin—following the protocol described by Fernandez-Valenzuela et al [18]. These antibiotics were applied to eliminate bacterial contaminants, followed by repeated washing to ensure algal viability. The purified cultures were then maintained individually on BG11 medium plates and liquid medium at 28°C. To ensure axenic purity, cultures were routinely examined using bright-field microscopy to observe cell morphology and detect any bacterial or fungal contaminants. Also the axenic microalgal culture was streaked on LB and NA medium to check contamination. All transfers were conducted under a laminar airflow hood using sterilized glassware and autoclaved media to minimize contamination risk.

2.3 Morphological and ultrastructural evaluation

Morphological characterization of the isolated microalga was carried out using both a light microscope (Lynx) and a Field Emission Scanning Electron Microscope (FESEM) (FEI Nova NanoSEM 450). For light microscopy, axenic cultures were placed on a glass slide, covered with a coverslip, and examined under 10X and 40X magnification to observe features such as cell shape, appearance, presence of pyrenoids, and motility. For FESEM analysis, the cultures were fixed overnight in 2.5% formaldehyde. After 24 hours, they were centrifuged at 10,000 rpm for 5 minutes and sequentially washed with ethanol solutions of increasing concentrations (10%, 30%, 50%, 70%, 90%, and 100%) for 5 minutes each. The prepared samples were then examined under FESEM using an Everhart–Thornley detector (ETD), and images were captured at magnifications of 5000X and 10000X under high vacuum conditions, with an operating voltage of 15 keV and a 3.5 µm spot size.

2.4 Molecular characterization and identification of isolated microalga

2.4.1. DNA Extraction from microalga

A single colony of the isolate *Chlorella sorokiniana* SM1 microalga was separately inoculated into BG-11 broth, and the resulting culture was used for DNA extraction. Prior to isolation, the cultures were examined for axenicity and confirmed to be free of contaminants. For DNA extraction, 60 ml of each culture was centrifuged at 4000 rpm for 10 minutes. Afterward, the supernatant was discarded, and the resulting pellet—typically weighing around 20-40 mg, was stored at -20°C. Genomic DNA was extracted using the Algae DNA Purification Kit (HiMedia) and stored at -20°C until further analysis. The DNA was eluted in an elution buffer, and a 5 µl sample was run on a 1% agarose gel prepared in 1× TAE buffer containing 0.5 µg/ml ethidium bromide to visualize and estimate its concentration.

2.4.2 Amplification and purification of 18s DNA from microalgal culture

The isolated genomic DNA was used for amplification using a Thermal Cycler (Bio-Rad). To target the 18S rDNA, 5.8S rDNA, and Internal Transcribed Spacer (ITS) regions, degenerate and universal primers specific to *Chlorella* were designed through multiple sequence alignment and Primer-BLAST tools (Supplementary Information 1 and 2). The forward primer (5′-TCCCGACTTCTGGAAGGGAC-3′) and reverse primer (5′-ATTCGATGGTTCACGGAATTC-3′) were used for amplification. PCR was performed in a 50 µl reaction containing approximately 50 ng of DNA, 10 µM of each primer (Sigma-Aldrich), 40 mM dNTPs (HiMedia), 10X buffer, Taq polymerase (HiMedia), and distilled water. The thermal cycling conditions included

an initial denaturation step at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute, and extension at 72°C for 2 minutes, with a final extension at 72°C for 5 minutes. The PCR product was analysed on a 1% agarose gel stained with 0.5 µg/ml ethidium bromide, and a 100 bp molecular marker (HiMedia) was used to estimate the size of the amplified 18S rDNA, which was approximately 2000 bp. The desired amplicon was purified using the HiMedia Quick Gel Purification Kit (MB539). The PCR product was sequenced and genetic identification was performed by comparing the sequence to entries in the GenBank database using BLAST, evaluating alignment scores, E-values, query coverage, and identity percentages to determine the closest match.

2.4.3 Phylogenetic Analysis

Phylogenetic analysis was conducted using MEGA 11 software to determine the evolutionary relationship of the isolated microalga. The neighbor-joining (NJ) method [19] was employed with a bootstrap value of 10,000 replicates to ensure statistical reliability, following the approach of Felsenstein (1985) [20]. To construct the phylogenetic tree, seven species showing the highest sequence similarity to the isolated microalga, as determined by BLAST results, were selected for comparison.

2.5 Growth analysis and study of the carbon sequestration potential of microalgal cultures

The axenic culture of *Chlorella sorokiniana* SM1 was inoculated into BG11 medium at an initial optical density of 0.1 (OD_{750}) and incubated at 28°C under continuous light with an intensity of 37 µE m^{-2} s^{-1} under static conditions. The flasks were manually shaken once daily, and optical density at 750 nm was recorded each day [21]. Upon completion of the growth cycle, pigment and biomass analyses were conducted following protocols from previous studies [22]. The carbon capture potential was assessed using established methods described in earlier studies [23,24].

2.6 Extraction of chlorophyll a, chlorophyll b and carotenoids from isolated microalgal species.

Pigment extraction from the isolated microalga *Chlorella sorokiniana* SM1 was carried out by centrifuging 1 ml of culture at 10,000 rpm for 5 minutes. After carefully discarding the supernatant, 1 ml of 99.9% methanol (Loba Chemie) was added to the pellet. The mixture was shaken vigorously for 1 minute and then incubated in the dark for 15 minutes. Following incubation, the samples were centrifuged again at 10,000 rpm for 5 minutes. The absorbance of the resulting supernatant was measured at wavelengths of 652 nm, 665 nm, and 480 nm to determine the chlorophyll and carotenoid concentrations [25].

2.7 Bioactive metabolites analysis from microalgae

2.7.1 Extract preparation

The isolated microalgal culture, *Chlorella sorokiniana* (SM1), grown in a 1000 mL volume, was harvested at the stationary phase and centrifuged at 10,000 rpm for 5 minutes. The biomass was washed three times with distilled water and dried in a microwave oven at 40°C for two days. After drying, 100 mg of the powdered microalgal biomass was soaked in 10 mL of 99.9% methanol and sonicated in a water bath for 20 minutes. It was followed by shaking on an orbital shaker for 1 hour at 25°C. The supernatant was then filtered using Whatman filter paper No. 1 and dried using a vacuum rotary evaporator. For sample preparation, 10 mg of the dried crude extract was dissolved in 10 ml of methanol to obtain a final concentration of 1 mg/ml. The methanolic extract of the microalga was then sent for LC-MS analysis at Venture Centre, Pune, Maharashtra, India.

2.7.2 LC-MS/MS operating conditions for untargeted metabolites analysis

Untargeted metabolite profiling of the *Chlorella sorokiniana* SM1 was performed using LC-MS/MS. The analysis was conducted at CAMS, Venture Centre, NCL Innovation Park, Pune, India, using an Agilent 1200 HPLC system coupled with an LC-MS/MS setup and an ESI-QTOF-MS (Electrospray Ionization–Quadrupole–Time-of-Flight Mass Spectrometer) from Agilent Technologies, USA. A Zorbax column (2.1 × 50 mm, 1.8 µm) was used to inject and separate a 10 µl sample at 25°C. The mobile phase consisted of water (A) and acetonitrile (B), both of which contained 0.1% formic acid. A gradient flow of 5% A and 95% B was maintained for 27 minutes, then reversed after 27.10 minutes for the remainder of the 30-minute run. Nitrogen gas was used for both nebulization and drying at a flow rate of 11 L/min in positive ionization mode. The capillary temperature was set at 290°C with a nebulizer pressure of 40 psi. Mass spectra were recorded across a range of 60–1600 m/z. Data acquisition was managed using Mass Hunter software, and molecular masses were analysed using Mass Hunter Qualitative Analysis Navigator B.08.0. Compound identification was performed by comparing the spectral data with the METLIN library database.

2.8 STATISTICAL ANALYSIS

All experiments were conducted in triplicate, and the resulting data were expressed as mean values accompanied by their respective standard deviations to ensure accuracy and reproducibility.

3. RESULTS AND DISCUSSION

3.1 Morphological observation and characterization of isolated microalgae

In the present study, a single microalgal strain, *Chlorella sorokiniana* SM1 was isolated from the study area and maintained as a monoculture. The culture was made axenic to ensure purity for further experiments. Scale-up was successfully carried out in BG-11 medium across various volumes ranging from 10 ml to 1000 ml, with each stage supporting satisfactory growth and used for subsequent analyses. BG11 medium was chosen for cultivating *Chlorella sorokiniana* because it is a well-established, nutrient-rich formulation optimized for cyanobacteria and green microalgae, providing balanced macronutrients and trace elements that support robust growth, high biomass yield, and reproducibility across studies. Its proven success in CO_2 bio-fixation and photobioreactor cultivation makes it preferable over other media**.** The *Chlorella sorokiniana* culture was checked for axenicity routinely. Axenic cultures consistently showed uniform algal cells without contaminants such as bacterial rods, cocci, or fungal hyphae, and periodic checks confirmed the absence of microbial colonies under microscope and on LB and NA plates. Morphological characterization of the dark green BMT isolate was conducted primarily using light microscopy and Field Emission Scanning Electron Microscopy (FESEM), as detailed in Table 1. FESEM imaging revealed key surface features and cell dimensions (Fig. 2). The cells appeared spherical with a wrinkled surface, and their average dimensions were recorded as 2.68 ± 0.43 µm in both width and length. These morphological traits, including shape, size, motility, and presence of pyrenoids, closely resembled those of *Chlorella* species, suggesting that *Chlorella sorokiniana* SM1 is related to *Chlorella*. Based on these observations, the culture was selected for molecular analysis.

3.2 Molecular characterization, identification and phylogeny

In this study, species-level identification was performed using 18S rDNA and ITS region sequencing with *Chlorella*-specific degenerate primers designed in-house. PCR amplification followed by BLAST analysis revealed a sequence similarity of 97.67%. Details of matched database sequences, percent similarity, accession numbers, and identity percentages are presented in Fig. 3 and summarized in Table 2. 97.67% similarity indicated newly isolated strain of *Chlorella sorokiniana* indicated by SM1 Based on the report from GeneOmbio Technologies Private Limited, Pune, Maharashtra, India, the isolate was identified as a novel strain and designated as *Chlorella sorokiniana* SM1. *C. sorokiniana* SM1 represents a novel strain that we have isolated from the local region. While *C. sorokiniana* is indeed a well-known and widely studied species, the SM1 strain described in our study is newly obtained and has not been previously reported. This strain was collected, purified, and characterized as part of our research, and therefore constitutes a distinct, locally isolated variant. The sequence was submitted to the NCBI GenBank with the accession number PQ778864.

3.3 Growth studies of isolated microalgal cultures and tentative C capture potential

To study the growth pattern of *C. sorokiniana* SM1, we used a known species, *C. sorokiniana*, procured from National Collection of Industrial Microorganisms (NCIM), Pune, India as a positive control. The growth of *C. sorokiniana* SM1 was monitored over 18 days (Fig. 4). Cultures were observed until they simultaneously reached the stationary phase. On day 18, the optical density at 750 nm of *C. sorokiniana* SM1 was approximately 37.5% higher compared to *C. sorokiniana*, indicating enhanced biomass accumulation and suggesting greater carbon capture potential. The biomass yield of *Chlorella sorokiniana* SM1 was recorded at 0.74 g/L, with a maximum optical density of 0.44 at 750 nm under static conditions, consistent with findings reported by [21]. The biomass of *Chlorella* sp. SM1 was 17.5% higher as compared to C. *sorokiniana*. The theoretical carbon capture potential of this species was estimated based on established methodologies from [23,24]. In short, theoretically 1gm of microalgae captures 1.8g of CO_2 thus 0.74g of *Chlorella sorokiniana* SM1 biomass captures 1.332g/g of CO_2. The results are summarized in Table 3.

3.4 Pigment analysis from microalgae

Pigment studies of microalgal species were conducted under controlled conditions, as outlined in Table 4. *C. sorokiniana* SM1 showed 13.94% increase in Chlorophyll a and 16.13% increase in Chlorophyll b compared to *C. sorokiniana.* It is also reflected in increased biomass production suggesting minimal physiological stress under the applied growth conditions, consistent with previously reported observations [25].

3.5 Bioactive metabolite analysis of microalgal extract

Untargeted metabolite profiling of methanolic extracts of *C. sorokiniana* SM1 was conducted using LC-MS/MS. The analysis revealed a distinct metabolite distribution pattern in *C. sorokiniana* SM1. In *C. sorokiniana* SM1, a total of 23 bioactive metabolites were recorded (Table 5). The identified compounds included derivatives of amino acids, fatty acids, terpenes, flavonoids, and phenolic acids. This microalga exhibited a unique chemical profile. These findings highlight the chemical diversity and suggest that different microalgae possess varying compositions of bioactive compounds. Literature review confirmed that many of these metabolites are known for their pharmacological properties, including antibacterial, antiviral, anticancer, antidiabetic, antioxidant, and anti-inflammatory activities (Table 5).

CONCLUSION

In this study, a novel single microalgal strain, *Chlorella sorokiniana* SM1 (BMT), was successfully isolated from agricultural soil samples, purified to axenic form, and scaled up in BG-11 medium across volumes up to 1000 ml. Morphological analysis revealed characteristics resembling *Chlorella* species, and molecular identification techniques confirmed its identity. Growth monitoring over 18 days showed that *C. sorokiniana* SM1 reached the stationary phase with approximately 37.5% higher biomass (OD_{750}) compared to the known *C. sorokiniana* control strain. Additionally, pigment content was significantly higher in *C. sorokiniana* SM1, indicating enhanced photosynthetic capacity. These traits suggest superior carbon assimilation. Carbon capture potential was theoretically estimated based on biomass productivity, highlighting its suitability for CO_2 sequestration. This parameter could be highly valuable for large-scale cultivation in the Baramati region of Maharashtra. Furthermore, LC–MS/MS profiling of methanolic extracts identified 23 bioactive metabolites, including amino acids, fatty acids, terpenes, flavonoids, and phenolic acids. Many of these compounds possess known pharmacological properties, such as antibacterial, antiviral, anticancer, antidiabetic, antioxidant, and anti-inflammatory activities. These findings establish *Chlorella sorokiniana* SM1 as a promising candidate for sustainable biotechnological, pharmaceutical, and environmental applications.

ACKNOWLEDGEMENT

The authors are thankful to the Shardabai Pawar Arts, commerce and Science College, Baramati and Agricultural Development Trust, Baramati for providing the infrastructure during the current work.

CONFLICT OF INTEREST DISCLOSURE

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

FUNDING SOURCE STATEMENT

External funding was not available for completing the current work.

ETHICAL APPROVAL STATEMENT

Not Applicable

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Figure 1.

Fig. 1 Google map view of sampling site (A), growth of microalga in medium (B), Microscopic image of microalga(C), growth of microalga on agar plate(D).

Figure 2.

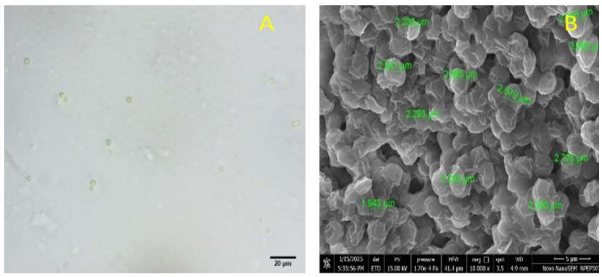

Fig. 2. Light microscopy and scanning electron microscopy photographs of the isolated microalga; the codes are indicated. A&B: *Chlorella sorokiniana* (BMT). Scale bars are visible in the lower section of each photograph.

Figure 3.

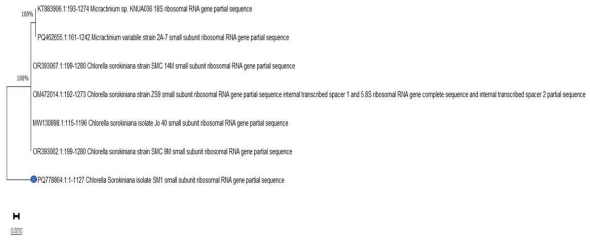

Fig. 3 Neighbor Joining consensus tree based on 18S rDNA gene. Coloured dots showed the new sequence derived from this study.

Figure 4.

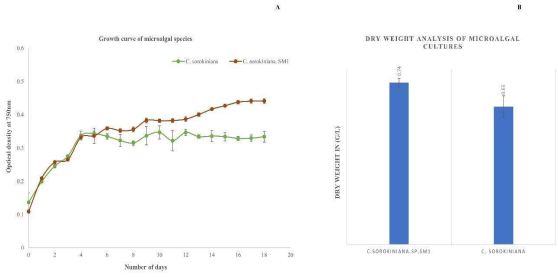

Fig. 4 Growth curve (A) and Dry weight analysis (B) of microalgal cultures. The values are mean±SD where n=3.

Table 1. Morphological observation and characterization of isolated microalga

Isolate	Shape	Width (µm)	Length (µm)	Motility	Pyrenoid	Special feature (SEM)	Tentative identification
BMT	Spherical	2.68±0.43	2.68±0.43	Non-Motile	No	Wrinkles all over the sphere	*Chlorella.* sp.

Table 2. Preliminary identification of isolated microalgae at Shardanagar, Baramati, India.

Strain ID	Closest match species	% similarity	Accesion number	New accession number (this work)
SM1	*Chlorella sorokiniana*	97.67%	LC505550	**PQ778864**

Table 3. Carbon capture potential of isolated microalgal species

Name of microalgae	Biomass (g/l)	C capture potential (g/g)
Chlorella sorokiniana SM1	0.74	1.332
Chlorella sorokiniana	0.63	1.134

Table 4: Pigment analysis of *Chlorella sorokiniana* SM1 microalgal culture (The values are mean±SD where n=3)

Name of microalgae	Chl a (ug/ml)	Chl b (ug/ml)	Carotenoid (ug/ml)
Chlorella sorokiniana SM1	24.52 ± 0.84	8.78 ± 0.2	2.63±0.35
Chlorella sorokiniana	21.52 ± 10	7.56 ± 0.22	1.97±0.09

Table 5. Bioactive metabolites of *Chlorella sorokiniana* SM1

Sr. No.	Name of the Compounds	Retention time (min.)	Molecular Mass	Molecular formula	Annotated Mass (m/z)	DB score (METLIN)	MSI Level	Medicinal importance	Refere
1.	Pro Gly Glu Asp	2.307	416.1551	$C_{15}H_{24}N_4O_9$	439.1443 (M+Na) +	90.61	METLIN Library database	Neuroprotectant	[26
2.	Capilliposide I	2.405	1064.2997	$C_{48}H_{56}O_{27}$	533.1571 (M+2H)+2	90.23	METLIN Library database	Anti-inflammatory property	[27
3.	Picolinic acid	3.044	123.0319	$C_6H_5NO_2$ (M+H)+	124.0392	99.97	METLIN Library database	Anti-cancer property	[28
4.	5'-Deoxy-5' (methylthio)adenosine	4.170	297.0904	$C_{11}H_{15}N_5O_3S$ (M+H) +	298.0976	97.11	METLIN Library database	Anti – cancer property	[29
5.	Strictosamide	12.098	498.2018	$C_{28}H_{34}N_2O_8$ (M+H)+	499.2090	93.03	METLIN Library database	Anti-inflammatory property	[30
6.	Phytosphingosine	12.735	317.2944	$C_{18}H_{39}NO_3$ (M+H)+	318.3018	91	METLIN Library database	Anti-cancer property	[31
7.	Omega-hydroxy behenic	14.251	356.3307	$C_{22}H_{44}O_3$ (M+NH4)+	374.3646	92.22	METLIN Library database	Skin care properties	[32
8.	Spisulosine	14.490	285.3044	$C_{19}H_{39}NO$ (M+H)+	286.3118	91.04	METLIN Library database	Anti-cancer property	[33
9.	Isomytiloxanthin	14.790	598.4039	$C_{40}H_{54}O_4$ (M+H)+	599.4110	93.13	METLIN Library database	Antioxidant property	[34
10.	Corynoline	14.904	367.1411	$C_{21}H_{21}NO_5$ (M+NH4)+	385.1749	93.52	METLIN Library database	Antioxidant property	[35]
11.	Armillaripin	15.112	414.2056	$C_{24}H_{30}O_6$ (M+Na)+	437.1947	93.85	METLIN Library database	Antibacterial property	[36]
12.	Militarinone A	15.623	459.2635	$C_{26}H_{37}NO_6$ (M+H)+	460.2708	93.04	METLIN Library database	Neuroprotectant	[37]
13	(S)-Nerolidol 3-O-[a-L Rhamnopyranosyl-(1 >4)-a-L rhamnopyranosyl-(1 >2)-b-D glucopyranoside	15.643	676.3685	C33 H56 O14 (M+NH4)+	694.4022	95.07	METLIN Library database	Anti-microbial, Antioxidant and Anticancer properties	[38]
14.	Chalcomycin	16.528	700.3670	C35 H56 O14	701.3741	95.95	METLIN Library database	Anti-microbial property	[39]
15.	12-HETE	17.538	320.2364	$C_{20}H_{32}O_3$ (M+H)+	320.2364	94.03	METLIN Library database	Anti-diabetic property	[40]
16.	Tokoronin	18.056	580.3611	$C_{32}H_{52}O_9$ (M+H)+	581.3685	90.68	METLIN Library database	Controls skin diseases	[41]
17.	Ivermectin B1a	18.932	874.5072	$C_{48}H_{74}O_{14}$ (M+H)+	875.5149	92.20	METLIN Library database	Anti-cancer property	[42]
18.	Ginsenoside F5	19.049	770.4826	$C_{41}H_{70}O_{13}$ (M+H)+	771.4902	90.97	METLIN Library database	Anti-cancer property	[43]
19.	Lucidenic acid M	19.208	462.2972	C27 H42 O6 (M+H)+	463.3045	94.54	METLIN Library database	Antitumor, anti-inflammatory, and antiviral properties	[44]
20.	Avermectin B2b aglycone	20.091	588.3316	$C_{33}H_{48}O_9$ (M+NH4)+	606.3655	92.81	METLIN Library database	Nematicides, Insecticides properties	[45
21.	Momordicoside G	23.853	632.4309	$C_{37}H_{60}O_8$ (M+Na)+	655.4202	92.98	METLIN Library database	Anti-diabetic, anti-cancer properties	[46
22.	Haplophytine	24.315	652.2914	$C_{37}H_{48}N_4O_7$ (M+Na)+	675.2808	92.64	METLIN Library database	Insecticidal property	[47
23.	E, e-Carotene-3,3'-dione	27.818	564.3978	$C_{40}H_{52}O_2$ (M+H)+	565.4050	95.89	METLIN Library database	Anti-cancer property	[48