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GC-MS Profiling of Bioactive Constituents in Methanolic Extracts from Stem and Seed of *Distimake* Species

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ABSTRACT

Phytochemical compounds play a crucial role in determining the therapeutic potential of plant species and significantly contribute to their ability to resist environmental stress factors, whether biotic or abiotic. Based on the specific presence of such bioactive molecules, plant genera are often classified into various medicinal categories such as anti-inflammatory, anti-cancer, anti-diarrhoeal, and anti-stomachic plants. Among them, the family Convolvulaceae, which encompasses the genus *Distimake* (previously classified under *Merremia*), is particularly recognized for its rich alkaloid content. The current research focused on the phytochemical investigation and profiling of bioactive compounds in two species belonging to this genus — *Merremia aegyptia* (L.) Urb and *Merremia dissecta* (Jacq.) Hallier f., which are now taxonomically updated as *Distimake aegyptius* (L.) A.R. Simoes and Staples and *Distimake dissectus* (Jacq.) A.R. Simoes and Staples, respectively. Methanolic extracts prepared from stem and seed samples of both species were subjected to gas chromatography-mass spectrometry (GC-MS) analysis to determine their phytochemical profiles. The findings showed that in the stem extract of *D. aegyptius*, 1,2,4-butane triol exhibited the highest peak area (26.84%). Meanwhile, the seed extract of the same species showed a predominant presence of 1,2-benzene dicarboxylic acid, and dibutyl ester (48.17%). For *D. dissectus*, the stem sample contained a significant amount of 1,3,4,5-tetrahydroxy cyclohexane carboxylic acid (quinic acid) with a peak area of 20.35%. Furthermore, the seed extract of *D. dissectus* emerged as a rich source of phytosterols, characterized by the presence of (3β)-Ergost-5-en-3-ol (Campesterol) (18.19%), stigmasta-5,22-dien-3-ol (19.23%), and gamma sitosterol (24.56%). These phytosterols are pharmacologically important due to their antioxidant, anti-inflammatory, anti-hypercholesterolemic, antidiabetic, and anticancer properties. The GC-MS analysis also revealed that both species shared certain common phytoconstituents, underscoring their potential as valuable medicinal resources.

Keywords: *Distimake dissectus*, Convolvulaceae, *Distimake aegyptius*, GC-MS

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Introduction

The genus *Distimake*, formerly categorized under Merremia of the family Convolvulaceae, is characterized by its strikingly colorful, tubular or funnel-shaped flowers. Although often perceived as a wild or weedy plant growing along roadsides, it is also cultivated in some regions for ornamental purposes and recognized among medicinal flora worldwide. Merremia, a flowering member of Convolvulaceae — a family known for species like morning glory — thrives predominantly in tropical environments, exhibiting rapid growth and colonizing disturbed habitats with ease. The family also comprises parasitic species with established therapeutic applications [1].

Historically, the taxonomic placement of the tribe Merremieae had not been universally accepted until more recent times [2]. Molecular studies, particularly chloroplast DNA (cpDNA) analysis, challenged the earlier assumption of its monophyletic lineage, revealing a moderately supported clade comprising most *Merremia* species alongside related genera such as *Xenostegia*, *Hewittia*, and *Operculina* [3].

Recent advancements in taxonomic revisions, notably by Simoes and Staples and recorded in the WCVP (World Checklist of Selected Plant Families) in 2020, have led to the reclassification of several endemic species within the tribe Merremieae. As a result of these updates, the species investigated in the current study — *Merremia aegyptia* (L.) Urb and *Merremia dissecta* (Jacq.) Hallier f. — have been reassigned to the genus *Distimake*, now recognized as *Distimake aegyptius* (L.) A.R. Simoes and Staples and *Distimake dissectus* (Jacq.) A.R. Simoes and Staples [4, 5].

Plant description

- *Distimake dissectus*

Family: Convolvulaceae
Common Name: Alamo Vine, Noyau Vine
Synonyms: *Merremia dissecta*, *Ipomoea dissecta*, *Convolvulus dissectus*
This species, native to tropical regions of America, is a white-flowered perennial morning glory that propagates through rhizomes, runners, and seeds. It is a climbing herbaceous plant with hairy stems and broad leaves. The leaves are simple, alternately arranged, and supported by smooth petioles measuring 5–7 cm in length. The leaf blade is divided and palmately lobed, reaching up to 4 × 6 cm to 8 × 12 cm in size. The flowers, mostly white and irregular in shape, have five loose sepals. The plant bears a single tubular flower surrounded by five corollas. The fruit is a capsule measuring 2 × 2 cm, containing five forked valves. Flowering typically occurs in February, April, July, and September.

- *Distimake aegyptius*

Family: Convolvulaceae
Common Name: Egyptian Woodpecker, Hairy Starling, Hairy Morning Glory, Mochukodi (Tamil)
Synonyms: Ipomoea aegyptia, Convolvulus vitifolius, Merremia aegyptia
This perennial creeping plant, commonly known as the Egyptian woody plant, is distinguished by its long, scattered hairs. The leaves are digitately arranged in a penta-foliate pattern, alternating along the stem. Each leaflet is broadly lanceolate in shape, measuring 5–10 × 2–5 cm. The flowers grow in hairy cymes resembling racemes and feature funnel-shaped white corollas accompanied by linear bracts 6–8 mm in length. The leaves are ovate-lanceolate, elongated, and become larger and densely hairy upon fruiting, except for two inner leaves that remain slightly smaller and smooth. The plant has five stamens with hairy filaments. The capsule fruit is long, oval, and papery, divided into four chambers with four valves, covered in silky sepals. It contains four smooth, shiny seeds. The flowering period spans from December to March.
At the molecular level, chemical compounds in complex mixtures can be identified and separated using gas chromatography-mass spectrometry (GC-MS). This analytical tool is used for determining the molecular weight of unknown substances within a sample. By comparing the generated spectra against reference data, the specific organic molecules present in the mixture can be accurately identified [6].
Previous research involving GC-MS has highlighted the presence of important metabolites in other species within the Merremia genus, such as *M. emarginata* and *M. tridentata*. In *M. emarginata*, compounds including D-mannitol, caryophyllene oxide, 9,12,15-octadecatrienoic acid, phytol, octadecanoic acid, and others like beta-tocopherol and stigmasterol were identified in the ethyl acetate extract [7]. Likewise, studies on *M. tridentata*'s methanolic root extract revealed key components such as fatty acids, including dodecanoic acid, hexadecanoic acid, and heptadecanoic acid, along with methyl ester derivatives like 16-methyl [8].
For *M. dissecta*, GC-MS analysis of its seed oil has shown a profile dominated by fatty acid methyl esters. Notable constituents identified included methyl stearate, 9-hexadecenoic acid methyl ester, 12-octadecadienoic acid methyl ester, and 9-octadecenoic acid methyl ester, which formed the bulk of the composition at varying concentrations [9].
The current study aims to analyze *M. aegyptia* and *M. dissecta* for bioactive components using GC-MS, providing insights into their potential therapeutic value.

Materials and Methods

- *Preparation of extracts*

The stems and seeds of the test plants were collected once fully mature and subsequently air-dried under shaded conditions. After drying, the material was ground into a fine powder. Soxhlet extraction was performed by adding 10 grams of the powdered plant material to 100 milliliters of 95% methanol, which was heated in a water bath for 24 hours. Once extraction was completed, the methanolic extracts were concentrated for further analysis.

- *GC-MS analysis*

A thermal desorption system, the TD 20 extractor, and a Shimadzu QP-2010 plus were used to perform the GC-MS analysis. Both polar and non-polar components present in the plant extracts were identified. A 2 µl aliquot of each extract was used for the GC-MS analysis [10].

- *GC-MS conditions*

The analytical column used was 30 meters in length, with a diameter of 0.25 mm and a film thickness of 0.25 µm. The stationary phase consisted of 5% diphenyl and 95% dimethyl polysiloxane. Helium gas, with a purity of 99.999%, was used as the carrier gas, maintained at a constant flow rate of 1 ml/min. A 2 µl sample was injected with a 10:1 split ratio. The initial injector temperature was set at 280 °C, and the ion source temperature was kept at 200 °C. The oven temperature began at 110 °C and was then increased to 200 °C at a rate of 10 °C/min, followed by a ramp to 280 °C at 5 °C/min. The final temperature of 280 °C was maintained throughout the isothermal phase. Each sample underwent a 45-minute analysis.

- *Mass spectrum interpretation*

The mass spectra obtained were compared with the NIST database to identify the components by matching their fragmentation patterns and retention indices.

Results and Discussion

The 2 species within the *Distimake* genus exhibit significant concentrations of active metabolites. These plants are well-known for their medicinal qualities, particularly in their anti-diabetic, anti-inflammatory, and anti-hypercholesterolemic actions. The active compounds found in these species present an opportunity for further exploration and potential extraction of their therapeutic ingredients.

Following the GC-MS analysis of the stem and seed samples from *D. aegyptius* and *D. dissectus*, spectrophotographic data was collected. The methanolic stem extract of *D. aegyptius* revealed 27 compounds, while the seed extract displayed 29 distinct peaks. In the case of *D. dissectus*, 29 compounds were detected in the stem extract, and the seed extract showed 20 distinct peaks.

The analysis identified a range of phytochemicals including tannins, alkaloids, flavonoids, phenolic compounds, sugars, fatty acids, steroids, amino acids, and various vitamins. **Table 1** provides the names of some significant compounds found in these species. Additionally, both species of Merremia shared the presence of several common phytochemicals, as evidenced by the GC-MS data.

Table 1. Some common names/scientific names of compounds isolated from these plant species

1	1,2-Benzene dicarboxylic acid, dibutyl ester	Dibutyl phthalate
2	1,3,4,5-Tetrahydroxy-cyclohexane carboxyl	Quinic acid
3	Dodecanoic acid, methyl ester	Lauric acid
4	9,12-Octadecadienoic acid	Linoelaidic acid
5	n-hexa decanoic acid	Palmitic acid
6	Stigmast-5-en-3-ol, (3-beta)	Y-Sitosterol, clionasterol
7	1-(+)-Ascorbic acid-2, 6-dihexadecanoate	L-Ascorbyl 2, 6-dipalmitate

The predominant peak area and percentage for 1,2,4-butanediol (26.84%) were observed in the stem extract, while in the seed extract of *D. aegyptius*, dibutyl phthalate (1,2-benzene dicarboxylic acid dibutyl ester) had the highest value at 48.17%. In *D. dissectus*, the stem extract exhibited 1,3,4,5-tetrahydroxy-cyclohexanecarboxyl (quinic acid) as the major compound (11.88%), while sitosterol was the dominant compound in the seed extract (24.56%) **(Figures 1 and 2; Tables 2-5)**.

1,2,4-Butanetriol is noted for being a precursor to cholesterol-lowering drugs and exhibits antibacterial, antiviral, candidicidal, and cholesterol-reducing properties. Lauric acid (decanoic acid) is recognized for its potent antibacterial characteristics and finds wide use in soap production and cosmetics.

Hexadecanoic acid methyl ester shows multiple bioactive effects, including antioxidant properties, inhibition of 5-alpha reductase, antibacterial, antifibrinolytic, hemolytic, nematicidal, and hypocholesterolemic actions. Quinic acid, known for its astringent nature, is used in the synthesis of oseltamivir, a drug for treating influenza A and B [11].

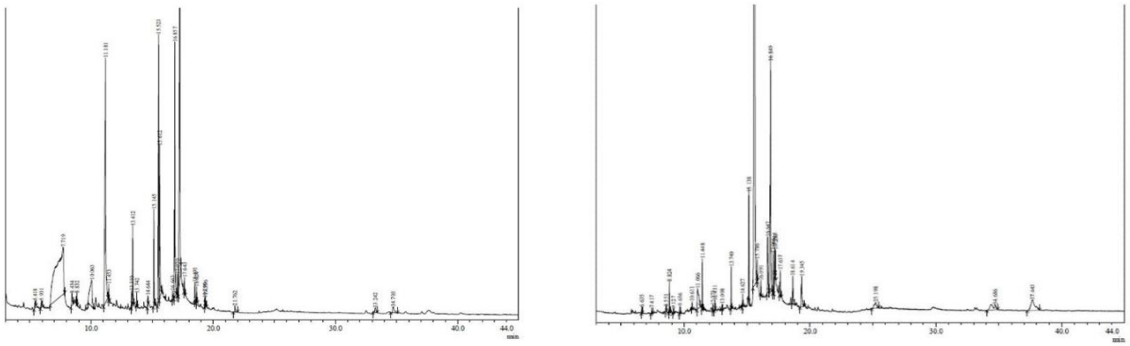
Dibutyl phthalate (DBP), a plasticizer commonly used to enhance PVC properties, is also noted for its role in preventing scrub typhus. However, due to its potential endocrine-disrupting effects, DBP is banned in cosmetic products in the European Union under Directive 76/768/EEC of 1976 [12-14].

Oleic acid methyl ester (9-octadecenoic acid methyl ester) has anti-inflammatory effects and serves as a cancer preventive, in addition to being a key ingredient in biodiesel production. 9-Octadecenamide exhibits both anti-inflammatory and antibacterial properties, with applications in treating mood disorders, depression, and sleep disturbances, potentially through cannabinoid regulation [15].

Stigmasterol is recognized for its diverse bioactivities, including anti-inflammatory, hypoglycemic, antioxidant, thyroid inhibition, and cancer-preventive effects, particularly for breast, colon, prostate, and ovarian cancers. It also has antiretroviral, antihypertensive, antidiabetic, and antineoplastic properties [16, 17]. The (z,z)-methyl ester of 9,12-octadecadienoic acid has a wide range of applications, including anti-inflammatory, anti-arthritic, hypocholesterolemic, hepatoprotective, nematocidal, antihistamine, and anti-acne activities.

Palmitic acid is used extensively in cosmetics, soaps, and industrial mold-release agents [18]. Methyl hexadecanoic acid, also known as methyl palmitate, exhibits anti-inflammatory and antifibrotic activities, along with potent antimicrobial properties against clinically relevant bacteria.

Other compounds such as 9,12,15-octadecatrienoic acid and octadecanoic acid are noted for their anti-inflammatory, antioxidant, and hepatoprotective effects. Additionally, octadecanoic acid shows antifungal and antitumor activity [19]. Ascorbic acid 2,6-dihexadecanoate (1-(+)-ascorbic-2,6-dihexadecanoate) is noted for its antioxidant properties, cardioprotective effects, anticancer activity, and ability to improve taste, while also exhibiting effects against infertility [20].



a) b)

Figure 1. GC-MS spectogram of a) *D. aegyptius* stem, and b) *D. aegyptius* seed

Table 2. Compounds detected in GC-MS analysis of *D.aegyptius* stem

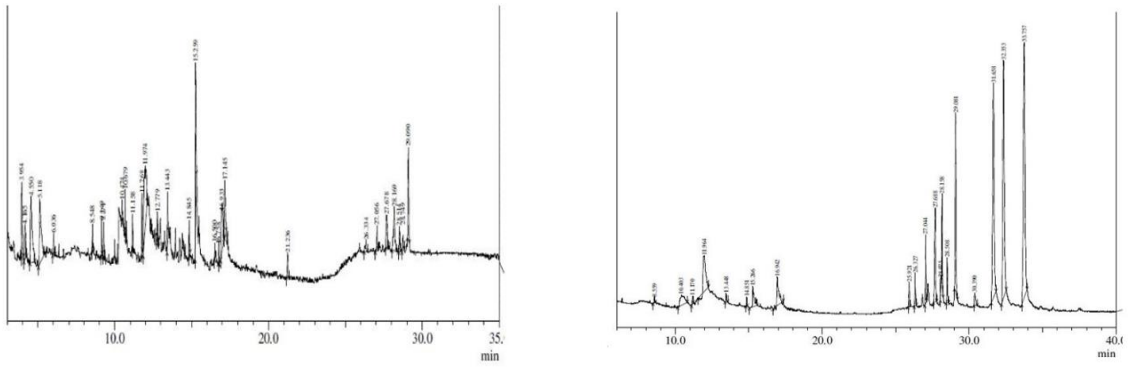
Peak#	Name	Retention time	Area (%)
1	Octanoic acid	5.418	0.37
2	1,2-Benzenediol	5.931	0.34
3	1,2,4-Butanetriol	7.719	26.84
4	Decanoicacid	8.434	0.61
5	1-Hexadecene	8.832	0.17
6	6, 6-Dideutero-nonen-1-ol-3	10.063	4.49
7	Dodecanoicacid	11.181	16.28
8	1-Octadecanol	11.453	0.34

9	4-((1e)-3-Hydroxy-1-propenyl)-2-methoxy phenol	13.310	0.43
10	Tetradecanoic acid	13.412	2.29
11	1-Octadecene	13.742	0.28
12	1, 2-Benzene dicarboxylic acid, diisononyl ester	14.644	0.30
13	Hexadecanoic acid, methyl ester	15.145	2.28
14	l-(+)-Ascorbic acid 2, 6-dihexadecanoate	15.523	9.04
15	1, 2-Benzene dicarboxylic acid, dibutyl ester	15.612	6.55
16	13-Hexyloxacyclotridec-10-en-2-one	16.663	0.28
17	9-Octadecenoic acid, methyl ester	16.857	8.46
18	Methyl stearate	17.070	0.45
19	6-Octadecenoic acid, (z)-	17.245	16.83
20	8, 11-Octadecadienoic acid, methyl ester	17.643	0.60
21	1, 3-Cyclohexadecanedione, 6-nitro-	18.493	0.56
22	9-Octadecenoic acid, 12-hydroxy-, methyl ester, [r-(z)]-	18.624	0.52
23	Methoxyethyl acetyl ricinoleate	19.290	0.20
24	9-Octadecenamide, (z)-	19.356	0.23
25	1, 2-Benzene dicarboxylic acid	21.762	0.24
26	Stigmasta-5,23-Dien-3-ol,(3.beta.)-	33.242	0.42
27	Stigmast-5-en-3-ol,(3.beta.)-	34.703	0.60
			100.00

Table 3. Compounds detected in GC-MS analysis of *D. aegyptius* seed

Peak#	Name	Retention time	Area (%)
1	Cyclohexane, octyl-	6.635	0.24
2	2-Undecanone	7.417	0.19
3	1, 7-Dimethyl-4-(1-methyl ethyl) cyclo decane	8.551	0.11
4	1-Tetradecene	8.824	0.99
5	Cyclohexane, 1,2,4,5-tetramethyl-, (1.alpha.,2.alpha.,4.alpha.,5.alpha.)-	9.127	0.21
6	Cyclo hexane, octyl-	9.656	0.22
7	Dodecanoic acid, methyl ester	10.611	0.18
8	Dodecanoic acid	11.066	3.12
9	1-Octadecene	11.448	1.56
10	Cyclohexane, decyl-	12.273	0.14
11	8-Pentadecanone	12.431	0.21
12	Methyl tetradecanoate	13.008	0.17
13	1-Octadecene	13.740	1.24
14	8-Pentadecanone	14.627	0.43
15	Hexadecanoic acid, methyl ester	15.138	3.45
16	1,2-Benzene dicarboxylic acid, dibutyl ester	15.539	48.17
17	Eicosyl trifluoroacetate	15.786	0.58
18	Isopropyl palmitate	16.091	0.43
19	13-Hexyloxacyclotridec-10-en-2-one	16.647	4.42
20	9-Octadecenoic acid (z)-, methyl ester	16.849	15.21
21	Methyl stearate	17.066	1.38
22	Heptadecene-(8)-carbonic acid-(1)	17.185	1.63
23	9,12-Octadecadienoic acid (z,z)-, methyl ester	17.250	0.84

24	8,11-Octadecadienoic acid, methyl ester	17.637	1.75
25	9-Octadecensaeure, 12-hydroxy-, methyl ester, (z)- (ricinolsaeuremet	18.614	1.97
26	9-Octadecenamide, (z)-	19.345	2.78
27	Octadeca-9,12-dienoic acid methyl ester	25.198	1.33
28	Stigmast-5-en-3-ol, (3.beta.)-	34.686	2.51
29	Octadeca-9,12-dienoic acid methyl ester	37.643	4.54
			100.00



a) b)

Figure 2. GC-MS spectrogram of a) *D.dissectus* stem, and b) *D. dissectus* seed

Table 4. Compounds detected in GC-MS analysis of *D. dissectus* stem

Peak#	Name	Retention time	Area (%)
1	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	5.076	8.18
2	Acetic acid,(1-methyl ethoxy)-,1-methyl ethyl ester	7.188	8.00
3	Tridec-1-ene<n->	8.537	0.95
4	Caryophyllene<(e)->	9.141	1.99
5	2-Nor pinene,2,6-dimethyl-6-(4-methyl-3-pentenyl	9.265	2.15
6	1,3-Propanediol,2-(hydroxymethyl)-2-nitro-	9.815	4.38
7	Isoamyl nitrite	10.276	1.39
8	Mentha-1(7),8-diene<p->	10.642	0.65
9	1-Hexadecene	11.149	1.73
10	Eudesmol<epi-gamma->	11.764	1.33
11	1,3,4,5-Tetrahydroxy-cyclohexane carboxyl	11.969	20.35
12	Hexadecanol<n->	13.435	1.31
13	7-Oxabicyclo[4.1.0]heptane, 1,3,3-trimethyl-2-(3-methyl-1,3-butadienyl)-, [1.alpha.,2. beta.(e),6.alpha.]-(.+-.)-	14.217	0.93
14	Docosanoicacid,methyl ester	14.835	2.65
15	N-hexadecanoicacid	15.254	18.91
16	1-Heneicosanol	15.476	0.56
17	9,12-Octadecadienoicacid(z,z)-	16.908	7.20
18	9,12,15-Octadecatrienoicacid,(z,z,z)-	16.975	4.12
19	Octadecanoicacid	17.130	6.61
20	1,2-Benzene dicarboxylic acid,diisooctyles	21.220	1.12
21	1,3,7,11-Tridecatetraene-1,1-d2,4,8,12-trimethyl	25.179	0.41
22	2-Isopropyl-5-methyl cyclohexyl 3-methyl-4-methylene cyclopentane carboxylate	28.719	0.55
23	Cyclopentane carboxylic acid, 3-methyl-4-methylene-, menthyl ester	31.639	0.86

24	Benzene, 1-(4'-pentyl[1,1'-bicyclohexyl]-4-yl)-4-(4-propyl cyclohexyl)-, [trans[trans(trans)]]-	32.326	1.20
25	(-)-5-Oxatricyclo[8.2.0.0(4,6)]dodecane,,12-trim	33.693	2.48
			100.00

Table 5. Compounds detected in GC-MS analysis of *D. dissectus* seed

Peak#	Name	Retention time	Area (%)
1	1-Undecene, 9-methyl-	8.559	0.15
2	1,6-Anhydro-beta-D-glucopyranose	10.403	2.38
3	9-Methyl-1-undecene	11.170	0.25
4	1,3,4,5-Tetrahydroxy-cyclohexane carboxylic acid	11.964	4.84
5	1-Heptanol, 3-methyl- $$ 3-methyl-1-heptanol	13.448	0.30
6	Undecanoicacid, methyl ester	14.851	0.20
7	Hexadecanoicacid	15.266	1.84
8	9,12-Octadecadienoicacid (z,z)-	16.942	2.52
9	3-Bromocholest-5-ene#	25.921	1.04
10	Stigmasterol	26.327	1.22
11	Cholest-5-en-3-ol(3.beta.)-,carbonochloridate	27.044	2.52
12	3-Bromocholest-5-ene#	27.688	5.42
13	Cholesta-2,4-diene $$ 17-(1,5-dimethyl-hexyl)-10,13-dimethyl-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-1h-cyclopenta	28.075	0.34
14	Stigmasta-5,22-dien-3-ol,acetate,(3.beta.)-	28.158	3.45
15	3-Bromocholest-5-ene#	28.508	1.90
16	Cholest-5-en-3-ol(3.beta.)-, carbonochloridate	29.081	8.83
17	26,26-Dimethyl-5,23-ergostadien-3beta-ol	30.390	0.81
18	Ergost-5-en-3-ol, (3beta)-	31.658	18.19
19	Stigmasta-5,22-e-dien-3-ol	32.353	19.23
20	Gamma-sitosterol	33.757	24.56
			100.00

Conclusion

The bioactive compounds identified in these plants have considerable potential for pharmaceutical, medicinal, and economic applications, making these species viable candidates for phytopharmaceutical development. Notable compounds like dibutyl phthalate, sitosterols, lauric acid, palmitic acid, ascorbic acid, quinic acid, and hexadecenoic acid provide a range of benefits. The use of GC-MS analysis is crucial for gaining insight into the active components of these plants, paving the way for future, more comprehensive investigations.

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Conflict of Interest: None

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Ethics Statement: None

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