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International Journal of Pharmaceutical Research & Allied Sciences, 2024, 13(1):39-46
https://doi.org/10.51847/dD8QR6U2EC

Original Article

ISSN : 2277-3657
CODEN(USA) : IJPRPM

GC-MS Analysis of Bioactive Compounds in Methanolic Extracts of Stem and Seed Samples of Distimake spp.

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ABSTRACT

The phytoconstituents of the plant are directly or indirectly linked to the medicinal and advantageous qualities of the genus and are essential to the plant's self-defense against a variety of biotic and abiotic challenges. Plant genera have been divided into subcategories depending on the presence of these compounds, such as medicinal, anti-inflammatory, anti-stomachic, anti-cancer, and anti-diarrhoeal. The Convolvulaceae family, which includes the genus Distimake formerly known as Merremia, is known for having high alkaloid content. The present investigation is centered on the identification and assessment of bioactive compounds from two distinct species in this genus—Merremia aegyptia and Merremia dissecta—using GC-MS analysis. Distimake aegyptius (L.) A.R. Simoes and Staples and Distimake dissectus (Jacq.) A.R. Simoes and Staples are the new names for these species. The highest peak area percentage was reported for 1,2,4 butane triol (26.84%)in D. aegyptius stem sample, 1,2-benzene dicarboxylic acid, dibutyl ester (48.17%) from D. aegyptius seed sample, 1,3,4,5-tetrahydroxy cyclohexane carboxylic acid (Quinic Acid) (20.35%) in D. dissectus stem sample and (3β)-Ergost-5-en-3-ol (Campesterol) (18.19%), stigmasta-5,22-dien-3-ol (19.23%), and gamma sitosterol (24.56%) in the D. dissectus seed sample making it a good source of phytosterols. These phytosterols possess antioxidant, anti-cancerous, anti-inflammatory, anti-hypercholesterolemic, and antidiabetic properties. GC-MS results revealed that some common phytochemicals were found in both the Merremia species.

Key words: *Convolvulaceae, GC-MS, Distimake aegyptius, Distimake dissectus*

INTRODUCTION

Distimake (formerly known as *Merremia* the family Convolvulaceae) genus consists of stunning and vibrant tubular/funnel blooms. It is commonly regarded as a roadside weed or grown as a subsistence ornamental among the medicinal plants found around the world. *Merremia* is a blooming plant of the Convolvulaceae family, which includes morning glory. A weedy tropical genus that spreads quickly across damaged regions. The family includes dodder, lovely garden plants like cornflowers, and weeds like *Convolvulus* and *Calystegia* that may be rather problematic. Certain parasite representatives have therapeutic uses also [1].

It is only lately that the tribe Merremieae has received official recognition [2]. A cpDNA analysis disproved the theory of its monophyletic origin. This phylum is composed of a modestly supported clade that includes the majority of *Merremia* species as well as clades including *Xenostegia, Hewittia, and Operculina* [3].

Recent classification developments in Simoes and Staples, 2017 and Staples in WCVP (World Checklist of Selected Plant Families), 2020 indicate that various endemic species of Merremieae have been reclassified. Both species in this study, *i.e. Merremia aegyptia* (L.) Urb and *Merremia dissecta* (Jacq.) Hallier f. were established in

the genus *Distimake* and reclassified as *Distimake aegyptius* (L.) A.R. Simoes and Staples and *Distimake dissectus* (Jacq.) A.R. Simoes and Staples [4, 5].

Plant description

- *Distimake dissectus* (Jacq.) A.R. Simoes and Staples

Family: Convolvulaceae
Common Name: Alamo Vine, Noyau Vine
Synonyms: *Merremia dissecta, Ipomoea dissecta, Convolvulus dissectus*
Description: Rhizomes, runners, and seeds are the means of propagation for this white, perennial morning glory, which is native to tropical America. a hairy-stemmed herbaceous climber with wide leaves. Simple, alternating leaves with glabrous, 5-7 cm long petioles are present. The segmented, palmately lobed leaf blade has a maximum size of 4*6 to 8*12 cm. With five loose sepals, the blooms are mostly white and asymmetrical. One tubular flower with five corollas adjacent. The fruit is a 2*2 centimetre capsule with five valves that are forked. Blooming months include February, April, July, and September.

- *Distimake aegyptius (Jacq.) A.R. Simoes and Staples*

Family: Convolvulaceae
Common Name: Egyptian Woodpecker, Hairy Starling, Hairy Morning Glory, Mochukodi in Tamil
Synonyms: *Ipomoea aegyptia, Convolvulus vitifolius, Merremia aegyptia*.
Description: The Egyptian woody plant is a creeper that blooms every year and has long, dispersed hairs. Penta foliate, alternating leaf form digitally. Leaflets are widely lanceolate, measuring 5–10 x 2–5 cm. The blooms have hairy cymes that resemble racemes. funnel-shaped white flower with linear, 6–8 mm-long bracts. The leaves are ovate-lanceolate, elongated, enlarged in fruit, and densely hairy, with two inner leaves that are somewhat shorter and hairless. Five stamens with hairy filaments. The capsules are long, oval, papery, four-chambered, four-valved, and covered in silky sepals. The seeds are four, glabrous, and shiny. Flowers: December to March.
A molecular level identification and separation of chemical mixtures is achieved using a gas chromatography-mass spectrometer. It is an analytical instrument for determining and examining a sample's unknown molecular weight. By analysing and contrasting the spectra with the reference, one may identify the unknown organic molecule present in the complex combination [6].
GC-MS analysis in other species of *Merremia* such as *M. emarginata* and *M. tridentata* have also been reported by various researchers indicating the presence of useful metabolites in them. D-Mannitol, 9,12,15-Octadecatrienoic acid, Caryophyllene oxide, Phytol, Octadecanoic acid, Methyl stearidonate, Eicosanoic acid, Gamolenic Acid, beta.-Tocopherol, Stigmasterol, beta.-Sitosterol, dl-.alpha.-Tocopherol have been reported in ethyl acetate extract of *M.emarginata* [7], whereas the major phytoconstituents reported in methanolic extract of roots of *M. tridentata* were Dodecanoic acid, Tetra decanoic acid, Hexadecanoic acid, Hepta decanoic acid,16-methyl methyl ester, etc [8]. *M. dissecta* seed oil was analysed by GC-MS revealing the presence of methyl stearate (13.12%); 9 hexadecanoic acid methyl ester (23.04%); 12- octadecadienoic acid methyl ester (32.78%); and 9-octadecenoic acid methyl ester (22.04%) as major constituents [9].
Research was conducted to determine possible bioactive components in these two species using GC-MS.

MATERIALS AND METHODS

- *Preparation of extracts*

Samples of the test plants' fully grown stems and seeds were gathered, and then dehydrated in the shade. After the material had dried, it was ground into a powder and 10 grams of Soxhlet extract was extracted in 100 millilitres of 95% methanol in a water bath for a whole day. Following that, the methanolic extracts were concentrated.

- *GC-MS analysis*

This was accomplished by employing a TD 20 thermal extractor and a Shimadzu QP-2010 plus thermal desorption system There were polar and non-polar phytocomponents in the plant extracts. Each extract was utilised in 2 µl quantity for the GC/MS analysis [10].

- *GCMS conditions*

Column size: 30 m × 0.25 mm ID × 0.25 µm, Film thickness: 0.25 mm, composed of 5MS (5% diphenyl/ 95% dimethyl polysiloxane), Carrier gas: helium gas (99.999%) at constant flow rate 1 ml/min. Injection volume:2µl with 10:1 split ratio, Initial injector temperature: 280 °C, Ion source temperature: 200 °C, 110 °C was the initial Oven temperature. This was gradually increased at a rate of 10 °C/min to 200 °C, after that at a rate of 5 °C/min

at 280 °C. The session was completed with the temperature kept at 280 °C isothermally. The run time of each sample was 45 minutes.

- *Mass spectrum interpretation*

The National Institute of Standards and Technology (NIST) database was consulted after a comparison between the fragmentation patterns of the mass spectra and the database patterns and the retention indices of each component.

RESULTS AND DISCUSSION

The two plant species of the *Distimake* genus are endowed with extremely potent metabolites. Their well-established anti-inflammatory, anti-diabetic, and anti-hypercholesterolemic properties can be further used to extract their active metabolites.

Spectrophotograms were produced for each sample after GC-MS analysis of stem and seed samples of *D. aegyptius* (L.) A.R. Simoes and Staples and *D. dissectus* A.R. Simoes and Staples were carried out. In the methanolic stem extract of *D. aegyptius*, the spectrogram revealed 27 compounds and 29 distinct peaks in the seed; in the methanolic stem extract of *D. dissectus*, the spectrogram revealed 29 components and 20 components in the seed. As phytoconstituents, these species exhibited the presence of tannins, alkaloids, flavonoids, phenols, sugars, steroids, fatty acids, amino acids, vitamins, and others. **Table 1** represents the common names of few important compounds isolated from these species. Certain common phytochemicals were also detected in both *Merremia* species, according to the obtained GC-MS data.

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

1	1,2 benzene di carboxylic acid, dibutyl ester	Dibutyl Phthalate
2	1,3,4,5-Tetrahydroxy-cyclohexanecarboxyl	Quinic acid
3	Dodecanoic acid,methylester	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 highest peak area and area percentage of 1,2,4-butanetriol (26.84%) were obtained in the stem sample, 1,2-benzenedicarboxylic acid dibutyl ester (dibutyl phthalate) (48.17%) in the *D. aegyptius* seed sample, while 1,3,4,5-tetrahydroxy-cyclohexanecarboxyl (quinic acid) (11.88%) in stem extract, sitosterol (24.56%) in *D. dissectus* seeds **(Figures 1a, 1b, 2a, 2b and Tables 2-5)**.

1,2,4 Butanetriol is a precursor of two recognised medications that decrease cholesterol. With its antibacterial, antiviral, candidicidal, and hypercholesterolemic qualities, decanoic acid (also known as Lauric acid) has prospective uses in the cosmetics sector it also possesses antibacterial qualities and is utilised in soaps.

Numerous actions, including antioxidant, 5-alpha reductase inhibitory, hemolytic, antifibrinolytic, antibacterial, nematicidal, and hypocholesterolemic, have been found for hexadecanoic acid methyl ester.

Quinic acid is an astringent substance that is also utilised in the production of the medication oseltamivir, which is used to treat influenza A and B [11].

Although 1,2-Benzenedicarboxylic acid dibutyl ester, or dibutyl phthalate (DBP), is a significant plasticizer that improves the usability of PVC and effectively prevents scrub typhus, its use in cosmetics is prohibited in the European Union by Directive 76/768/EEC of 1976 due to DBP's potential endocrine disruptor status [12-14].

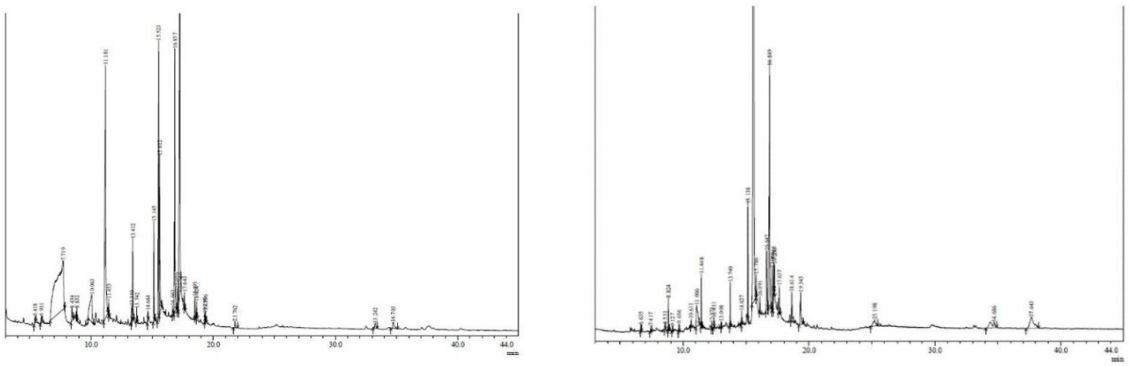
9-Octadecenoic Acid, Methyl Ester (Oleic Acid, Methyl Ester) has a preventive anti-inflammatory character against cancer, it is also the main component of biodiesel.

9-Octadecenamide has anti-inflammatory and antibacterial properties; it is used to treat mood, sleep, and depression problems regulated by cannabinoids [15]. Stigmasterol is recognised for its anti-inflammatory, hypoglycemic, antioxidant, thyroid-inhibiting, and pro-inflammatory properties. It may also help prevent breast, colon, prostate, and ovarian cancers. Antiretroviral, antihypertensive, antidiabetic, and antineoplastic properties [16, 17]. Anti-inflammatory, anti-arthritic, anti-androgenic, hypocholesterolemic, hepatoprotective, nematocidal, 5-alpha-reductase inhibitory, antihistamine, anticoronary, insecticidal, anti-eczematic, and anti-acne properties are exhibited by the (z,z)-methyl ester of 9,12-octadecadienoic acid.

Cosmetics, soaps, and industrial mould separators are all made with palmitic acid [18]. Methyl hexadecanoic acid, also known as methyl palmitate or hexadecanoate methyl ester showed anti-inflammatory, antifibrotic activity and highest antimicrobial efficacy against clinically pathogenic bacteria.

9,12,15-octadecatrienoic acid - anti-inflammatory, preventive against cancer, hepatoprotective, antioxidant. Octadecanoic acid has antifungal, antitumor, antibacterial activity [19].

1-(+)-ascorbic-2,6-dihexadecanoate acid has antioxidant, cardioprotective, anticancer, and taste effects. It also acts against infertility [20].



a) b)

Figure 1. GC-MS Spectogram of a) *D. aegyptius* stem b) *D. aegyptius* seed

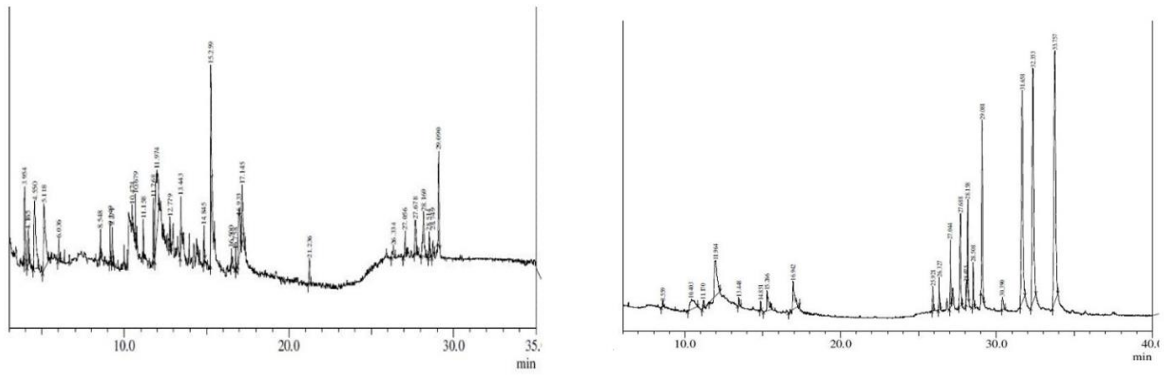
Table 2. Compounds detected in GC-MS analysis of *D.aegyptius* stem

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-methoxyphenol	13.310	0.43
10	tetradecanoic acid	13.412	2.29
11	1-octadecene	13.742	0.28
12	1,2-benzenedicarboxylic 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-benzenedicarboxylic 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 acetylricinoleate	19.290	0.20
24	9-octadecenamide, (z)-	19.356	0.23
25	1,2-benzenedicarboxylic 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-methylethyl)cyclo decane	8.551	0.11
4	1-tetradecene	8.824	0.99
5	cyclohexane,1,2,4,5-tetraethyl-,(1.alpha.,2.alpha.,4.alpha., 5.alpha.)-	9.127	0.21
6	Cyclo hexane,octyl-	9.656	0.22
7	Dodecanoic acid, methylester	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-benzenedicarboxylic 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-, methylester, (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	aceticacid,(1-methylethoxy)-,1-methylethylester	7.188	8.00
3	tridec-1-ene<n->	8.537	0.95
4	caryophyllene<(e)->	9.141	1.99
5	2-norpinene,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-cyclohexanecarboxyl	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,methylester	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-benzenedicarboxylicacid,diisooctyles	21.220	1.12
21	1,3,7,11-tridecatetraene-1,1-d2,4,8,12-trimethy	25.179	0.41
22	2-isopropyl-5-methylcyclohexyl 3-methyl-4-methylenecyclopentanecarboxylate	28.719	0.55
23	cyclopentanecarboxylic acid, 3-methyl-4-methylene-, menthyl ester	31.639	0.86
24	benzene, 1-(4'-pentyl[1,1'-bicyclohexyl]-4-yl)-4-(4-propylcyclohexyl)-, [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	.beta.-d-glucopyranose, 1,6-anhydro-	10.403	2.38
3	1-undecene, 9-methyl-	11.170	0.25
4	1,3,4,5-tetrahydroxy-cyclohexanecarboxylic acid	11.964	4.84
5	1-heptanol, 3-methyl- $$ 3-methyl-1-heptanol	13.448	0.30
6	undecanoicacid,methylester	14.851	0.20
7	Hexadecanoicacid	15.266	1.84
8	9,12-octadecadienoicacid(z,z)-	16.942	2.52
9	3-bromocholest-5-ene#	25.921	1.04
10	Stigmasterol	26.327	1.22
11	cholest-5-en-3-ol(3.beta.)-,carbonochloridate	27.044	2.52
12	3-bromocholest-5-ene#	27.688	5.42
13	cholesta-2,4-diene $$ 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-3.beta.-ol	30.390	0.81
18	ergost-5-en-3-ol,(3.beta.)-	31.658	18.19
19	stigmasta-5,22-dien-3-ol	32.353	19.23
20	gamma.-sitosterol	33.757	24.56
			100.00

CONCLUSION

All these bioactives identified from these species have possible economic, pharmaceutical, or medicinal importance and therefore these species can be recommended as phytopharmaceuticals. Some of the important compounds that were identified are dibutyl phthalate, sitosterols, lauric acid, palmitic acid, ascorbic acid, quinic acid and hexadecenoic acid having multiple benefits. This type of GC-MS analysis will also help to understand the active ingredient nature in these two medicinal plants and would be useful for further detailed analyses.

ACKNOWLEDGMENTS : I would like to acknowledge the JNU-AIRF facility for providing the GC-MS result and would also like to thank the Department of Botany, University of Rajasthan for providing the necessary facilities for the experiment.

CONFLICT OF INTEREST : None

FINANCIAL SUPPORT : I am thankful to UGC for providing a Senior Research Fellowship that helped in conducting this study.

ETHICS STATEMENT : None

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