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GC-MS Analysis of acetone extract of leaves of *Morinda reticulata* Gamble

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ABSTRACT

Majority of world population still rely on drugs of plant origin. Many plants belonging to *Morinda* species show medicinal activities and *Morinda reticulata* Gamble, one among them, is used as a folklore medicine. Literature review reveals that various stomach ailments can be cured by the decoction of leaves of *Morinda reticulata* Gamble. The present study aims to disclose the active components present in the acetone extract of leaves of *Morinda retculata* Gamble by GC-MS analysis. GC-MS analysis showed that1-Pentadecene,Ethyl alpha-d-glucopyranoside, (trans)-2-nonadecene,(E)-Phytol,2-Nonyloxirane,9-Heptadecanone, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Phthalate, 9-Octodecanoicacid (Z)-,Furan, 2, 5dimethyl-, 1-Heneicosanol, isophytol, acetate, Palmitaldehyde, Diallylacetal, 1-Nonadecene, 1,2-Benzenedicarboxylicacid, Octane, Squalene were the phytochemicals present in the acetone extract. Squalene, 1,2-Benzenedicarboxylic acid, Hexadecanoic acid & 9-Octadecanoic acid are the bio-active compounds which exhibit various pharmacological activities such as anti- bacterial, antitumour, antioxidant, immunostimulant, lipoxygenase inhibitor etc. Its bio-active components rationalize its use as herbal remedies.

INTRODUCTION

The plant kingdom plays a vital role in the existence of human life on earth. The history of phytomedicine is as old as civilization. The major advantages of herbal medicine are affordability, and fewer side effects. Plants are biosynthetic lab, produces primary metabolites and secondary metabolites which possess wide range of pharmacological activities such as anti-cancer, antioxidant, anti-inflammatory, hepatoprotective, analgesic, antimicrobial etc. The important secondary metabolites derived from plants are alkaloids, glycosides, tannins, steroids, flavonoids etc. The community of developing countries mainly depends on traditional herbal medicine for their primary health care. Plants are constantly screened to check their pharmacological activity and to establish the bioactive chemicals. The secondary metabolites may be polar or nonpolar in nature. Successive solvent extraction method is used to extract the active constituents and phytochemical screening helps to identify the secondary metabolites present in different extract.

Gas chromatography plays a vital role to separate the volatile constituents and mass spectrometry helps to identify the compounds. GC-MS is a hyphenated technique in which separation and identification occurs simultaneously. This process occurs rapidly and only picogram of sample is needed. The unknown organic compound can be detected by spectral interpretation and comparing the spectra obtained with reference spectra.[1] GC-MS helps in chemical profiling which helps to establish standardization parameters and prevents adulteration. Moreover, it justifies its use as a traditional medicine.

The genus, *Morinda* species has several medicinal properties and is widely used as herbal remedies for various ailments. *Morinda reticulata* Gamble (Family Rubiaceae), is an arborescent climber [2]. Rice mixed with powdered leaves of *Morinda reticulata* Gamble is used for purification of blood. Leucorrhea and back pain can be healed by fresh leaves of *Morinda reticulata* Gamble. Leaves are used as decoction for various stomach ailments[3]. The replaced name of *Morinda reticulata* Gamble is *Gynochthodes ridsdalei*[4]. Literature reviews reveals that there are no published reports on the phytochemicals present in the acetone extract of leaves of *Morinda reticulata* Gamble.

The present study aims to determine the bioactive phytochemicals present in the acetone extract of leaves of *Morinda reticulata* Gamble using GC -MS technique.

Fig. 1 : *Morinda reticulata* Gamble

MATERIALS AND METHODS

Collection of specimens

The leaves for the proposed study were collected from localities of Palode and Kallar Thiruvananthapuram. The collected specimen leaves from TBGRI garden were carefully examined and authenticated by Dr. A.G Pandurangan, Taxonomist and Former Director, Nehru Tropical Botanical Garden and Research Institute, Palode. A voucher specimen (voucher No 76866) has been deposited there for future use.

Successive SolventExtraction[5]

In a soxhlet extractor,50g of the air- dried powdered plant material was extracted successively with petroleum ether 60-80^0 C, chloroform, acetone, ethanol. Each time before extracting the next solvent, dried the powdered material in hot air oven below 50^{0}C and finally the marc was macerated with chloroform-water for 24 hours to obtain the aqueous extract. The extracts were concentrated by distilling off the solvent and evaporated to dryness on the water- bath. The preliminary phytochemical screening revealed that acetone extract showed the presence of bioactive phytochemicals that exhibit remarkable pharmacological activity. The extract revealed the presence of phytosterols, phenolic compounds, tannins and flavonoids. To explore the volatile compounds present in the extract, the acetone extract was subjected to GC-MS analysis

GC--MS Analysis

The acetone extract obtained by successive solvent extraction of air dried leaves of *Morinda reticulata* Gamble was subjected to GC-MS analysis. Gas Chromatography- Mass spectrometric analysis (GC-MS) was carried out in Shimadzu GC-MS Model Number QP2010S, Shimadzu equipped with column Rxi-5SilMS, 30m length, 0.25mm internal diameter, 0.25 micrometer thickness. The settings were as follows., column oven temperature: 60.0 °C, injection temperature: 260.00 °C, injection mode: split, flow control mode: linear velocity, Pressure:57.4 kPa, total Flow:24.0 ml/minute, column flow :1.00 ml/minute, linear velocity:36.5 cm/sec,oven temperature program:60^{0}C holds for 2min:10^{0}C/min to 260^{0}C holds for 10 min and 5^{0}C/min to 280^{0}C holds for 6minute.In the mass spectrometer part, start time :7.00min,end time :42.00minute, ACQ Mode :Scan, event time :0.50sec, scan speed :1000, start m/z :50.00, end m/z :500.00 . Software used to handle mass spectra and chromatograms were GC-MS Software: GC-MS Solutions. Interpretation of mass spectra of acetone extract of leaves of *Morinda reticulata* Gamble was conducted using the data bases of National Institute of Standard and technology (NIST 11) and WILEY 8 libraries.

RESULTS

The bioactive phytoconstituents present in acetone extract of leaves of *Morinda reticulata* Gamble were determined by analyzing the sample through GC-MS technique.

The total ion chromatogram of acetone extract of leaves of *Morinda reticulata* leaves was shown in (Figure 2) and the compounds identified during the analysis were shown in (Table 1). The GC- MS analysis results of acetone extract of leaves of *Morinda reticulata* Gamble disclosed the presence of 17 phytochemicals. The interpretation of mass spectra was done by data bases of NIST 11 and WILEY 8 libraries. (Table 1) showed the name of compounds, its retention time, peak area, peak height, percentage of peak area, percentage of peak height and base mass to charge ratio.

DISCUSSION

Thepreliminary phtochemical screening showed that acetone extract of leaves of *Morinda reticulata* Gamble contain bioactive constituents such as phytosterol, phenolic compounds , tannins and flavonoids. To detect the volatile compound present in the extract GC-MS analysis was conducted. The GC MS analysis of acetone extract revealed the presence of phytochemicals like 1-Pentadecene, Ethyl alpha-d-glucopyranoside, (trans)-2-nonadecene, octane, (E)-Phytol, 2-Nonyloxirane, 9-Heptadecanone, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Phthalate, 9-Octodecanoicacid, Furan, 2, 5-dimethyl-, Heneicosanol, isophytol, acetate, Palmitaldehyde, Diallylacetal, 1-Nonadecene, 1,2-Benzenedicarboxylicacid, squalene. The retention time showed that1-Pentadecene eluted first and squalene, a pharmacologicaly active compound, eluted last. The

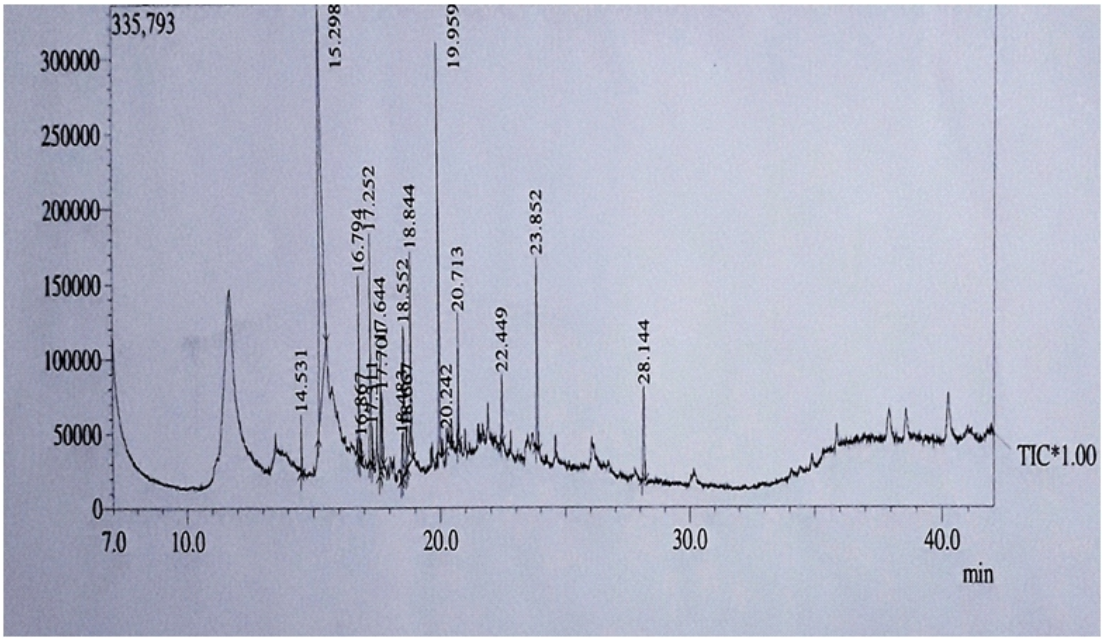

Fig. 2 : TIC Chromatogram of acetone extract of leaves of *Morinda reticulata* Gamble

Table 1 : Compounds identified on the acetone extract of leaves of *Morinda reticulata* Gamble

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	14.531	58244	1.05	39533	2.36	1-Pentadecene	83.10
2	15.298	2317343	41.97	269284	16.10	Ethyl .alpha.-d-glucopyranoside	60.00
3	16.794	194549	3.52	121844	7.28	(TRANS)-2-NONADECENE	57.05
4	16.867	25533	0.46	14875	0.89	OCTANE	57.05
5	17.252	330023	5.98	153444	9.17	(E)-PHYTOL	68.05
6	17.311	51808	0.94	26080	1.56	2-NONYLOXIRANE	70.10
7	17.644	181511	3.29	86399	5.16	9-HEPTADECANONE	71.05
8	17.701	101547	1.84	51577	3.08	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	82.05
9	18.483	75384	1.37	29138	1.74	PHTHALATE	149.00
10	18.552	425015	7.70	100547	6.01	9-OCTADECENOIC ACID (Z)-	73.05
11	18.667	74499	1.35	27836	1.66	FURAN, 2,5-DIMETHYL-	58.10
12	18.844	342122	6.20	140520	8.40	1-Heneicosanol	57.05
13	19.959	498294	9.03	273930	16.37	Isophytol, acetate	71.05
14	20.242	87619	1.59	12071	0.72	PALMITALDEHYDE, DIALLYL ACETAL	84.00
15	20.713	140854	2.55	88780	5.31	1-NONADECENE	57.10
16	22.449	75618	1.37	45644	2.73	(TRANS)-2-NONADECENE	57.05
17	23.852	300780	5.45	128475	7.68	1,2-BENZENEDICARBOXYLIC ACID	149.00
18	28.144	240315	4.35	62912	3.76	Squalene	69.05
		5521058	100.00	1672889	100.00		

Spectrum

Table 2 : Pharmacological activities of bioactive phytochemicals

Serial No	Name of compound	Biological Activity
1	Squalene	Antibacterial, Antioxidant, Antitumour, Immunostimulant, Lipoxygenase inhibitor-[6]
2	1,2-Benzenedicarboxylic Acid	Antibacterial[7]
3	9-Octadecanoic acid	Antibacterial, Antifungal[8]
4	Hexadecanoic acid	Antimicrobial, antioxidant, hypocholesterolemic, antiandrogenic, nematicide, pesticide[8][9][10]

peak area and peak height revealed that isophytol acetate and Ethyl alpha-d-glucopyranoside were present in larger amounts. Literature review also revealed that Squalene possess antibacterial, antioxidant, antitumour, immunostimulant, lipoxygenase inhibitor activity. 1,2-Benzenedicarboxylic acid has antibacterial activity and Hexadecanoic acid possess anti microbial, antioxidant, hypocholestermic, antiandrogenic, nematicidal and pesticidal activity. 9-Octadecanoic acid exhibited antibacterial and antifungal activity. The bio-active compounds and the reported pharmacological activities are presented in (Table 2)

CONCLUSION

The natural plant species show diversity and are complex in nature. New technology reveals the complexity and helps in the discovery of new drugs. The hyphenated techniques GC - MS plays a major role in this field. Gas chromatography separates volatile and stable compounds and mass spectrometry helps in identification of components since the fragmentation pattern of mass spectra of a particular compound is unique in nature. When gas chromatography coupled with mass spectrometer is used , separation and identification are possible. *Morinda reticulata* Gamble, a folklore medicine, is used in various stomach ailments. The preliminary phytochemical analysis of acetone extract of leaves of *Morinda reticulata* Gamble revealed that, it is a rich source of phyto active chemicals. GC-MS analysis of *Morinda reticulata* Gamble revealed the presence of 17 phytochemicals. Squalene, 1, 2-Benzenedicarboxylic acid, Hexadecanoic acid & 9-Octadecanoic acid are the compounds which show remarkable pharmacological activities such as antitumour, antioxidant, antibacterial, antifungal, antimicrobial etc. So it's use as conventional medicine is justified. The present investigation point out that further studies are needed to isolate the bioactive compound and check the medicinal property of each component by *in vitro* and *in vivo* methods. Moreover this, isolated molecules may act as a lead molecule for the development of new drugs.

CONFLICT OF INTEREST

We declare that there are no conflicts of interest.

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REFERENCES

1. Elizabeth T, Aneesh TP, Della T, Anandan R. GC-MS Analysis of phytochemical compounds present in the rhizomes of *Nervilia aragoana* Gaud. Asian Journal of Pharmaceutical and Clinical Research. 2013:6(3):68-74.
2. Nair, Rahul, Kavitha M, Sethuraman T, Doss G. Conservation and invitro multiplication of highly endangered Indian traditional plant (*Morinda reticulata* Gamble) through nodal explants. Plant Knowledge Journal. 2012:1(2):46-51.
3. IjinuT, Anish N, Shiju H, George V, Pushpaganadan P. Home gardens for nutritional and primary health security of rural poor of South Kerala. Indian Journal of Traditional Knowledge. 2011:10(3):413-428.
4. Razafimandibison S, G, Bremer B. Nomenclatural changes and taxonomic notes in the tribe Morindaceae (Rubiaceae) Adansonia. 2011:33(2):283-309.
5. Kokate CK: Practical Pharmacognosy, Vallabh Prakashan; Delhi, India 2014.p.108.
6. Meenatchi Ammal R,Viji Stella Bai G. GC-MS Determination of bioactive constituents of *Heliotropium indicum* leaf. Journal of Medicinal Plants Studies. 2013:1 (6):30-33.
7. Singh R, Dar S, Sharma P. Antibacterial activity and toxicological evaluation of semi purified hexane extract of *Urtica dioica* leaves. Research Journal of Medicinal Plants. 2012:6(2):123-135.
8. Irawan C, Foliatini, Hanafi, Sulistiawaty L, Sukiman M. Volatile compound analysis using GC-MS, Phytochemical screening and antioxidant activities of the husk of "Julang-Jaling" (*Archidendron bubalinum* (Jack) I.C Nielsen) From Lampung, Indonesia. Pharmacog J. 2018:10(1):92-98.
9. Deepa P, Kaleena P, Valvittan K. GCMS Analysis and antibacterial activity of chromatographically separated pure fractions of leaves of *Sansevieria roxburghiana.* Asian J Clin Res. 2011:4 (4):130-133.
10. Awa EP, Ibrahim S. Ameh DA. GC/MS Analysis and antimicrobial activity of diethyl ether fraction of methanolic extract from the stem bark of *Annona senegalensis* Pers. Int J Pharm Sci Res.2012:3(11):4213-4218.