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IDENTIFICATION AND CHARACTERIZATION OF NOVEL PHYTOCONSTITUENTS FROM THE FERMENTED EXTRACT OF LEAVES OF *MORINGA OLEIFERA* BY GC-MS ANALYSIS

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Keywords:

Moringa oleifera, Moringaceae, Fermentation, Phytoconstituents, GCMS analysis

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ABSTRACT: *Moringa oleifera*is a well-known plant that can be used for the treatment of various disorders or nutritional deficiencies; all parts of this plant possess various activities such as antitumor, antipyretic, antiepileptic, anti-inflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, cholesterol-lowering, antioxidant, antidiabetic, hepatoprotective, antibacterial and antifungal activities and are being employed for the treatment of different ailments. Numerous bioactive compounds were found to be present in the fermented extract of *Moringa oleifera* with therapeutic activity having great research value by pharmaceutical industries. The fermented extract of *Moringa oleifera* leaves was analyzed by gas chromatography-mass spectroscopy for the identification and characterization of phytoconstituents and their therapeutic efficacy. The existing compounds identified in extract were Diethyl Phthalate (56.05%), dl-Mevalonic acidlactone (10.40%), 4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl (8.88%), Bis(2-Ethylhexyl) phthalate (2.99%), 5-Fluoro-6-methyl-5-hepten-2-one (2.42%). Beta-D-,glycopyranoside, methyl (1.94%), glyceraldehydes (1.71%), 2-propen-1-one, 3-[(1,1-dimethylethyl) thio]-1(1.67%), 1,2,3-propanetriol, diacetate 1.16%), 2,4-dihydroxy-2,5-dimethyl-3(2h)-furan-3-one(1.12%), 2-cyclopenten-1-one, 2-hydroxy-(0.99%), 4-(1-hydroxy-ethyl). gamma. Butanolacton (0.98%), 1,3,5-triazine-2,4, 6-triamine (0.92%), 2(3h)-furanone, 5-acetyldihydro(0.86%), dihydro-5-(1-hydroxyethyl)-2(3h)-furanone (0.85%), docosyl ethyl ether(0.77%), benzeneethanol, 4-hydroxy(0.67%), benzene acetic acid (0.61%), benzene, 1,1'-sulfonylbis-(0.59%), 2-propanamine, n-methyl-n-nitroso (0.59%), 1-heptanol(0.58%), 3,4,5-trimethoxyphenol (0.41%), phenol, 5-ethenyl-2-methoxy (0.41%), 2-oxepanone (0.37%), 3,3-dimethyl-1-(2-naphthyl) butanone (0.31%), furaneol (0.28%), 3(2h)-furanone, 4-hydroxy-5-methyl(0.24%), butanoic acid, 3,3-dimethyl (0.19%), 2-propanamine, n-methyl-n-nitroso (0.08%). These compounds were established qualitatively *via* GC-MS evaluation. GC-MS reports will be auspicious in the preparation of herbal nutraceuticals, cosmeceuticals and the pharmaceutical area.

INTRODUCTION: *Moringa oleifera*, commonly known as moringa, belongs to the family Moringaceae. Nowadays, moringa is referred to as a miracle tree due to its potency to overcome malnourishment.

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Every part of the plant, from leaf to root, is a good source of vitamins, amino acids, and many other essential nutrients and minerals.

Besides nutritional uses, moringa is used to boost immunity and possesses anti-tumor, antipyretic, antiepileptic, anti-inflammatory, antiulcer, antihypertensive, cholesterol-lowering and antidiabetic activity. The seeds of moringa are considered to be antipyretic, acrid, bitter [1] and reported to show antimicrobial activity [2]. Niazimicin has been proposed to be a potent

chemopreventive agent in chemical carcinogenesis [3]. This study explores and analyzes moringa leaves' chemical constituents after fermentation. In the given study, fermented moringa leaves extract was conducted, which was further got analyzed for the identification of phytoconstituents by Gas Chromatography-Mass Spectrometry (GC/MS). The major biologically active compounds obtained from fermentation can be used in pharmaceuticals and nutraceuticals to overcome malnourishment, vitamins & amino acid deficiency.

MATERIALS AND METHODS:

Procurement and Verification of Plant Materials: In July 2019, *Moringa oleifera* Lam [Moringaceae] leaves were obtained from an online market in Tamilnadu (India). The sample of leaves was authenticated from CIMAP, Lucknow, India-226026. (Reference no. CISIR/CIMAP/Moringa0254).

Equipment: An earthen pot sufficiently large and glazed, a porcelain jar of suitable size, a lid to close the vessel is used as basic equipment.

Fermentation Method: In this process, the freshly collected leaves of *Moringa oleifera* were cleaned and dried under shade at 25°C; after drying, the leaves drug is boiled in a specified volume of water for a defined time; it is then cooled and strained or filtered. The starting ratio of crude drug to water is fixed, *e.g.*, 1:4 or 1:16. The volume is then brought down to one-fourth its original volume by boiling during the extraction procedure. Then, the concentrated extract is decanted and filtered.

Sample Processing: The specified volume of the extract is taken in an earthen pot sufficiently large and glazed then jaggery is added (A medium of sugars is required for fermentation). The pot is filled up to three fourth of its capacity. The void space provides space for the fermenting liquid when it rises due to the frothing and evolving of many gases.

Inoculum: Now, in sweetened extract, the inoculum of yeasts comes from the fire flame flowers (Dhataki flowers) are added to initiate fermentation. These flowers are nectareous and highly titaniferous. The flowers contain yeast spores in the dry nectariferous region [4]. The yeasts multiply rapidly by division in a short time [5]. Finally, the vessel should be closed and sealed. Sealing is done by winding around a long ribbon of cloth smeared with clay on one surface.

Fermentation Process: The duration of fermentation varies with season during autumn and summer seasons; fermentation takes place in 10 days. In winter, it takes 15-20 days. During the rainy season and spring, fermentation occurs in 8 days [6].

In the current study, the fermentation vessel is left undisturbed for 15 days and then opened. There are claims that yeast cell walls naturally bind heavy metals and pesticide residues and act as a natural cleaning system, making self-fermentation of herbal products safer than powder decoctions [7, 8, 9].

GC-MS Analysis of Phytoconstituents: A fermented extract of *Moringaoleifera* leaves (FEMO) was examined using Gas Chromatography-Mass Spectroscopy (GC-MS). The (GC-MS) instrument was equipped with Shimadzu QP-2010 Ultra with capillary standard, a non-polar column 60 M TRX 5-MS. Helium gas was employed as vehicle gas with a mobile phase flow rate kept at 1.25 ml min^{-1} [10].

RESULT AND DISCUSSION:

Identification of Phytocomponent by GC MS of Aqueous Extract of *M. oleifera* Leaves: The GC-MS profile of the aqueous extract of *M. Oleifera* leaves is shown in **Fig. 1**, which reflects 31 peaks of biomolecules.

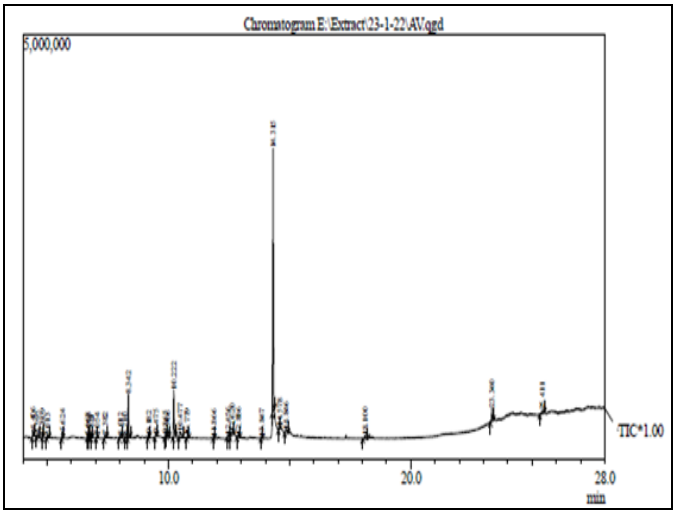
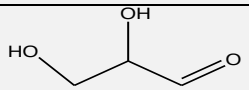
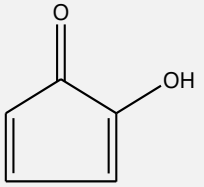
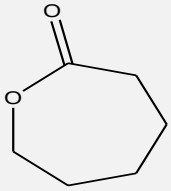
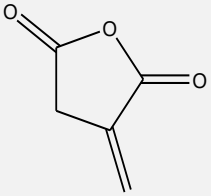
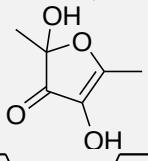
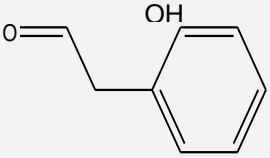
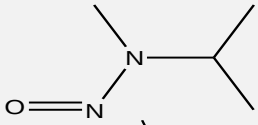
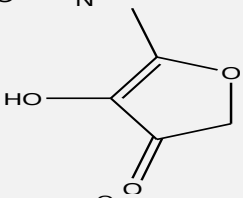
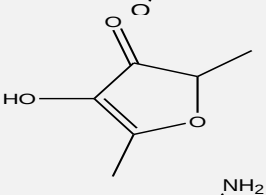
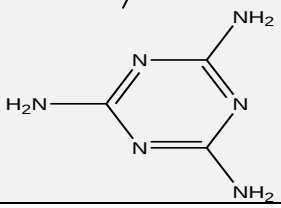
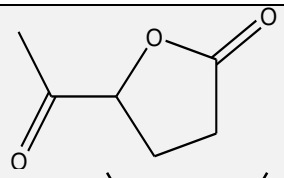
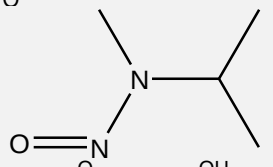
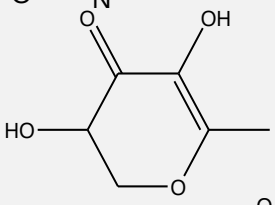
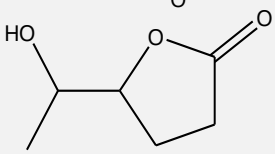
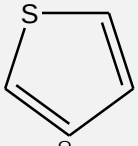
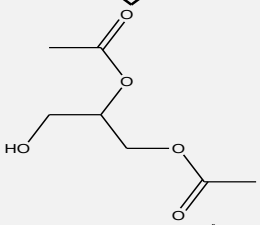
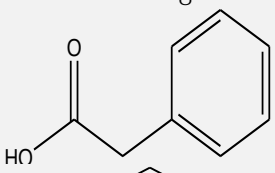
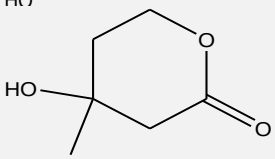
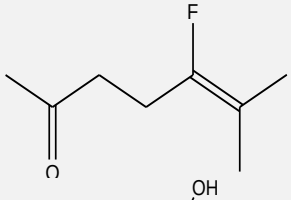
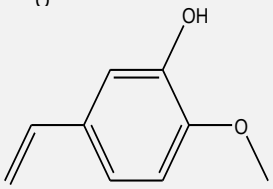
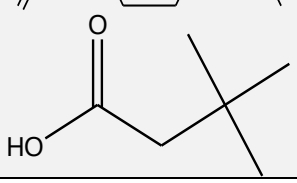


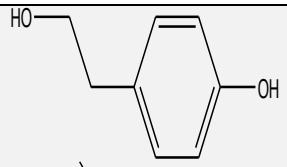
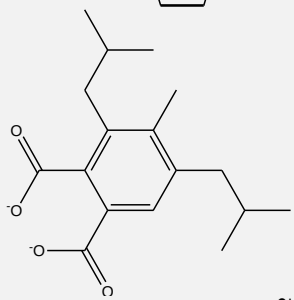
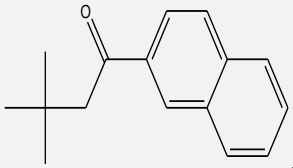
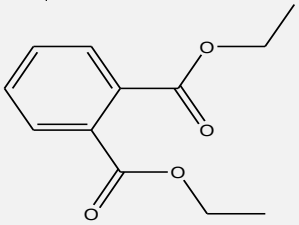
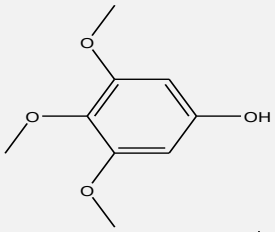
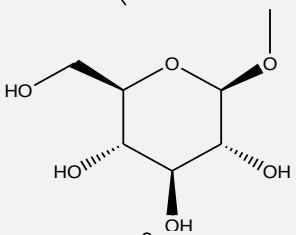
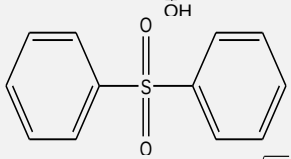
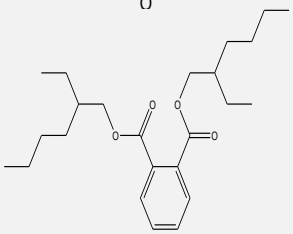
FIG. 1: GC MS OF *M. OLEIFERA* LEAVES AQUEOUS EXTRACT

They present the phytocomponents, their retention time, peak area percentage, and Molecular weight. The chemical structure of active components and their known key applications, like medicinal, cosmetics, *etc*. are tabulated in **Table 1.**

TABLE 1: A COMPOUND PRESENT IN FERMENTED EXTRACT OF *MORINGA OLEIFERA* LEAVES USING GC-MS ANALYSIS

S. no.	R. Time	Peak Area Z.	Name of compound	MF	MW	Nature of compound	Chemical structure	Cas No.
1.	4.406	1.71	Glyceraldehyde	$C_3H_6O_3$	90	Aldehyde		56-82-6
2.	4.595	0.37	2-Oxepanone	$C_6H_{10}O_2$	114	ketone		502-44-3
3.	4.809	0.99	2-Cyclopenten-1-one,2-hydroxy-	$C_5H_6O_2$	98	ketone		10493-98-8
4.	5.013	0.74	3-METHYLENEDIHYDRO-2,5-FURANDIONE#	$C_5H_4O_3$	112	ketone		2170-03-8
5.	5.624	1.12	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	144	ketone		10230-63-3
6.	6.668	0.24	Benzeneacetaldehyde	C_8H_8O	120	aldehyde		122-78-1
7.	6.725	0.08	2-Propanamine,N-Methyl-N-Nitroso-	$C_4H_{10}N_2O$	102			30533-08-5
8.	6.821	0.24	3(2H)-Furanone,4-hydroxy-5-methyl-	$C_5H_6O_3$	114	ketone		19322-27-1
9.	7.054	0.28	Furaneol	$C_6H_8O_3$	128	alcohol		3658-77-3
10.	7.382	0.92	1,3,5-Triazine-2,4,6-triamine	$C_3H_6N_6$	126	amine		108-78-1

11.	8.012	0.86	2(3H)-Furanone,5-acetyldihydro-	$C_6H_8O_3$	128	ketone		29393-32-6
12.	8.210	0.59	2-Propanamine,N-Methyl-N-Nitroso-	$C_4H_{10}N_2O$	102	amine		30533-08-5
13.	8.342	8.88	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144			28564-83-2
14.	9.182	0.98	4-(1-Hydroxy-Ethyl).Gamma.Butanol acton	$C_6H_{10}O_3$	130			0-00-0
15.	9.475	0.85	Thiophene	C_4H_6S	86	Thiophene		1708-32-3
16.	9.865	1.16	1,2,3-Propanetriol, Diacetate	$C_7H_{12}O_5$	176	Alcohol		25395-31-7
17.	9.966	0.61	Benzeneaceticacid	$C_8H_8O_2$	136			103-82-2
18.	10.222	10.40	dl-Mevalonicacidlactone	$C_6H_{10}O_3$	130			674-26-0
19.	10.477	2.42	5-Fluoro-6-methyl-5-hepten-2-one	$C_8H_{13}FO$	144	ketone		0-00-0
20.	10.779	0.41	Phenol,5-ethenyl-2-methoxy-	$C_9H_{10}O_2$	150	phenol		621-58-9
21.	11.866	0.19	Butanoicacid,3,3-Dimethyl-	$C_6H_{12}O_2$	116	alcohol		1070-83-3

22.	12.456	0.67	Benzeneethanol,4-hydroxy-	$C_8H_{10}O_2$	138	alcohol		501-94-0
23.	12.620	1.67	Bis(2-Methylpropyl)4-Methylbenzene-1,2-Dicarboxylate	$C_{17}H_{24}O_4$	292			66286-98-4
24.	12.886	0.58	1-Heptanol	$C_7H_{16}O$	116			111-70-6
25.	13.847	0.31	3,3-Dimethyl-1-(2-Naphthyl) Butanone	$C_{16}H_{18}O$	226			0-00-0
26.	14.315	56.05	Diethyl Phthalate	$C_{12}H_{14}O_4$	222			84-66-2
27.	14.578	0.41	3,4,5-Trimethoxyphenol	$C_9H_{12}O_4$	184	phenol		30225-76-4
28.	14.846	1.94	. beta. -D-Glucopyranoside,methyl	$C_7H_{14}O_6$	194			709-50-2
29.	18.100	0.59	Benzene,1,1'-Sulfonylbis-	$C_{12}H_{10}O2S$	218	sulphonamide		127-63-9
30.	23.340	2.99	Bis(2-Ethylhexyl)phthalate	$C_{24}H_{38}O_4$	390			117-81-7
31.	25.411	0.77	Docosylethylether	$C_{24}H_{50}O$	354	ether		0-00-0

CONCLUSION: The fermented extract of the Moringa oleifera was analyzed to develop herbal nutraceuticals to ensure uniformity and quality control. For the same objective, the current paper was designed to identify the biologically active compounds extant in the fermented extract of moringa leaves with the aid of Gas chromatography-Mass spectroscopy (GC-MS). The analysis revealed diverse medicinal compounds like phthalates, mevalonic acid lactone, glyceraldehyde, fatty acids, ketone, arachnoids, and different types of ester compounds. Previous research studies have reported potential pharmacological activities of these compounds, including antidiabetic, antirheumatic, anthelminthic, antipsoriatic, antioxidant, anti-epileptic, antigonorrheal, analgesic, anti-inflammatory, and pesticidal activity.

They are also exercised in the cosmetics industry. GC-MS will be promising in the pharmaceutical sector in identifying a variety of Phytoconstituents in distinct plant extracts, polyherbal extracts, and the standardization of particular plant materials. The benefits of identifying Phytoconstituents are not just limited to the Pharma arena but also to health sectors involved in manufacturing dietary supplements to support overall health and other products and the cosmetic industry. Academically, this analysis unlocks more opportunities to study plants for their diverse contents and improved awareness and knowledge of the traditional application. Furthermore, it helps in a scientific backup of indigenous use and creates a chance to use modern data with traditional formulations and produce unique, safe, and effective medicines. Yet, more research is necessary to prove the efficacy of the bioactive compound; this will be the objective of our future studies.

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