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GC-MS analysis of methanol extract of leaves of *Mallotus repandus* and *Helicterus isora*

Reshmi Akter[1], Anamul Hasan Saikat[1], Md. Shahed-Al-Mahmud[2], Anawara Begum[3*]

[1]Department of Pharmacy, Stamford University Bangladesh, 51, Siddeswari Road, Dhaka-1217, Bangladesh

[2]Genomics Research Center, Academia Sinica, Taipei 115, Taiwan.

[3]Department of Pharmacy, Dhaka International University, Satarkul, Badda, Dhaka -1212.

[*]**ORCID iD: https://orcid.org/0009-0004-0756-2986**

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Abstract: **Background: *Mallotus repandus* belonging to the euphorbiaceae family is an important medicinal plant which is widely distributed in tropical and subtropical regions of Asia. Traditionally, It has been reported to show antioxidant, antiviral, antimicrobial, cytotoxicity, anti-inflammatory, anti-ulcerogenic, hepatoprotective and hypoglycemic activities in various country of the world.**

Helicterus isora **belonging to the sterculiaceae family is a small tree, found in Australia, Saudi Arabia, India, South China, and other parts of Asia. Demulcent, astringent, hepatoprotective, antidiabetic, expectorant, antioxidant, antibacterial etc activities have been reported to show by various parts of this plant.**

The aim of this study was to detect some of the phytochemicals of both plants which could justify their use in various ailments and thus could be potential source of novel drug.

Methods: GC MS analysis of methanol extract of leaves of *Mallotus repandus* and *Helicterus isora* was performed to detect phytochemicals.

Result: GC MS analysis of MEMR detected twenty four (24) chemical compounds such as : Caryophyllene; n-Hexadecanoic; 9,12,15-Octadecatrienoic acid; methyl ester (Linolenic acid, methyl ester); 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Phytol); Vitamin E (α-Tocopherol); 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (Linoleic acid); 13-Docosenamide etc. On the other hand, 2,4(1H,3H)-Pyrimidinedione, 5-methyl- (Thymine); Xanthine riboside 9-beta-D- Ribofuranosylxanthine (Xanthosine); cis-9-Hexadecenal; 13-Docosenamide, (13Z)- (Erucamide); α-Tocopherol (Vitamin E); Stigmasta-5,22-dien-3-ol, (3beta,22E)- etc have been detected in GC MS analysis of MEHI.

Conclusion: The identification of biologically active compounds may explain the scientific reasons for their use as traditional medicine and can open a path to formulate them as medicine.

Keywords: **GC MS, MEMR, MEHI, Traditional medicine, phytochemicals.**

1. INTRODUCTION

Mallotus repandus is regionally known as Gunti, Jhante, or Bon Natai, belonging to the family Euphorbiaceae is widely distributed and naturally grown at Sundarban, Savar, and Sylhet, in Bangladesh. The genus *Mallotus* consists of around 150 species widely distributed in tropical and subtropical regions in Asia (Cambodia, China, India, Laos, Malaysia, Sri Lanka, Thailand, and Vietnam). A small number of species are found in the North and East of Australia and the Pacific-Ocean Archipelago (the East of Fiji). Earlier researcher have reported diverse natural compounds of *Mallotus* species such as bergenin (1); diterpenic lactones (2) ; δ-lactone triterpenes (3); mallorepine, a cyano-γ-pyridone (4); hydrolyzable tannins (5); polyphenols (6); and benzopyrans (7); Three new triterpenoids 3α-hydroxy-13α-ursan-28,12β-olide 3-benzoate, 3α-hydroxy-28β-methoxy-13α-ursan-28,12β-epoxide 3-benzoate and, 3α-hydroxy-13α-ursan-28-oic acid were isolated from the stem and root bark of *M. repandus* (8).

Antioxidant, antiviral, antimicrobial, cytotoxicity and anti-inflammatory properties are different sorts of therapeutic properties shown by the isolated compounds from the extracts of *Mallotus* genus. *M. repandus* has been traditionally used for the relief of myalgia in Thailand(9). In Taiwan, the leaves of *M. repandus* have been used as anti-inflammatory drugs (10). Additionally, it has used as a folk medicine to stop itching and as a remedy for fever, insecticide, snake-bite, treatment in rheumatic arthritis, hepatitis(11), and liver cirrhosis (12). The anti-inflammatory effect against xylene induced ear edema (13), and CCl4-induced acute hepatitis in rats (14), has reported. Bergenin, a polyphenolic compound in the stem of *M. repandus* has reported as natural antioxidant supplements (15). The aerial part of *M. repandus* has shown a remarkable anti-ulcerogenic activity in stressed mice ulcers(4). The stem of *M. repandus* has processes α-amylase inhibitory activity and in vivo hypoglycemic effect (16).

Helicteres isora Linn (*Sterculiaceae*) called 'Marod Phali', a species of a small tree, found in Australia, Saudi Arabia, India, South China, and other parts of Asia (17). Traditionally, the roots and bark have been used as expectorant, demulcent, astringent and skin diseases such as scabies. Fruits are demulcent, mildly astringent and useful in flatulence. Juice of roots has useful in empyema, stomach problems, and diabetes(18). The fruit (aqueous) extracts of *H. isora* has antibacterial activity against *E. coli*, *Staphylococcus epidermidis*, *Salmonella typhimurium*, *Proteus vulgaris*, *Salmonella typhi*, *Staphylococcus aureus*, *Enterobacter aerogenes*, and *Pseudomonas aeruginosa* (19). Some researches have demonstrated that *H. isora* possesses anti-tumor (20), anti-diabetic (21), hepatoprotective (22) and antioxidant (23) activities.

An earlier study has shown the presence of cucurbitacin B and isocucurbitacin B in *H.isora roots* (20). Fruits of *H. isora* comprises flavonoid glucuronides, isoscutellarein-4´-methylether-8-O-β-D-glucuronide-2´, isoscutellarein-4´-methylether-8-O-β-D-glucuronide-6´´-n-butylester, 4´´-disulfate and isoscutellarein-8-O-β-D-glucuronide-2´, 4´´-disulfate (24); rosmarinic acid and isorinic acid derivatives (25); helisterculin, helisorin, 4´-methyl-3´,5,7,8-tetrahydroxyflavone(26); tannins, cardiac glycosides, sterols, triterpenes, a and β–amyrins, lupeol, friedelin, taraxerone, β-sitosterol and volatile oil were reported(27). From the aerial parts, chloroplast pigments, phytosterol, saponins, sugars, phlobotannins, lignins; β-sitosterol, tertratricontanes; triterpenoid and its acetates; flavonoids; trifolin, hibifolin; 4´,7-dimethoxy-5,8-dihydroxy flavone were reported(28).

To the best of our knowledge, though, various phytochemical investigations have been done on both plants, no GC MS analysis of methanol extract of leaves has been found to detect phytochemicals. Thus present study was undertaken to check out the phytochemicals in leaves of this two plants to justify their traditional use in multiple ailments.

2. MATERIALS AND METHODS

Collection and extraction of leaves of *mallotus repandus*:

Leaves of *Mallotus repandus* was procured from Dhaka (Gazipur) Bangladesh during February, 2023. Responsible scientific officer in National Harberium of Bangladesh accomplished identification with a DACB no. 47532 and a specimen has been kept in the department of Pharmacy of Dhaka International University for future reference.

The stem and other adulterants were removed from the leaves of *Mallotus repandus*. The fresh leaves were separated, rinsed and allowed for air drying at ambient temperature (25±2˚C) until the leaves became dry for grinding. The dried leaves were grounded to the coarse powder by dint of blender equipment and before grinding of the sample, the grinder was completely cleaned to restrict contamination with any other materials grounded beforehand. Maceration of 450 g fresh powdered leaves in 3 liters methanol was performed for seven (07) days under occasional stirring. Seven days later, filtration of the mixture followed by concentrating using rotary evaporator (<40˚C) yielded 30.8 g (yield 10.2 %) of semisolid methanol extract of leaves of *Mallotus repandus* **(MEMR).** The fresh MEMR was subjected to the GC-MS analysis

Collection and extraction of leaves of *Helicterus Isora*:

The *Helicterus isora* leaves were collected from the Jahangir Nagar University, Savar, Dhaka, Bangladesh. The plant leaves were collected at the day time at march in 2023. *H. isora* leaves were identified by Bangladesh National Herbarium institute, Mirpur, Dhaka. The accession number of *H. isora is* DACB 48987 and the voucher specimen is deposited at the Pharmacy Department, Dhaka International University.

H. isora leaves were washed with distilled water to avoid the contamination of the fresh sample. The collected leaves sample were dried under shade at room temperature for ten days. The dried samples were grounded to a coarse powder with Blender Machine (NOWAKE, JAPAN). The powdered samples were stored in an autoclaved glass container. Before grinding the sample, the grinder was thoroughly cleaned to avoid contamination with any other substance that had been grounded

previously. The obtained dried coarse powder was again grounded to fine powder by commercial grinder (Hammer mill) and about 400g powdered materials were macerated with 2.0 liters methanol in a round bottom flask for 14 days. The flask was shaken twice daily in a vigorous manner. The whole mixtures were filtered by a piece of sterilized white cotton material followed by Whatman filter paper (No.1). The solvent from the filtrate was evaporated by air until a semisolid mass is obtained and finally 25gm greenish black crude extract was obtained. This fresh methanol extract of leaves of *Helicterus isora* **(MEHI)** was subjected to the GC-MS analysis.

GC-MS (Gas Chromatography-mass Spectroscopy) analysis of MEMR and MEHI

GC-MS (Gas chromatography- mass spectroscopy) analysis of fresh MEMR and MEHI was done separately. The GC-MS analysis was performed using Agilent Technologies 7890A capillary gas chromatograph, directly coupled to a mass spectrometer system (Model: 5975C inert XL EI/CI MSD with triple axis detector). A fused silica capillary column of 5% phenyl, 95% dimethyl-poly-silloxane (HP-5MSI; length: 90 m, diameter: 0.250 mm and film: 0.25 µm) was used. The GC parameter was set as follows: the inlet temperature was set at 250ºC and oven temperature was programmed as 90ºC for 0 min, then 3ºC/min to 200ºC for 2 min and then 15ºC/min to 280ºC for 2 min. Total run time was 46 min and column flow rate was 1.1 mL/min Helium gas. The auxiliary (GC to MS interface) temperature was set to 280ºC. The MS parameter was set as the MS was in scan mode. The ionization mode was EI (electron ionization) type. The mass range was set in the range of 50– 550 *m/z*. MS quad temperature and source temperature was set at 150ºC and 230ºC respectively.

Identification of compounds: Each component was searched and identified by using National Institute of Standards and Technology ("NIST-MS Library 2009"). Peak area of the total ionic chromatogram (TIC) was used to determine the relative percentage amounts of separated compounds and calculation were done automatically.

3. RESULT

Methanol extract of leaves of *Mellotus repandus*: The GC MS of MEMR has revealed almost twenty four (24) compounds which have been reported to show specific biological activity/activities **(Table 1 and Figure 1)**. For instance : Caryophyllene (Antibacterial, antioxidant, gastroprotective, anxiolytic, anti-inflammatory, antiaging and neuroprotective (29)); α-Humulene (Anti-inflammatory and antimicrobial (30)); Hexadecane (antibacterial, antioxidant

(31)); Neophytadiene (analgesic, antipyretic, anti-inflammatory, antimicrobial, and antioxidant (32)); 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Phytol) (Anti-inflammatory, antithrombotic, antimicrobial, and antitumor effects (33)); β-Tocopherol (Antioxidant activity (34)); Vitamin E (α-Tocopherol) (Antioxidant (35)).

Methanol extract of leaves of *Helicterus isora*: Total thirty two (32) compounds have been detected in GC MS analysis of MEHI **(Table 2 and Figure 2)** . 2,4(1H,3H)-Pyrimidinedione, 5-methyl- (Thymine) (Antibacterial (36)); 4-Methylquinazoline (Lepidine) Anticancer (37)); Hexanoic acid (Antifungal(38)); 1-Deoxy-d-mannitol (Antipyretic and antiparasitic (39)); 4-Hydroxy-4-methyl tetrahydro-2H-pyran-2-one (Mevalonolactone) (Antiaging effects (40)); Hexadecanoic acid, methyl ester (Hepatoprotective (41)); Hexadecanoic acid (Palmitic acid) (Antioxidant, hypercholesterolemic, nematicide, pesticide (42), Antibacterial (43), Algicidal activity (44)); 8-Heptadecenoic acid (Anticancer (45)); 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (Methyl linoleate) (Antifungal (46)); 9-Octadecenoic acid, methyl ester, (E)- (Methyl elaidate) (Anticancer (47)); cis-9-Hexadecenal (Antifungal, Anticancer (48)); Stearic acid (Antibacterial(43)); 1-Docosene (Antibacterial (49)); cis-9-Hexadecenal (Antimelanogenic (50)); Squalene (anticancer, antioxidant, drug carrier, detoxifier, skin hydrating, and emollient (51)); 1-Nonadecene (Antituberculosis, anticancer, antioxidant, antimicrobial (52)); Octatriacontyl pentafluoropropionate (Anti covid 19 (53)); ((14β)-Pregnane immunosuppressant (54), cytotoxic (55), antidepressants (56), anti-inflammatory (57), anti-oxidants (58), antibacterial agents (59), antifungal agents (60), anti-proliferative activity (61), anti-tumor agents (62), anti-tobacco mosaic virus (63)); α-Tocopherol (Vitamin E) antioxidant (64)); Ergost-5-en-3-ol, (3beta,24R)- (Campestral) (65).

4. DISCUSSION

According to our review, no such study like GC MS analysis of methanol extract of leaves of *Mallotus repandus* and *Helicterus isora* have been undertaken yet. Though it is a preliminary study but based on this results further elaborative research can be done to isolate phytochemicals mentioned **(Table 1, Figure 1 and Table 2, figure 2)**. In addition, synthesis of particular phytoconstituents and valuation of corresponding biological response can open the pathway to produce new API (active pharmaceutical ingredient) and formulate new medicine by the API company and the pharmaceutical company respectively.

5. CONCLUSION

GC MS analysis of MEMR detected various types of phytochemicals which have been recorded to demonstrate biological activities like anti-inflammatory, antimicrobial, antioxidant, anticancer, gastroprotective, antitumor, anxiolytic, neuroprotective, antithrombotic and antiaging. MEHI also checked out multiple phytoconstituents those have been reported to show anti-inflammatory, antitumor, anticancer, antioxidant, antibacterial, antimelanogenic, antituberculosis,, antibacterial, antitumor, antiproliferative, antiangiogenic etc activities.Thus, further research can be done to isolate the respective phytoconstituents from this two plants with an intention to produce new drugs.

List of abbreviations:

MEMR = Methanol extract of leaves of *Mallotus repandus*

MEHI = Methanol extract of leaves of *Helicterus isora*

GC MS = Gas Chromatography-Mass Spectroscopy

RT = Retention time

PA = Peak area

SL = Serial

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Consent for publication

All authors hereby consents for publication

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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APPENDICES - A

List of Table:

Table 1: Identification of the compounds of MEMR by GC-MS analysis and their biological activity.

SL no.	RT(min)	% PA	Compound Name	Mol. Formula	Mol. Wt.	Compound nature	Reported Bioactivity
1	10.3509	0.6368	Copaene	$C_{15}H_{24}$	204	Sesquiterpenoids	Not found
2	10.9632	8.4721	Caryophyllene	$C_{15}H_{24}$	204	Sesquiterpenoids	Antibacterial, antioxidant, gastroprotective, anxiolytic, anti-inflammatory, antiaging and neuroprotective. (29)
3	11.4267	0.837	α-Humulene	$C_{15}H_{24}$	204	Sesquiterpenoids	Anti-inflammatory and antimicrobial (30)
4	12.1419	0.6955	Delta-Cadinene	$C_{15}H_{24}$	204	Sesquiterpenoids	Not found
5	13.0116	0.7421	Hexadecane	$C_{16}H_{34}$	226	Diterpenoids	antibacterial, antioxidant (31)
6	13.7612	1.1579	Mome Inositol	$C_7H_{14}O_6$	194	Carbohydrate	Not found
7	15.9985	3.8253	Neophytadiene	$C_{20}H_{38}$	278	Sesquiterpenoids	analgesic, antipyretic, anti-inflammatory, antimicrobial, and antioxidant (32)
8	16.6394	1.2376					
9	17.2631	2.0963	n-Hexadecanoic (Palmitic acid)	$C_{16}H_{32}O_2$	256	Saturated fatty acid	Not found
10	17.6865	2.0886					

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11	19.2601	1.2854	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294	Fatty acid methyl ester	Not found
12	19.323	5.7483	9,12,15-Octadecatrienoic acid, methyl ester (Linolenic acid, methyl ester)	$C_{19}H_{32}O_2$	292	Fatty acid methyl ester	Not found
13	19.426	18.5026	3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Phytol)	$C_{20}H_{40}O$	296	Diterpene alcohol	Anti-inflammatory, antithrombotic, antimicrobial, and antitumor effects (33)
14	19.5919	1.126	Octadecanoic acid (Stearic acid)	$C_{18}H_{36}O_2$	284	Unsaturated fatty acid	Not found
15	19.6377	0.7929	9,12-Octadecadienoic acid (Z,Z)	$C_{18}H_{32}O_2$	280	Unsaturated fatty acid	Not found
16	19.6949	7.3428	9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (Linoleic acid)	$C_{18}H_{30}O_2$	278	Unsaturated fatty acid	Not found
17	22.0295	0.7735	unidentified				
18	22.842	0.806	15-Hydroxypentadecanoic acid	$C_{15}H_{30}O_3$	258	Saturated fatty acid	Not found
19	24.6387	14.3765	13-Docosenamide	$C_{22}H_{43}NO$	337	Unsaturated fatty acid	Not found
20	24.8561	0.7079	unidentified				
21	26.6013	1.7702	β-Tocopherol	$C_{28}H_{48}O_2$	416		Antioxidant activity (34)
22	27.5397	17.5708	Vitamin E (α-Tocopherol)	$C_{29}H_{50}O_2$	430		Antioxidant (35)
23	28.7814	2.2001	Ergost-5-en-3-ol (3β)	$C_{28}H_{48}$	400	Steroidal	Not found
24	29.1018	4.4676	Stigmasta-5,22-dien-3-ol	$C_{29}H_{48}$	412	Steroidal	Not found

Table 2: Identification of the compounds of *Helicterus isora* by GC-MS analysis and their biological activity.

SL no.	RT (min)	% PA	Compound Name	Mol. Formula	Mol. Wt.	Reported Bioactivity
1	6.0253	1.5967	2,4(1H,3H)-Pyrimidinedione, 5-methyl- (Thymine)	$C_5H_6N_2O_2$	126	Antibacterial(36)
2	7.0495	0.4789	4-Methylquinazoline (Lepidine)	$C_9H_8N_2$	144	Anticancer (37)
3	7.6046	0.2666	Ethanol, 2-(2-butoxyethoxy)-	$C_8H_{18}O_3$	162	Not found
4	8.1653	4.9128	5-Hydroxymethyl-2-furancarboxaldehyde	$C_6H_6O_3$	126	Not found
5	9.3154	0.205	5-Hydroxy-2-(hydroxymethyl)-2,3-dihydro-4H-pyran-4-one	$C_6H_8O_4$	144	Not found
6	11.0549	0.4409	3,5-Dimethylanisole	$C_9H_{12}O$	136	Not found
7	11.7759	5.3449	Xanthine riboside 9-beta-D-Ribofuranosylxanthine (Xanthosine)	$C_{10}H_{12}N_4O_6$	284	Not found
8	12.0792	1.2256	Hexanoic acid	$C_6H_{12}O_2$	116	Antifungal (38)

9	13.7042	2.431	1-Deoxy-d-mannitol	$C_6H_{14}O_5$	166	Antipyretic and antiparasitic(39)
10	13.8472	2.5481	D-Mannonic acid	$C_6H_{12}O$	196	Not found
11	14.1162	1.7156	4-Hydroxy-4-methyl tetrahydro-2H-pyran-2-one (Mevalonolactone)	$C_6H_{10}O_3$	130	Antiaging effects(40)
12	14.6083	0.4582	Methyl 4-hydroxybutanoate	$C_5H_{10}O$	118	Not found
13	17.2632	0.4964	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	Hepatoprotective(41)
14	17.6981	2.7822	Hexadecanoic acid (Palmitic acid)	$C_{16}H_{32}O_2$	256	Antioxidant, hypercholesterolemic,nematicide, pesticide (42), antibacterial (43), algicidal activity (44)
15	18.5049	0.3252	8-Heptadecenoic acid	$C_{17}H_{32}O_2$	268	Anticancer (45)
16	19.2545	0.9489	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (Methyl linoleate)	$C_{19}H_{34}O_2$	294	Antifungal (46)
17	19.3231	1.2025	9-Octadecenoic acid, methyl ester, (E)- (Methyl elaidate)	$C_{19}H_{36}O_2$	296	Anticancer (47)
18	19.6436	3.804	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	Not found
19	19.7123	4.1841	cis-9-Hexadecenal	$C_{18}H_{34}O_2$	282	Antifungal, anticancer (48)
20	19.924	1.6841	Stearic acid	$C_{18}H_{36}O_2$	284	Antibacterial (45)
21	22.8364	1.3365	Hexadecanoic acid, 2,3-dihydroxypropyl ester	$C_{19}H_{38}O_4$	330	Not found
22	23.6146	1.0435	1-Docosene	$C_{22}H_{44}$	308	Antibacterial (49)
23	24.0896	2.2553	cis-9-Hexadecenal	$C_{16}H_{30}$	238	Antimelanogenic(50)
24	24.2898	1.3814	(3E)-3-Icosene	$C_{20}H_{40}$	280	Not found
25	24.6389	4.6031	13-Docosenamide, (13Z)- (Erucamide)	$C_{22}H_{43}NO$	337	Not found
26	24.8563	1.109	Squalene	$C_{30}H_{50}$	410	Anicancer, antioxidant, drug carrier, detoxifier, skin hydrating, and emollient (51)
27	25.3083	1.618	1-Nonadecene	$C_{19}H_{38}$	266	Antituberculosis, anticancer, antioxidant, antimicrobial(52)
28	25.4628	2.8622	Octatriacontyl pentafluoropropionate	$C_{41}H_{77}F_5O_2$	697	Anti covid 19 (53)
29	26.241	0.9498	(14β)-Pregnane	$C_{21}H_{36}$	288	Immunosuppressant (54) ,cytotoxic (55), antidepressants (56), anti-inflammatory (57), anti-oxidants(58), antibacterial agents(59) , antifungal agents (60) anti-proliferative activity (61), anti-tumor agents (62) and anti-tobacco mosaic virus agents (63)
30	27.5342	1.2777	α-Tocopherol (Vitamin E)	$C_{29}H_{50}O_2$	430	Antioxidant (64)
31	28.7701	0.8113	Ergost-5-en-3-ol, (3beta,24R)- (Campestral)	$C_{28}H_{48}O$	400	Cholesterol lowering and cancer prevention, Anti angiogenic (65)
32	29.102	1.3104	Stigmasta-5,22-dien-3-ol, (3beta,22E)-	$C_{29}H_{48}O$	412	Not found

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List of Figure:

Figure 1: Total ionic chromatogram (TIC) of MEMR obtained by GC-MS with the energy of ionization of 70 eV.

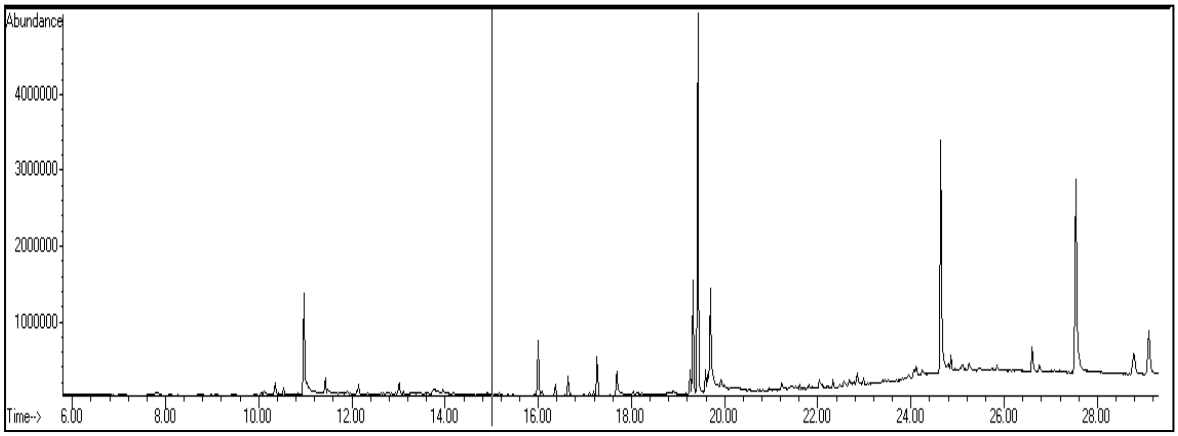

Figure 2: Total ionic chromatogram (TIC) of MEHI obtained by GC-MS with the energy of ionization of 70 eV.