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Comparative GC-MS Analysis of Methanol, Ethyl Acetate, and n-Hexane Extracts of *Melia azedarach* L. Fruit

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Abstract

Melia azedarach L. is a medicinal plant traditionally used in various communities and is known to contain chemically diverse phytoconstituents. This study aimed to characterize and compare the phytochemical profiles of methanol, ethyl acetate, and *n*-hexane extracts of *M. azedarach* fruit using gas chromatography–mass spectrometry (GC-MS). The three extracts showed distinct chemical profiles that reflected differences in solvent polarity. The methanolic extract produced 14 chromatographic peaks corresponding to 42 possible compounds and was dominated by cis-vaccenic acid (55.35%), 17-octadecynoic acid (19.11%), and *n*-hexadecanoic acid (8.71%). The ethyl acetate extract contained 15 peaks corresponding to 45 possible compounds, with cis-vaccenic acid (30.23%), heptadecane, 2,6,10,15-tetramethyl- (16.38%), and β-sitosterol-related compounds (9.26%) as the major constituents. The *n*-hexane extract showed the greatest number of chromatographic peaks, with 28 peaks corresponding to 84 possible compounds, and was dominated by hexadecanoic acid methyl ester (29.67%), 9-octadecenoic acid methyl ester (19.42%), farnesyl bromide (12.49%), and cis-vaccenic acid (7.55%). Overall, the extracts were characterized predominantly by fatty acids, fatty acid esters, and lower-abundance terpenoid-, triterpenoid-, and sterol-related constituents. The differences among the three extracts demonstrate the influence of solvent polarity on the phytochemical composition of *M. azedarach* fruit. Because compound assignments were based primarily on GC-MS library matching, the identified constituents should be considered tentative until confirmed using authentic standards or complementary structural techniques. These findings provide a comparative chemical profile of *M. azedarach* fruit and a basis for further compound confirmation and targeted biological evaluation.

1. Introduction

Indonesia is recognized as one of the world's megadiverse countries and possesses a rich diversity of plant species with potential medicinal value [1–3]. Traditional use of plant-derived materials remains important in many Indonesian communities, particularly in regions where ethnobotanical knowledge has been transmitted across generations. In Aceh, for example, numerous herbs and spices continue to be used as

traditional remedies for a variety of health conditions, demonstrating the continuing importance of local plant resources in community-based healthcare [4].

Melia azedarach L., a member of the Meliaceae family, is a medicinal plant distributed across tropical and subtropical regions. In Aceh, Indonesia, the plant is locally known as *boh putek uteun*. Different parts of *M. azedarach*, including its leaves, twigs, bark, and fruits, have attracted scientific interest because they contain structurally diverse secondary metabolites. Previous phytochemical investigations have reported phenolic compounds, flavonoids, sterols, limonoids, and triterpenoids in different plant parts. M'rabet et al. [5], for example, identified multiple phenolic acids and flavonoids in *M. azedarach* leaves and fruits, with the chemical composition varying according to plant organ and maturity stage. More specifically, Wang et al. [6] isolated triterpenoids, an aromatic ester, and lignans from *M. azedarach* fruits, demonstrating the chemical diversity of this plant part. More recently, Cao et al. [7] isolated tirucallane-type triterpenoid and limonoid constituents from the fruits and demonstrated anti-inflammatory activity for selected isolated compounds. These findings indicate that *M. azedarach* fruit is a chemically complex natural material that warrants further phytochemical characterization.

The pharmacological relevance of *M. azedarach* has also been investigated in several experimental models. Jeba Malar et al. [8] reported the presence of steroids, alkaloids, phenols, flavonoids, saponins, tannins, anthraquinones, and amino acids in extracts of *M. azedarach* and demonstrated biological activities in in vitro experiments. In studies related to glucose regulation, Khan et al. [9] reported that treatment with an ethanol extract of *M. azedarach* significantly reduced blood glucose levels by 14.8% in streptozotocin-induced diabetic rats. Similarly, Seifu et al. [10] demonstrated that an aqueous leaf extract of *M. azedarach* reduced plasma glucose and insulin concentrations in rodent models of type 2 diabetes and influenced gastric emptying. Although these findings suggest potential metabolic relevance, they are predominantly derived from preclinical models and should not be interpreted as evidence of clinical efficacy.

The investigation of plant-derived compounds with possible metabolic relevance is particularly important given the increasing global burden of diabetes. Diabetes mellitus comprises a heterogeneous group of metabolic disorders characterized by hyperglycemia resulting from disturbances in insulin secretion, insulin action, or both [11, 12]. Type 2 diabetes is characterized by a progressive loss of adequate β-cell insulin secretion, commonly occurring in the context of insulin resistance [13]. According to the Global Burden of Disease Study 2021, approximately 529 million people were living with diabetes worldwide in 2021, and this number is projected to exceed 1.31 billion by 2050 [14]. These trends support continued investigation of natural products as potential sources of compounds for subsequent pharmacological research, while recognizing that chemical identification alone does not demonstrate therapeutic activity.

Previous work by Aini et al. [15] specifically investigated the methanolic extract of *M. azedarach* fruit and demonstrated the presence of terpenoids, steroids, flavonoids, and phenolic constituents. GC-MS profiling of that methanolic fraction identified multiple fatty acid-, terpenoid-, and steroid-related compounds. At the same time, complementary antioxidant and in silico analyses suggested that selected constituents warranted further investigation for their potential biological relevance. However, that study focused primarily on the methanolic extract. Because solvent polarity strongly influences the extraction of plant metabolites, characterization using solvents with different polarities is necessary to obtain a broader understanding of the chemical composition of *M. azedarach* fruit.

Despite previous reports describing individual phytochemical constituents of *M. azedarach* fruit, direct comparison of methanol, ethyl acetate, and *n*-hexane extracts under the same GC-MS analytical framework remains limited. Such a comparative approach can provide information on how solvent polarity affects the number, relative abundance, and chemical classes of detected constituents. This is particularly relevant for distinguishing relatively polar constituents from lipophilic fatty acids, fatty acid esters, terpenoid-related compounds, triterpenoids, and sterol-related compounds.

Therefore, the present study aimed to characterize and compare the phytoconstituent profiles of methanol, ethyl acetate, and *n*-hexane extracts of *M. azedarach* L. fruit using gas chromatography–mass spectrometry (GC-MS). Particular attention was given to differences in the major compounds and chemical classes detected among the three solvent fractions. The identified constituents were further discussed in relation to previously reported biological properties of their respective chemical classes. However, because the present study is primarily a chemical profiling investigation, the detected compounds are considered tentative GC-MS identifications, and their pharmacological relevance requires confirmation through compound isolation, structural characterization, and targeted biological assays.

2. Materials and Methods

2.1. Plant Material Collection and Identification

The fruits of *M. azedarach* L. were collected from Gampong Teu Dayah, Kuta Malaka District, Aceh Besar Regency, Aceh Province, Indonesia. The sampling location was geographically located in the Aceh Besar region, characterized by tropical climatic conditions with high biodiversity. Mature fruits were selected based on morphological characteristics, including yellowish mature fruit color, intact surface structure, and absence of fungal contamination or physical damage.

The collected plant materials were transported to the laboratory in clean polyethylene bags and washed thoroughly with running distilled water to remove soil particles and surface contaminants. The fruits were subsequently air-dried at room temperature (25–30°C) in a shaded and well-ventilated area to prevent degradation of thermolabile phytochemicals caused by direct sunlight exposure.

The dried fruits were ground into a fine powder using an electric grinder and stored in airtight containers until extraction. Plant identification was confirmed based on morphological characteristics using standard botanical references.

2.2. Chemicals and Reagents

Analytical-grade solvents including methanol, ethyl acetate, and n-hexane were used as extraction solvents based on their different polarity characteristics. All solvents were purchased from certified chemical suppliers. Distilled water was used throughout the sample preparation process.

2.3. Extraction Procedure

Dried *M. azedarach* fruit powder was extracted separately using methanol, ethyl acetate, and *n*-hexane by maceration. The powdered samples were soaked in each solvent and maintained for a sufficient period with occasional stirring to facilitate the extraction process.

After extraction, the mixtures were filtered using Whatman No. 1 filter paper. The filtrates were concentrated under reduced pressure using a rotary evaporator at a controlled temperature (≤40°C) until dry crude extracts were obtained. The extracts were then stored in amber glass bottles at 4 °C until GC-MS analysis.

2.4. GC-MS Instrumentation and Analytical Conditions

The phytochemical composition of M. azedarach fruit extracts was analyzed using GC-MS. The instrument used was a GC-MS Gas Chromatography system equipped with an Auto Sampler 5975A coupled with an Agilent Technologies 7890A GC system.

The GC-MS analysis was performed using an AS 2000 autosampler. A sample volume of 1 µL was injected using the hot-needle injection technique with a split ratio of 25:1. Separation of compounds was achieved using a SPB-50 capillary column (Supelco, Bellfonte, CA, USA) with a length of 30 m, internal diameter of 0.25 mm, and film thickness of 0.25 µm.

Helium was used as the carrier gas with a constant flow rate of 1 mL/min. The injector temperature was maintained at 230°C, while the interface temperature was set at 250 °C and the ion source temperature was maintained at 200°C.

The oven temperature program was initiated with an isothermal temperature of 70°C for 5 min, followed by an increasing temperature gradient of 5°C/min until reaching 310°C. The final temperature was maintained at 310°C for 1 min. Before the next injection, the column was equilibrated at 70°C for 6 min.

Mass spectra were recorded at a scan rate of two scans per second within the mass range of m/z 50–600. The chromatographic peaks and mass spectra obtained were processed using MASSLAB software (Thermo Quest, Manchester, UK). Compound identification was carried out by comparing mass spectra fragmentation patterns and retention indices with the National Institute of Standards and Technology (NIST) database and PubChem database.

2.5. Data Processing and Compound Identification

The detected compounds were identified based on their retention time, molecular fragmentation pattern, and similarity index (SI). The peak area percentage was calculated automatically from the chromatogram and used to estimate the relative abundance of each compound.

The identified phytoconstituents were classified according to their chemical groups, including fatty acids, fatty acid esters, terpenoids, triterpenoids, steroids, and other secondary metabolites. The compounds with the highest peak area percentages were considered the major constituents of each extract.

3. Results and Discussion

3.1. Methanolic Extract

The chemical constituents of the methanolic extract of *M. azedarach* L. fruit were analyzed using GC-MS. As presented in Table 1 and Figure 1, the analysis generated 14 chromatographic peaks representing 42 possible

Table 1. GC-MS results of phytoconstituents of methanolic extract of *M. azedarach* L. fruit.

Peak No.	Retention Time (min)	Hit 1	SI	Secondary Metabolite Group	Area (%)
1	15.38	*2(5H)-Furanone, 3,5,5-trimethyl-*	695	Lactone (Furanone), Terpenoid (oxygenated volatile compound)	0.76
2	35.75	*n-Hexadecanoic acid*	830	Palmitic acid, Lipid	8.71
3	38.22	*Methyl 10-trans,12-cis-octadecadienoate*	828	Polyunsaturated fatty acid ester (Lipid / PUFA)	0.47
4	38.31	*6-Octadecenoic acid, methyl ester, (Z)-*	833	Unsaturated fatty acid ester (Lipid)	1.44
5	39.03	*17-Octadecynoic acid*	842	Unsaturated fatty acid (Lipid)	19.11
6	39.14	*cis-Vaccenic acid*	923	Unsaturated fatty acid (Lipid / MUFA)	55.35
7	39.46	*Octadecanoic acid*	783	Saturated fatty acid (Lipid)	4.61
8	40.91	*12-Methyl-E,E-2,13-octadecadien-1-ol*	779	Unsaturated aliphatic alcohol (lipid derivative / volatile compound)	0.49
9	43.82	*9,17-Octadecadienal, (Z)*	796	Unsaturated aliphatic aldehyde (lipid derivative)	1.33
10	47.45	*n-Propyl 11-octadecenoate*	765	Unsaturated fatty acid ester (Lipid)	1.26
11	52.5	*Farnesyl bromide*	700	Terpenoid (Sesquiterpene)	4.72
12	52.8	*Cucurbitacin B, 25-desacetoxy-*	622	Triterpenoid (Cucurbitacin)	0.53
13	53.46	*13,14-Epoxyoleanan-3-ol, acetate*	568	Triterpenoid (terpene derivative)	0.69
14	53.65	*Ergosta-5,22-dien-3-ol, acetate, (3β,22E)-*	595	Steroid / Phytosterol (steroidal lipid)	0.54

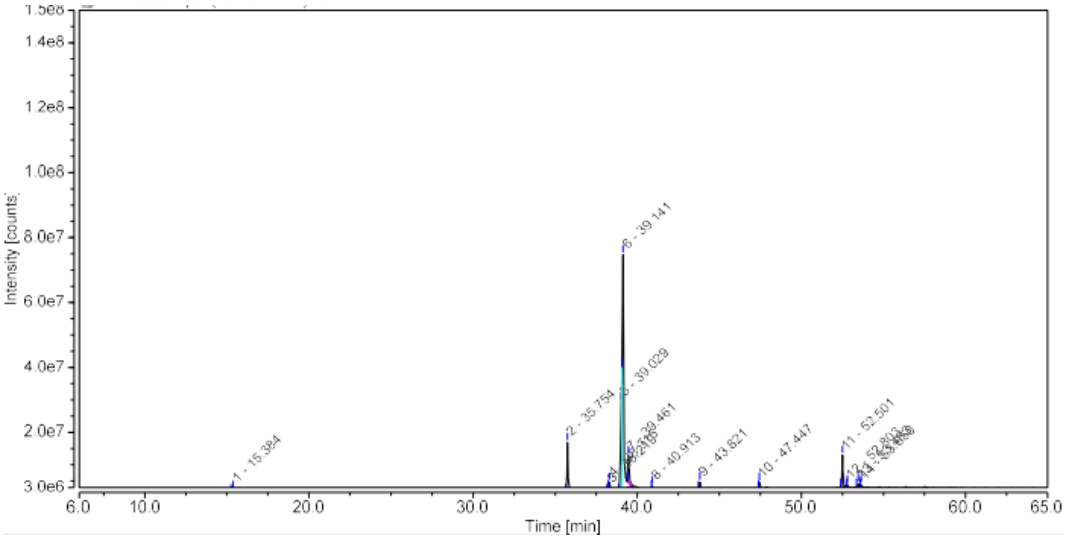

Figure 1. GC-MS chromatogram results results of methanol extract of *M. azedarach* L fruit.

compounds with different similarity indices. The chemical profile was dominated by lipid-related constituents, particularly saturated and unsaturated fatty acids and their derivatives.

The major tentatively identified compounds were cis-vaccenic acid (55.35%), 17-octadecynoic acid (19.11%), and *n*-hexadecanoic acid (8.71%). Together, cis-vaccenic acid and 17-octadecynoic acid accounted for more than 70% of the total chromatographic area, indicating that unsaturated fatty acids constitute the predominant chemical fraction of the methanolic extract.

Several lower-abundance constituents were also detected. Farnesyl bromide was tentatively identified at a retention time of 52.50 min with a relative area of 4.72%. In contrast, a cucurbitacin-related compound, identified by the GC-MS library as cucurbitacin B, 25-desacetoxy-, was detected at 52.80 min with an area of 0.53%. Other minor compounds included 13,14-epoxyoleanan-3-ol acetate and ergosta-5,22-dien-3-ol acetate, suggesting the presence of triterpenoid- and sterol-related constituents. Because these identifications were based on mass spectral library matching, they should be considered tentative until confirmed using authentic standards or complementary spectroscopic techniques.

Cis-vaccenic acid was the most abundant compound detected in the methanolic extract. Fatty acids are involved in several physiological processes, including membrane structure, intracellular signaling, gene expression, and energy metabolism, although their

biological effects differ according to individual molecular structure [16]. Therefore, the high abundance of cis-vaccenic acid observed in the present study should primarily be interpreted as an important compositional characteristic of the extract rather than direct evidence of a particular pharmacological effect.

The detection of 17-octadecynoic acid further demonstrates the diversity of unsaturated fatty acids present in the extract. The biological properties of free fatty acids are influenced by factors such as carbon-chain length, degree of unsaturation, and the presence of specific functional groups. Previous studies have demonstrated that several free fatty acids exhibit antimicrobial activity through interactions with microbial cell membranes and other cellular targets [17, 18]. However, identification by GC-MS alone is insufficient to establish the biological contribution of 17-octadecynoic acid in the present extract.

Saturated fatty acids, including *n*-hexadecanoic acid and octadecanoic acid, were also detected. These compounds contribute to the overall lipid composition of the methanolic fraction. Fatty acids and their derivatives have been widely investigated for antimicrobial activity, although their effects vary substantially depending on molecular structure and experimental conditions [17, 18].

Several fatty acid esters, including methyl 10-trans,12-cis-octadecadienoate, 6-octadecenoic acid methyl ester, and *n*-propyl 11-octadecenoate, were detected at relatively low abundance. Fatty acid esters are commonly reported during GC-MS profiling of medicinal plant extracts and contribute to the overall chemical diversity of plant-derived fractions [19]. Their actual contribution to biological activity would require further experimental investigation.

In addition to lipid-derived constituents, the methanolic extract contained several minor terpenoid-, triterpenoid, and sterol-related compounds. The cucurbitacin-related compound tentatively identified as cucurbitacin B, 25-desacetoxy-, belongs to a chemical class that has been extensively investigated for anticancer and anti-inflammatory properties [20]. Nevertheless, biological properties reported for authentic cucurbitacin B should not automatically be attributed to the derivative tentatively detected in the present study.

Sterol-related compounds were also observed in the methanolic fraction. Phytosterols are naturally occurring plant sterols structurally related to cholesterol and have important applications in food, nutraceutical, and pharmaceutical research [21]. Their presence further demonstrates the chemical diversity of the *M. azedarach* fruit extract.

Overall, the methanolic extract was predominantly characterized by unsaturated fatty acids, particularly cis-vaccenic acid and 17-octadecynoic acid, together with smaller proportions of saturated fatty acids, fatty acid esters, and terpenoid-, triterpenoid-, and sterol-related constituents. These findings provide a chemical basis for further investigation, although confirmation of individual compounds and targeted biological assays are necessary before specific pharmacological activities can be established.

3.2. Ethyl Acetate Extract

The GC-MS profile of the ethyl acetate extract differed from that of the methanolic extract, indicating that solvent polarity influenced the extraction of phytochemical constituents. As summarized in Table 2 and Figure 2, 15 chromatographic peaks corresponding to 45 possible compounds were detected.

The major constituent of the ethyl acetate extract was cis-vaccenic acid, with a relative area of 30.23%. Other prominent peaks included heptadecane, 2,6,10,15-tetramethyl- (16.38%), β-sitosterol-related compounds (9.26%), tetradecane, 2,6,10-trimethyl- (7.61%), and an androstane-related compound (7.31%). These results indicate that the ethyl acetate fraction contained a mixture of fatty acid derivatives, hydrocarbon- or terpenoid-related compounds, and sterol-related constituents.

The high abundance of cis-vaccenic acid confirms that unsaturated fatty acids remain important constituents of the ethyl acetate extract. Awais et al. [22] previously reported that oil obtained from *M. azedarach* contained a high proportion of oleic acid, further indicating that unsaturated fatty acids are major constituents of lipid fractions derived from this species.

The detection of β-sitosterol-related compounds is also noteworthy from a phytochemical perspective. β-Sitosterol belongs to the phytosterol class, which is structurally related to cholesterol and is widely investigated for applications in functional foods, nutraceuticals, and pharmaceutical products [21]. In the present study, however, its detection should be regarded primarily as evidence of the sterol composition of the ethyl acetate fraction.

Previous GC-MS studies also support the chemical diversity of *M. azedarach*. Naveed et al. [23] identified multiple constituents in crude *M. azedarach* extracts, including aromatic and nitrogen-containing compounds.

Table 2. GC-MS results of phytoconstituents of ethyl acetate extract of *M. azedarach* L fruit.

Peak No.	Retention Time (min)	Hit 1	SI	Secondary Metabolite Group	Area (%)
1	35.988	*n-Hexadecanoic acid*	708	Saturated fatty acid (Lipid)	4.01
2	36.556	*9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione*	707	Oxygenated phenolic compound / cyclic ketone (oxygenated secondary metabolite)	5.72
3	39.27	*cis-Vaccenic acid*	834	Monounsaturated fatty acid (Lipid / MUFA)	30.23
4	39.725	*E-11-Hexadecenoic acid, ethyl ester*	796	Unsaturated fatty acid ester (Lipid)	2.05
5	40.144	*Heptadecanoic acid, heptadecyl ester*	773	Saturated fatty acid ester (Lipid)	2.45
6	41.021	*Heptadecanoic acid, heptadecyl ester*	694	Saturated fatty acid ester (Lipid)	1.91
7	43.899	*9-Octadecenoic acid, 2,2,2-trifluoroethyl ester*	743	Unsaturated fatty acid ester (Lipid)	4.21
8	45.946	*Diisooctyl phthalate*	681	Aromatic ester (phthalate ester)	4.51
9	50.439	*Androstane-11,17-dione, 3-[(trimethylsilyl)oxy]-, 17-[O-(phenylmethyl)oxime], (3α,5α)-*	572	Steroid (steroidal lipid)	2.62
10	50.755	*Tetradecane, 2,6,10-trimethyl-*	652	Terpenoid / aliphatic isoprenoid	7.61
11	53.412	*Heptadecane, 2,6,10,15-tetramethyl-*	674	Terpenoid (aliphatic isoprenoid / diterpenoid hydrocarbon)	16.38
12	53.718	*W-18*	579	Aliphatic hydrocarbon (Lipid-related compound)	0.82
13	56.741	*Androstane-11,17-dione, 3-[(trimethylsilyl)oxy]-, 17-[O-(phenylmethyl)oxime], (3α,5α)-*	564	Steroid (steroidal lipid)	7.31
14	57.911	*β-Sitosterol*	660	Steroid / Phytosterol (major secondary metabolite)	9.26
15	59.8061	*W-18*	579	Aliphatic hydrocarbon (Lipid-related compound)	0.91

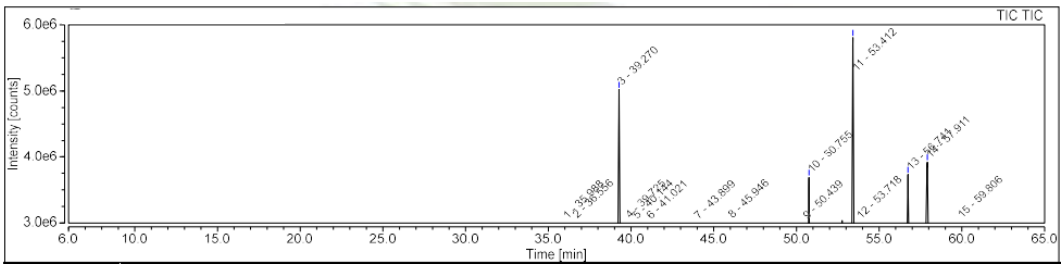

Figure 2. GC-MS chromatogram results of ethyl acetate extract of *M. azedarach* L fruit.

Similarly, Jawad and Al Garaawi [24] reported several fatty acid derivatives, sterol-related compounds, and other phytoconstituents in *M. azedarach* leaf extracts. Although the specific chemical profiles vary among studies, likely because of differences in plant part, geographical origin, extraction solvent, and analytical conditions, these reports support the presence of chemically diverse metabolites in *M. azedarach*.

Overall, the ethyl acetate extract showed a more balanced distribution of fatty acid-, hydrocarbon-, terpenoid-, and phytosterol-related compounds than the methanolic extract. The results indicate that ethyl acetate extracted moderately non-polar constituents that were less prominent in the methanol fraction.

3.3. n-Hexane Extract

The *n*-hexane extract exhibited the greatest number of chromatographic peaks among the three extracts. GC-MS analysis generated 28 peaks corresponding to 84 possible compounds with different similarity indices, as presented in Table 3 and Figure 3.

The major constituent was hexadecanoic acid methyl ester, accounting for 29.67% of the chromatographic area, followed by 9-octadecenoic acid methyl ester (19.42%), farnesyl bromide (12.49%), and cis-vaccenic acid (7.55%). The predominance of fatty acid methyl esters is consistent with the non-polar character of *n*-hexane and its ability to extract lipophilic constituents.

Hexadecanoic acid methyl ester and 9-octadecenoic acid methyl ester together represented almost half of the chromatographic area. Fatty acid methyl esters derived from plant oils are widely investigated as potential biodiesel constituents, and previous research has demonstrated the suitability of *M. azedarach* oil as a feedstock for biodiesel production [22]. In the context of the present study, their detection primarily demonstrates the lipid-rich nature of the *n*-hexane fraction.

Table 3. GC-MS results of phytoconstituents of n-hexane extract of *M. azedarach* L. fruit.

Peak No.	Retention Time (min)	Hit 1	SI	Secondary Metabolite Group	Area (%)
1	10.765	*Cyclohexene, 4-methylene-1-(1-methylethyl)-*	713	Terpenoid (monoterpene hydrocarbon)	0.43
2	19.55	*2-Methylbicyclo[4.3.0]non-1(6)-ene*	784	Terpenoid (sesquiterpene hydrocarbon)	1.85
3	22.499	*cis-β-Farnesene*	814	Terpenoid (sesquiterpene hydrocarbon)	1.45
4	31.155	*Methyl 8-methyl-nonanoate*	752	Lipid (aliphatic fatty acid ester)	0.8
5	33.509	*E-2-Tetradecen-1-ol*	786	Lipid (unsaturated aliphatic alcohol / fatty alcohol)	0.35
6	35.223	*Hexadecanoic acid, methyl ester*	879	Lipid (saturated fatty acid ester)	29.67
7	35.971	*Octadecanoic acid, 2-(2-hydroxyethoxy)ethyl ester*	704	Lipid (fatty acid ester / glycol ester)	0.86
8	36.525	*9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione*	697	Oxygenated phenolic compound / polyketide (cyclic ketone)	0.26
9	38.416	*9,12-Octadecadienoic acid, methyl ester*	888	Lipid (polyunsaturated fatty acid ester)	4.49
10	38.512	*9-Octadecenoic acid (Z)-, methyl ester*	924	Lipid (monounsaturated fatty acid ester)	19.42
11	38.954	*Heptadecanoic acid, 10-methyl-, methyl ester*	797	Lipid (branched-chain fatty acid ester)	2
12	39.222	*cis-Vaccenic acid*	819	Lipid (monounsaturated fatty acid / MUFA)	7.55
13	44.052	*9,17-Octadecadienal, (Z)*	717	Lipid (unsaturated aliphatic aldehyde)	0.4
14	49.984	*Farnesyl bromide*	732	Terpenoid (sesquiterpene / halogenated terpene)	12.49
15	50.296	*β-Acorenol*	652	Terpenoid (sesquiterpene alcohol)	0.76
16	50.443	*Cedran-diol, (8S,14)-*	633	Terpenoid (sesquiterpene diol)	0.49
17	50.715	*9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3β,5Z,7E)-*	575	Steroid (secosteroid / sterol-derived triterpenoid)	0.53
18	51.324	*Cedran-diol, (8S,14)-*	684	Terpenoid (sesquiterpene diol)	2.2
19	51.708	*Stigmasta-3,5-diene*	668	Steroid (unsaturated phytosterol / sterol-derived triterpenoid)	1.87
20	52.728	*(3S,3aR,3bR,4S,7R,7aR)-4-Isopropyl-3,7-dimethyloctahydro-1H-cyclopenta[1,3]cyclopropa[1,2]benzen-3-ol*	609	Terpenoid (sesquiterpene alcohol, tricyclic skeleton)	1.21
21	53.028	*Cedran-diol, (8S,14)-*	601	Terpenoid (sesquiterpene diol)	0.29
22	53.388	*4-Methyldocosane*	565	Lipid-related aliphatic hydrocarbon (branched alkane)	0.84
23	53.898	*Androstane-11,17-dione, 3-[(trimethylsilyl)oxy]-, 17-[O-(phenylmethyl)oxime], (3α,5α)-*	600	Steroid (androstane derivative / derivatized steroid)	0.35
24	56.092	*β-Sitosterol*	693	Steroid (phytosterol / sterol-derived triterpenoid)	3.15
25	56.497	*β-Acorenol*	620	Terpenoid (sesquiterpene alcohol)	2.31
26	56.905	*β-Amyrin*	740	Terpenoid (pentacyclic triterpenoid alcohol)	2.6
27	58.007	*Lupeol*	621	Terpenoid (pentacyclic triterpenoid alcohol)	1.11
28	59.799	*W-18*	593	Synthetic compound	0.27

Several terpenoid- and sesquiterpenoid-related compounds were also tentatively identified, including cis-β-farnesene, β-acorenol, and cedran-diol. In addition, sterol- and triterpenoid-related constituents such as β-sitosterol, β-amyrin, and lupeol were detected at lower relative abundances. These findings indicate that *n*-hexane was effective in recovering a wider range of lipophilic phytoconstituents than the more polar extraction solvents.

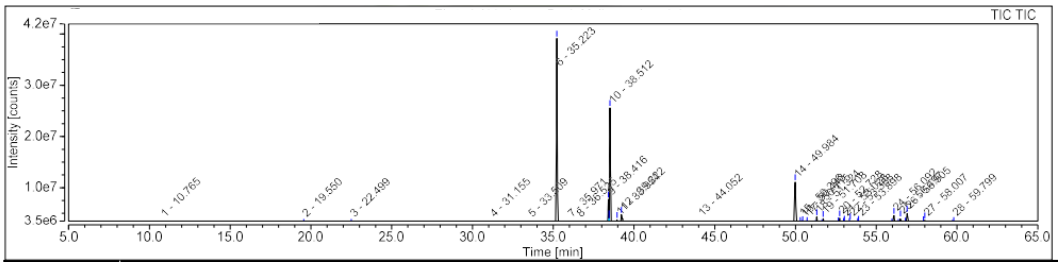

Figure 3. GC-MS chromatogram results of n-hexane extract of *M. azedarach* L fruit.

β-Sitosterol accounted for approximately 3.15% of the chromatographic area, while β-amyrin and lupeol represented 2.60% and 1.11%, respectively. These compounds belong to the sterol and pentacyclic triterpenoid classes that are frequently investigated in natural-product research. However, their biological activities in *M. azedarach* fruit cannot be inferred solely from their tentative GC-MS identification. Isolation, structural confirmation, and biological testing would be required to determine their specific contributions.

Farnesyl bromide represented 12.49% of the chromatographic area and was one of the dominant tentative library matches in the *n*-hexane extract. Because direct evidence concerning its occurrence and biological role in *M. azedarach* remains limited, the present study does not assign a specific pharmacological activity to this compound. Additional analytical confirmation is particularly important for compounds identified solely through mass spectral library matching.

Overall, the *n*-hexane extract showed the greatest chemical complexity among the three solvent fractions, particularly with respect to fatty acid esters and lipophilic terpenoid-, triterpenoid-, and sterol-related constituents. This pattern is consistent with the low polarity of *n*-hexane and its preferential extraction of hydrophobic compounds.

3.4. Comparison of the Three Extracts

Comparison of the three solvent extracts demonstrated clear differences in phytochemical composition. The methanolic extract was strongly dominated by unsaturated fatty acids, particularly cis-vaccenic acid and 17-octadecynoic acid. The ethyl acetate extract contained a broader mixture of fatty acid-, hydrocarbon-, terpenoid-, and phytosterol-related compounds. In contrast, the *n*-hexane extract yielded the highest number of chromatographic peaks and was particularly rich in fatty acid methyl esters and other lipophilic constituents.

These differences demonstrate the importance of solvent polarity in determining the chemical profile obtained from *M. azedarach* fruit. Polar and semi-polar solvents preferentially recovered different proportions of fatty acids and moderately non-polar compounds. In contrast, n-hexane favored the extraction of hydrophobic lipids, terpenoid-related compounds, triterpenoids, and sterol-related constituents.

The present GC-MS findings therefore provide a comparative chemical profile of *M. azedarach* fruit obtained using three extraction solvents. Although several tentatively identified compounds belong to chemical classes previously associated with biological activities, the present results should not be interpreted as direct evidence of antioxidant, antimicrobial, anti-inflammatory, or antidiabetic efficacy. Further studies involving confirmation with authentic standards, complementary structural characterization, quantitative analysis, and targeted biological assays are required to establish the pharmacological significance of these phytoconstituents.

4. Conclusions

GC-MS analysis of methanol, ethyl acetate, and *n*-hexane extracts of *M. azedarach* L. fruit revealed chemically distinct phytoconstituent profiles that were influenced by solvent polarity. The extracts were dominated primarily by fatty acids and fatty acid esters, accompanied by lower-abundance terpenoid-, triterpenoid-, and sterol-related constituents. Major detected compounds included cis-vaccenic acid, hexadecanoic acid, and their derivatives, while β-sitosterol, lupeol, β-amyrin, and cucurbitacin-related constituents were tentatively identified in selected extracts.

Several of these compounds belong to chemical classes previously reported to possess biological activities. However, the present GC-MS results represent chemical profiling and do not directly demonstrate antioxidant, anti-inflammatory, antimicrobial, or antidiabetic efficacy. Consequently, the findings should be considered a basis for subsequent phytochemical and pharmacological investigations rather than evidence of therapeutic activity. Future studies should include confirmation of major compounds using authentic standards or complementary analytical techniques, followed by

isolation and targeted biological assays to evaluate the pharmacological potential of *M. azedarach* fruit extracts.

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