A Preliminary Analysis of Chemical Profiles in Methanolic Crude Extracts of Euphorbia Milii Flowers via GC-MS

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Abstract

The Euphorbia genus has been widely used, particularly in the treatment of constipation, inflammation, and diarrhea, due to its reported pharmacological activities such as anti-inflammatory, antimicrobial, antifungal, antidiabetic, and antitumor properties. This research aims to identify the chemical profiles and investigate the biological activities of compounds detected in the methanolic (MeOH) crude extract of *Euphorbia milii* flowers using gas chromatography-mass spectrometry (GC-MS). The methanolic extract was analyzed using an Agilent 5975C inert MSD with a triple-axis detector, which combines a gas chromatograph (GC) directly coupled to a mass spectrometer (MS). The GC-MS analysis identified 56 chemical compounds in the methanolic extract of *Euphorbia milii*. Among these, 16 significant compounds were selected for further study. These include three saturated fatty acid esters, five saturated fatty acids, one unsaturated fatty acid, one phthalate ester, four terpenoids, one carboxylic acid anhydride, and one carboxylic ester. The biological properties of these compounds were evaluated, revealing notable activities such as antimicrobial, antioxidant, anti-inflammatory, antifungal, and antibacterial effects. The presence of bioactive compounds in *Euphorbia milii* flowers highlights its potential contribution to the medicinal field, offering a range of pharmacological benefits.

Keywords: Euphorbia milii, GC-MS analysis, secondary metabolites, biological activities

Introduction

Plants have long served as a vital source of medicinal compounds, utilized in the treatment of various ailments in both humans and animals. According to Newman and Cragg (2020), over 50% of modern clinical drugs are derived from natural sources, underscoring their significant role in the pharmaceutical

industry. Among these plants, *Euphorbia milii* is recognized for its notable medicinal properties. The increasing costs, inaccessibility, and adverse effects associated with current synthetic drugs have accentuated the need for new pharmaceutical agents that exhibit minimal side effects (Farnsworth et al., 1985). Prior research on *Euphorbia milii* has predominantly concentrated on its latex, with limited investigation into its flowers. This gap in research presents an opportunity for further exploration. Metabolite determination in natural products and metabolomic studies requires the application of various precise and accurate analytical methods. Techniques such as gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), nuclear magnetic resonance (NMR), Fourier-transform infrared spectroscopy (FT-IR), and mass spectrometry (MS) are essential for analyzing the chemical constituents of plants (Van Der Kooy & Verpoorte, 2011). These methods facilitate the examination of environmental responses, nutritional profiles, cancer research, drug discovery, and disease diagnosis. Consequently, this study aims to identify the compounds present in the methanolic extract of *Euphorbia milii* flowers using gas chromatography-mass spectrometry (GC-MS) and to explore their potential medicinal properties.

Experimental

Plant Material

The sample of *Euphorbia milii* flowers was collected from Kota Sarang Semut, Kedah (February 2021). The botanical identity of the plant specimen was confirmed by the Biological Laboratory, Universiti Malaysia Terengganu.

Extraction and Sample Preparation

The flowers of *Euphorbia milii* were dried without exposure to sunlight to prevent degradation of the phytochemicals. Subsequently, the dried samples were ground and stored in a labeled, airtight container in preparation for further analysis. The ground samples were then soaked in methanol, and the mixture was subjected to sonication for approximately 40 minutes at a temperature of 40 °C to enhance the extraction of bioactive compounds (Duran & Marcato, 2013). Following sonication, the samples were filtered to separate the solid residues from the liquid extract, which was then stored securely for subsequent analyses. To concentrate the crude extracts, a rotary evaporator was employed under reduced pressure at a temperature range of 35-40 °C. The resulting crude extracts were stored in a refrigeration unit to minimize the risk of contamination and to preserve the integrity of the samples for further analysis (Mena & Gutiérrez, 2016).

GC-MS Analysis

GC-MS analysis was performed using an Agilent 7890 gas chromatograph (GC) coupled directly to an Agilent 5975C inert mass spectrometer system (MS) with a triple-axis detector. The system was equipped with a fused silica gel column (30 m length, 0.25 mm diameter, 0.25 μm film thickness) and operated in electron ionization mode at 70 electron volts (eV). The injector temperature was maintained at 280°C, using a split injection mode. Initially, the GC oven temperature was held at 50°C for 2 minutes, then ramped up to 275°C at a rate of 6°C per minute, holding for 10 minutes (Karki et al., 2020). A 1 μL aliquot of each plant extract was injected into the GC using a split less injection mode. The column head pressure was set at 50.0 kPa, with helium as the carrier gas at a linear velocity of 36.445 cm/second. The total flow rate was programmed at 52 mL/minute, with a column flow of 1.00 mL/minute. The raw GC chromatogram was obtained and processed using the Agilent 7890A GC system, with peak identification performed using MSD Chem-Station software. A library search was conducted for all peaks using the NIST/EPA/NIH version 2.0 database to identify the molecules corresponding to the GC-MS spectra. The results were further compared with GC-MS spectra from the Human Metabolome Database (HMDB) and consolidated into a single table (Tiwari et all, 2011).

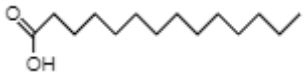
Results And Discussion

Based on previous studied carried out on *Euphorbia milii* extracts, it is reported that to show presence of terpenoids, phenolic compounds, flavonoids and steroids. In addition, a previous GCMS analysis carried out by Rautela et al (2020), it is also reported that *Euphorbia milii* extracts contain saturated fatty acids, unsaturated fatty acids, saturated fatty acid esters, carboxylic acid compounds and phthalate compounds. With the obtained raw GC chromatogram data, a library search was carried out for all the peaks using the NIST/EPA/NIH version 2.0 and HMDB.

The obtained peaks were compared and analyzed to validate the data obtained. Based on raw GC-MS chromatogram, 56 compounds are shown to be detected through the analysis. However, only 16 compounds of the detected compounds are being studied in this research. Those compounds have been identified and quantified based on the valid source of previous studies. The compound nature and biological properties were identified in details based on information reported in previous studies. The Table 1 shows the peaks and retention time for the selected metabolites.

Table 1: The peaks and retention time for selected metabolites

No	Peak	Retention Time (min)	Area (%)	Compound Name
(1)	3	25.189	1.3129	Tetradecanoic acid
(2)	8	27.624	0.6353	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione
(3)	10	27.927	2.7302	Hexadecanoic acid, methyl ester
(4)	12	28.684	20.4245	n-Hexadecanoic acid
(5)	13	29.037	0.6514	Hexadecanoic acid, ethyl ester
(6)	14	29.466	0.7435	Cyclandelate
(7)	18	31.119	1.1505	Methyl stearate
(8)	19	31.359	1.1944	2-Methyl-Z, Z-3,13-octadecadienol
(9)	20	31.737	4.9929	Octadecanoic acid
(10)	22	32.400	0.8887	Citronellal
(11)	23	32.677	1.8997	(Z, Z)-9,12-Octadecadienoic acid
(12)	35	36.772	2.8196	Bis(2-ethylhexyl) phthalate
(13)	36	37.270	0.635	Docosanoic acid
(14)	47	40.942	0.5327	2-Dodecen-1-yl (-) succinic anhydride
(15)	55	48.102	3.0452	Ursa-9(11),12-dien-3-one
(16)	56	48.834	1.9596	Cholesta-22,24-dien-5-ol, 4,4-dimethyl



(1)

Tetradecanoic acid ($C_{14}H_{28}O_2$) **(1),** which is known as myristic acid is a saturated fatty acid. The molecular weight of the compound is 228.3709 g/mol. This compound has been reported to have antioxidant (Rosy et al., 2020), anticancer, hypercholesterolemic (Gomathi et al., 2015) and nematicide (Yasin et al., 2019). Figure 1 shows the GC-MS spectrum of tetradecanoic acid obtained through this GC-MS analysis.

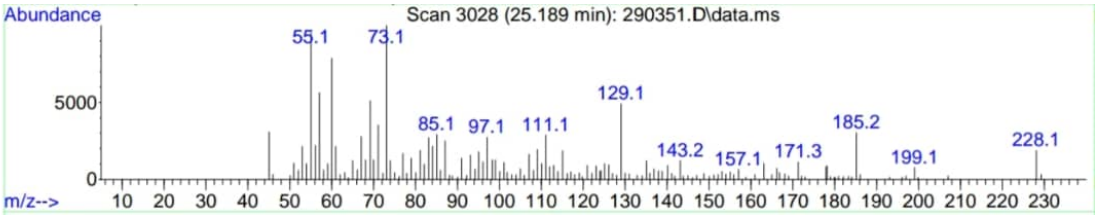
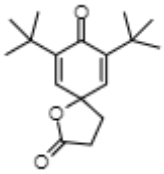


Figure 1: GC-MS spectrum of **(1)**



(2)

7,9-Ditert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione ($C_{17}H_{24}O_3$) **(2)**, is an oxaspiro compound which carries two additional tert-butyl substituents at positions C7 and C9. It is also a compound with a lactone, an enone and a cyclic ketone. The molecular weight of the compound is 276.4 g/mol (Figure 2). 7,9-Ditert-butyl-1-oxaspiro(4,5)deca-6,9 -diene-2,8-dione is a flavonoid compound. This compound is reported to show antimicrobial properties against bacteria such as *Staphylococcus aureus* which is Gram positive bacteria and *Escherichia coli, Salmonella typhi,* and *Pseudomonas aeruginosa* are Gram negative bacteria (Sharif et al., 2015). Other than that, the compound also reported to exhibit antioxidant properties (Arora et al., 2017).

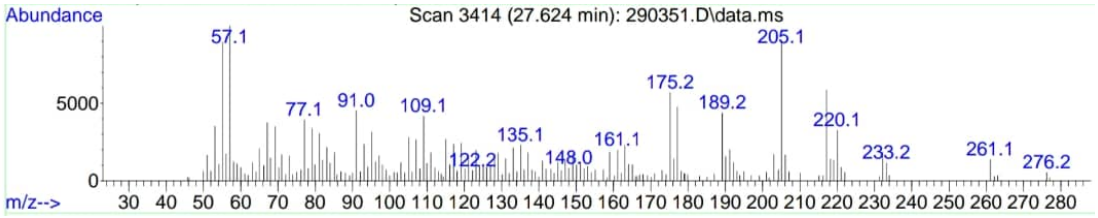
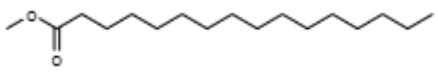


Figure 2: GC-MS spectrum of **(2)**



(3)

Hexadecanoic acid, methyl ester ($C_{17}H_{34}O_2$) (3), is a saturated fatty acid ester. The **m**olecular weight **of the compound is** 270.4507g/mol (Figure 3). The compound is also known as methyl palmitate. Based on previous carried on this compound, it was reported that hexadecanoic acid, methyl ester exhibits antioxidant, antiandrogenic, pesticide, hemolytic, nematicide and hypocholesterolemic (Arora et al., 2017). Hexadecanoic acid, methyl ester was also reported for its antifungal, antimicrobial 5-alpha reductase inhibiting properties (Krishnaveni et al., 2016). Hexadecanoic acid, methyl ester reacts against to bacteria fungi. *Staphylococcus aureus, Streptococcus mutans, and Bacillus cereus are* Gram positive bacteria whereas*, Escherichia coli* and *Pseudomonas aeruginosa* Gram-negative bacteria which the compound react against to. It is stated that the react antagonistically towards *Aspergillus parasiticus* and *Candida albicans* fungi (Davoodbasha et al., 2018).

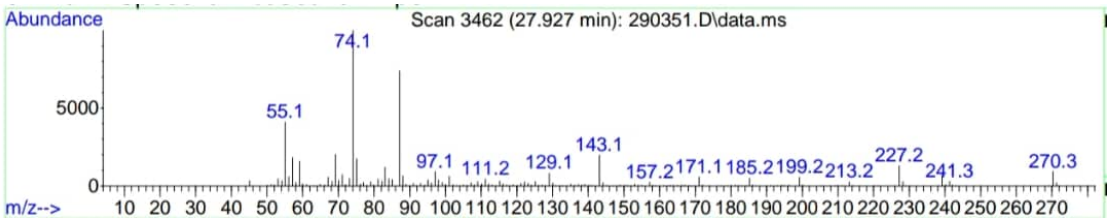
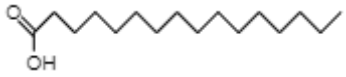


Figure 3: GC-MS spectrum of **(3)**



(4)

n-Hexadecanoic acid ($C_{16}H_{32}O_2$) **(4)**, which is also known as palmitic acid is a saturated fatty acidThe molecular weight of the compound is 256.4 g/mol (Figure 4). Based on previous research done on this bioactive compound, it was reported to have anticancer properties. Besides, n-Hexadecanoic acid is also reported to have activities like antibacterial, antioxidant, antiandrogenic, hemolytic, nematicide, hypocholesterolemic and 5-alpha reductase inhibitor (Gomathi et al., 2015). n-hexadecanoic acid shows a significant antibacterial activity against *Staphylococcus aureus*, which is Gram positive bacteria. n-Hexadecanoic acid also reported to show anti-inflammatory and cytotoxic activity against human colorectal carcinoma cells (Rosy et al., 2020).

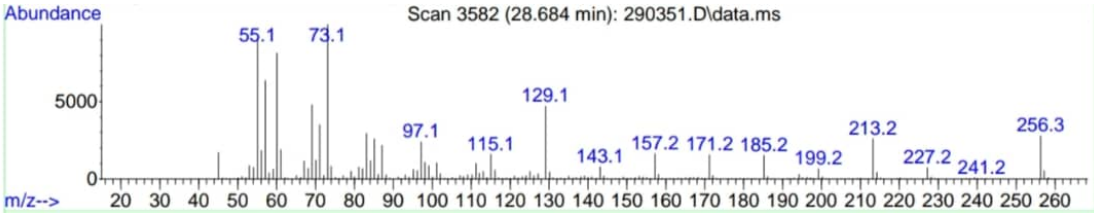
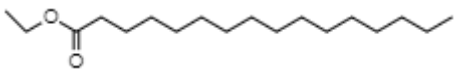


Figure 4: GC-MS spectrum of **(4)**



(5)

Hexadecanoic acid, ethyl ester ($C_{18}H_{36}O_2$) **(5)**, is a saturated fatty acid ester. The molecular weight of the compound is 284.5 g/mol. Based on previous studies, this compound has been reported to show antioxidant, hemolytic, hypocholesterolemic, nematicide, anti-androgenic properties (Tyagi et al., 2017). Hexadecenoic acid ethyl ester also acts as flavouring agent used in foods including condiments and seasonings, hair and skin conditioning agent. and Other than that, previous studies also reported that this compound has anti-inflammatory and 5-alpha reductase inhibitor (Grace et al., 2020). Figure 5 shows the GC-MS spectrum of Hexadecanoic acid, ethyl ester obtained through this GC-MS analysis.

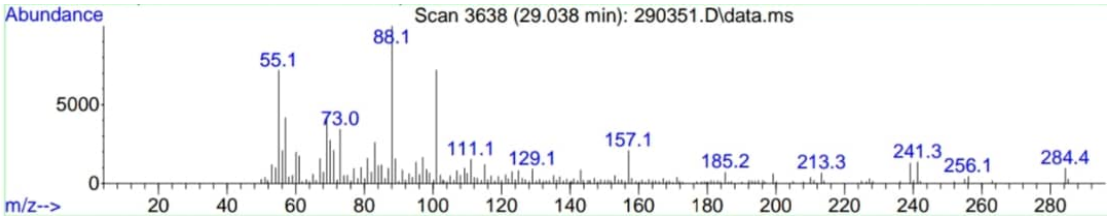
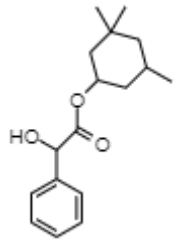


Figure 5: GC-MS spectrum of **(5)**



(6)

Cyclandelate ($C_{17}H_{24}O_3$) **(6)**, which is also known as (3,3,5trimethylcyclo hexyl) 2-hydroxy-2-phenylacetate, is a carboxylic ester. The molecular weight of the compound is 256.4 g/mol (Figure 6). This compound has been widely used as drug in medicinal field. Cyclandelate is used to treat disease resulting from bad blood circulation. Their function is to increase the size of blood vessels (Gooriah et al., 2015). Cyclandelate relaxes and making veins and arteries wider, allowing blood to pass through easier. Cyclandelate is also currently applied in treating migraine, arteriosclerosis, and nighttime leg cramps. It is mainly applied in treating cardiovascular diseases (Gooriah et al., 2015).

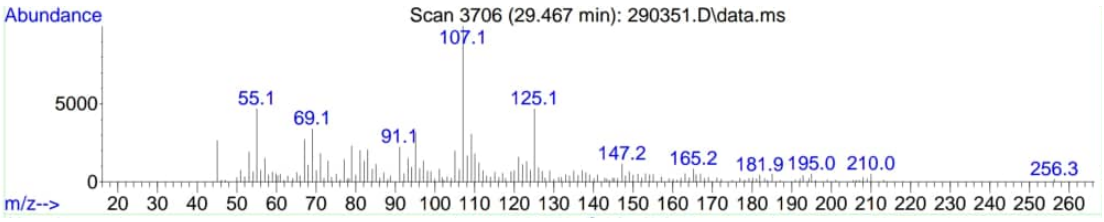
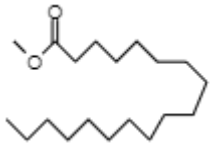


Figure 6: GC-MS spectrum of **(6)**



(7)

Octadecanoic acid, methyl ester ($C_{19}H_{38}O_2$) **(7)**, is a saturated fatty acid ester, which is also known as methyl stearate. The molecular weight of the compound is 298.5 g/mol (Figure 7). Based on previous studies, this compound has been reported to show antifungal, antibacterial and antimicrobial properties. This compound exhibited antifungal activity against diverse pathogenic fungus such as *Candida glabrata, Candida albicans, Candida krusei, Candida parapsilosis, Paracoccidioides brasiliensis Paracoccidioides lutzii, Saccharomyces cerevisiae* and *Aspergillus niger* (Pinto et al., 2017). Octadecanoic acid, methyl ester also reported to exhibit antibacterial activity against *Staphylococcus aureus, Bacillus megaterium* (Ebrahim et al., 2020) that are Gram positive bacteria and *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae* which are Gram negative bacteria (Cabral et al., 2013). Despite antibacterial and antifungal properties, methyl stearate also exhibits antidiarrheal, cytotoxic and antiproliferative properties (Sunita et al., 2018). Methyl stearate is a nematicidal compound that inhibited *Meloidogyne incognita* infection by reducing egg hatching and nematodes in soil (Lu et al., 2020).

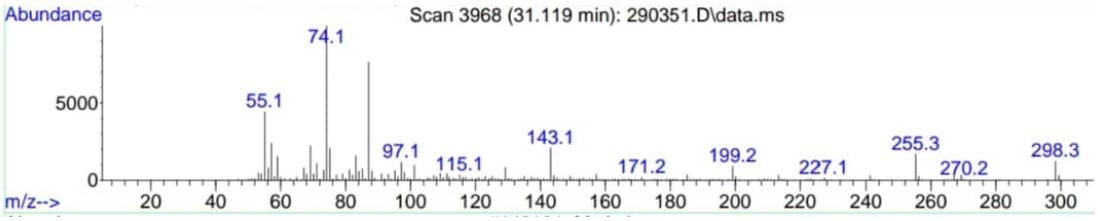
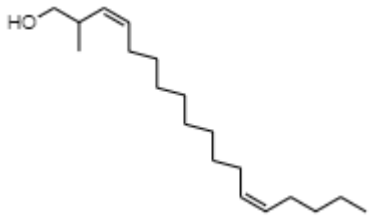


Figure 7: GC-MS spectrum of **(7)**



(8)

2-Methyl-Z,Z-3,13- octadecadienol ($C_{19}H_{36}O$) **(8)**, is a terpenoid compound. The molecular weight of the compound is 280.5 g/mol (Figure 8). The properties that exhibited by the compound are pesticide, herbicide, insecticide and pheromone (Adeyemi et al., 2018). This compound mainly beneficial in pesticidal uses (Krishnaveni et al., 2016).

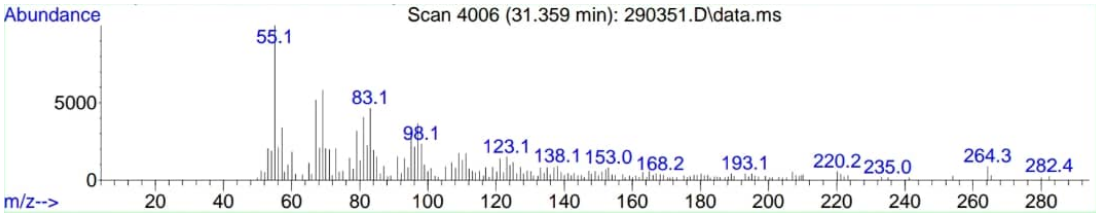
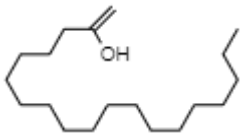


Figure 8: GC-MS spectrum of **(8)**



(9)

Octadecanoic acid ($C_{18}H_{36}O_2$) **(9)**, which is known as stearic acid (saturated fatty acid). The molecular weight of the compound is 284.48 g/mol (Figure 9). Based on previous study, this compound exhibits 5-α reductase inhibitor and hypocholesterolemic (Gomathi et al., 2015). Other than that, stearic acid also has been reported to show antifungal, antitumor and antibacterial (Arora et al., 2017). This compound shows antibacterial activity against Gram positive bacteria such as *Bacillus subtilis*, *B. pumilus*, *Micrococcus luteus*, *Staphylococcus aureus* and Gram-negative bacteria such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae, Escherichia coli.* As for the antifungal properties, it is reported the compound act against yeasts such as *Candida albicans*, *Candida krusei, Candida tropicalis* and *Candida parapsilosis* (Agoramoorthy et al., 2007). Octadecanoic acid compound also acts as an antioxidant (Rosy et al., 2020).

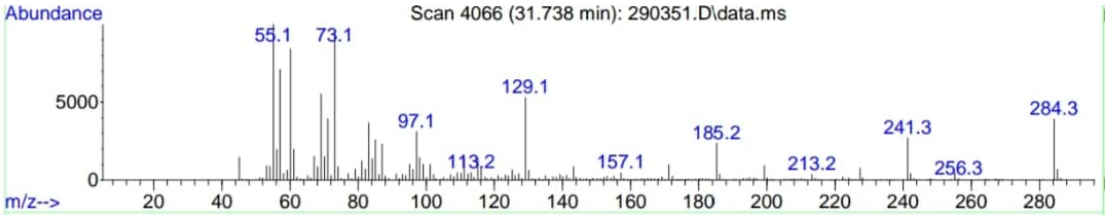
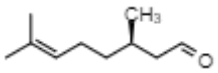


Figure 9: GC-MS spectrum of **(9)**



(10)

Citronellal ($C_{10}H_{18}O$) **(10)**, which is also known as 3,7-dimethyloct-6-enal, is a monoterpenoid aldehyde. The molecular weight of the compound is 154.25 g/mol. Citronellal is a compound that has been used as drug due to it pharmalogical benefits. This compound is also reported to give lemon aroma. It exhibits central nervous system depressant and antinociceptive properties. Other than that, Citronellal was effective as an anti-inflammatory and analgesic compound (Melo et al., 2010). The compound is extensively used in studies assessing the anti-inflammatory effect of steroidal and non-steroidal drugs. Citronellal can also reduce oxidation of lipids and protein in liver, which shows it also can act as antioxidant (Melo et al., 2011).

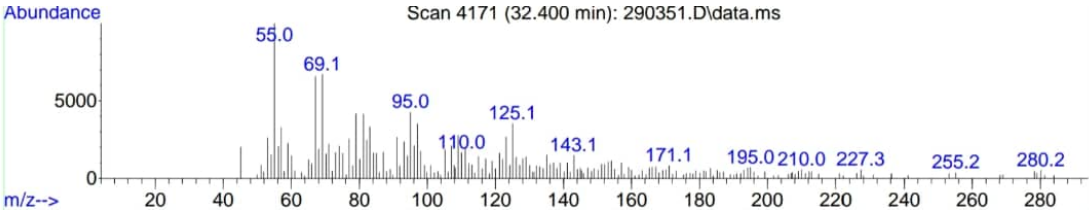
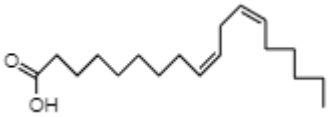


Figure 10: GC-MS spectrum of **(10)**



(11)

(Z,Z)-9,12-Octadecadienoic acid ($C_{18}H_{32}O_2$) **(11)**, is an unsaturated fatty acid. The molecular weight of the compound is 280.4 g/mol (Figure 11). This compound has been reported to have anti-inflammatory and antibacterial properties (Rossellia et al., 2007). (Z,Z)-9,12-Octadecadienoic acid is reported to exhibit antibacterial activity against *Staphylococcus aureus*, which is Gram positive bacteria and *Klebsiella pneumonia, Proteus mirabilis, Escherichia coli, Pseudomonas aeuroginos*, which are Gram negative bacteria. Besides it also shows antifungal activity against *Epidermaphyton floccosum* and *Tricophyton rubrum* fungi (Peters & Omeodu, 2016).

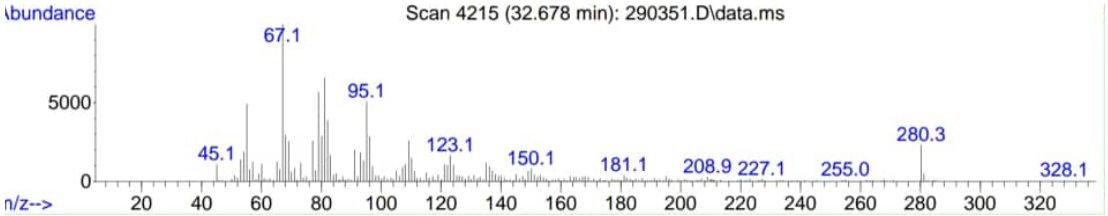
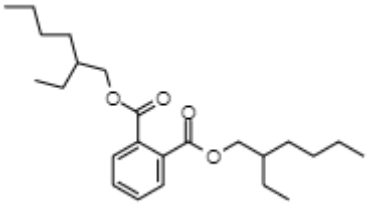


Figure 11: GC-MS spectrum of **(11)**



(12)

Bis(2-ethylhexyl) phthalate ($C_{24}H_{38}O_4$) **(12)**, is a phthalate ester. The molecular weight of this compound is 390.6 g/mol. This compound has reported to exhibit antifungal and antibacterial properties. Based on previous research, the compound shows significant activity against *Candida albicans* fungus. This compound also shows antibacterial activity against both Gram positive which are *Staphylococcus aureus, Bacillus subtilis,* and *Sarcina lutea* bateria and Gram negative which are *Escherchia coli, Shigella sonnei, Shigella shiga* and *Shigella dysenteriae* bacteria (Habib & Karim, 2009). Other than that, the compound also showed cytotoxic activity against some carcinoma cell lines, which are mainly human breast carcinoma and liver carcinoma cells (Lotfy et al., 2018). In addition, there are some studies showed that this compound has anti-inflammatory properties (Habib & Karim, 2009). Figure 12 shows the GC-MS spectrum of Bis(2-ethylhexyl) phthalate of obtained through this GC-MS analysis.

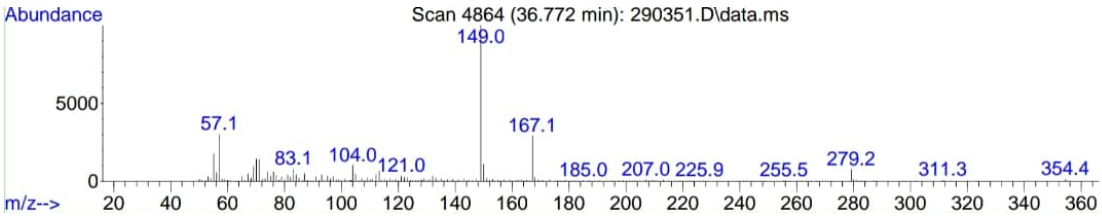
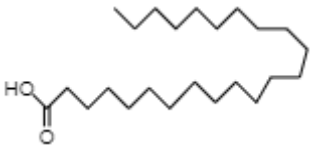


Figure 12: GC-MS spectrum of **(12)**



(13)

Docosanoic acid ($C_{22}H_{44}O_2$) **(13)**, which is also known as behenic acid is a saturated fatty acid. The molecular weight of the compound is 340.6 g/mol (Figure 13). This compound had reported to show significant α-glucosidase inhibitor properties, which proves it has anti diabetic properties (Zhang et al., 2020).

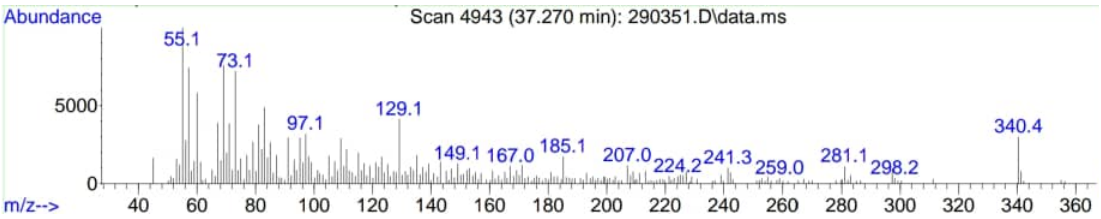
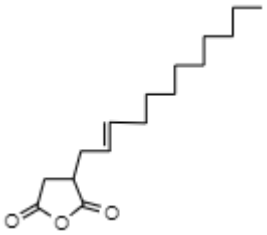


Figure 13: GC-MS spectrum of **(13)**



(14)

2-Dodecen-1-yl (-) succinic anhydride ($C_{16}H_{26}O_3$) **(14)**, is a carboxylic acid anhydride. The molecular weight of the compound is 266.38 g/mol. This compound had reported to show antineoplastic, antioxidants and antimicrobial properties (Tanod et al., 2019). Based on previous research, this compound shows strong antibacterial against *Staphylococcus aureus,* a Gram-positive bacterium. However, this compound shows moderate antibacterial effect on *Escherichia coli, Pseudomonas aeruginosa* and *Acinetobacter baumanii,* which are Gram negative bacteria (Narimane et al., 2017). Figure 14 shows the GC-MS spectrum of 2-Dodecen-1-yl (-) succinic anhydride obtained through this GC-MS analysis.

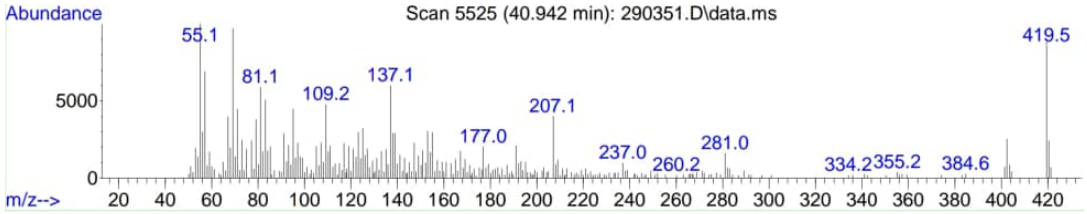
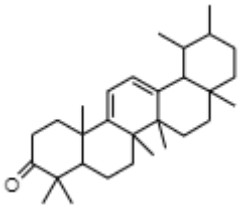


Figure 14: GC-MS spectrum of **(14)**



(15)

Ursa-9(11),12-dien-3-one($C_{30}H_{46}O$) **(15)**, is a triterpene ester that belong to terpenoid group. The molecular weight of this compound is 422.7 g/mol. Based on studies done on this compound, this compound has showed to have anti-inflammatory and antioxidant (Pal Singh et al., 2018). Figure 15 shows the GC-MS spectrum of Ursa-9(11),12-dien-3-one obtained through this GC-MS analysis.

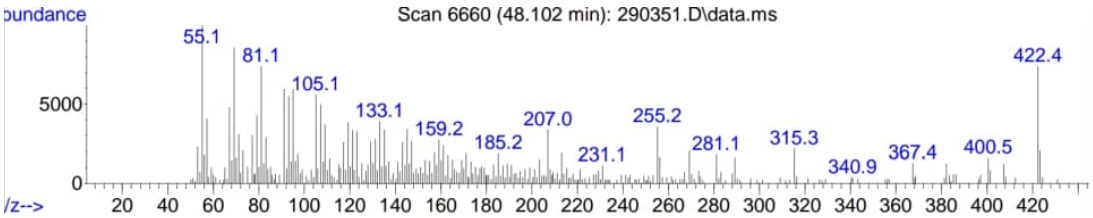
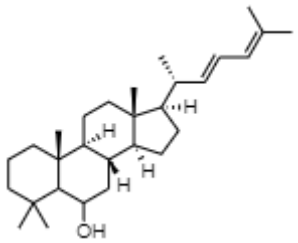


Figure 15: GC-MS spectrum of **(15)**



(16)

Cholesta-22,24-dien-5-ol, 4,4-dimethyl ($C_{29}H_{48}O$) **(16)**, is sterol compound that belongs to triterpenoid group. The molecular weight of the compound is 412.7g/mol. This compound exhibits antimicrobial and trypanocidal activity based on previous research done (Dandekar et al., 2015). Cholesta-22, 24-dien-5-ol, 4,4-dimethyl is reported to show antibacterial activity against *Staphylococcus aureus* (Gram positive bacteria) (Abreu et al., 2011) and *Salmonella typhi* (Gram negative bacteria). Other than that, this compound also exhibits antifungal activity against *Candida albicans* fungi (Putra & Hadi, 2017). Cholesta-22,24-dien-5-ol,4,4-dimethyl was also identified to have anti-inflammatory, anticancer, anti-arthritic, anti-asthmatic properties. This proves to be a beneficial metabolite in pharmaceutical field (Krishnaveni et al., 2016). Figure 16 shows the GC-MS spectrum of Cholesta-22,24-dien-5-ol, 4,4-dimethyl obtained through this GC-MS analysis.

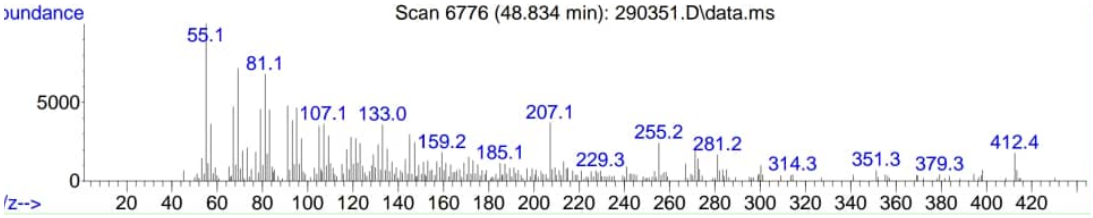


Figure 16: GC-MS spectrum of **(16)**

Conclusion

In this study, the methanolic extract of *Euphorbia milii* flowers was analyzed using GC-MS to identify the chemical compounds present. From the GC-MS data, 16 compounds were selected for further analysis to determine their biological properties. These compounds included three saturated fatty acid esters, five saturated fatty acids, one unsaturated fatty acid, one phthalate ester, four terpenoids, one carboxylic acid anhydride, and one carboxylic ester. The biological properties of these compounds were evaluated based on previously reported studies on each detected chemical compound. The detected metabolites of *Euphorbia milii* reveals the presence of chemical compounds with medicinal properties. Each metabolite exhibits unique biological activities, making these compounds valuable for use in the pharmaceutical and cosmetic industries, where they could be applied in the development of medicines and cosmetic products.

Conflict of Interest

All authors declare that they have no conflicts of interest.

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