



GC-MS Analysis of Phytochemical Constituents in Methanol Extract of Wood Bark from *Durio Zibethinus* Murr

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Abstract: *Durio zibethinus* Murr. (Durian), a medicinally important plant belongs to the family Bombacaceae. Traditionally the decoctions of the leaf and root have believed to show antipyretic effect and can also be used as a febrifuge and anti-malarial agent. In the present study, the preliminary phytochemical screening of secondary metabolites in the wood bark of *Durio zibethinus* was investigated using standard methods. The methanol extract of the wood bark was fractionated into four combined fractions (F1- F4) and the bioactive compounds in the fractions were evaluated using Gas Chromatography–Mass Spectrometry. Phytochemical screening on this crude extract revealed the presence of phenols, alkaloids, steroids, tannins, terpenes, saponins and flavonoids. Gas chromatography-mass spectrometry (GC-MS) analysis revealed the presence of 2, 6, 5 and 4 compounds in fractions 1-4 respectively. The major chemical constituents identified are (Z)-9-Octadecenoic acid (Oleic Acid) (91.36 %), (E)-9-Octadecenoic acid (Elaidic Acid) (56.10 %), (Z)-6-Octadecenoic acid (Petroselinic acid) (41.19 %) and (E)-9-Octadecenoic acid (Elaidic Acid) (42.20 %) in fractions 1-4 respectively. The presence of these major constituents in the plant extract provides the scientific evidences for its biological and therapeutic properties related to health. Thus this research was aimed to investigate the secondary metabolites present in the wood bark of *Durio zibethinus* Murr that could have medicinal values and application in industries, as well as, to identify the phytoconstituents by using GC-MS analysis.

Keywords: *Durio zibethinus* Murr.; Phytochemical screening; Secondary metabolites; GC-MS

1. INTRODUCTION

Medicinal plants and herbs have been proved to be of great importance to the health of the individuals and Communities (Alves *et al.*, 2000). The evaluation of all the drugs is based on phytochemical and pharmacological approaches which leads to the drug discovery referred as natural product screening (Foye *et al.*, 2008). Any part of the plant may contain active components such as bark, leaves, flowers, roots, fruits and seeds (Gordon and David. 2001). Phytochemicals are the chemicals produced by various parts of the plants. These bioactive compounds have various activities such as antimicrobial and antibacterial some have been reported to exhibit hemolytic and foaming activity reported by Sanjeeb *et al.* (2011).

Durian (*Durio zibethinus* Murr.; Family Bombacaceae) is a tropical fruit plant cultivated in Malaysia and the Southeast Asian countries (Bhore *et al.*, 2018). Durian is considered as “King of Tropical Fruit” due to its high nutritional status and with its appearance that resembles the thorny thrones of Asian kings (Subhadrabandhu and Ketsa, 2001). Scientific interest lies in the fact that durian is considered by some botanists to be one of the most primitive of the trees in the tropical rain forest (Bautista *et al.*, 2012). Fruit pulp of this fruit is an excellent source of nutrients as it contains proteins, dietary fat, fibers, and carbohydrates (Bhore *et al.*, 2018).

The durian fruit is believed to have warming properties on the body; however, it has not been clinically investigated (Brown, 1997). Durian fruit is considered to have potential medicinal and therapeutic properties that include its ability to boost the immune system and wound healing (Chansiripornchai and Pongsamart, 2008). It is also widely believed that durian pulp contains strong

aphrodisiac properties and local community believes that consumption of fruits in conjunction with alcohol will lead to death. However, there is no evidence to support these claims (Ho and Bhat, 2015). It is reported that durian has anti-oxidant (Ang, 2018), anti-cancer, anti-cardiovascular, anti-diabetic (Leontowicz *et al.*, 2007; Siburian *et al.*, 2019) and anti-obesity properties (Leontowicz *et al.*, 2008), and can improve digestion, cure insomnia, lower the blood pressure and relieve the symptoms of depression, anxiety, and stress disorders (Kumar *et al.*, 2005; Haruenkit *et al.*, 2007). Previous studies have also reported the potential use of durian fruit pulp as fertility enhancing agent and studies were conducted to find out its effectiveness to treat infertility in PCOS (polycystic ovarian syndrome) (Ansari, 2016). Although the fruit is effective against various components of metabolic syndrome, specific studies of the mechanism of ovulation and menstrual disturbances need to be conducted. Durian fruits have also shown anti-proliferative activities as being reported by Jalil and Aziz (2019). With this background, the present study was aimed to investigate the secondary metabolites present in the wood bark of *Durio zibethinus* Murr. which have medicinal values and application in industries, as well as, to identify the phytoconstituents by using GC-MS analysis.

2. MATERIALS AND METHODS

2.1. Plant Collection and Identification

Fresh plant materials of *Durio zibethinus* Murr., were collected from the premises of Igbinedion University, Okada in Ovia North-East Local Government Area of Edo State, Nigeria. The plant materials were identified by the herbarium curator of Department of Plant Biology, University of Ilorin and authenticated by comparing it with authentic specimen at the Botanical Survey of India, Coimbatore with Voucher No. UILH/001/1371.

2.2. Plant Preparation

The plant materials were thoroughly washed with distilled water, chopped into smaller pieces to increase the surface area of the parts to allow for a quicker drying period and air-dried at room temperature (26°C) for 12 weeks. An electrical grinder was used to grind the dried material into fine powders. During the course of this study, the powdered plant material was stored in air-tight glass containers and kept away from sunlight to prevent possible photo-oxidation reactions.

2.3. Qualitative Phytochemical Screening

Qualitative phytochemical screening of the extract was carried out for various plant constituents. The crude extract was screened for the presence or absence of secondary metabolites such as alkaloids, saponins, tannins, flavonoids, steroids, terpenoids, cardiac glycosides and phenolic compounds using standard procedures with minor modifications where necessary.

2.3.1. Detection of Alkaloids

A 100 mg of each stem bark extract was diluted with 5 ml of hydrochloric acid, boiled and filtered. To 2 ml of the filtrate was added to 1 ml of dilute ammonia. 2ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 5 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Dragendoff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Dragendoff's reagent) was regarded as positive for the presence of alkaloids (Ejikeme *et al.*, 2017).

2.3.2. Detection of Saponins

Froth test: 0.5 g of each stem bark extract dissolved in 10 ml of distilled water in a test tube, stoppered, shaken vigorously for about 20 seconds, allowed to stand in a vertical position and observed over a 30 minute period of time for a "honey comb" froth above the surface of liquid after 30 min shall be taken as a positive test for the presence of saponins (Ejikeme *et al.*, 2014).

2.3.3. Detection of Tannins

Ferric chloride test: A portion of each stem bark extract dissolved in distilled water and clarified by filtration. A bluish black colouration after the addition of 10 % ferric chloride solution indicated the presence of tannins (Santhi and Sengottuvel, 2016).

2.3.4. Detection of Flavonoids

0.5 g of each stem bark extract was dissolved in diluted NaOH and HCl was added. A yellow solution that turned colourless indicated the presence of flavonoids (Gothandam *et al.*, 2010).

2.3.5. Detection of Steroidal Compounds

Lieberman's test: 0.5 g of each stem bark extract was dissolved in 2 ml of acetic anhydride and cooled well in an ice-bath. A colour changed from purple to blue to green when concentrated sulphuric acid was carefully added, indicating the presence of a steroid nucleus (Seema and Parwez, 2011).

2.3.6. Detection of Terpenoids

Salkowski's test: 0.5 g of each stem bark extract was dissolved in 2 ml of chloroform. Concentrated H₂SO₄ (3 ml) was carefully added to form a layer. A reddish brown colouration at the interface indicated the presence of terpenoids (Rahman *et al.*, 2017).

2.3.7. Detection of Cardiac Glycosides

Keller-Killiani's test: 0.5 g of each stem bark was dissolved in water and 2 ml of glacial acetic acid containing one drop of ferric chloride solution was added. 1 ml of concentrated sulphuric acid was carefully added to form a brown ring at the interface indicating the presence of Deoxysugar characteristic of Cardenolides. A violet ring will appear below the brown ring, while in the acetic acid layer a greenish ring will form just above the brown ring and gradually spread throughout this layer (Aiyelaagbe and Osamudiamen, 2009).

2.3.8. Detection of Phenolic Compounds

Each stem bark extract was dissolved in distilled water, and a few drops of 1% lead acetate were added to form a bulky white precipitate which indicated the presence of phenolic compounds (Prabhavathi *et al.*, 2016).

2.4. GC-MS Analysis

2.4.1. Instruments and Chromatographic Conditions

The GC-MS analysis was performed on an Agilent Technologies interfaced [Model: 7890A (GC)] with Mass Selective Detector model: 5975C (MSD). The electron ionization was at a 70v with an ion source temperature at 250 °C. Highly pure helium gas (99.9% purity) was used as carrier gas, while HP-5ms (30mm X 0.25mm X 0.320µm) was used as the stationary phase. The oven temperature program initial temperature is 140 °C held for 5 minutes at 4°C per minute to the final temperature of 240 °C to hold for 15minute at the rate of 3.5 °C/minutes holding for 6 minutes. 1µl of each fraction of the methanolic extract of wood bark from *D. zibethinus* was auto injected.

2.4.2. Identification of Phytochemical Compounds

Identification of phytochemical compounds and interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components using computer searches on a NIST Ver.2.1 MS data library. The name, molecular weight and structure of the components of the test materials were established.

3. RESULTS AND DISCUSSION

3.1. Qualitative Phytochemical Screening

Qualitative determination of phytochemicals as seen in Table 1 showed various plant secondary metabolites in which there was presence of alkaloids, saponins, tannins, flavonoids, terpenoids, glycosides and phenolic compounds, while steroids were found to be absent in wood bark of *D. zibethinus*.

Plants have been shown to contain secondary metabolites that can also protect humans against diseases (Doughari *et al.*, 2009). To these effects most of the phytochemicals have been reported by several authors for specific functions. For instance, Agu and Thomas (2012) reported that alkaloids have defense mechanisms through which plants ward off pests. This suggests the medicinal properties (such as analgesic, antispasmodic and bactericidal effects) of alkaloids from plants (Kigigha *et al.*, 2015; Eparti *et al.*, 2016; Doherty *et al.*, 2010; Osuntokun and Oluwafoise, 2015). Alkaloids are also essential for the treatment of cardiovascular and kidney disorders (Sweetman, 2005). It was also reported that alkaloids have a wide range of pharmacological activities including antimalarial (e.g.,

quinine), anticancer (e.g., homoharringtonine) (Kittakoop *et al.*, 2014), antibacterial (e.g., chelerythrine) (Cushnie *et al.*, 2014), and antihyperglycemic activities (e.g., piperine) (Qiu *et al.*, 2014).

Saponins in the wood bark is an indication that the plant could be used as expectorant, cough suppressant and it also has hemolytic activity (Kigigha *et al.*, 2015; Okwu, 2005; Osuntokun and Oluwafoise, 2015; Epedi *et al.*, 2016). Saponins have been reported to have antimicrobial (Soetan *et al.*, 2006), antifungal (Hazem *et al.*, 2012) and anti-inflammatory properties (Patel and Patil, 2012). Brusotti *et al.* (2013) also reported that saponin has been demonstrated to be a good candidate for the control of a pathogen of rice blast disease, *Pyricularia grisea*.

Tannins are known to occur abundantly in the bark of trees where they act as a barrier to microorganisms. Tannin is astringent in nature and has the ability to bind or precipitate proteins and various other organic compounds making them unavailable for absorption. Plants rich in Tannin are said to have several medical applications (Okuda, 2005; Doughari *et al.*, 2009). Tannins have health importance such as wound healing, varicose ulcers, hemorrhoids, frostbite and burns, and it has the ability to regenerate skin, as well as anti-inflammatory and anti-diuretics activity (Okwu and Okwu, 2004; Khanbabaee and van Ree, 2001).

TABLE1. Qualitative phytochemical screening of methanolic extract of wood bark from *Durio zibethinus*

Secondary Metabolites	Stem bark
Alkaloids	+
Saponins	+
Tannins	+
Flavonoids	+
Steroids	-
Terpenoids	+
Glycosides	+
Phenolic compounds	+

Key: Present: +; Absent: –.

Flavonoids have been reported to possess antioxidant, anticarcinogenic, antimicrobial antitumor, allergenic, anti-inflammatory and antidiarrheal properties (Yamamoto and Gaynor 2001; Cushnie and Lamb 2005; Chahar *et al.*, 2011; Yao *et al.*, 2011). Flavonoids are also essential for the treatment of ulcer (Ateufack *et al.*, 2015).

Naturally occurring terpenoids often exhibit a variety of biological activities such as anti-inflammatory, anti-HIV, anti-tumour-promoting, ichthyotoxic and antimycobacterial activities (Cantrell *et al.*, 2001; Rajic *et al.*, 2000; Villar *et al.*, 2003).

Reports have confirmed the antiproliferative and apoptotic effects of cardiac glycosides in several cancer cell lines, including breast (Kometiani *et al.*, 2005; Bielawski *et al.*, 2006; Lopez-Lazaro *et al.*, 2005), prostate (McConkey *et al.*, 2000; Huang *et al.*, 2004; Yeh *et al.*, 2003), melanoma (Newman *et al.*, 2006), pancreatic (Newman *et al.*, 2007), lung (Mijatovic *et al.*, 2006; Frese *et al.*, 2006), leukaemia (Raghavendra *et al.*, 2007; Daniel *et al.*, 2003) and renal adenocarcinoma (Lopez-Lazaro *et al.*, 2005)78. Cardiac glycosides have been a cornerstone of the treatment of heart diseases (Ahmed *et al.*, 2008). Phenols have been reported to possess antioxidant (Heima *et al.*, 2002), antibacterial and antifungal (Alasalvar *et al.*, 2001; Acamovic and Brooker, 2005; Edreva *et al.*, 2008). All these facts may be linked to the biological activities of wood bark from *D. zibethinus*.

3.2. GC-MS Analysis

GC-MS is one of the best techniques to identify the constituents of volatile matter, long chain, branched chain hydrocarbons, alcohols acids, esters etc. The GC-MS analysis of fractions of *D. zibethinus* wood bark revealed the presence of two, six, five and four compounds (phytochemical constituents) in fractions 1, 2, 3, and 4 respectively. The peaks in the chromatogram were integrated and compared with the database of spectrum of known components stored in the GC-MS library. The chemical constituents identified in fraction 1 are Heptadecanoic acid, heptadecyl ester (8.64 %) and (Z)-9-Octadecenoic acid [Oleic acid] (91.36 %). Fraction 2 consists of Furo [2,3-c] pyridine, 2,3-

dihydro-2,7-dimethyl- (0.90 %), Hexadecanoic acid, methyl ester (8.28 %), 3-Pyrrolidinol (0.58 %), Hexadecanoic acid, ethyl ester (13.13 %), (Z)-6-Octadecenoic acid, methyl ester (21.01 %) and (E)-9-Octadecenoic acid [Elaidic Acid] (56.10 %). Fraction 3 has (E)-9-Octadecenoic acid [Elaidic Acid] (14.78 %), 2-Ethylacridine (9.79 %), (Z)-6-Octadecenoic acid [Petroselinic acid] (41.19 %) and trans-13-Octadecenoic acid, methyl ester (29.34 %) and 2,3-Dihydroxypropyl (E)-9-Octadecenoate (4.89 %) while fraction 4 consists of Hexadecanoic acid, methyl ester (17.89 %), Hexadecanoic acid, ethyl ester (21.33 %), (Z)-9-Octadecenoic acid, methyl ester (18.56 %) and (E)-9-Octadecenoic acid [Elaidic acid] (42.20 %).

GC-MS chromatograms of the peak of the compounds detected in fractions 1-4 were shown in Figures 1, 2, 3 and 4 respectively. The identification of the phytochemical compounds was confirmed based on the peak area, retention time and molecular formula. The active principles with their Retention time (RT), Molecular formula, Molecular weight (MW) and peak area in percentage are presented in Tables 2 & 3. The phytochemicals identified through GC-MS analysis showed many biological activities relevant to this study are listed in Table 3. The biological activities listed are based on Dr. Duke's Phytochemical and Ethnobotanical Databases created by Dr. Jim Duke of the Agricultural Research Service/USDA.

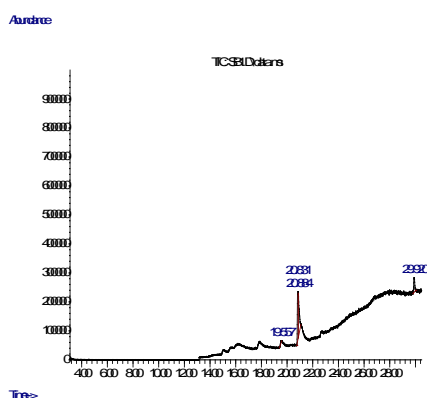


Figure1. GC-MS Chromatogram of fraction 1 of methanolic wood bark extract of *D. zibethinus*

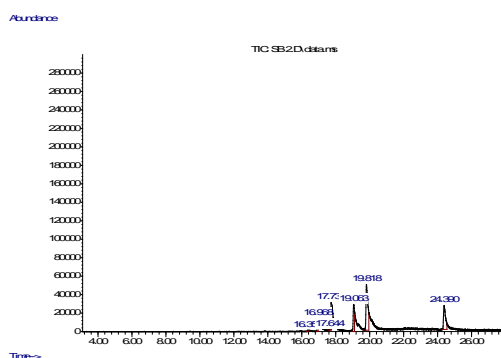


Figure2. GC-MS Chromatogram of fraction 2 of methanolic wood bark extract of *D. zibethinus*

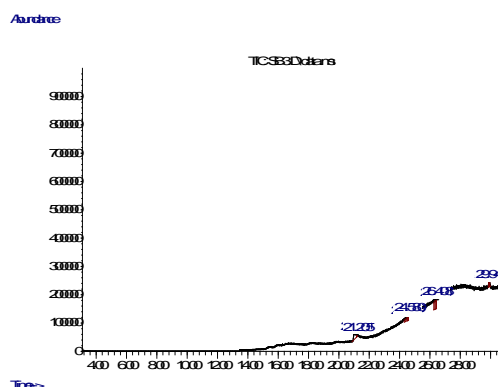


Figure3. GC-MS Chromatogram of fraction 3 of methanolic wood bark extract of *D. zibethinus*

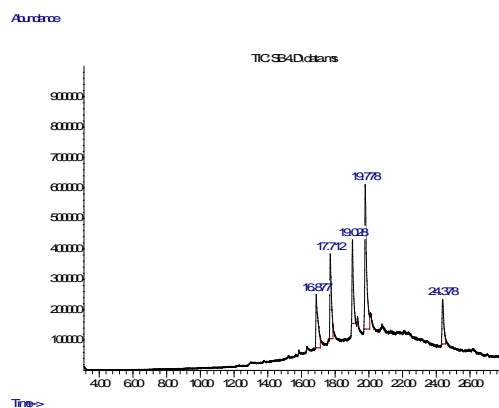


Figure4. GC-MS Chromatogram of fraction 4 of methanolic wood bark extract of *D. zibethinus*

Table2. GC-MS analysis revealed the presence of phytochemical compounds in methanol wood bark extract of *D. zibethinus*

S/N	R/T	Name of Compound	Molecular Formular	Molecular Weight	Peak Area %
Fraction 1					
1	19.557	Heptadecanoic acid, heptadecyl ester	C ₃₄ H ₆₈ O ₂	508	8.64
2	29.923	(Z)-9-Octadecenoic acid (Oleic Acid)	C ₁₈ H ₃₄ O ₂	282	91.36
Fraction 2					
3	16.356	Furo [2,3-c] pyridine, 2,3-dihydro-2,7-dimethyl-	C ₉ H ₁₁ NO	149	0.90
4	16.968	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270	8.28
5	17.644	3-Pyrrolidinol	C ₄ H ₉ NO	87	0.58
6	17.730	Hexadecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	284	13.13
7	19.063	(Z)-6-Octadecenoic acid, methyl ester	C ₁₉ H ₃₆ O ₂	296	21.01
8	19.818	(E)-9-Octadecenoic acid (Elaidic Acid)	C ₁₈ H ₃₄ O ₂	282	56.10
Fraction 3					
9	24.527	(E)-9-Octadecenoic acid (Elaidic Acid)	C ₁₈ H ₃₄ O ₂	282	14.78
10	24.579	2-Ethylacridine	C ₁₅ H ₁₃ N	207	9.79
11	26.307	(Z)-6-Octadecenoic acid (Petroselinic acid)	C ₁₈ H ₃₄ O ₂	282	41.19
12	26.410	trans-13-Octadecenoic acid, methyl ester	C ₁₉ H ₃₆ O ₂	296	29.34
13	29.946	2,3-Dihydroxypropyl (E)-9-Octadecenoate	C ₂₁ H ₄₀ O ₅	373	4.89
Fraction 4					
14	16.877	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270	17.89
15	17.712	Hexadecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	284	21.33
16	19.028	(Z)-9-Octadecenoic acid, methyl ester	C ₁₉ H ₃₆ O ₂	296	18.56
17	19.778	(E)-9-Octadecenoic acid (Elaidic Acid)	C ₁₈ H ₃₄ O ₂	282	42.20

Table3. GC-MS analysis showed phytochemical compounds, their nature and their biological activitie methanol wood bark extract of *D. zibethinus*

S/N	R/T	Peak Area %	Name of Compound	Compound Nature	Activity
Fraction 1					
1	19.557	8.64	Heptadecanoic acid, heptadecyl ester	Fatty acid	Acidifier, acidulant, arachidonic acid-inhibitor, inhibit production of uric acid.
2	29.923	91.36	(Z)-9-Octadecenoic acid (Oleic Acid)	Fatty acid	Anticancer, Anemiagenic, Insectifuge, Antiandrogenic, Dermatitigenic.
Fraction 2					
3	16.356	0.90	Furo [2,3-c] pyridine, 2,3-dihydro-2,7-dimethyl-	Pyridine derivative	Use in dyes, flavor, fragrances, Pharmaceuticals, agrochemicals and veterinary products

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4	16.968	8.28	Hexadecanoic acid, methyl ester	Fatty acid ester	Antioxidant, hypocholesterolemic, antiandrogenic, flavor, nematocide, hemolytic, 5-alpha reductase inhibitor
5	17.644	0.58	3-Pyrrolidinol	Pyrrolidine derivative	No activity reported
6	17.730	13.13	Hexadecanoic acid, ethyl ester	Fatty acid ester	Antioxidant, flavor, hypocholesterolemic, nematocide, pesticide, lubricant, antiandrogenic, hemolytic, 5-alpha reductase inhibitor
7	19.063	21.01	(Z)-6-Octadecenoic acid, methyl ester	Fatty acid ester	Antioxidant, Antimicrobial.
8	19.818	56.10	(E)-9-Octadecenoic acid (Elaidic Acid)	Fatty acid	Acidifier, acidulant, anticancer, antitumor, arachidonic acid-inhibitor.
Fraction 3					
9	24.527	14.78	(E)-9-Octadecenoic acid (Elaidic Acid)	Fatty acid	Acidifier, acidulant, anticancer, antitumor, arachidonic acid-inhibitor.
10	24.579	9.79	2-Ethylacridine	Heterocyclic compound	No activity reported
11	26.307	41.19	(Z)-6-Octadecenoic acid (Petroselinic acid)	Fatty acid	Cancer preventive, Insectifuge.
12	26.410	29.34	trans-13-Octadecenoic acid, methyl ester	Fatty acid ester	Acidifier, acidulant, arachidonic acid-inhibitor, inhibit production of uric acid.
13	29.946	4.89	2,3-Dihydroxypropyl (E)-9-Octadecenoate	Fatty acid ester	Anticancer, antitumor, ectoparasiticide, edemagenic
Fraction 4					
14	16.877	17.89	Hexadecanoic acid, methyl ester	Fatty acid ester	Antioxidant, hypocholesterolemic, antiandrogenic, flavor, nematocide, hemolytic, 5-alpha reductase inhibitor
15	17.712	21.33	Hexadecanoic acid, ethyl ester	Fatty acid ester	Antioxidant, flavor, hypocholesterolemic, nematocide, pesticide, lubricant, antiandrogenic, hemolytic, 5-alpha reductase inhibitor
16	19.028	18.56	(Z)-9-Octadecenoic acid, methyl ester	Fatty acid ester	Acidifier, acidulant, arachidonic acid-inhibitor, increase zinc bioavailability.
17	19.778	42.20	(E)-9-Octadecenoic acid (Elaidic Acid)	Fatty acid	Acidifier, acidulant, anticancer, antitumor, arachidonic acid-inhibitor.

**Source: Dr. Duke's Phytochemical and Ethnobotanical Databases

Most phytochemical constituents identified in *D.zibethinus* wood bark are fatty acids and fatty acid esters that contribute to the antioxidant, antimicrobial, antitumor, cancer-preventive, hypocholesterolemic, nematocide, pesticide, antiandrogenic, hemolytic, 5-alpha reductase inhibitor, lubricant and flavor activities. Hence, the wood bark of *D.zibethinus* Murr is worthy for further investigation on isolation of individual phytochemical constituents and toxicological aspects for the development of new lead of therapeutic interest.

4. CONCLUSION

The wood bark of *D.zibethinus* contains many important phytochemical components such as alkaloids, saponins, tannins, flavonoids, terpenoids, glycosides and phenolic compounds. However, the presence of these phytochemicals in this plant suggests its vast pharmacological potentials. The GC-MS analysis of methanolic extract showed a number of medicinal active components. Meanwhile, further studies are needed to isolate the bioactive components in the plant. Hence, this study may be useful to explore the pharmacological and biosynthetic activity of *D.zibethinus* Murr further.

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