Original Research Article

GC-MS Analysisand BiologicalRoles of PhytochemicalCompoundsinn-HexaneExtract of*Duriozibethinus*Murr. Seeds

ABSTRACT

Aim: The current study was to assess the phytochemical compounds in *Durio zibethinus* seeds that can be used for medicinal and industrial purposes.

Methodology: The n-hexane extract of the seeds was analyzed using Gas Chromatography–Mass Spectrometry. The Gas Chromatography–Mass Spectrometry (GC-MS) analysis of n-hexane extract from D. zibethinus seeds revealed the presence of twelve compounds.

Results: The chemical constituents identified are beta-Guaiene (0.85 %), Hexadecanoic acid, methyl ester (3.82 %), 9,12-Octadecadienoic acid, methyl ester (1.26 %), Phytol (1.36 %), Methyl stearate (2.08 %), Lupeol (1.97 %), n-Tetracosanol-1 (17.34 %), Squalene (32.75 %), Triacontanediol (34.47 %), 1-Heptacosanol (1.89 %), Eicosane (0.88 %) and Hexadeca-2,6,10,14-tetraen-1-ol, 3,7,11,16-tetramethyl- (29.34 %).

Conclusion: The presence of these chemical constituents in the seed extract has authenticated the scientific evidences for its physiological properties relating to human health.

Keywords:*Durio zibethinus* Murr.; Phytochemicals; GC-MS analysis; Biologicalactivities

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1. INTRODUCTION

The adoption of medicinal plants as a source of possible medications has become incredibly successful. Most plants are extremely helpful in the drug development process since they naturally create chemical compounds or molecules with pharmacological or bioactive qualities [1]. The broad category of "medicinal plants" includes a wide range of botanical species that produce compounds that are beneficial to human fitness and wellbeing. In the past, the creation of pharmaceutical medications for diseases including cancer, infections, digestive problems, diabetes, and hypertension has benefited significantly from using medicinal plants [2]. Different parts of plants, such as leaves, fruits, roots and seeds have been recognized for their medicinal activities [3].

A tropical fruit plant called durian (*Durio zibethinus* Murr.; Family Bombacaceae) is grown in Malaysia and other Southeast Asian countries. Based on its appearance like the thorny thrones of Asian kings and its high nutritional status, it is referred to as the “King of Tropical Fruit” [4]. Durian is said to have anti-oxidant, anti-cancer, anti-heart disease, anti-diabetic, and anti-obesity properties [5]. The capacity to enhance the immune system is one of the purported medicinal and therapeutic benefits of durian fruit. Its fruit pulp maybe a great source of nutrients like proteins, carbohydrates, fibers and dietary fat [6]. It is also disclosed that durian seed, pulp and peel flour possess nutritional, structural, antioxidant and anti-inflammatory properties [7]-[8].

Natural chemical substances (phytochemicals) are essential for promoting health benefits. [9]. Considering their roles in plant metabolism, phytochemicals can be categorized as primary or secondary metabolites. For plants to grow and develop, primary metabolites such as sugars, amino acids, proteins, nucleic acids' purines and pyrimidines, and chlorophylls are essential. Moreover, in addition to safeguarding plants from environmental hazards including pollution, stress, UV exposure, and illnesses, they also contribute to the colour, aroma, and flavour of plants [10]. As opposed to that, secondary metabolites such as carotenoids, phytosterols, alkaloids, terpenes and polyphenols (including flavonoids, phenolic acids, tannins, stilbenes, coumarins, and lignans) have different roles in a plant's environment, including luring pollinators and functioning as natural defenses against pathogens and predators [11]-[12]. Therefore, this study uses Gas Chromatography-Mass Spectrometry (GC-MS) to thoroughly investigate the bioactive compounds found in the n-hexane extract of *Durio zibethinus* Murr. seeds, helping to further our understanding of the chemical makeup of this less-studied area of the *Durio zibethinus* Murr. plant. There are attempts to study the biological activities of the detected bioactive compounds present in the n-hexane extract of *Durio zibethinus* Murr. seeds.

2.MATERIALS AND METHODS

2.1Sample Collection and Authentication

The plant materials, *Durio zibethinus* Murr. seeds were collected at Crown Estate of Igbinedion University, Okada, Edo State, Nigeria in the month of April, 2023. At the Taxonomy section, Biological Sciences Department, Igbinedion University, Okada, Nigeria, the seeds samples were identified and authenticated. The Voucher No. IUOH/001/1371 was assigned.

2.2Preparation and Extraction of Plant Materials

The *Durio zibethinus* Murr. seeds were washed and air-dried, the seed coats were carefully peeled off manually from the seeds. These seeds were appropriately ground into little particles using a mortar and pestle and stored in a polythene bag. By soaking 2.0 kg of the crushed seeds in 10.0 L of methanol at ambient temperature for 72 hours, the extract was obtained. The extracts were filtered with Whatmann filter paper. The extract was separated from the solvent using a Soxhlet apparatus. The resulting extract was stored in an air-tight container in a refrigerator until it was required for GC-MS analysis [13].

2.3GC-MS Analysis

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 maintained at 7 eV with an ion source temperature stationed at 250 °C. Highly pure helium gas (99.9 % purity) was utilized as carrier gas, while HP-5ms (30mm X 0.25mm X 0.320µm) was utilized as the stationary phase. The oven temperature was programmed to begin with a temperature of 140 °C, held for 5 minutes at 4 °C per minute and allowed to reach a

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maximum temperature of 240 °C for 15 minutes, thereafter the temperature was maintained for another 6 minutes at the rate of 3.5 °C/minute.

2.4Identification of Phytochemical Compounds

Identification of phytochemical compounds and interpretation of mass spectrum GC-MS was conducted using the National Institute of Standard and Technology (NIST) database which contain more than 62,000 patterns. This was followed by comparing the spectrum of unknown component with the spectrum of the known component using computer searches on a NIST Ver.2.1 MS data library. Hence process establishes the name, structure and molecular weight of the components of the test materials

3. RESULTS

Table 1: GC-MS analysis of the n-hexane extract from *D. zibethinus* seeds

Number	Retention Time (min)	Name of the Compound	Molecular Formula	Composition (%)	Mass Spectral Data
1	14.684	Beta-Guaiene	$C_{15}H_{24}$	0.85	119,133,147,**161**,175
2	17.498	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	3.82	15,27,41,57,**74**
3	19.88	9,12-Octadecadienoic acid (ZZ), methyl ester	$C_{19}H_{34}O_2$	1.26	25,27,41,55,**67**
4	20.262	Phytol	$C_{20}H_{40}O$	1.36	27,41,57,**71**,95
5	20.382	Methyl stearate [Octadecanoic acid, methyl ester]	$C_{19}H_{38}O_2$	2.08	25,41,43,57,**74**
6	26.535	Lupeol	$C_{30}H_{50}O$	1.97	41,**43**,68,81,95
7	27.437	n-Tetracosanol-1	$C_{24}H_{50}O$	17.34	27,41,**55**,83,97
8	29.351	Squalene	$C_{30}H_{50}$	32.75	27,41,55,**69**,81
9	30.2345	1,30-Triacontanediol	$C_{30}H_{62}O_2$	34.47	25,41,**55**,69,82
10	30.348	1-Heptacosanol	$C_{27}H_{56}O$	1.89	27,41,**55**,83,97
11	30.635	Eicosane	$C_{20}H_{42}$	0.88	27,41,43,**57**,71
12	31.054	Hexadeca-2,6,10,14-tetraen-1-ol, 3,7,11,16-tetramethyl-	$C_{20}H_{34}O$	1.34	14,27,**41**,55,69

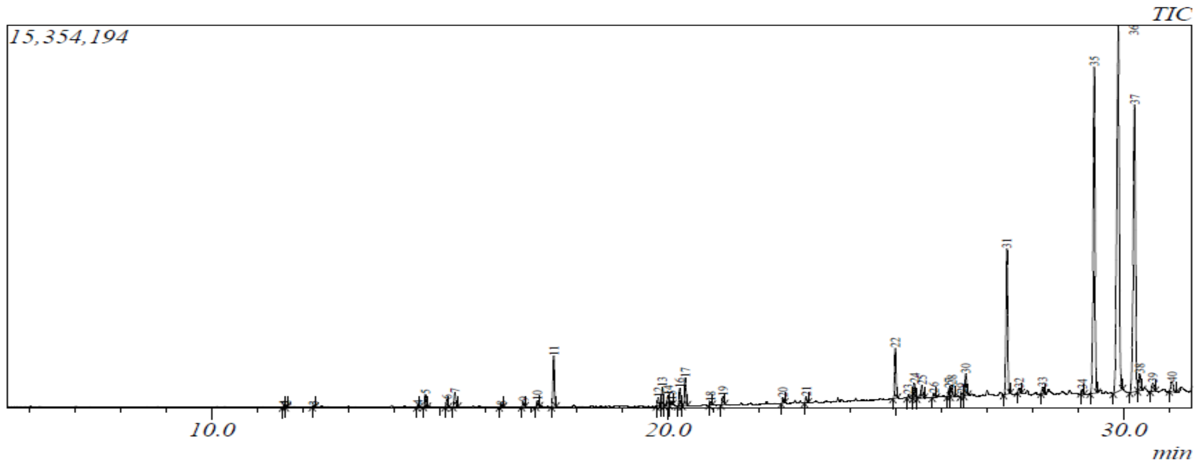

Figure1: GC-MS Chromatogram of n-hexane extract of *D. zibethinus* seeds

The mass spectra of the compounds with the highest percentage peak areas were also represented in *Figures2-13* respectively.

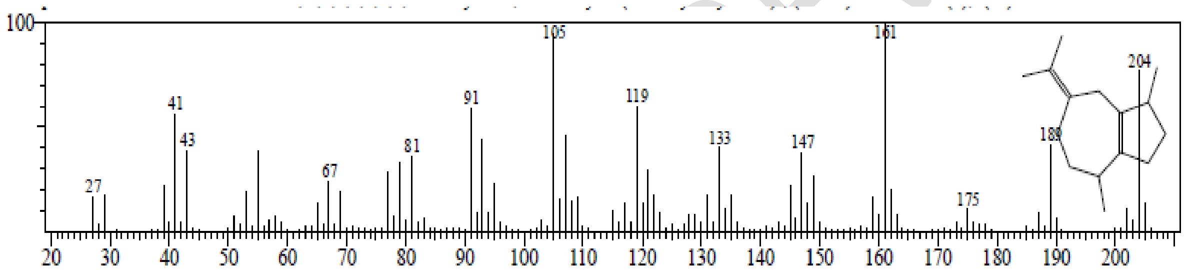

Figure2: Mass spectrum of Beta-Guaiene (RT: 14.684, Area % = 0.85) from*D. zibethinus* seeds

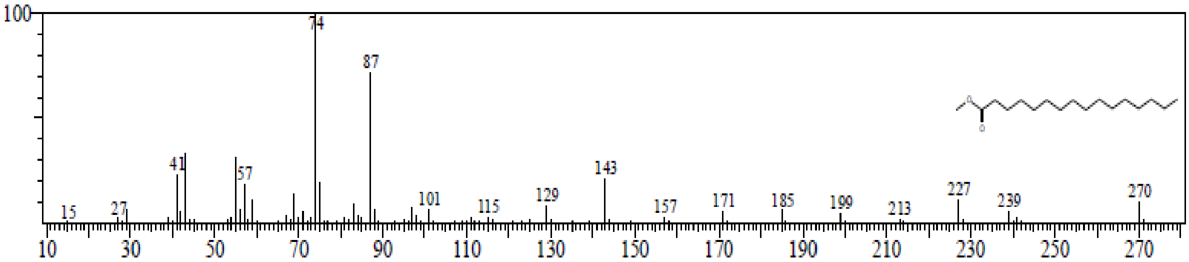

Figure3: Mass spectrum of Hexadecanoic acid, methyl ester (RT: 17.498, Area % = 3.82) from *D. zibethinus* seeds

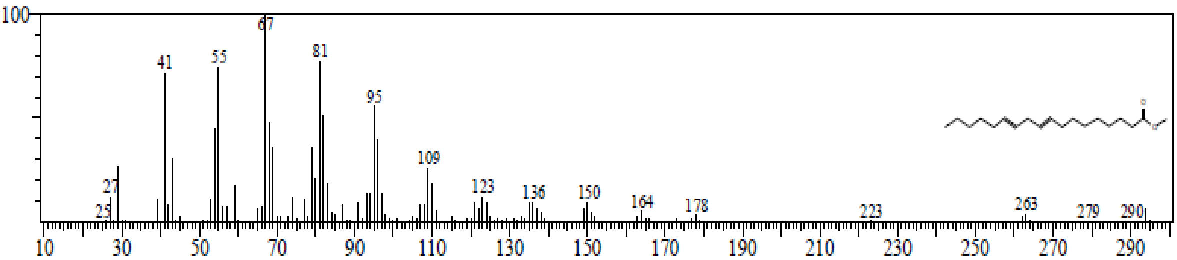

Figure4: Mass spectrum of 9,12-Octadecadienoic acid, methyl ester (RT: 19.881, Area % = 1.26) from *D. zibethinus* seeds

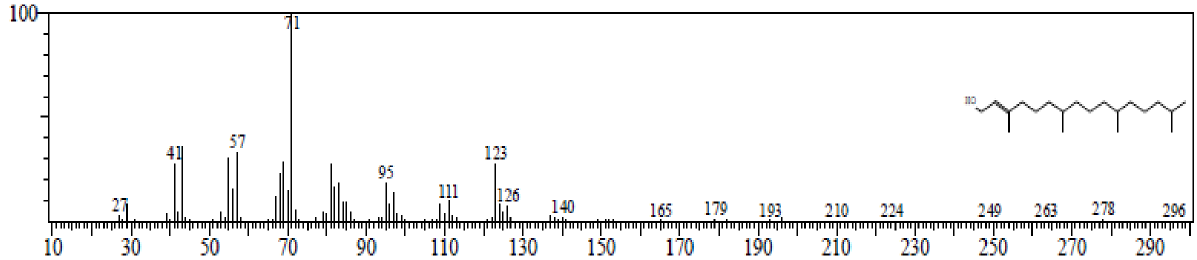

Figure5: Mass spectrum of Phytol (RT: 20.262, Area % = 1.36) from *D. zibethinus* seeds

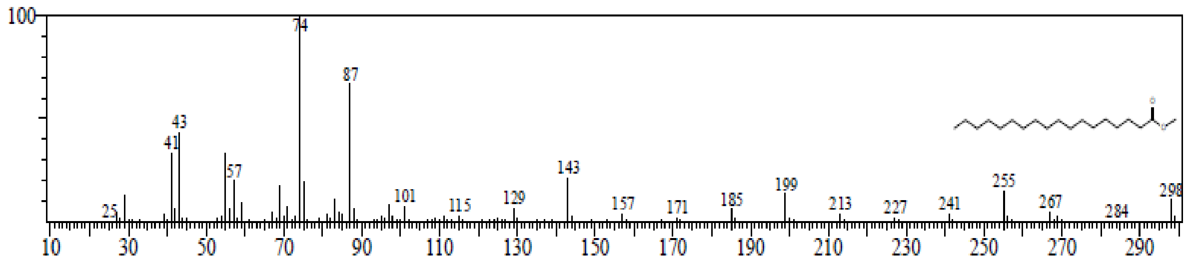

Figure6: Mass spectrum of Methyl stearate (RT: 20.382, Area % = 2.08) from *D. zibethinus* seeds

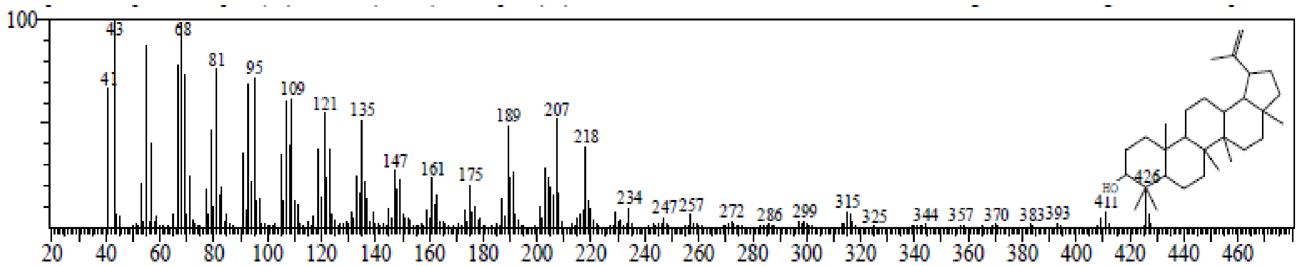

Figure7: Mass spectrum of Lupeol(RT: 26.535, Area % = 1.97) from *D. zibethinus* seeds

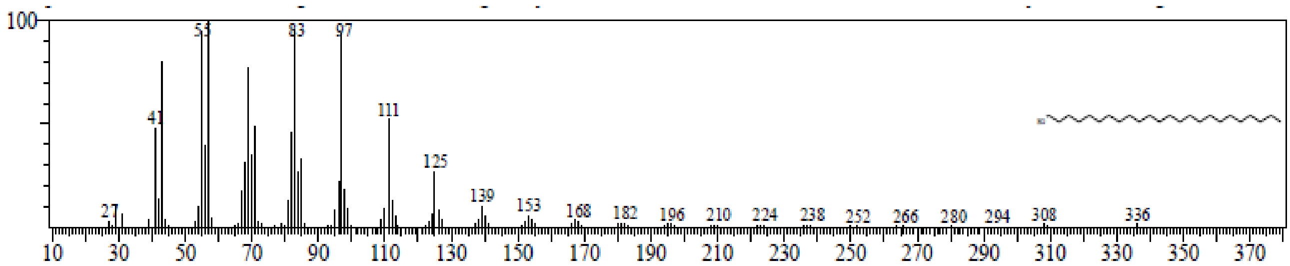

Figure8: Mass spectrum of n-Tetracosanol-1 (RT: 27.437, Area % = 17.34) from *D. zibethinus* seeds

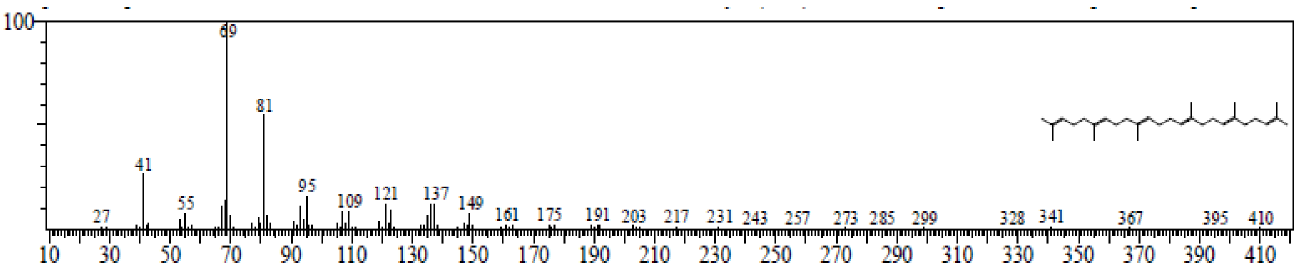

Figure9: Mass spectrum of Squalene (RT: 29.351, Area % = 32.75) from *D. zibethinus* seeds

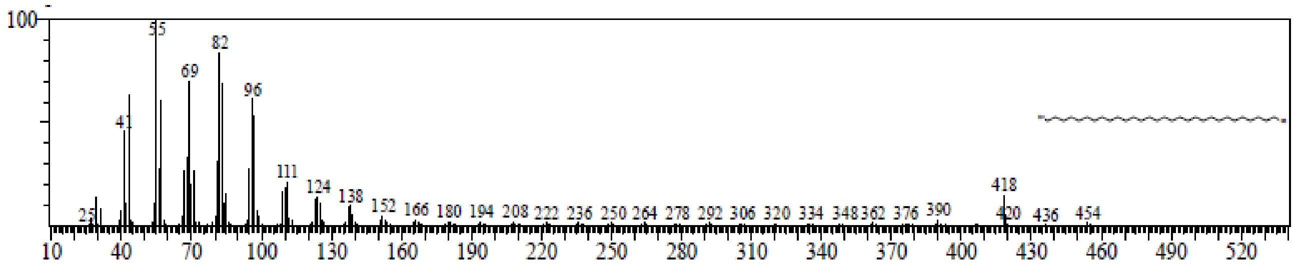

Figure10: Mass spectrum of 1,30-Triacontanediol (RT: 30.234, Area % = 34.47) from *D. zibethinus* seeds

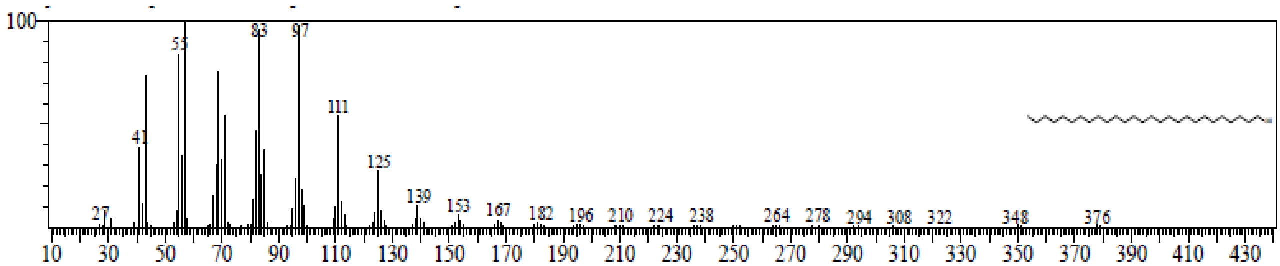

Figure11: Mass spectrum of 1-Heptacosanol (RT: 30.348, Area % = 1.89) from *D. zibethinus* seeds

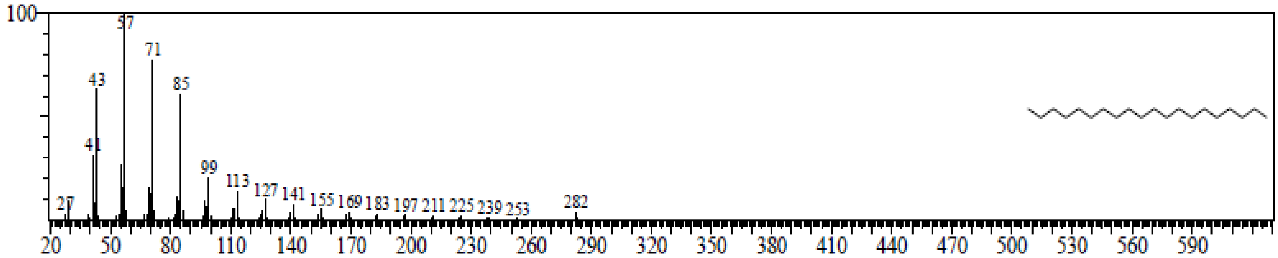

Figure12: Mass spectrum of Eicosane (RT: 30.635, Area % = 0.88) from *D. zibethinus* seeds

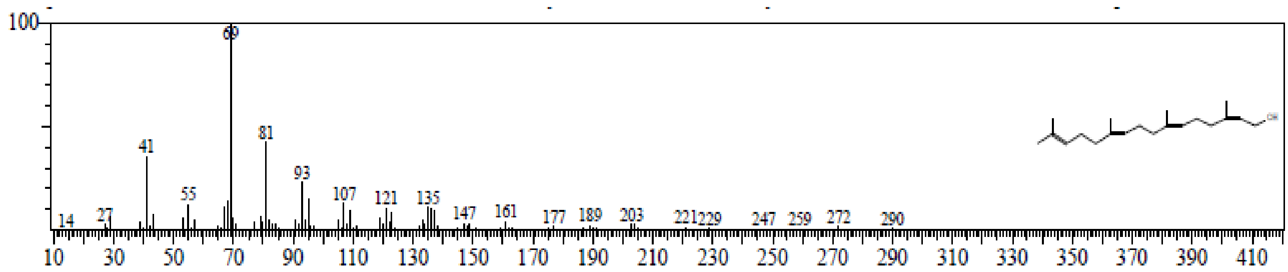

Figure13: Mass spectrum of Hexadeca-2,6,10,14-tetraen-1-ol, 3,7,11,16-tetramethyl- (RT: 31.054, Area % = 1.34) from *D. zibethinus* seeds

4. DISCUSSION

4.1 GC-MS Analysis

GC-MS is one of the leading techniques to identify the constituents of volatile matter, long chain, branched chain hydrocarbons, alcohols, acids, esters etc. Twelve compounds (phytochemical constituents) were identified in the n-hexane extract of *D. zibethinus* seeds through GC-MS analysis. The chromatogram peaks were integrated and thereafter compared with the database of spectrum of known compounds stored in the GC-MS NIST library.The chemical constituents identified are beta-Guaiene (0.85%), Hexadecanoic acid, methyl ester (3.82%), 9,12-Octadecadienoic acid, methyl ester (1.26 %), Phytol (1.36 %), Methyl stearate (2.08 %), Lupeol (1.97 %), n-Tetracosanol-1 (17.34 %), Squalene (32.75 %), Triacontanediol (34.47 %), 1-Heptacosanol (1.89 %), Eicosane (0.88 %) and Hexadeca-2,6,10,14-tetraen-1-ol, 3,7,11,16-tetramethyl- (29.34 %).

Figure 1 shows the GC-MS chromatogram of the peak of the compounds detected. The fragmentation pattern and molecular formula were used to confirm the identification of the phytochemical compounds. The retention time (RT), percentage composition and mass spectral data of active principles are presented in *Table 1*.

4.2 Biological Activities of the Phytochemicals Detected through GC-MS Analysis

The biological roles of phytochemicals in human metabolism has long been established. Phytochemicals are very active constituents and are abundant in nature. They are grouped and have significant roles in preventing various diseases. The use of these phytochemicals is done in a combination of multiple phytochemicals and other drugs as well [14, 15].

Phytochemicals exhibit a wide range of therapeutic roles, including antioxidant, anti-inflammatory, anti-diabetic, analgesic, anti-cancer, neuroprotective, and anti-microbial activities [16,17,18,19]. Most phytochemical constituents identified in *D. zibethinus* seeds are terpenoids, fatty acid esters, terpene alcohols,triterpenoids, and fatty alcohols that contribute to the antibacterial,antifungal, antioxidant, anti-inflammatory activities etc. Among the phytochemicals identified in *D. zibethinus* seed oil are as follows:

Beta-Guaiene (**1**)is a sesquiterpenoid. It has a bicyclic structure with a cyclohexene ring and a cyclopropane ring. Beta-Guaiene has been shown to exhibit various biological activities, including antimicrobial, anti-inflammatory, and anticancer activities [20].

Hexadecenoic acid methyl ester (**2**)is a saturated fatty acid methyl ester that shows antioxidant, antitumor, immunostimulant, chemopreventive and lipoxygenase inhibitory, antimicrobial activities and it is a potent mosquito larvicide [21].9,12-octadecadienoic acid

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(Z,Z)-, methyl ester (**3**) is also a saturated fatty acid methyl ester that shows antioxidant [22] and anti-inflammatory activities [23,24]. Methyl stearate or Octadecanoic acid, methyl ester (**5**)is another saturated fatty acid methyl ester identified in *D. zibethinus* seed oil. Mazumder et al. [25] have reported that methyl stearate or octadecanoic acid, methyl ester has anti-inflammatory, intestinal lipid metabolism regulation, nematicidal, antinociceptive, antioxidant, antimicrobial and antifungal activities.

Phytol (**4**) is a diterpene member of the long-chain unsaturated acyclic alcohols and exert a wide range of biological effects. It has been reported that phytol is a potential candidate for a broad range of applications in the pharmaceutical and biotechnological industry. Recent investigations with phytol demonstrated anxiolytic, metabolism-modulating, cytotoxic, antioxidant, autophagy- and apoptosis-inducing, antinociceptive, anti-inflammatory, anti-cancer, immune-modulating, and antimicrobial effects [26,27,28].Another diterpenoid alcohol detected in *D. zibethinus* seed oil wasHexadeca-2,6,10,14-tetraen-1-ol, 3,7,11,16-tetramethyl- (**12**). It has been reported that Hexadeca-2,6,10,14-tetraen-1-ol, 3,7,11,16-tetramethyl- shows antimicrobial, diuretic and anti- inflammatory activities [29,30].

Lupeol is apentacyclic triterpenoid that showsanti-malarial, anti-microbial, anti-angiogenic, as well as anti-inflammatory and antiarthritic activities. Lupeol has also been reported as an effective higher blood-brain barrier permeability [31]. Lupeol inhibits neuroinflammation in cerebellar cultures and induces neuroprotection associated with the modulation of astrocyte response and expression of neurotrophic and inflammatory factors [32].

The presence of important alcoholic compound detected in *D. zibethinus* seed oil was n-Tetracosanol-1 (**7**). This is a fatty acid derivative of lignoceric acid that shows antimicrobial, antioxidant, anticancer, antimutagenic and nematicidal activities [33]. It also lowers cholesterol and enhances immune functions [34]. n-Tetracosanol-1 is one of the constituents of policosanol which modifies several cardiovascular disease risk factors by reducing LDL oxidation, platelet aggregation and endothelial cell damage [35]. Another alcoholic compound detected in *D. zibethinus* seed oil was1-Heptacosanol (**10**). Researchers have observed that 1-heptacosanolhave antimicrobial and antioxidant activities [36,37].

Squalene (**8**)belongs to the class of triterpene hydrocarbon, and its known to be a biochemical precursor for all steroids in plants and animals. It has several applications in the food, pharmaceutical, and medical sectors. It is essentially used as a dietary supplement, vaccine adjuvant, moisturizer, cardio-protective agent, anti-tumor agent and antioxidant [38]. Squalene has been reported to help in the synthesis of cholesterol, effective as skin emollient, scavenging free radicals, and inhibit the fungal growth [39].

Eicosane (**11**) is a long-chain hydrocarbon and has been reported for its antibacterial,anti-inflammatory, analgesic, and antipyretic activity [40,41,42,43]. In addition, eicosane may promote wound healing due to their potent free radical scavenging, hydroxyproline and glutathione action (antioxidant) [44,45]. From GC-MS Analysis results, it is obvious that *D. zibethinus seed* accumulates essential phytochemicals, hence, the essential oil extracted from the seed of *D. zibethinus* Murr. can be used in developing new leads of therapeutic interest.

5. CONCLUSION

In conclusion,the bioactive phytochemicals found in*D. zibethinus* Murr. seeds are abundant and essential for maintaining human health and wellbeing. According to these studies, the essential oil extracted from the seeds of *D. zibethinus* Murr.may be useful in the formulation of nutritional and/or therapeutic products. This research has contributed to the global effort to encourage the usageof herbal drugs from natural sources which has been generally accepted as safer drugs to synthetic ones.

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