

**Tropical Journal of Phytochemistry & Pharmaceutical Sciences**Available online at <https://www.tjpps.org>**Original Research Article****Phytochemical Constituents, GC-MS Analysis, and Antifungal Activity of Ethanol Extract of *Enantia chlorantha* Stem Bark**Ezekiel G. Adeyeni<sup>1\*</sup>, Ezekiel T. Ayodele<sup>2</sup>, Olusola N. Majolagbe<sup>3</sup>, Ibidotun T. Olawoore<sup>4</sup>, Ruth A. Adeyeni<sup>2</sup><sup>1</sup>Department of Chemistry, Hallmark University, Ijebu-Itele, Nigeria<sup>2</sup>Department of Pure and Applied Chemistry, Ladoké Akintola University of Technology, Ogbomoso, P.M.B. 4000, Nigeria<sup>3</sup>Department of Pure and Applied Biology, Ladoké Akintola University of Technology, Ogbomoso, P.M.B. 4000, Nigeria<sup>4</sup>Department of Chemistry, Ahmadu Bello University, Zaria, Kaduna State, Nigeria**ABSTRACT**

The use of plant-derived compounds in traditional medicine has a long history. One such plant, the *Enantia chlorantha* tree, which can grow up to thirty meters tall, is known for its wide range of medicinal uses. This study investigated the potential antifungal effects of an ethanol extract from the stem bark of *Enantia chlorantha*, along with the phytochemicals contained in the extract. The extraction was performed using ethanol through maceration of the powdered bark. After conducting qualitative phytochemical analysis, the extract was further examined using gas chromatography-mass spectrometry (GC-MS) to identify the compounds present. To assess the antifungal effectiveness of the extract, the mycelial growth inhibition method was used against three fungal species: *Aspergillus flavus*, *Aspergillus fumigatus*, and *Aspergillus niger*. Phytochemical screening revealed several classes of bioactive compounds, including alkaloids, glycosides, flavonoids, tannins, anthraquinones, reducing sugars, phenols, steroids, phlobatannins, and terpenoids. GC-MS analysis identified 28 different compounds in the ethanol extract. Among the most abundant were 1-octadecene (8.24%), 7-hexadecene (6.59%), 9-eicosene (7.52%), bis(2-ethylhexyl) phthalate (5.40%), cyclotetradecane (5.51%), caryophyllene oxide (6.10%), and 1,2-benzenedicarboxylic acid dipropyl ester (8.56%). The extract exhibited strong antifungal activity, inhibiting the growth of *Aspergillus flavus* (85.96%), *Aspergillus fumigatus* (75.67%), and *Aspergillus niger* (85.04%). These findings suggest that *Enantia chlorantha* could be a promising natural source of antifungal agents, particularly effective against *Aspergillus* infections.

**Keywords:** *Enantia chlorantha*, Phytoconstituents, Antifungal Activity, GC-MS

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Herbal medicines have been a part of traditional medicine for a very long time, and they have been used to treat a wide variety of illnesses. For a very long time, many traditional medicinal practices have relied on herbs<sup>1</sup>. Since the dawn of human civilisation<sup>2</sup>, people have been using natural remedies that are produced from plants for the goal of providing medical care. Two of the many low-cost goals of medicinal plant research are the development of therapeutic chemicals and the discovery of prototype pharmaceuticals. Also included in this list is the development of therapeutic chemicals<sup>3</sup>. It is believed that the secondary metabolites of plants, which are complex chemical components, are the source of the medicinal effects that plants have. Flavonoids, essential oils, glycosides, triterpenes, saponins, tannins, and polysaccharides are some of the components that fall into this category<sup>4,5</sup>.

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It has been proven in a number of studies that extracts from medicinal plants may possess antibacterial activities against a wide variety of *fungus* and antibiotic-resistant bacteria<sup>6</sup>. The ornamental tree *Enantia chlorantha*, which belongs to the family *Annonaceae* and is indigenous to the woodlands of Nigeria, may be found primarily along the beaches of Central and West Africa<sup>7</sup>. It is a member of the *Annonaceae* family. With its eye-catching yellow slash and distinctive black fruits, this ornamental tree has the potential to grow to a height of thirty meters and is well-known for its appearance<sup>8</sup>. As a result of the herb's purported medical characteristics, it has been utilised by individuals to treat a variety of ailments, including typhoid fever, jaundice, rickettsia fever, cough, and wounds<sup>9</sup>. In an aqueous extract, the water-soluble alkaloids of the plant have demonstrated capabilities that include the ability to alleviate pain, reduce fever, combat germs, and effectively combat malaria. Additionally, the extracts have the ability to heal wounds, ulcers, and leprosy areas<sup>8</sup>. Using *Aspergillus flavus*, *Aspergillus fumigatus*, and *Aspergillus niger* as test subjects, an ethanol extract was prepared from the stem bark of *Enantia chlorantha* and examined for its ability to inhibit the growth of these three types of black mould. A GC-MS analysis was performed in order to ascertain the chemical composition of the extract.

## Materials and Methods

### Collection and identification of plant material

A collection of stem bark from the *Enantia chlorantha* tree was collected by researchers at Ijebu Itele, which is located in Ogun State, southwest Nigeria, in the month of November 2022. The FRIN Medicinal Plant Section in Jericho, Ibadan, Oyo State, Nigeria, was responsible for cataloguing the botanical specimens. A voucher with the number FRI NO 382 is used to identify the specimen of herbarium that was just deposited.



**Figure 1:** *Enantia chlorantha* in its natural habitat showing the leaves and stem<sup>7</sup>

### Preparation of extract

A coarse powder was then milled from the stem bark after it had been washed with distilled water, let to air-dry for a period of 28 days, and then eventually milled. The extraction of 500 grammes of powdered stem bark was accomplished by macerating the material in four litres of ethanol at room temperature for three days while continuously agitating the mixture. To obtain a concentrated extract, the remaining liquid was subjected to a reduction in pressure and then evaporated in a rotary evaporator. This was done after the ethanol extract had been filtered.

### Qualitative phytochemical screening of plant extract

In order to evaluate the phytochemical components of the *Enantia chlorantha* extract, the same methodologies<sup>10</sup> outlined were utilised. There were ten different phytochemicals that were investigated, and some of them include tannins, phlobatannins, steroids, terpenoids, saponins, alkaloids, glycosides, phenols, flavonoids, anthraquinones, and reducing sugars.

### GC-MS analysis

For the purpose of analysing the extract, Shimadzu's QP-2010 Ultra, which is a gas chromatography-mass spectrometry (GC-MS) apparatus, was utilised. For the purpose of conducting the analysis in scan mode, the DB-5MS capillary column was utilised. This column had a film thickness of 0.25 µm, an internal diameter of 0.25 mm, and a length of 30 meters. Helium was used as the carrier gas at a flow rate of 0.72 mL/min, with a total flow rate of up to 31.8 mL/sec. This allowed for the specified linear velocity to be maintained. One litre per minute was the flow rate that was used for purging. The temperature at the injection port was kept at 250 degrees Celsius, while the temperature at the detector was kept at 300 degrees Celsius. The temperature of the oven was raised from sixty to three hundred degrees Celsius at a pace of ten degrees Celsius per minute without any breaks, and then it was kept at that temperature for sixteen minutes. The volume of the injection was 1 microlitre, and it was administered using a splitless injection technique.

### Evaluation of antifungal activity

#### Collection and maintenance of test microbes

This investigation made use of three different types of fungi: *Aspergillus fumigatus*, *Aspergillus niger*, and *Aspergillus flavus*. These

fungi were taken from organisms that had been cultured in the past and were stored in a controlled environment at the Ladoké Akintola University of Technology (LAUTECH) Teaching Hospital in Ogbomoso, which is located in the state of Oyo in Nigeria. Both the fungus and the agar slants were stored in McCartney bottles, and the bottles were placed in the refrigerator.

#### Antifungal activity evaluation of *Enantia chlorantha* stem bark extract

The effectiveness of the plant extract as an antifungal agent was evaluated by the use of the mycelial growth inhibition method<sup>11</sup>. The extract was added to each of the potato dextrose agar (PDA) plates at a concentration of three hundred and fifty microgrammes per millilitre. The plates were then infected with agar plugs of 6 millimetres in diameter that contained cultures of *Aspergillus niger*, *Aspergillus flavus*, and *Aspergillus fumigatus* that had been incubated for a period of 48 hours. Individuals who were assigned to the negative control group were given plates that were devoid of any extract. Every plate was maintained at a temperature of 28 ± 2 degrees Celsius for a period of seventy-two hours. Following the completion of the incubation time, we determined the percentage of mycelial development inhibition by measuring the diameter of the mycelial growth on the PDA plates and then applying the calculation that is presented below.

$$\text{Percentage of mycelial growth inhibition} = \frac{D_{\text{control}} - D_{\text{test}}}{D_{\text{control}}} \times \frac{100}{1}$$

Where: D is the Diameter of fungal growth on the plates

## Results and Discussion

### Phytochemical constituents of *Enantia chlorantha* stem bark extract

Table 1 presents the results of a phytochemical analysis conducted on the ethanol extract of the stem bark of *Enantia chlorantha*. The analysis revealed the presence of a variety of compounds, including metabolites, alkaloids, glycosides, tannins, anthraquinones, reducing sugars, phenols, steroids, phlobatannins, and terpenoids. It is common knowledge that these phytochemical components possess antioxidant properties in addition to an assortment of other beneficial qualities. Research has demonstrated that flavonoids have the ability to efficiently neutralise a number of oxidising chemicals, such as free radicals and singlet oxygen<sup>12</sup>, which are linked to a wide range of disorders. Flavonoids have been demonstrated in a number of studies to possess protection against mucosal inflammation<sup>13</sup> and antioxidant properties<sup>14, 15</sup>.

**Table 1:** Phytochemical composition of ethanol extract of *Enantia chlorantha* stem bark

| Phytoconstituent | Inference |
|------------------|-----------|
| Alkaloids        | +         |
| Glycosides       | +         |
| Flavonoids       | +         |
| Tannins          | +         |
| Saponins         | +         |
| Terpenoids       | +         |
| Phenols          | +         |
| Steroids         | +         |
| Anthraquinones   | +         |
| Reducing Sugars  | +         |
| Phlobatannins    | +         |

‘+’ indicate the presence of phytoconstituents

Vegetables that are high in flavonoids are widely employed as functional meals because of the potential impact they could play in the treatment of complications related to the cardiovascular system<sup>16</sup>. It is common knowledge that these vegetables have a high bioavailability,

and it is also recognised that frequent ingestion is connected with considerable plasma concentrations of flavonoids, which have a number of positive impacts on health<sup>17</sup>. Additional studies have demonstrated that flavonoids have the potential to shield the heart from the effects of ischaemia reperfusion<sup>18, 19</sup>. How many there are Tannins lessen the mucosa's sensitivity to chemical irritation, and saponins activate protective mechanisms in the membrane. Tannins and saponins work together to protect mucosa against chemical irritation. Not only does this process lessen the amount of acid that is produced, but it also lessens inflammation, has astringent properties, and safeguards the lining of the stomach membrane. It has been shown that terpenoids and alkaloids are quite effective in treating stomach ulcers<sup>20, 21</sup>. Terpenoids have been proven to have the ability to relax the smooth muscles of the cardiovascular system in two different ways. The first method involves blocking calcium ions from entering these muscles. The second method involves reducing the activity of reactive oxygen species (ROS) and enhancing the creation of nitric oxide of the cardiovascular system<sup>22</sup>.

*Compounds identified from the GC-MS analysis of ethanol extract of Enantia chlorantha stem bark*

Figure 2 is a chromatogram that illustrates the results of the GC-MS analysis performed on the ethanol extract of the stem bark of *Enantia chlorantha*. It was found that there were twenty-eight peaks, each of which indicated a different phytochemical organisation. Through the process of determining the area percentage and retention time (RT), the peaks were ultimately discovered. Take a look at the phytoconstituents that are reported in Table 2 for the ethanol extract of the stem bark of *Enantia chlorantha*. There have been isolated the following chemical

substances: The compounds 7-hexadecene, 5-methyl-2-ethenyl-cyclohexane, 9-octadecene, and hexadecan-2-one are discussed in Section 13.1.0 of the bicyclo section. Listed below are the chemicals that are present: Cyclopentanol, cis-Z-alpha, 9-eicosene, 1,5-heptadiyne, and 1-(1-methylene-2-propenyl) are the compounds in question. -. 2S, 3S, 6S bisabolene epoxide 2, 3-dimethylpentane-2-(1-propen-2-yl) was (1-Formyl-2, 2, 6-trimethyl), Alloaromadendrene oxide-(1), 1, 2-Di-but-2-enyl-cyclohexane, 1-Octadecene, Diazoprogerone, Caryophyllene oxide, and -3-vinylcyclohexanone. Three-methyl-but-2-enyl, also known as -6-cyclohexene Cyclotetradecane, Aminosalicic Acid, and 1, 2-Benzenedicarboxylic Acid are the components that make up this product. Oleic Acid, 3-Octadecene, Dipropyl Ester 8-Hexadecenal, 14-methyl-,

Cyclododecanone, 2-methylene-, and Bis (2-ethylhexyl) phthalate are the compounds that are included in this compound. Some data suggests that these compounds may have potential applications in medicine, including the ability to act as antioxidants, anti-ulcer agents, and anti-inflammatory agents. Several of the bioactive components that have been reported in the literature 4-methyl-2-ethenyl-cyclohexane-1-carboxylic acid, cis-Z-alpha is the chemical formula. The compounds bisabolene epoxide, caryophyllene oxide, and oleic acid each have their own unique set of properties, including analgesic, anti-inflammatory, antibacterial, cytotoxic, anticancer, and antioxidant properties. A substance known as<sup>23, 24, 25</sup> Bis (2-ethylhexyl) phthalate is utilised in order to safeguard reproductive and endocrine processes and to alleviate issues regarding health<sup>26</sup>.

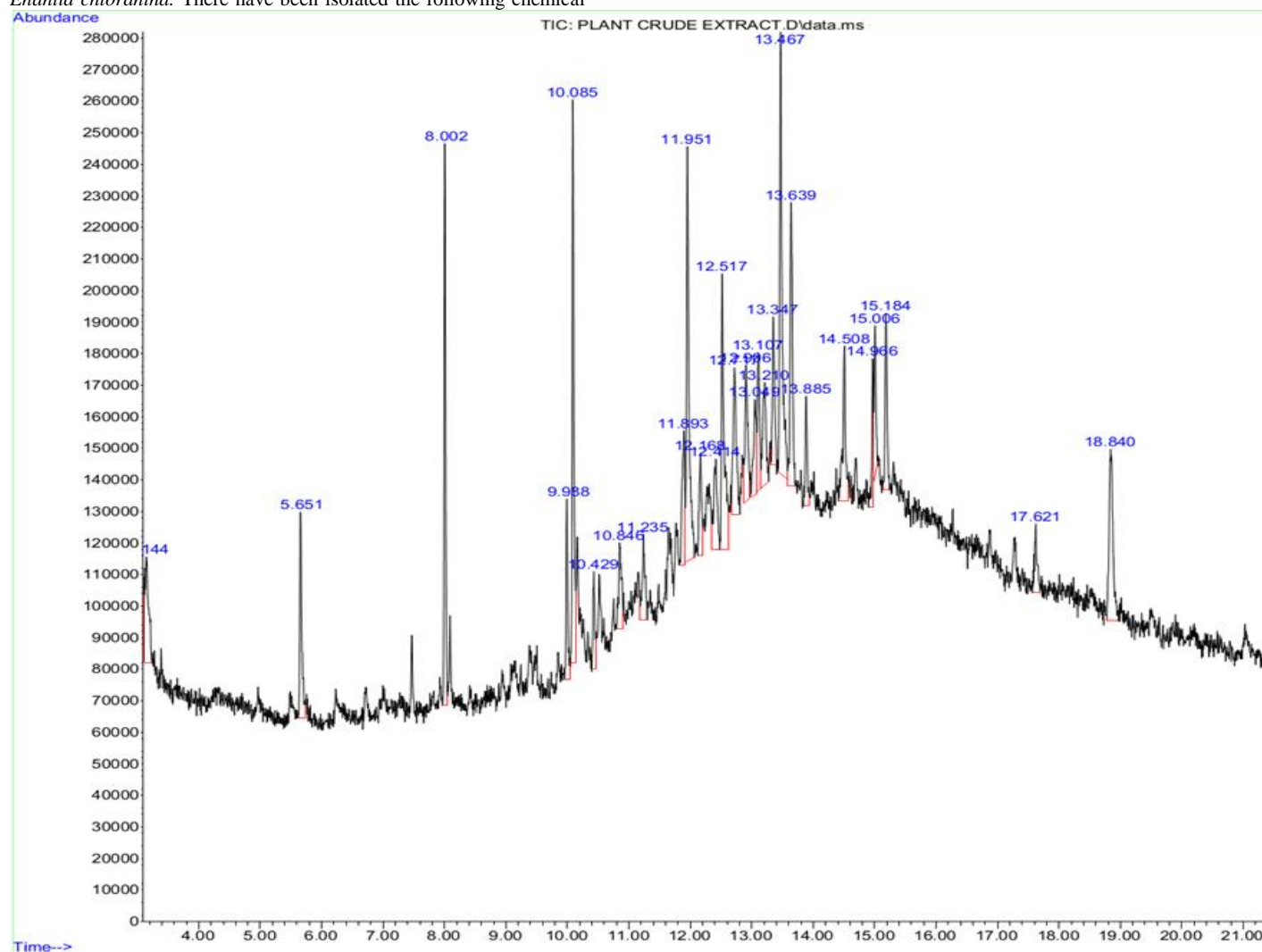
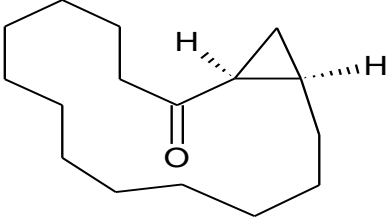
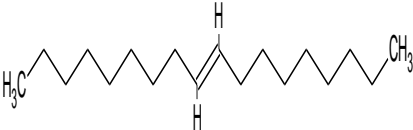
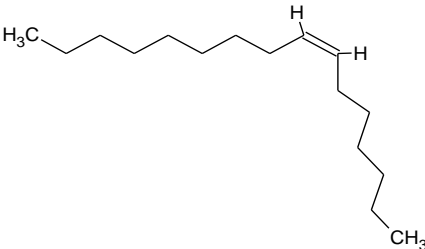
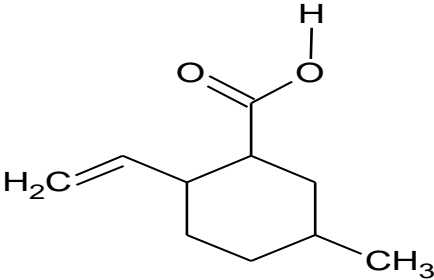
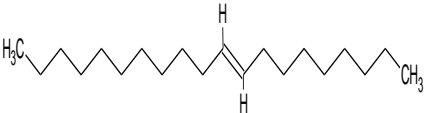
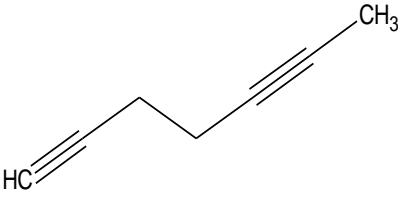

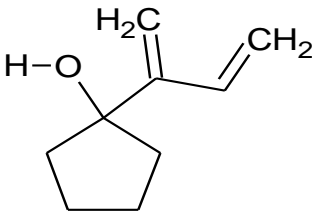
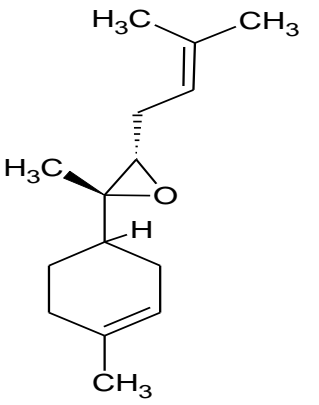
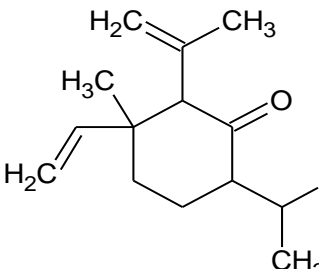
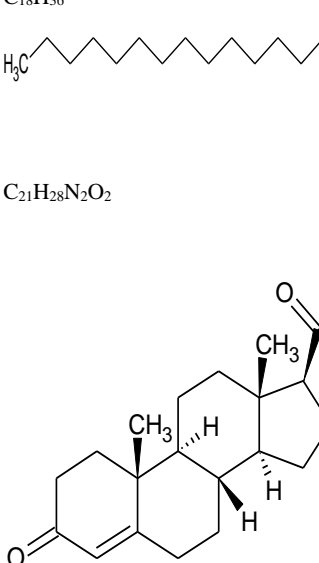

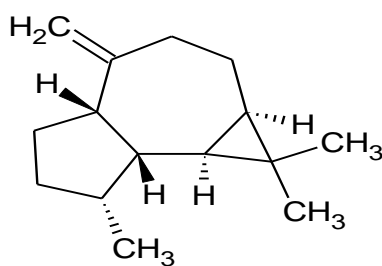
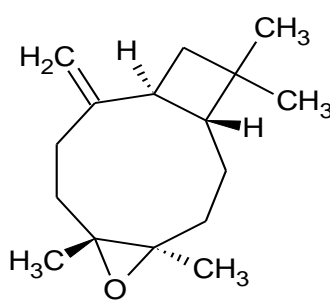
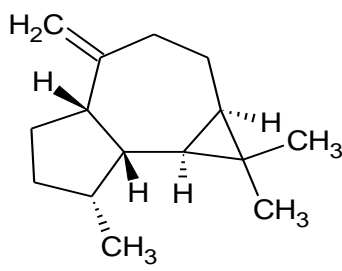
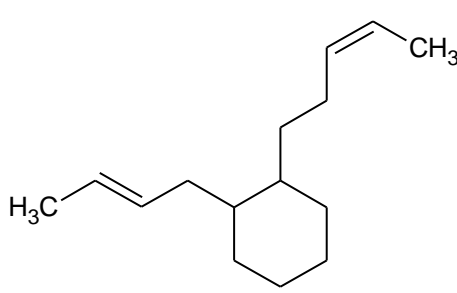
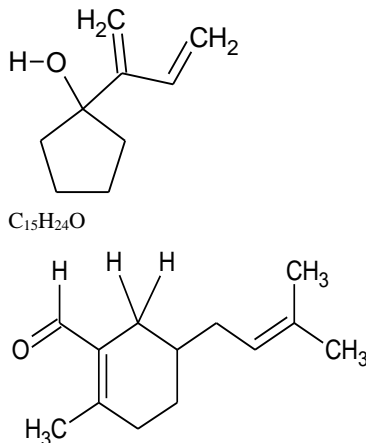


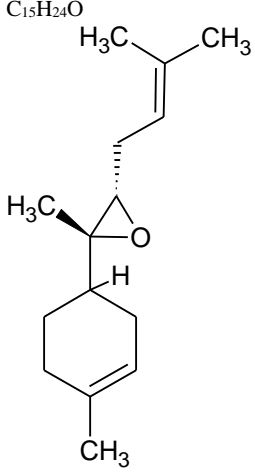
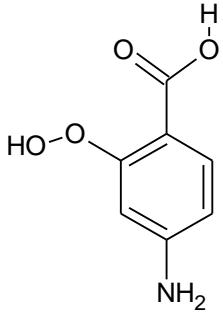
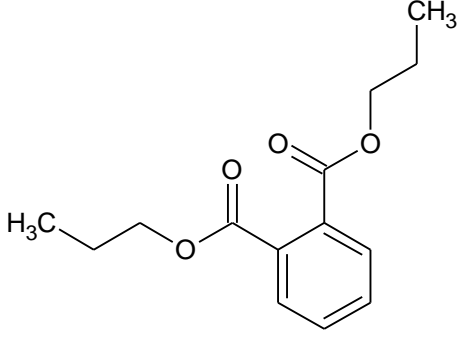
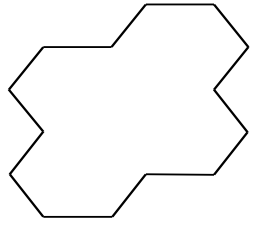
Figure 2: GC chromatogram of ethanol extract of *Enantia chlorantha* stem bark

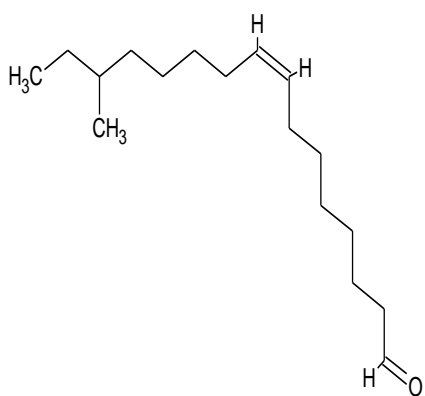
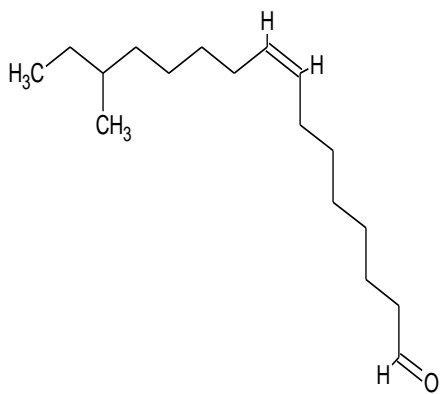
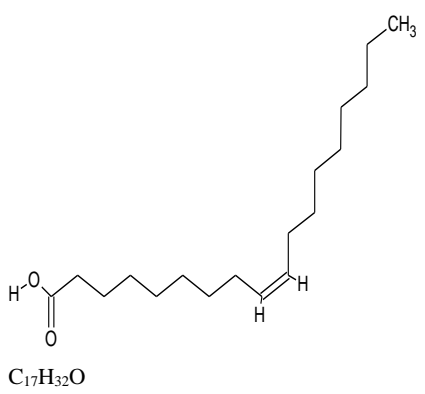
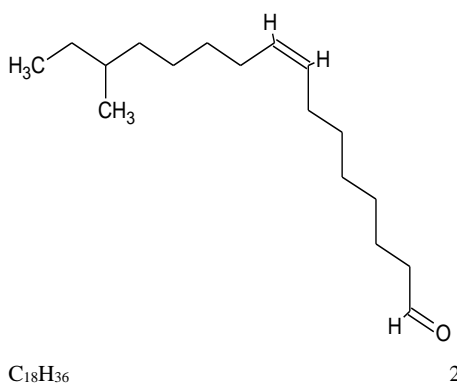

**Table 2:** Compounds identified from the GC-MS analysis of the ethanol extract of *Enantia chlorantha* stem bark

| S/N | RT     | Name of Compound                                 | Molecular Formula/<br>Chemical Structure   | Molecular Weight | Peak<br>Area<br>(%) |
|-----|--------|--|--|------------------|---------------------|
| 1   | 3.144  | (1S,15S)-Bicyclo[13.1.0]hexadecan-2-one          |    | 236.39           | 2.97                |
| 2   | 5.651  | 9-Octadecene                                     |    | 252.5            | 3.35                |
| 3   | 8.002  | 7-Hexadecene                                     |   | 224.42           | 6.59                |
| 4   | 9.988  | 5-Methyl-2-ethenyl-cyclohexane-1-carboxylic acid |  | 168.23           | 2.41                |
| 5   | 10.085 | 9-Eicosene                                       |  | 280.5            | 7.52                |
| 6   | 10.429 | 1, 5-Heptadiyne                                  |  | 92.14            | 1.47                |
| 7   | 10.846 | Cyclopentanol, 1-(1-methylene-2-propenyl)-       |   | 138.21           | 2.12                |

|    |        |   |  |                      |        |      |
|----|--------|---|--|----------------------|--------|------|
| 8  | 11.235 | cis-Z-.alpha.-Bisabolene epoxide  |    | $C_{15}H_{24}O$      | 220.25 | 1.79 |
| 9  | 11.893 | (2S,3S,6S)-6-Isopropyl-3-methyl-2-(prop-1-en-2-yl)-3-vinylcyclohexanone |    | $C_{15}H_{24}O$      | 220.35 | 2.87 |
| 10 | 11.951 | 1-Octadecene  |   | $C_{18}H_{36}$       | 220.35 | 8.24 |
| 11 | 12.168 | Diazoprogestosterone  |  | $C_{21}H_{28}N_2O_2$ | 340.5  | 2.01 |
| 12 | 12.414 | Alloaromadendrene oxide-(1)   |  | $C_{15}H_{24}$       | 204.35 | 2.60 |

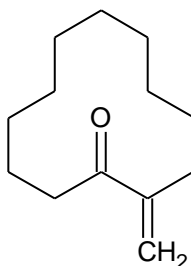
|    |        |  |                 |  |      |  |
|----|--------|--|-----------------|--|------|--|
|    |        |  |                 |    |      |  |
| 13 | 12.517 | Caryophyllene oxide  | $C_{15}H_{24}O$ | 220.35   | 6.10 |  |
|    |        |  |                 |    |      |  |
| 14 | 12.717 | Alloaromadendrene oxide-(1)                                    | $C_{15}H_{24}$  | 204.35   | 3.84 |  |
|    |        |  |                 |   |      |  |
| 15 | 12.906 | 1,2-Di-but-2-enyl-cyclohexane                                  | $C_{14}H_{24}$  | 192.34   | 2.83 |  |
|    |        |  |                 |  |      |  |
| 16 | 13.049 | Cyclopentanol, 1-(1-methylene-2-propenyl)-                     | $C_9H_{14}O$    | 138.21   | 2.31 |  |
|    |        |  |                 |  |      |  |
| 17 | 13.107 | 1-Formyl-2,2,6-trimethyl-3-(3-methyl-but-2-enyl)-6-cyclohexene | $C_{15}H_{24}O$ | 220.35   |      |  |

|  |        |   |  |        |      |
|--|--------|---|--|--------|------|
| 18   | 13.210 | cis-Z-.alpha.-Bisabolene epoxide            | C <sub>15</sub> H <sub>24</sub> O              | 220.35 |      |
|     |        |   |  |        |      |
| 19   | 13.347 | Aminosalicylic Acid                         | C <sub>7</sub> H <sub>7</sub> NO <sub>3</sub>  | 153.14 | 2.61 |
|    |        |   |  |        |      |
| 20   | 13.467 | 1,2-Benzenedicarboxylic acid dipropyl ester | C <sub>14</sub> H <sub>18</sub> O <sub>4</sub> | 250.29 | 8.56 |
|  |        |   |  |        |      |
| 21   | 13.639 | Cyclotetradecane                            | C <sub>14</sub> H <sub>28</sub>                | 196.37 | 5.51 |
|   |        |   |  |        |      |
| 22   | 13.885 | 8-Hexadecenal, 14-methyl-, (Z)-             | C <sub>17</sub> H <sub>32</sub> O              | 252.4  | 1.77 |

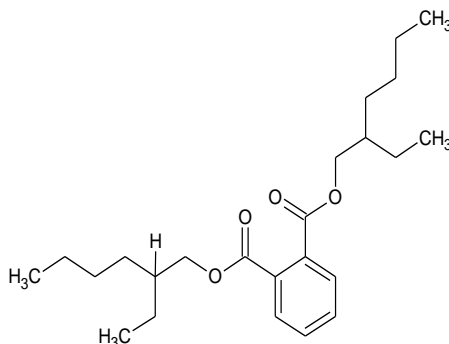
|    |        |                        |             |  |  |      |  |
|----|--------|------------------------|-------------|--|--|------|--|
|    |        |                        |             |  |    |      |  |
| 23 | 14.508 | 8-Hexadecenal,<br>(Z)- | 14-methyl-, | MW: 252.4<br>C <sub>17</sub> H <sub>32</sub> O | 252.4  | 3.58 |  |
|    |        |                        |             |  |   |      |  |
| 24 | 14.966 | Oleic Acid             |             | C <sub>18</sub> H <sub>34</sub> O <sub>2</sub> | 282.46   | 2.03 |  |
|    |        |                        |             |  |  |      |  |
| 25 | 15.006 | 8-Hexadecenal,<br>(Z)- | 14-methyl-, | C <sub>17</sub> H <sub>32</sub> O              | 252.4  | 2.01 |  |
|    |        |                        |             |  |  |      |  |
| 26 | 15.184 | 3-Octadecene, (E)-     |             | C <sub>18</sub> H <sub>36</sub>                | 252.5  | 2.89 |  |
|    |        |                        |             |  |  |      |  |



27      15.184      Cyclododecanone, 2-      C<sub>13</sub>H<sub>22</sub>O      194.31      1.48  
methylene-



28      17.621      Bis(2-ethylhexyl) phthalate      C<sub>14</sub>H<sub>38</sub>O<sub>4</sub>      390.6      5.40



#### Antifungal activity of ethanol extract of *Enantia chlorantha* stem bark

An ethanol extract of the stem bark of *Enantia chlorantha* was found to have a significant inhibitory effect on the mycelial growth of the three test fungi, namely *Aspergillus flavus*, *Aspergillus fumigatus*, and *Aspergillus niger*. The level of inhibition that the *E. chlorantha* stem bark extract had on the fungus that was being tested was significantly higher than that of the control, which was the normal therapy. The control group that was administered according to standard methods demonstrated a growth suppression of 73.50 percent against *Aspergillus flavus*, 67.33 percent against *Aspergillus fumigatus*, and 72.88 percent against *Aspergillus niger*. The results shown in Table 3 demonstrate that the extract of *E. chlorantha* has a much stronger inhibitory effect against *Aspergillus fumigatus* (84.96%), *Aspergillus niger* (84.94%), and *Aspergillus flavus* (75.67%). In many cases, the antifungal activities of plant extracts are attributed to the bioactive components those extracts contain. The antifungal properties of plant extracts have been demonstrated through the rupture of fungal cell membranes, the inhibition of enzyme function, and the inhibition of the formation of fungal cells. Because of the wide variety of chemical components that they contain, plant extracts have the potential to treat a wide range of fungal infections<sup>27</sup> and <sup>28</sup>. The results of this investigation indicate that the ethanol extract of *E. chlorantha* stem bark has the potential to serve as a viable alternative or supplemental treatment for *Aspergillus* infections. This is due to the fact that it demonstrated antifungal activity at the concentration that was assessed, which was 150 µg/mL.

**Table 3:** Antifungal activities of ethanol extract of *E. chlorantha* stem bark

| Test Organism                | Percentage growth inhibition (%) |         |
|------------------------------|----------------------------------|---------|
|                              | Control                          | Extract |
| <i>Aspergillus flavus</i> ,  | 73.50                            | 85.96   |
| <i>Aspergillus fumigates</i> | 67.30                            | 73.67   |
| <i>Aspergillus niger</i>     | 72.80                            | 85.94   |

#### Conclusion

According to the findings of the research, the stem bark of *E. chlorantha* contains a wide range of secondary metabolites that exhibit a variety of pharmacological actions, including antioxidant activity. When the extract was subjected to GC-MS analysis, the results revealed the presence of twenty-eight (28) different compounds. The antibacterial, antioxidant, anticancer, hypercholesterolemic, and anti-inflammatory activities of these compounds have been extensively reported in the literature pertaining to the pharmaceutical industry. The presence of these compounds is one of the possible explanations for the therapeutic qualities of *E. chlorantha*. It is necessary to do additional study in order to investigate the possibility of developing innovative pharmaceutical compounds from certain bioactive components that have been found in *E. chlorantha*.

#### Conflict of interest

The authors declare no conflict of interest

#### Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them

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