

# NUTRITIVE COMPOSITION AND GC- MS ANALYSIS OF BIOACTIVE PHYTOCHEMICALS FROM THE METHANOLIC EXTRACTS OF THE STEM AND ROOT OF *Tephrosiavogelii*

## ABSTRACT

**Aims:** This study was aimed at investigating the nutritive composition and bioactive compounds in the methanol extracts of root and stem of *Tephrosiavogelii*.

**Place and Duration of Study:** Sample: Samples were collected in K- Vom community in Jos, Plateau state Nigeria between April and May 2023.

**Methodology:** Sample extractions were carried out using maceration method. phytochemical screening and nutritive composition were carried out using standard methods while the bioactive compounds were detected using GC-MS technique. Phytochemicals were ascertained based on molecular weights (m/z) acquired from GC-MS chromatograms. Phytocompounds were established through interpretation of spectral peaks and comparing data with stored databases from the National Institute Standard and Technique (NIST) library.

**Results:** The extracts had variable percentage yield with methanol root extract having the highest (3.80%). The results of the phytochemical screening showed the presence and absence of some phytochemicals while the proximate composition varied significantly (P=0.002). The moisture content was in the range of (6.75 to 9.50%), protein (8.99 to 11.56%), crude fiber (2.00 to 7.33%), fat content (47.33 to 51.06%), ash content (15.80 to 17.60%) and carbohydrate (8.33 to 13.84%) in the methanol extracts of the root and stem. Gas Chromatography-Mass Spectrometry, (GC-MS) determined some specific phytocompounds in the extracts, GC-MS analysis furnished a combined total of 14 phytocompounds in the two extracts with unsaturated aliphatic hydrocarbons and fatty acids being the major families detected.

**Conclusion:** The extracts upon analysis revealed high potential for a vast number of bioactive compounds which justifies its use for various ailments by traditional practitioners. Phytochemical components identified in this study advocate the presence of ethnomedical and phytopharmaceutical versatility of each of the extracts which could be used in the therapeutic drug formulation studies.

**Keywords:** *Tephrosiavogelii*, Gas Chromatography-Mass Spectrometry (GC-MS), nutritive composition, Phytocompounds, GC-MS chromatograms

## 1.0 Introduction

Native to West Africa, many ethnomedical uses have been advocated for

*Tephrosiavogelii*. Around the middle belt area of Nigeria, it is cultivated on a

commercial scale for killing fish and, to a lesser extent, as part of medicament for

bone-setting.<sup>[1]</sup> Grounded leaves and stem bark are mixed with vegetable oil and

rubbed on the skin around fractured limb; pieces of cut stem are used to hold broken limb in position roots are boiled in water and, when warm, feet with localized fungal infections are immersed therein for some minutes.<sup>[1]</sup> The sap is added to palm-wine to treat diarrhea.<sup>[2]</sup> In view of its great potential in the therapy and prophylaxis of disease, efforts have been made to identify and isolate the active compounds contained in the plant. Compounds isolated from *Tephrosiavogelii* include flavonoids, glycosides, steroids, tannins, and reducing sugars.<sup>[1]</sup> Bioactive phytochemicals from diverse herbal plants are known regarding their ability to fight against pathogens causing human and animal diseases.<sup>[2][3]</sup> Notably, such ability possessions of the medicinal plants have attracted researchers to exploit their lead compounds for devising the synthesis of the modern pharmaceuticals. Henceforth, this may describe why more than 25% of the pharmaceutical drugs available in the pharmaceutical market today are derived from the medicinal plants.<sup>[4][5]</sup> Therefore, drug discovery from medicinal plants involves extensive studies to investigate and determine bioactive compounds from traditionally and locally-used medicinal plants.

## **2.0 Materials and Methods**

### **2.1 Plant collection and authentication**

The stem and root of *Tephrosiavogelii* was collected in K-Vom, Jos South Local Government Area of Plateau State, Nigeria. Authentication was by Mr. Sale Mohammed (a taxonomist) from the College of Animal Health and Production Technology, Vom, Plateau State, Nigeria.

### **2.2 Sample preparation and extraction**

The stem and root of the plant was washed properly and dried separately at room temperature and pulverized using a pestle and mortar for extraction. The powdered stem and root of the plant (particle size of 0.75mm) was macerated separately in methanol in a solid-solvent ratio of 1:10 (100g in 1L) for 48 hours at room temperature and filtered to obtain the filtrates. Filtrates were completely evaporated using a hot air oven at 45°C. The evaporation afforded the methanol extracts of the stem and root of the plant.

### **2.3 Phytochemical screening of the methanol extracts of the stem and root of *Tephrosiavogelii***

The methanol extracts of the stem and root of the plant was analyzed for their phytochemical using standard qualitative procedures as described by Dubey<sup>[6]</sup> Soni & Sosa,<sup>[7]</sup>

### **2.4 Nutritive Composition Determination**

The nutritive composition of the methanol extracts of the stem and root of the plant was determined as described by<sup>[8][9]</sup>.

### **2.5 GC-MS analysis of the methanol extracts of the stem and root of *Tephrosiavogelii***

Standard method according to Konappaet *al.*,<sup>[10]</sup> and Shalini *et al.*,<sup>[11]</sup> was adopted using GC-MS QP 2010 Plus Shimadzu system and Gas chromatography interfaced to a mass spectrometer instrument.

## 2.6 Identification of phytochemicals

The identification of the compounds was based on the comparisons of their mass spectra with NIST Ver. 2.0 Year 2008 library WILEY8, FAME.

## 3.0 RESULTS AND DISCUSSION

### 3.1 Results

**Table 1: Yield of the extraction of methanol extracts of the root and stem of *Tephrosiavogelii***

| Extract       | Weight (g) | % Yield |
|---------------|------------|---------|
| Root methanol | 5.7        | 3.8     |
| Stem methanol | 3.8        | 2.5     |

**Table 2: Qualitative phytochemical composition of the methanol extracts of the root (MRE) and stem (MSE) of *Tephrosiavogelii***

| Phytoconstituent   | MRE | MSE |
|--------------------|-----|-----|
| Tannins            | +   | +   |
| Saponins           | +   | +   |
| Reducing sugar     | -   | -   |
| Alkaloids          | +   | +   |
| Terpenoides        | +   | +   |
| Flavonoids         | +   | +   |
| Cardiac glycosides | +   | +   |
| Anthraquinones     | +   | +   |
| Phenols            | +   | +   |
| Steroids           | +   | +   |
| Volatile oil       | +   | +   |
| Glycosides         | +   | +   |
| Calcones           | +   | -   |
| Quinones           | -   | +   |

Keys: - = Absent; + = present

**Table 3: Nutritive compositions of the methanol extracts of the root (MRE) and stem (MSE) of *Tephrosiavogelii***

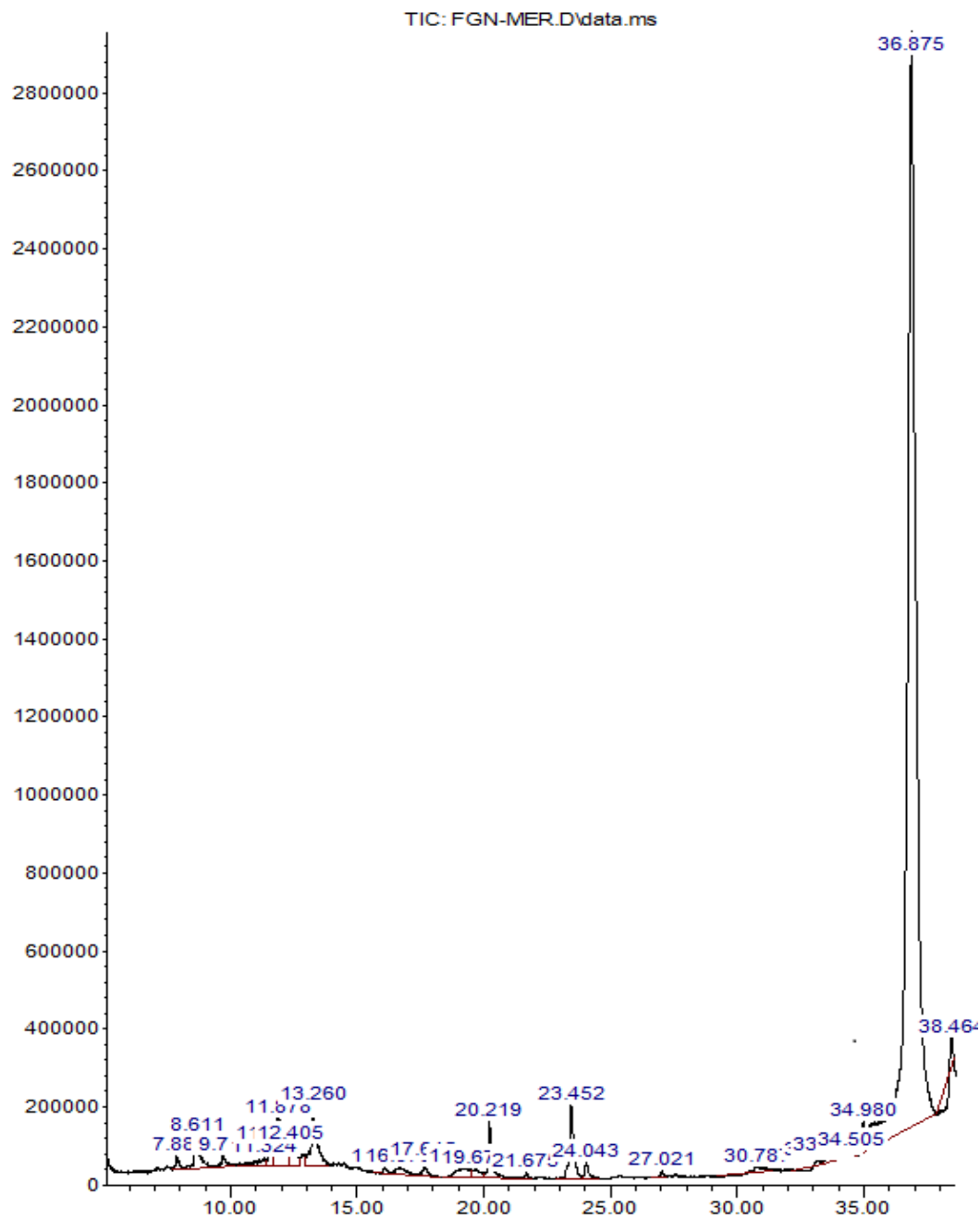
| <b>Nutritive composition</b>  | <b>MRE</b> | <b>MSE</b> |
|-------------------------------|------------|------------|
| %Moisture                     | 6.75±0.21  | 9.50±0.21  |
| %Fat                          | 51.06±0.56 | 47.33±0.56 |
| %Protein                      | 8.99±0.18  | 11.56±0.18 |
| %Ash                          | 17.60±0.30 | 15.80±0.30 |
| %Fibre                        | 7.33±0.02  | 2.00±0.02  |
| %Carbohydrate<br>(Calculated) | 8.33±0.12  | 13.84±0.12 |

Key: MRE = Methanol Root Extract; MSE = Methanol Stem Extract

UNDER PEER REVIEW

# GC-MS Analysis for the whole methanol root extract of *Tephrosiavogelii*

Abundance



Time-->

Fig. 1: GC-MS chromatogram for the whole methanol root extract of

*Tephrosiavogelii*

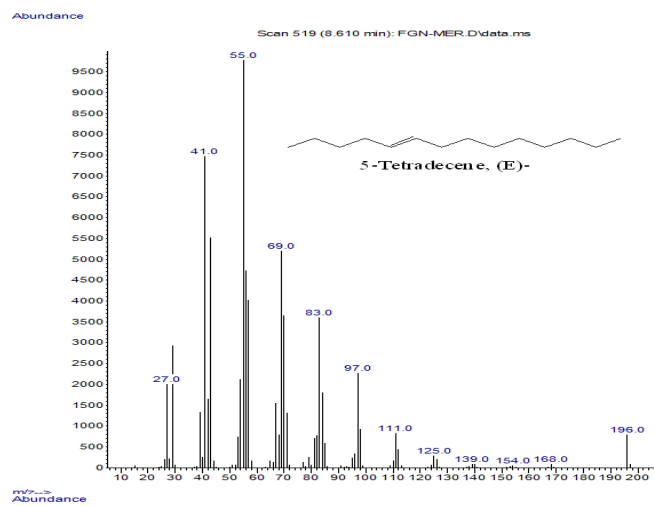
**Table 4 Bioactive compounds detected in methanol root extract of *Tephrosiavogelii***

| <b>Peak</b> | <b>Retention Time</b> | <b>% Peak Area</b> | <b>Compound</b>                    | <b>Ref</b> | <b>CAS</b>  | <b>Similarity index</b> |
|-------------|-----------------------|--------------------|------------------------------------|------------|-------------|-------------------------|
| <b>1</b>    | 8.6108                | 1.4874             | 5-Tetradecene, (E)-                | 61866      | 041446-66-6 | 93                      |
| <b>2</b>    | 11.8782               | 3.6657             | 2,4-Di-tert-butylphenol            | 70634      | 000096-76-4 | 94                      |
| <b>3</b>    | 13.2602               | 2.826              | Cetene                             | 87833      | 000629-73-2 | 98                      |
| <b>4</b>    | 17.645                | 0.5065             | 1-Tridecene                        | 49686      | 002437-56-1 | 93                      |
| <b>5</b>    | 20.2193               | 1.9583             | Hexadecanoic acid, methyl ester    | 130822     | 000112-39-0 | 98                      |
| <b>6</b>    | 23.452                | 2.6794             | 10-Octadecenoic acid, methyl ester | 155731     | 013481-95-3 | 99                      |
| <b>7</b>    | 24.0427               | 0.5587             | Methyl stearate                    | 157879     | 000112-61-8 | 98                      |

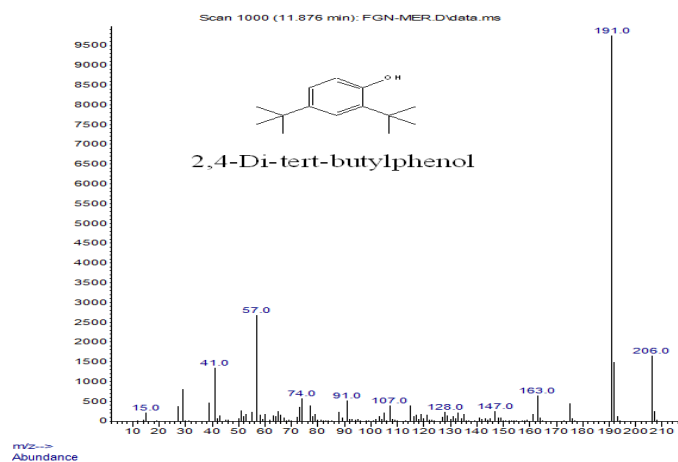
UNDER PEER REVIEW

**Fig. 2a-g** Mass Spectra of Compounds Detected in Methanol Root Extract of *Tephrosiavogelii*

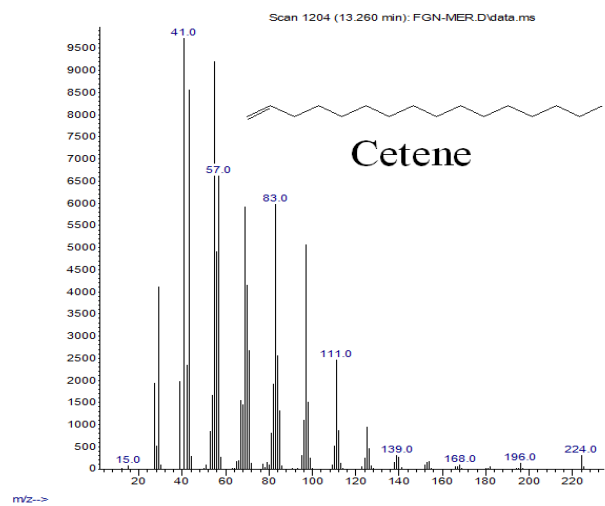
**Fig. 2a**



**Fig. 2b**



**Fig. 2c**



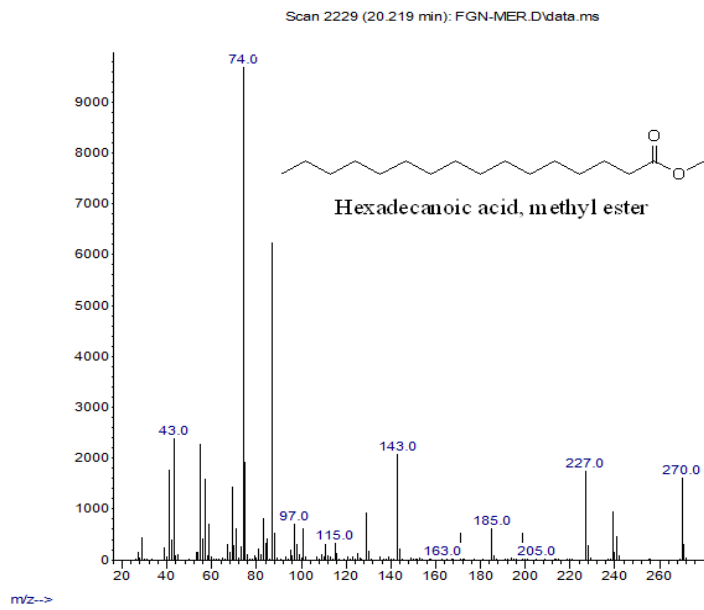
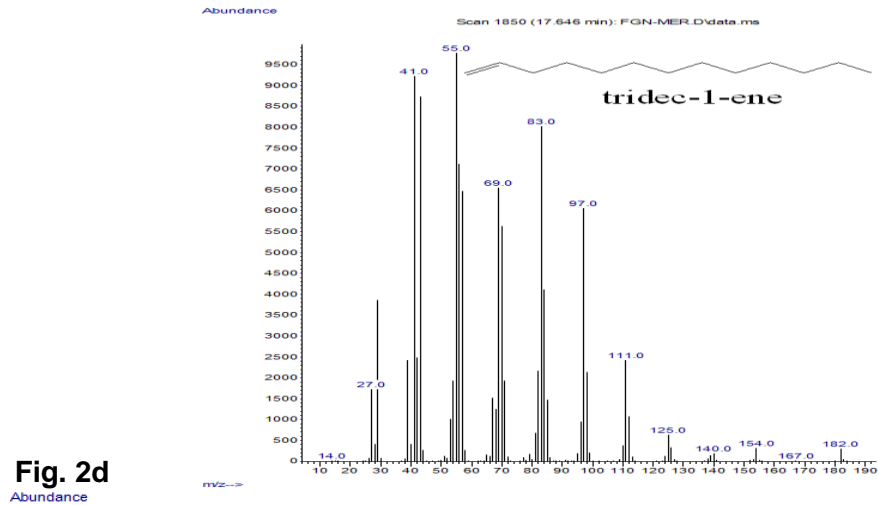
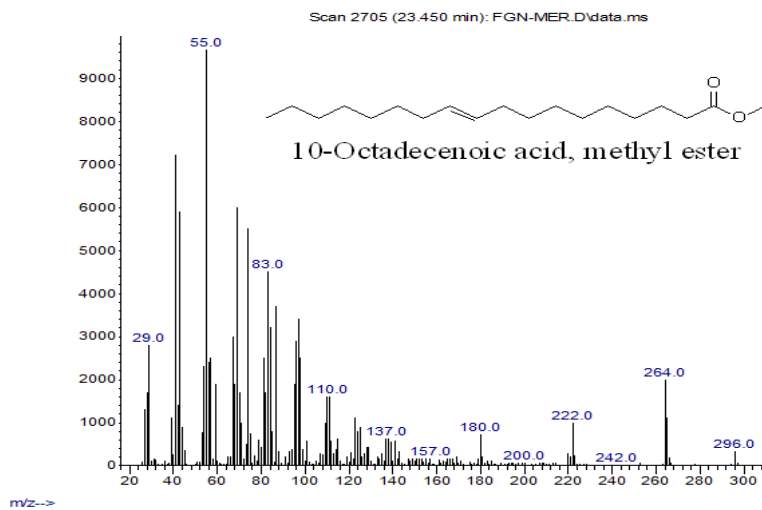


Fig. 2g



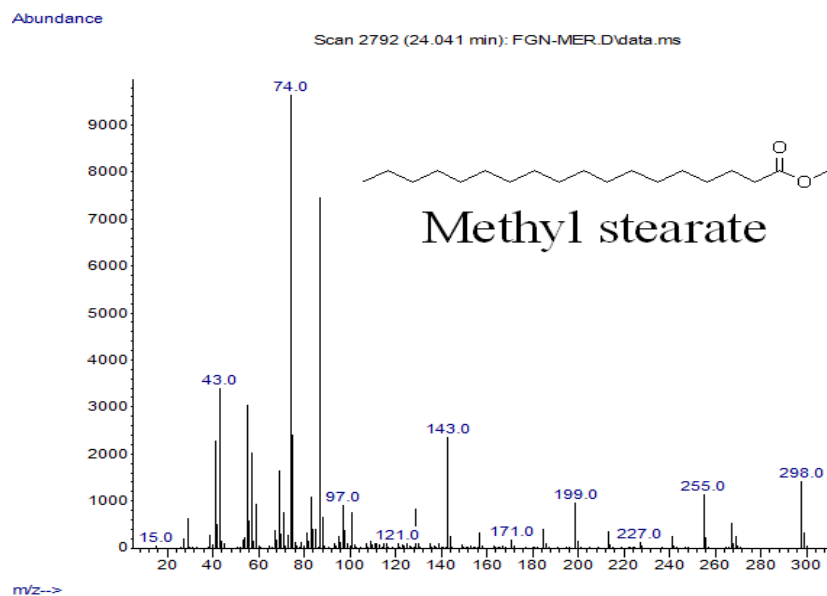
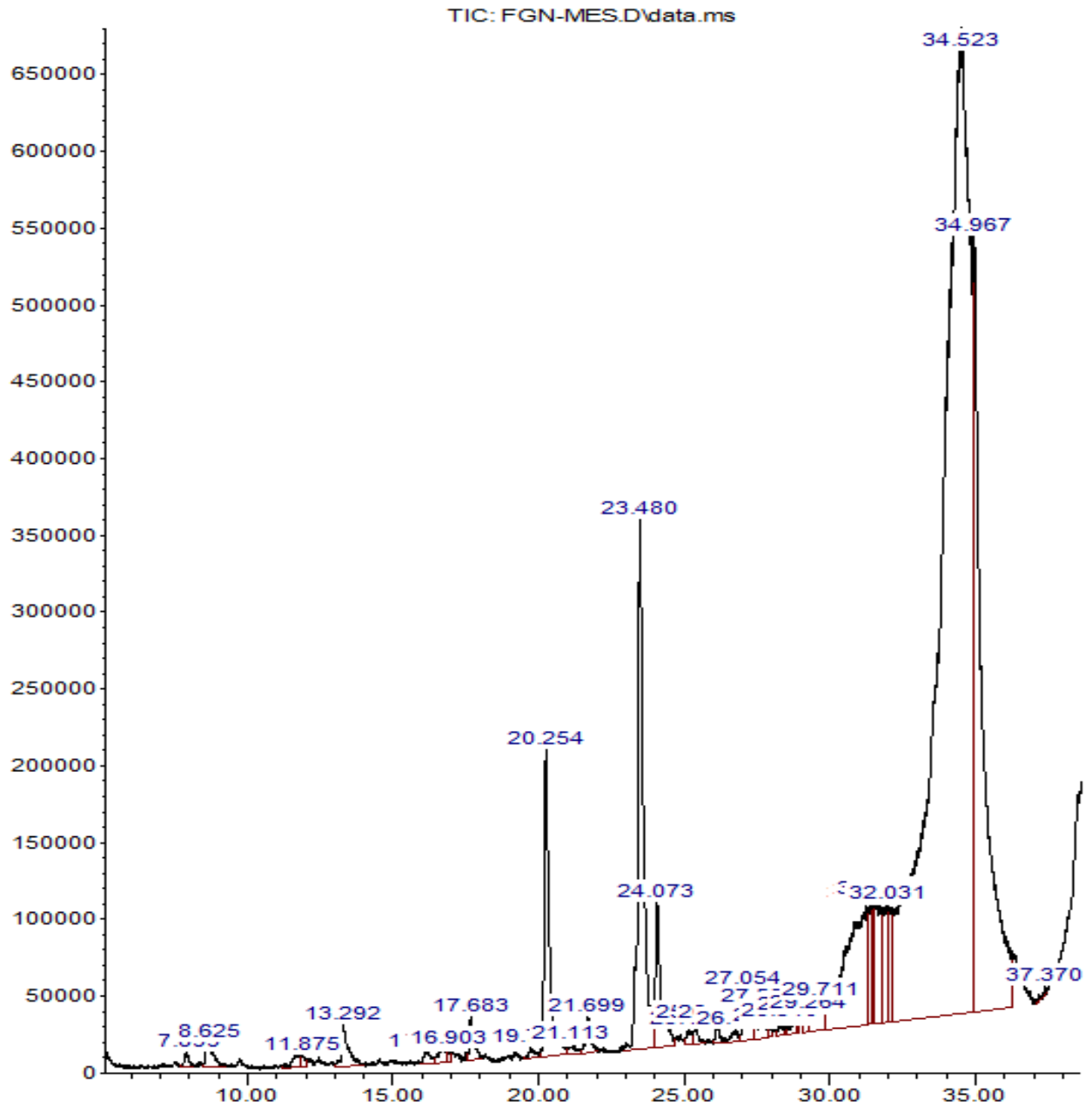


Fig. 2h

UNDER PEER REVIEW

# GC-MS Analysis for the whole methanol stem extract of *Tephrosiavogelii*

Abundance



Time-->

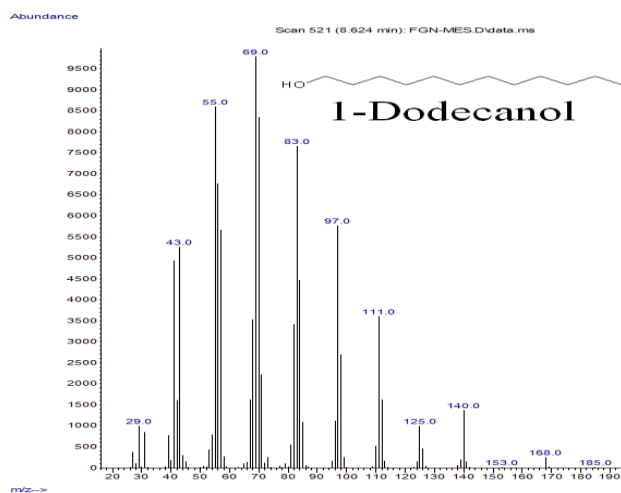
Fig 3: GC-MS chromatogram for the whole methanol stem extract of

*Tephrosiavogelii*

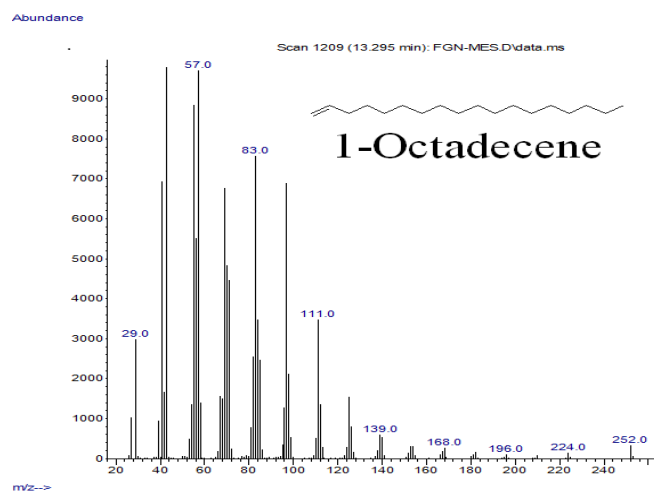
**Table 5: Bioactive compounds detected in methanol stem extract of *Tephrosiavogelii***

| Peak | Retention Time | % Peak Area | Compound                                       | Ref    | CAS          | Similarity index |
|------|----------------|-------------|--|--------|--------------|------------------|
| 1    | 8.6247         | 0.3545      | 1-Dodecanol                                    | 53012  | 000112-53-8  | 91               |
| 2    | 13.2919        | 0.5976      | 1-Octadecene                                   | 113634 | 000112-88-9  | 90               |
| 3    | 17.6829        | 0.3998      | 1-Nonadecene                                   | 126870 | 018435-45-5  | 91               |
| 4    | 20.2536        | 3.079       | Hexadecanoic acid, methyl ester                | 130813 | 000112-39-0  | 99               |
| 5    | 23.4805        | 5.9298      | 11-Octadecenoic acid, methyl ester             | 155737 | 052380-33-3  | 99               |
| 6    | 24.0726        | 1.566       | Methyl stearate                                | 157879 | 000112-61-8  | 99               |
|      | 25.1501        | 0.1522      | 9,12-Octadecadienoic acid (Z,Z)-, methyl ester | 153891 | 000112-63-0  | 92               |
| 7    |                |             |  |        |              |                  |
| 8    | 25.395         | 0.1453      | Dichloroacetic acid, heptadecyl ester          | 217449 | 1000282-98-2 | 90               |
| 9    | 34.5228        | 58.869      | .beta.-Sitosterol                              | 245059 | 000083-46-5  | 90               |

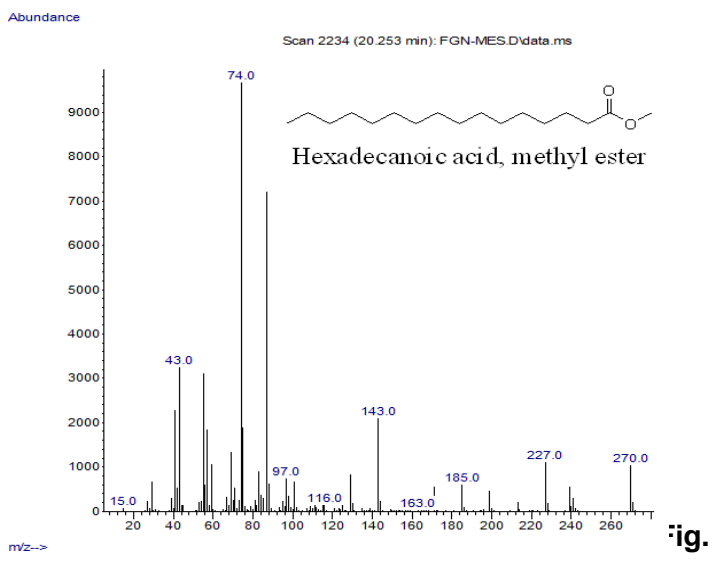
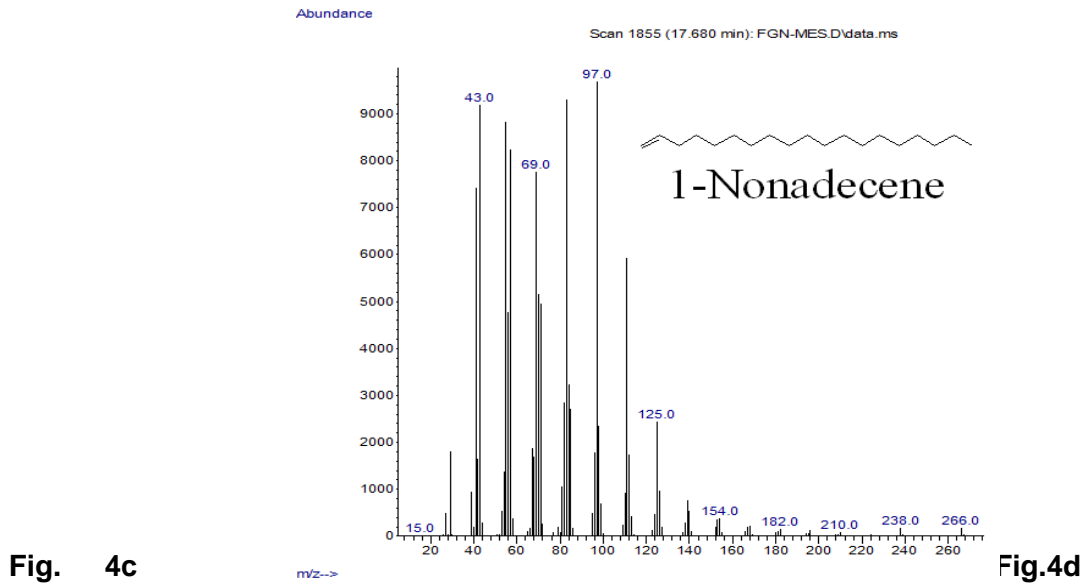
**Fig. 4a – iMass Spectra of Compounds Detected in Methanol Root Extract of *Tephrosiavogelii***

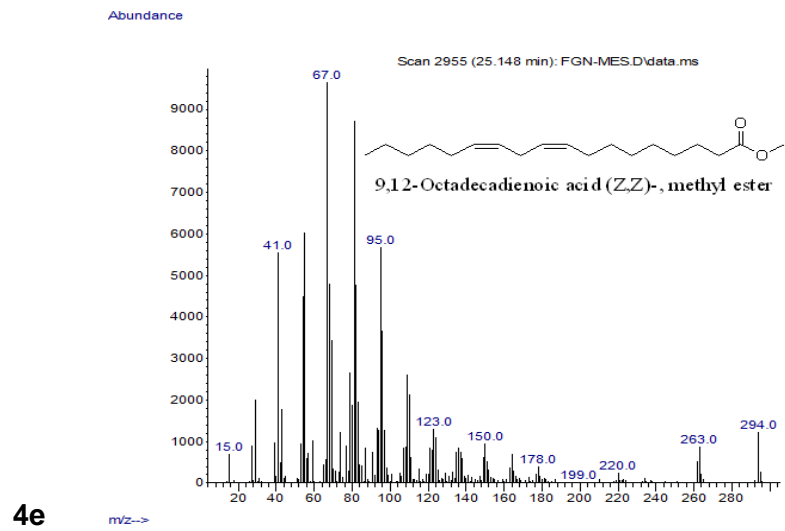


**Fig. 4a**



**Fig. 4b**





4e

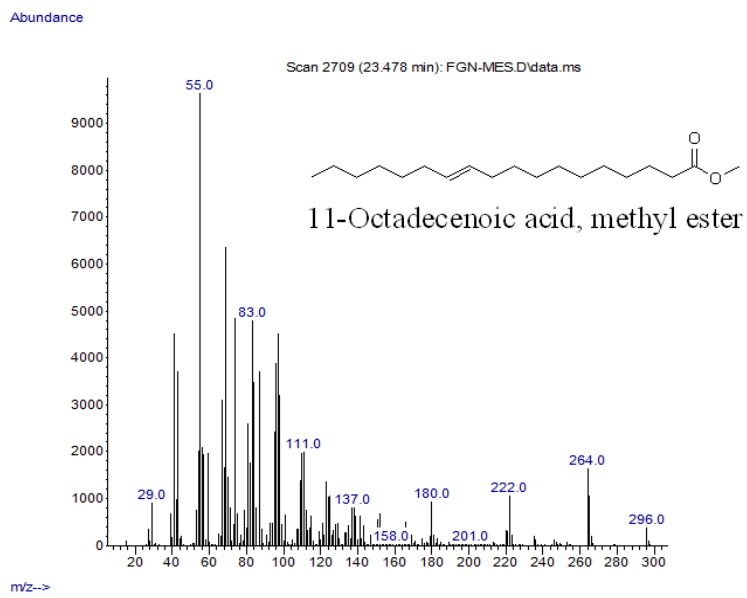


Fig. 4f

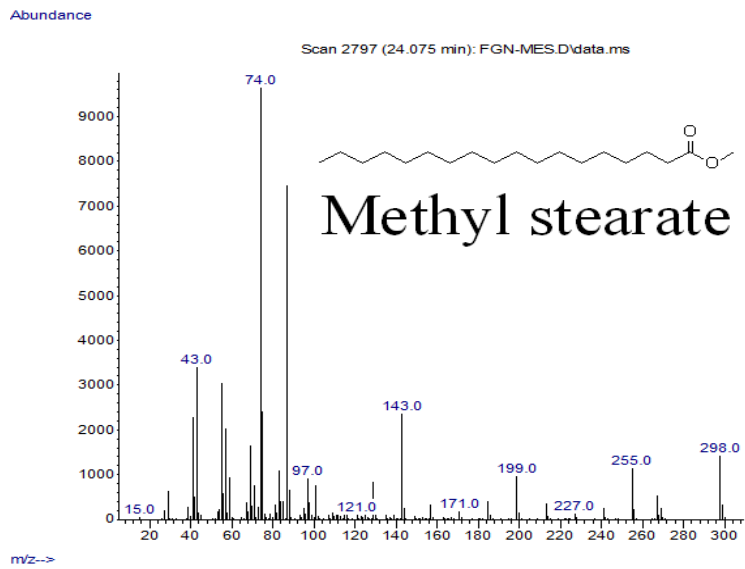


Fig. 4g

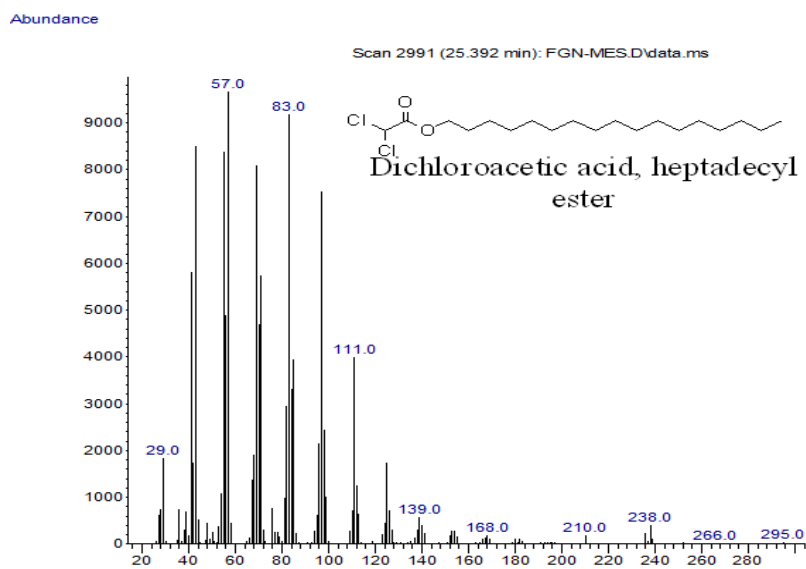
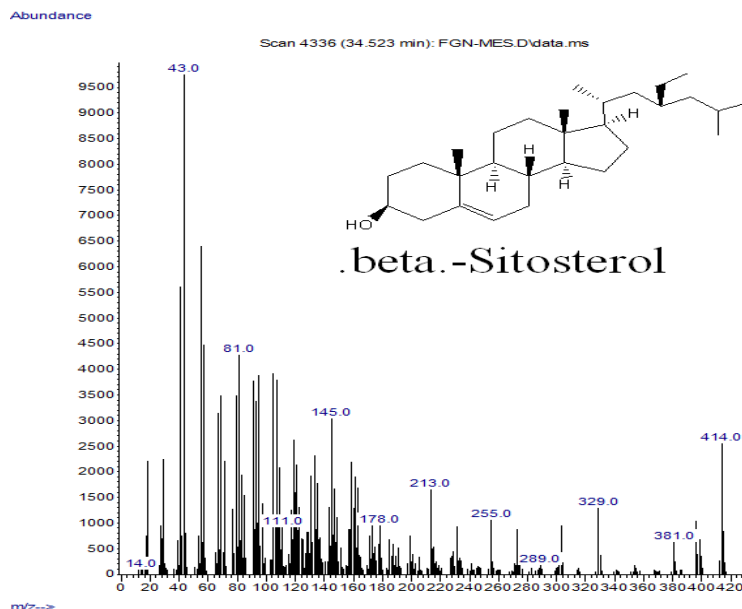


Fig. 4h



**Fig.4i**

#### **4.0 Discussion**

##### **4.1 Extraction**

In the results of extraction (Table 1), the highest yield (3.80%) was in methanol root extract while the stem yielded lower with 2.53%. This yield for the root is in agreement with the 3.00% reported by Mlozi et al. <sup>[12]</sup> although slightly lower while the yield of the stem does not agree with the 0.40% reported by Tole and Neme <sup>[13]</sup>. This significant difference in yield may be due to difference in geographical area and climatic conditions.

##### **4.2 Qualitative phytochemical screening of the extracts**

The results of the qualitative phytochemical screening (Table 2), showed the presence of terpenoids, anthraquinones, Tannins, saponins, flavonoids, phenols, glycosides, Alkaloids, cardiac glycosides, steroid, volatile oils and calcones while quinones and reducing sugars are absent in the methanol root extract. The methanol stem extract revealed the presence of terpenoids, flavonoids, phenols, Tannins, alkaloids, cardiac glycosides, anthraquinones, steroids, Quinones, volatile oils and saponins while reducing sugars and calcones are

absent. These findings do agree with the findings of Kabera *et al.*<sup>[14]</sup> and Mlozi *et al.*<sup>[12]</sup> who reported similar presence for the methanol leaves and root extracts of *Tephrosia Vogellii*.

#### **4.3 Nutritive composition of the extracts**

Table 3 showed the nutritive compositions of the root and stem extracts, one of these is the moisture content which has effect on the susceptibility of samples to spoilage by microbial actions.<sup>[15]</sup> This study revealed that the methanol stem extract and methanol roots extracts had a moisture content of 6.75 and 9.50 % respectively whose difference is not statistically significant ( $p \geq 0.05$ ). However, the amount of moisture in the methanol root and stem extracts are quite higher than 2.40% reported by Arukwe *et al.*<sup>[16]</sup> for Avocado seed. The results of this study also revealed that the ash contents of methanol root extract and methanol stem extract were 17.60% and 15.80% respectively. This clearly showed that methanol root and stem extract contain similar mineral contents. These results are not comparable to 1.31% reported by Gumte *et al.*<sup>[17]</sup> for mango kernel flour. Methanol root extract had the highest fat content (51.06%) than methanol stem extract (47.33%). The results for methanol root extract (MRE) and methanol stem extract (MSE) are higher than the 30.83% reported by Justina *et al.*<sup>[18]</sup>. The percentage crude fibre for MRE and MSE is 7.33% and 2.00% respectively showing significant difference in the amount of fibre in each extract. The MRE crude fibre values are quite higher than 3.96% reported by Kittiphoom,<sup>[19]</sup> for mango seed while that of MSE (2.00%) is much lower than it. The difference in values may largely be due to difference in plant and/or geographical location. In result of the protein content of the MRE and MSE (Table 3), the MSE (11.56%) had the highest value when compared to MRE (8.99%), although they do not agree with the higher 15.23% and 15.55% reported by Justina *et al.*<sup>[18]</sup> in avocado seed. The results of the Carbohydrate content (calculated) showed MSE (13.83%) and MRE (8.33%) respectively. These are although, quite lower than 48.11% reported by Arukwe *et al.*<sup>[16]</sup> for Avocado seed. Since carbohydrate generates energy, the

findings are an indication that the sample could only fairly produce energy to power the cells and tissues of the body on consumption.

#### 4.4 GC-MS Analysis

**Table 6: Compounds detected with their biological/medicinal activity in methanol root extract of *Tephrosiavogelii***

| S/N | Compounds                       | Molecular formula                              | Molecular weight (g/mol) | Family of compounds               | Medicinal/Biological activity  |
|-----|---------------------------------|--|--------------------------|-----------------------------------|--|
| 1   | 5-Tetradecene, (E)-             | C <sub>14</sub> H <sub>28</sub>                | 196.37                   | Unsaturated aliphatic hydrocarbon | Antibacterial, ant tuberculosis activities(kuppuswamy <i>et al.</i> ,2013) <sup>[20]</sup>                                     |
| 2   | 2,4-Di-tert-butylphenol         | C <sub>14</sub> H <sub>22</sub> O              | 206.32                   | Phenol                            | Antioxidant, antibacterial and antifungal activities (Kontham <i>et al.</i> ,2015) <sup>[21]</sup>                             |
| 3   | Cetene                          | C <sub>16</sub> H <sub>32</sub>                | 224.42                   | Unsaturated aliphatic hydrocarbon | Antimicrobial and antioxidant effect, also had highest value of antifungal activity (Edet <i>et al.</i> ,2023) <sup>[22]</sup> |
| 4   | 1-Tridecene                     | C <sub>13</sub> H <sub>26</sub>                | 182.34                   | Unsaturated aliphatic hydrocarbon | Antibacterial activity (Kumar <i>et al.</i> ,2011) <sup>[23]</sup>   |
| 5   | Hexadecanoic acid, methyl ester | C <sub>17</sub> H <sub>34</sub> O <sub>2</sub> | 270.45                   | Fatty acid methyl esters          | Antioxidant, decrease blood cholesterol, anti-inflammatory activities (Hema.,2011) <sup>[24]</sup>                             |
| 6   | Methyl stearate                 | C <sub>19</sub> H <sub>38</sub> O <sub>2</sub> | 298.50                   | Fatty acid methyl esters          | Anti-diarrheal, cytotoxic and anti-proliferative activities (Ayoola <i>et al.</i> ,2020) <sup>[25]</sup>                       |

**Table 7: Compounds detected with their biological/medicinal activity in methanol stem extract of *Tephrosiavogelii***

| S/N | Compounds                                      | Molecular formula  | Molecular weight | Family of compounds             | Medicinal/Biological activity   |
|-----|--|--|------------------|---------------------------------|---|
| 1   | 1-Dodecanol                                    | C <sub>12</sub> H <sub>26</sub> O                              | 186.33           | Fatty alcohol                   | Antibacterial activity (Farina <i>et al.</i> ,2014) <sup>[26]</sup>   |
| 2   | 1-Octadecene                                   | C <sub>18</sub> H <sub>36</sub>                                | 252.28           | Long chain hydrocarbon (alkene) | Antibacterial, antioxidant and anticancer (Lee <i>et al.</i> ,2007) <sup>[27]</sup>   |
| 3   | Hexadecanoic acid, methyl ester                | C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>                 | 270.45           | Fatty acid esters               | Antioxidant decrease blood cholesterol and anti-inflammatory activities (Hema 2011) <sup>[24]</sup>   |
| 4   | 11-Octadecenoic acid, methyl ester             | C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>                 | 296.49           | Fatty acid esters               | Anti-cholesterolemic and anticancerogenic (Asghar <i>et al.</i> ,2011) <sup>[28]</sup>  |
| 5   | Methyl stearate                                | C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>                 | 298.50           | Fatty acid methyl esters        | Anti-diarrheal, cytotoxic and antiproliferative activities (Ayoola <i>et al.</i> ,2020) <sup>[25]</sup>   |
| 6   | 9,12-Octadecadienoic acid (Z,Z)-, methyl ester | C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>                 | 294.47           | Fatty acid methyl esters        | Insecticidal (Christiana <i>et al.</i> ,2019) <sup>[29]</sup> anti-inflammatory and anticancer activities (Adeyemi <i>et al.</i> ,2017) <sup>[30]</sup>                         |
| 7   | Dichloroacetic acid, heptadecyl ester          | C <sub>19</sub> H <sub>36</sub> Cl <sub>2</sub> O <sub>2</sub> | 367.40           | Fatty acid ester                | Anti-inflammatory, antioxidant and hypocholesterolemia activities (Reddy <i>et al.</i> ,2020) <sup>[31]</sup>   |
| 8   | .beta.-Sitosterol                              | C <sub>29</sub> H <sub>50</sub> O                              | 414.71           | Steroid                         | Anti-cancer, antioxidant, anti-diabetic, antimicrobial, anti-inflammatory, anti-tuberculosis, anti-HIV, anti-arthritic and antipyretic activities (Khaled 2020) <sup>[32]</sup> |

The active principles in Table 4 and 5 detected 14 bioactive phytochemical compounds in the two extracts of *Tephrosiavogelii*. The major family of bioactive compounds detected in the MRE (Methanol Root Extract) and MSE (Methanol Stem Extract) of *Tephrosiavogelii* are unsaturated aliphatic hydrocarbons (21.42%) and fatty acid esters (35.71%) respectively. Table 6 and 7 captured the bioactive compounds detected in MRE and MSE, molecular formula, molecular weight, family of compounds and their biological/medicinal activity. In the MRE, 6 compounds were detected (Table 6) while 8 compounds were detected in the MSE (Table 7). Since fatty acids have many unique and important biological properties such as antifungal, anti-inflammation, anticancer and antibacterial activity,<sup>[33]</sup> Heterocyclic Compounds have antifungal, anti-inflammatory, antioxidant, anticancer, herbicidal, antiallergic and antibacterial activities<sup>[34]</sup> and fatty acids ester like methyl stearate are used as Flavor component in food, lubricant, used in the manufacture of pharmaceuticals, cosmetic

and soap, surfactant and softening agents.<sup>[35]</sup> Phenolic compounds showed antioxidant activity and significant effects on chronic degenerative diseases, such as central neurodegenerative disorders, cataracts, macular degeneration (age-related), diabetes mellitus, cardiovascular Complication, and cancer.<sup>[36]</sup> Plant steroids possess many interesting medicinal, pharmaceutical and agrochemical activities like anti-tumor, immunosuppressive, hepatoprotective, antibacterial, plant growth hormone regulator, sex hormone, anti-helminthic, cytotoxic and cardiogenic activity.<sup>[37]</sup> Esters such as Heptadecylheptafluorobutyrate shows biological activity of antioxidant, antibacterial, antifungal, hepatoprotective, anticancer, anti-inflammatory agent.<sup>[38]</sup> Hydrocarbons such as Cetene shows antimicrobial and antioxidant effect, also had highest value of antifungal activity.<sup>[22]</sup> Fatty alcohols such as 1-dodecanol shows antibacterial activity and also used as chemical to remove flower buds and suckers from tobacco plants.<sup>[26]</sup> These medicinal values and/or biological characteristics of the extracts points to the fact that MRE and MSE of *T.vogelii* could serve as alternative remedies in ethnopharmacology and also supports the use of the plant in traditional medicine in Nigeria orally or externally since Nabukenya et al., 2014 reported very low toxicity of the aqueous leaf extracts of *Tephrosiavogelii* at high doses makes them safe at currently non-standardized doses used for animal treatment.

#### 4.6 CONCLUSION

The many reports suggesting that *Tephrosiavogelii* are quite rich in useful metabolites are further strengthened by the findings of this study. The extracts have shown very high potential for a vast number of bioactive compounds which affirms why it is used for various ailments by traditional practitioners. Furthermore, it is safe to suggest that there are much more possible therapeutic characteristics of the plant than already put into use even orally since Mlozi et al., 2020 [39] ascertained that in vivo toxicity evaluation of the methanolic extracts of the leaf and root of *Tephrosiavogelii* in animal models showed no significant

toxicity and highlighted its safety orally which agrees with the report of Nabukenya et al., 2014.

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