International Research Journal of Pure and Applied Chemistry

Volume 25, Issue 2, Page 11-27, 2024; Article no.IRJPAC.112156
ISSN: 2231-3443, NLM ID: 101647669

Nutritive Composition and GC- MS Analysis of Bioactive Phytochemicals from the Methanolic Extracts of the Stem and Root of *Tephrosia vogelii*

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Authors' contributions

This work was carried out in collaboration among all authors. Authors UII and OAO designed the experiments. Authors GFN and IEL carried out the determination of the nutritive composition, authors EFC and GFN analyzed the results of the GC-MS. Authors UII prepared the manuscript with contributions from all co-author. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IRJPAC/2024/v25i2847

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/112156

Original Research Article

Received: 19/11/2023
Accepted: 22/01/2024
Published: 13/03/2024

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ABSTRACT

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

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 versality of each of the extracts which could be used in the therapeutic drug formulation studies.

Keywords: Tephrosia vogelii; Gas Chromatography-Mass Spectrometry (GC-MS); nutritive composition; phytocompound; GC-MS chromatograms.

1. INTRODUCTION

Native to West Africa, many ethnomedical uses have been advocated for *Tephrosia vogelii.* 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 [1]. 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 [1]. The sap is added to palm-wine to treat diarrhea [2]. 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 *Tephrosia vogelii* include flavonoids, glycosides, steroids, tannins, and reducing sugars [1]. Bioactive phytocompounds from diverse herbal plants are known regarding their ability to fight against pathogens causing human and animal diseases [2,3]. 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 [4,5]. Therefore, drug discovery from medicinal plants involves extensive studies to investigate and determine bioactive compounds from traditionally and locally-used medicinal plants.

2. MATERIALS AND METHODS

2.1 Plant Collection and Authentication

The stem and root of *Tephrosia vogelii* 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 *Tephrosia vogelii*

The methanol extracts of the stem and root of the plant was analyzed for their phyto-chemical using standard qualitative procedures as described by Dubey [6] Soni & Sosa [7].

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 AOAC [8]; AOAC [9].

2.5 GC-MS Analysis of the Methanol Extracts of the Stem and Root of *Tephrosia vogelii*

Standard method according to Konappa et al. [10] and Shalini et al. [11] was adopted using GC-MS QP 2010 Plus Shimadzu system and Gas chromatography interfaced to a mass spectrometer instrument.

2.6 Identification of Phytocompounds

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. RESULTS AND DISCUSSION

3.1 Results

3.1.1 GC-MS Analysis for the whole methanol root extract of *Tephrosia vogelii*

The result of the GC-MS analysis of whole methanol root extract of Tephrosia vogelii revealed varying families of bioactive compounds as shown in Fig. 1 and the subsequent mass spectra of the detected compounds in Fig. 2 (a – g).

3.1.2 GC-MS Analysis for the whole methanol stem extract of *Tephrosia vogelii*

Varying bioactive compounds were shown in the GC-MS analysis chromatogram of the whole extract of the methanol stem of Tephrosia vogelii as shown in Fig. 3 and followed by the Mass spectra of the bioactive compounds detected in it (Fig. 4a-4i).

3.2 Discussion

3.2.1 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. [12] although slightly lower while the yield of the stem does not agree with the 0.40% reported by Tole and Neme [13]. This significant difference in yield may be due to difference in geographical area and climatic conditions.

3.2.2 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. [14] and Mlozi et al. [12] who reported similar presence for the methanol leaves and root extracts of *Tephrosia Vogelii*.

3.2.3 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[15]. 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. [16]. 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. [17] 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. [18]. 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,[19] 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. [18] 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. [16] 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.

Table 1. Yield of the extraction of methanol extracts of the root and stem of *Tephrosia vogelii*

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 *Tephrosia vogelii*

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 *Tephrosia vogelii*

Nutritive composition	MRE	MSE
%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 (Calculated)	8.33±0.12	13.84±0.12

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

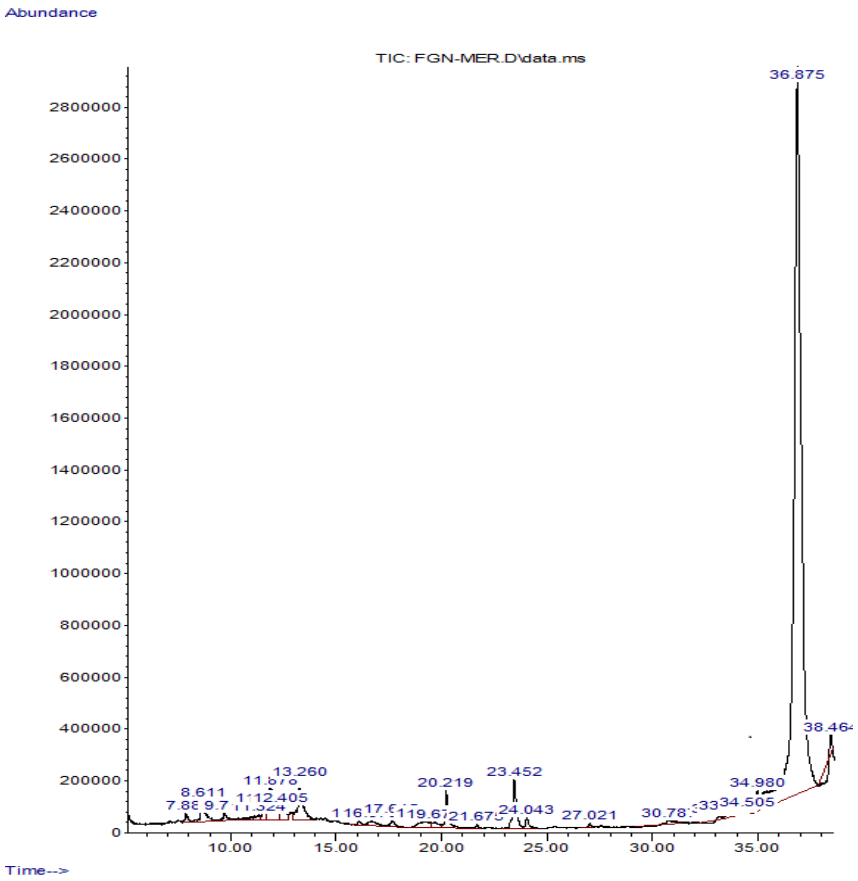

Fig. 1. GC-MS chromatogram for the whole methanol root extract of *Tephrosia vogelii*

3.2.4 GC-MS analysis

The active principles in Table 4 and 5 detected 14 bioactive phytochemical compounds in the two extracts of *Tephrosia vogelii*. The major family of bioactive compounds detected in the MRE (Methanol Root Extract) and MSE (Methanol Stem Extract) of *Tephrosia vogelii* 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,[33] Heterocyclic Compounds have antifungal, anti-inflammatory, antioxidant, anticancer, herbicidal, antiallergic and antibacterial activities [34] 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 [35]. 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 [36]. 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 cardiotonic activity [37]. Esters such as Heptadecyl heptafluorobutyrate shows biological activity of antioxidant, antibacterial, antifungal, hepatopreservative, anticancer, anti-inflammatory agent [38]. Hydrocarbons such as Cetene shows antimicrobial and antioxidant effect, also had highest value of antifungal activity [22]. Fatty alcohols such as 1-dodecanol shows antibacterial activity and also used as chemical to remove flower buds and suckers from tobacco plants [26]. 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. [39] reported very low toxicity of the aqueous leaf extracts of *Tephrosia vogelii* at high doses makes them safe at currently non-standardized doses used for animal treatment.

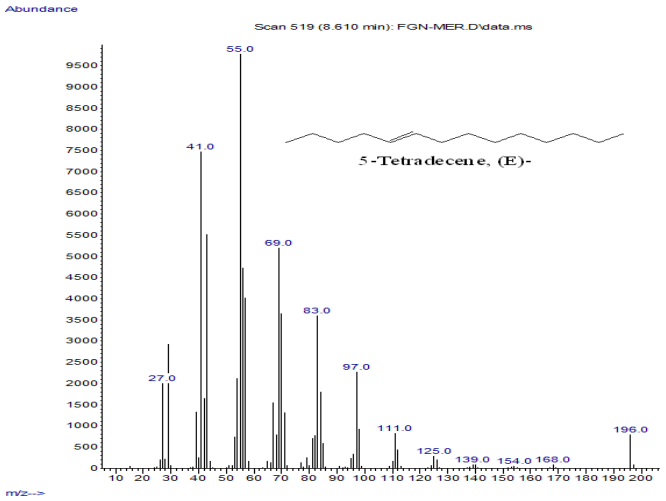

Fig. 2a

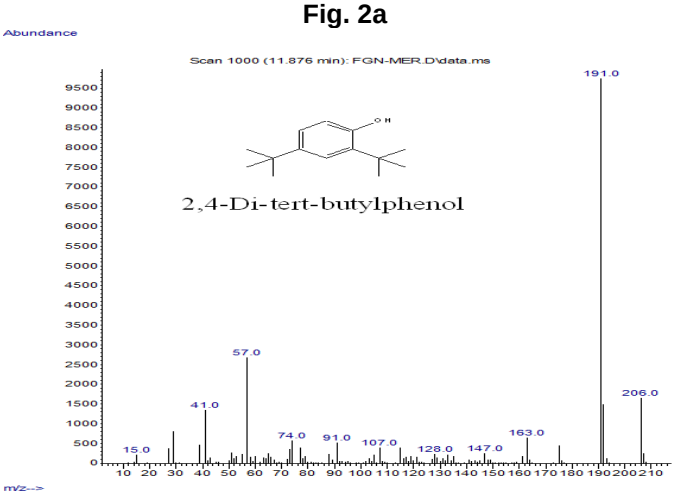

Fig. 2b

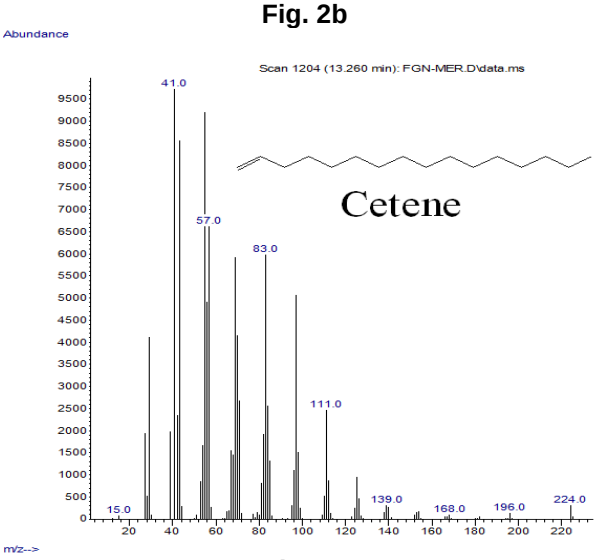

Fig. 2c

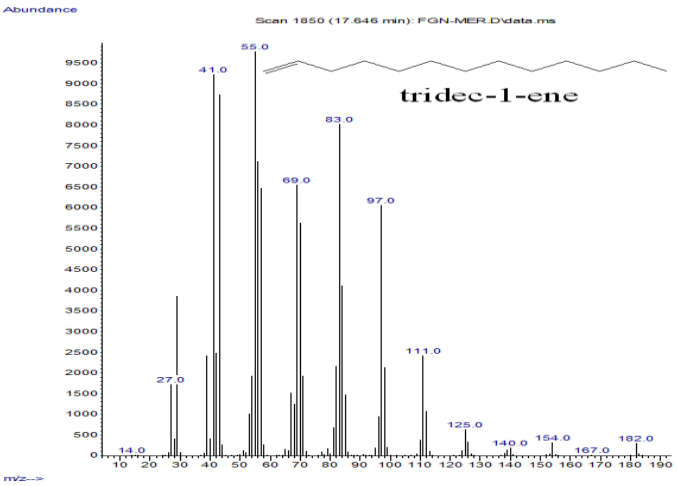

Fig. 2d

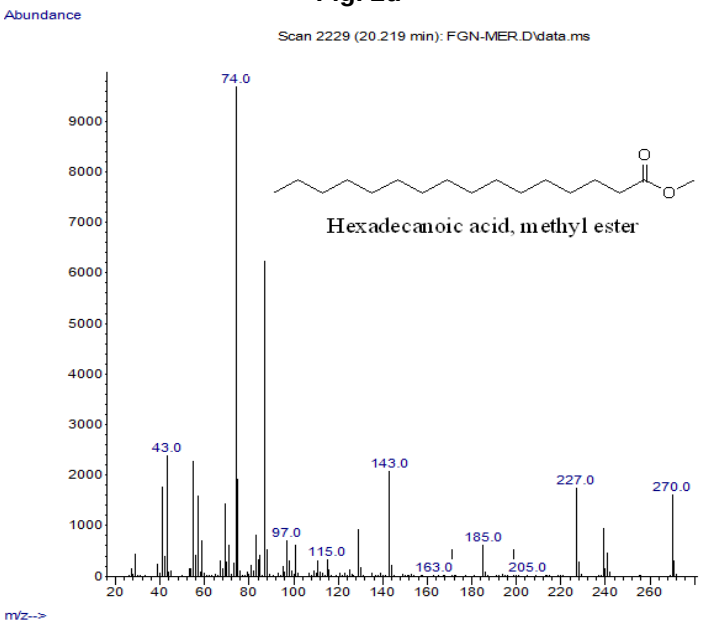

Fig. 2e

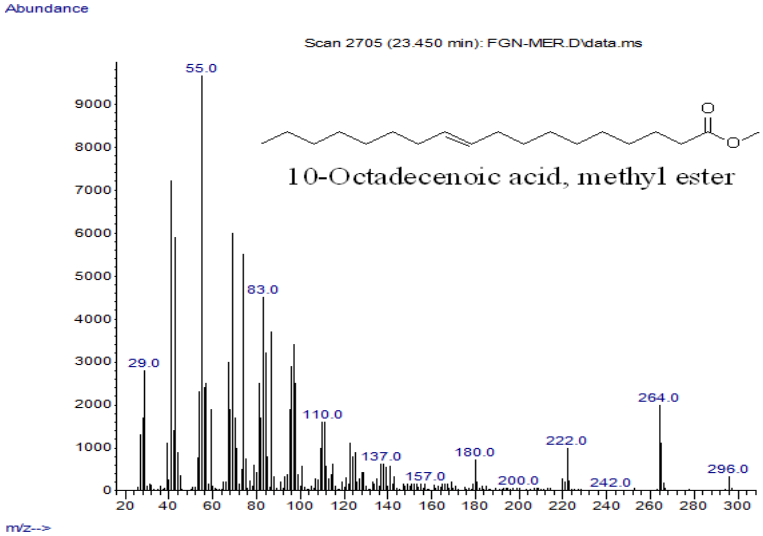

Fig. 2f

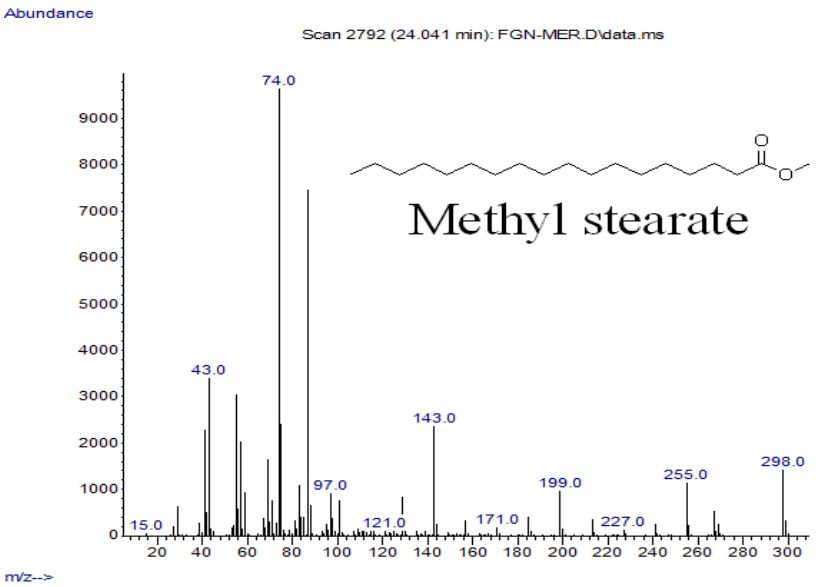

Fig. 2g

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

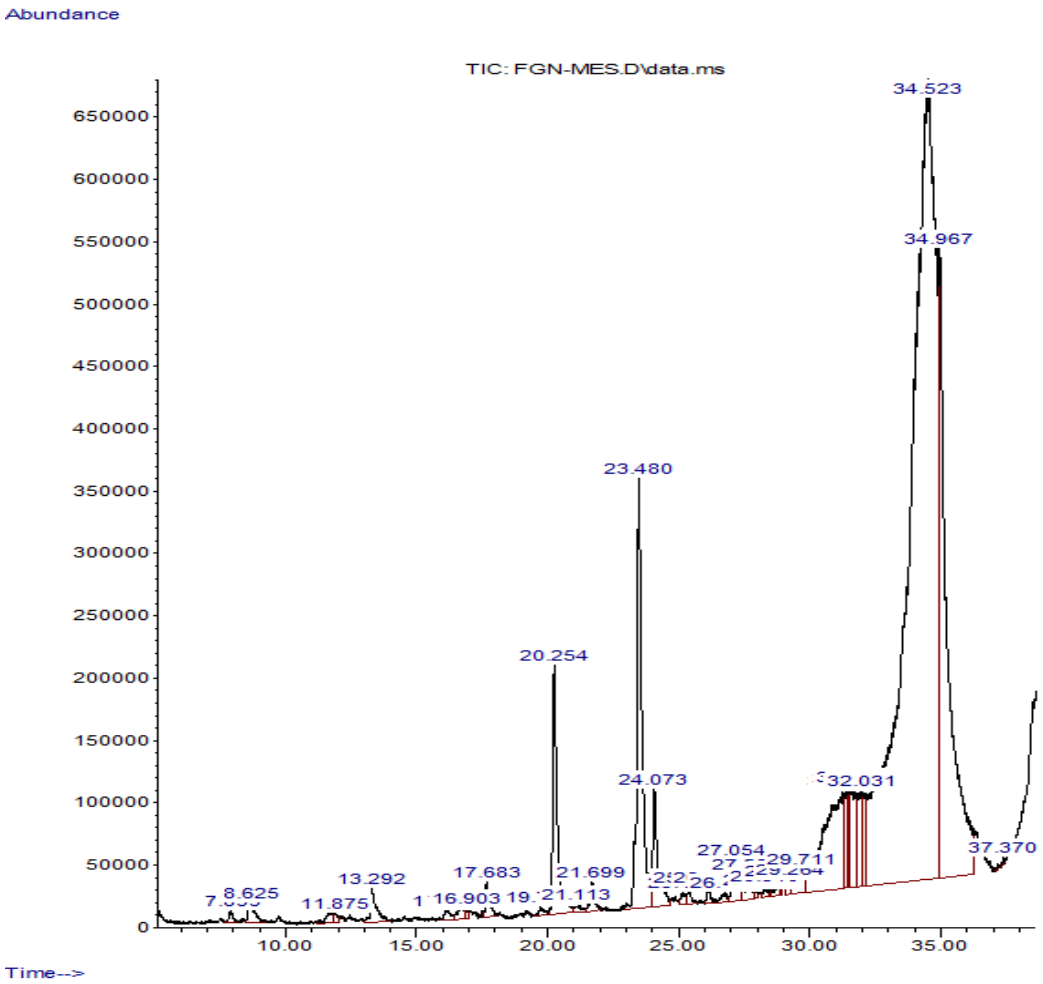

Fig. 3. GC-MS chromatogram for the whole methanol stem extract of *Tephrosia vogelii*

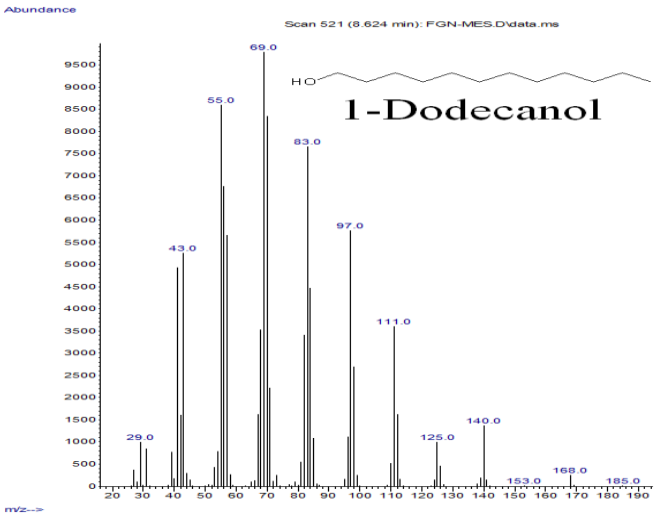

Fig. 4a

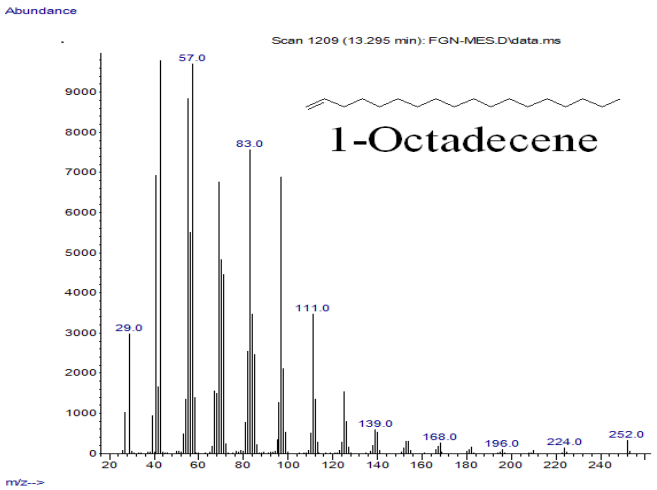

Fig. 4b

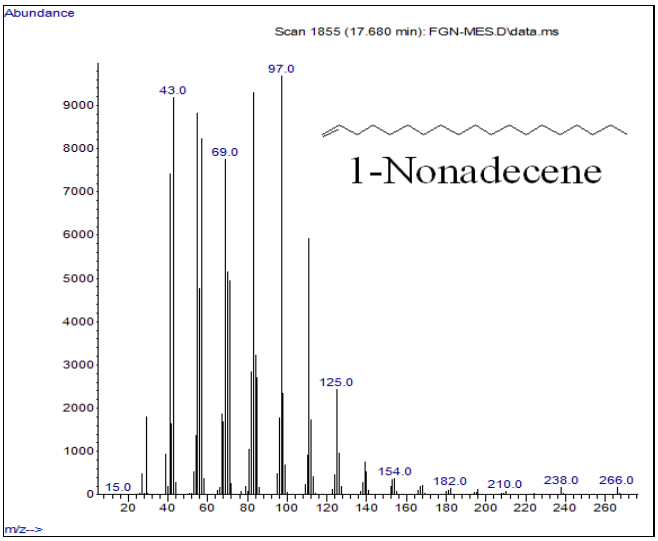

Fig. 4c

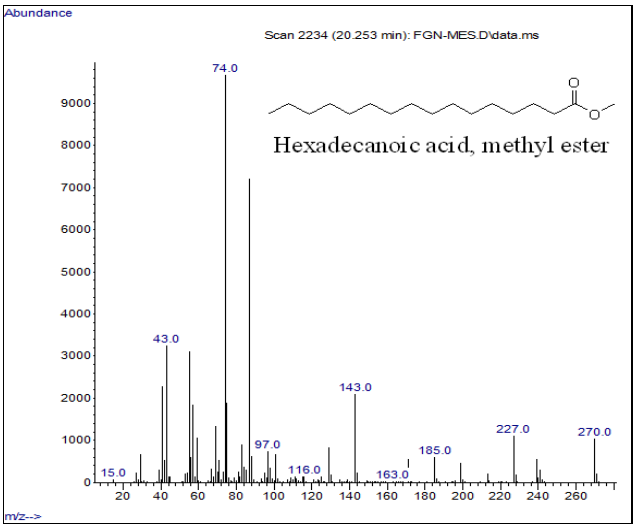

Fig. 4d

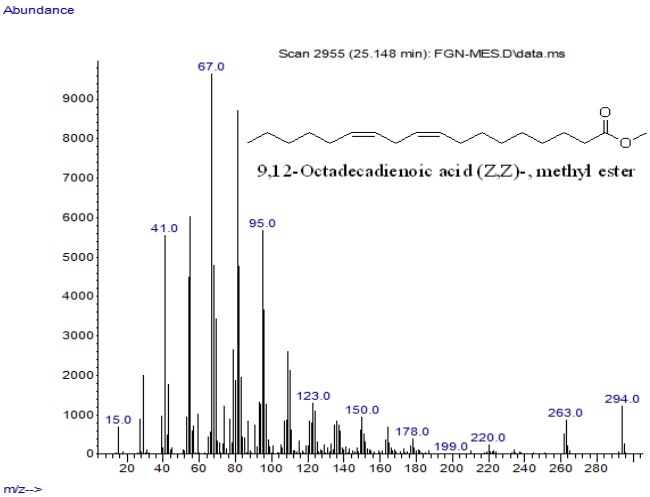

Fig. 4e

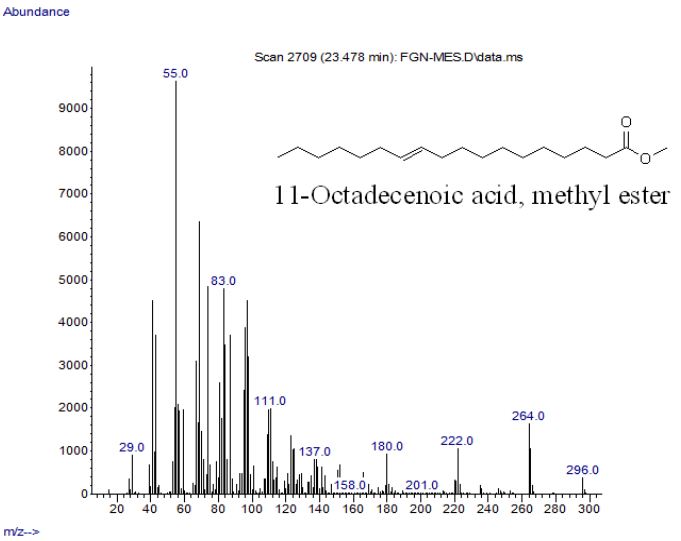

Fig. 4f

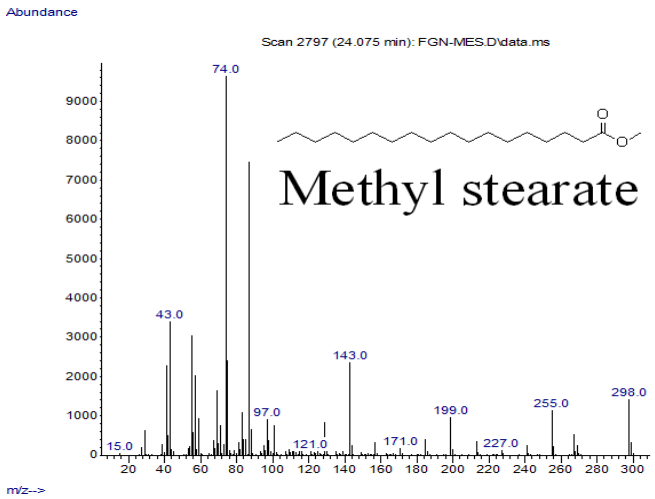

Fig. 4g

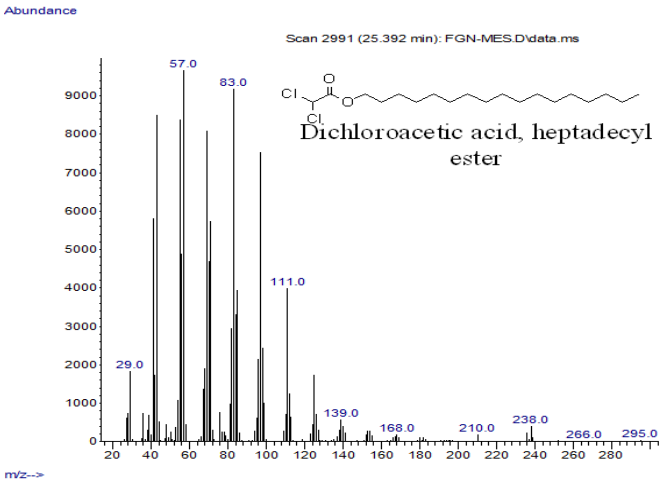

Fig. 4h

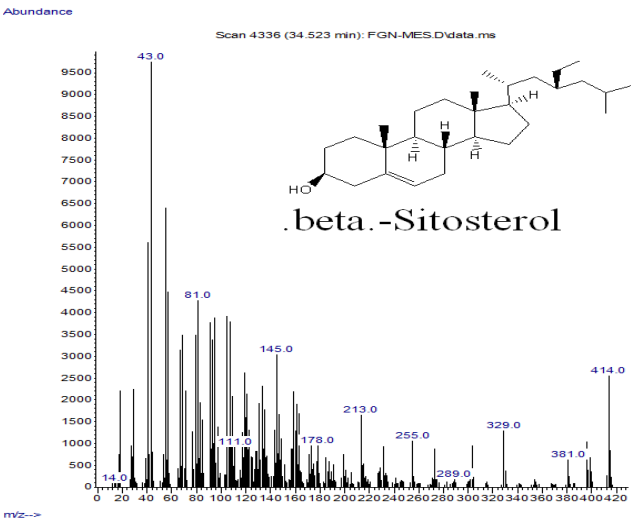

Fig. 4i

Fig. 4a – i Mass Spectra of Compounds Detected in Methanol Root Extract of *Tephrosia vogelii*

Table 4. Bioactive compounds detected in methanol root extract of *Tephrosia vogelii*

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

Table 5. Bioactive compounds detected in methanol stem extract of *Tephrosia vogelii*

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
7	25.1501	0.1522	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	153891	000112-63-0	92
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

Table 6. Compounds detected with their biological/medicinal activity in methanol root extract of *Tephoresia vogelii*

S/N	Compounds	Molecular formula	Molecular weight (g/mol)	Family of compounds	Medicinal/Biological activity
1	5-Tetradecene, (E)-	$C_{14}H_{28}$	196.37	Unsaturated aliphatic hydrocarbon	Antibacterial, ant tuberculosis activities Kuppuswamy et al. [20]
2	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206.32	Phenol	Antioxidant, antibacterial and antifungal activities Kontham et al. [21]
3	Cetene	$C_{16}H_{32}$	224.42	Unsaturated aliphatic hydrocarbon	Antimicrobial and antioxidant effect, also had highest value of antifungal activity Edet et al. [22]
4	1-Tridecene	$C_{13}H_{26}$	182.34	Unsaturated aliphatic hydrocarbon	Antibacterial activity Kumar et al. [23]
5	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45	Fatty acid methyl esters	Antioxidant, decrease blood cholesterol, anti-inflammatory activities Hema [24]
6	Methyl stearate	$C_{19}H_{38}O_2$	298.50	Fatty acid methyl esters	Anti-diarrheal, cytotoxic and anti-proliferative activities (Ayoola et al. [25]

Table 7. Compounds detected with their biological/medicinal activity in methanol stem extract of *Tephoresia vogelii*

S/N	Compounds	Molecular formula	Molecular weight	Family of compounds	Medicinal/Biological activity
1	1-Dodecanol	$C_{12}H_{26}O$	186.33	Fatty alcohol	Antibacterial activity Farina et al. [26]
2	1-Octadecene	$C_{18}H_{36}$	252.28	Long chain hydrocarbon (alkene)	Antibacterial, antioxidant and anticancer Lee et al. [27]
3	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45	Fatty acid esters	Antioxidant decrease blood cholesterol and anti-inflammatory activities Hema [24]
4	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.49	Fatty acid esters	Anti-cholesterolemic and anticancerogenic Asghar et al. [28]
5	Methyl stearate	$C_{19}H_{38}O_2$	298.50	Fatty acid methyl esters	Anti-diarrheal, cytotoxic and antiproliferative activities Ayoola et al. [25]
6	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294.47	Fatty acid methyl esters	Insecticidal Christiana et al. [29] anti-inflammatory and anticancer activities Adeyemi et al. [30]
7	Dichloroacetic acid, heptadecyl ester	$C_{19}H_{36}Cl_2O_2$	367.40	Fatty acid ester	Anti-inflammatory, antioxidant and hypocholesterolemia activities Reddy et al. [31]
8	.beta.-Sitosterol	$C_{29}H_{50}O$	414.71	Steroid	Anti-cancer, antioxidant, anti-diabetic, antimicrobial, anti-inflammatory, anti-tuberculosis, anti-HIV, anti-arthritic and antipyretic activities Khaled [32]

4. CONCLUSION

The many reports suggesting that *Tephrosia vogelii* 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. [39] ascertained that in vivo toxicity evaluation of the methanolic extracts of the leaf and root of *Tephrosia vogeliiin* animal models showed no significant toxicity and highlighted it safety orally which agrees with the report of Nabukenya et al. [40].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/112156