Original Research Article

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

ABSTRACT

Aims: This study was aimed at investigating the nutritive composition and bioactive compounds in the methanol extracts of root and stem of *Tephoresiavogelii.*
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 61 phytocompounds in the two extracts with fatty acid esters 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.

KeywordsTephrosia vogelii, 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 *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] Elliptone and Tephrosin have been reported present by Sharma and Khanna [3] in the stem and root while Rotenone was also reported by Barnes and Fryere[4] in the same plant parts. Bioactive phytocompounds from diverse herbal plants are known regarding their ability to fight against pathogens causing human and animal diseases.[5][6] 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.[6][7]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 *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 was macerated separately in methanol in the ratio of 1:10 for 48hours 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.

Comment [BD1]: What is the powder size? 40 mesh? 60 mesh? Or other?
What is the weight of the test sample used?

Comment [BD2]: Does petroleum ether use the same method?

2.3 Phytochemical screening of the methanol and petroleum ether extracts of the stem and root of *Tephrosia vogelii*

The methanol and petroleum ether extracts of the stem and root of the plant was analyzed for their phytochemical using standard qualitative procedures as described by Dubey [8] Soni & Sosa, [9].

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 [10][11].

2.5 GC-MS analysis of the methanol and petroleum ether extracts of the stem and root of *Tephrosia vogelii*

Standard method according to Konappa*et al*., [12] and Shalini *et al*., [13]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.

3.0 RESULTS AND DISCUSSION

3.1 Results

Table 1: Yield of the extraction of methanol and petroleum ether 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

Comment [BD3]: Where do petroleum ether extractions yield?

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

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

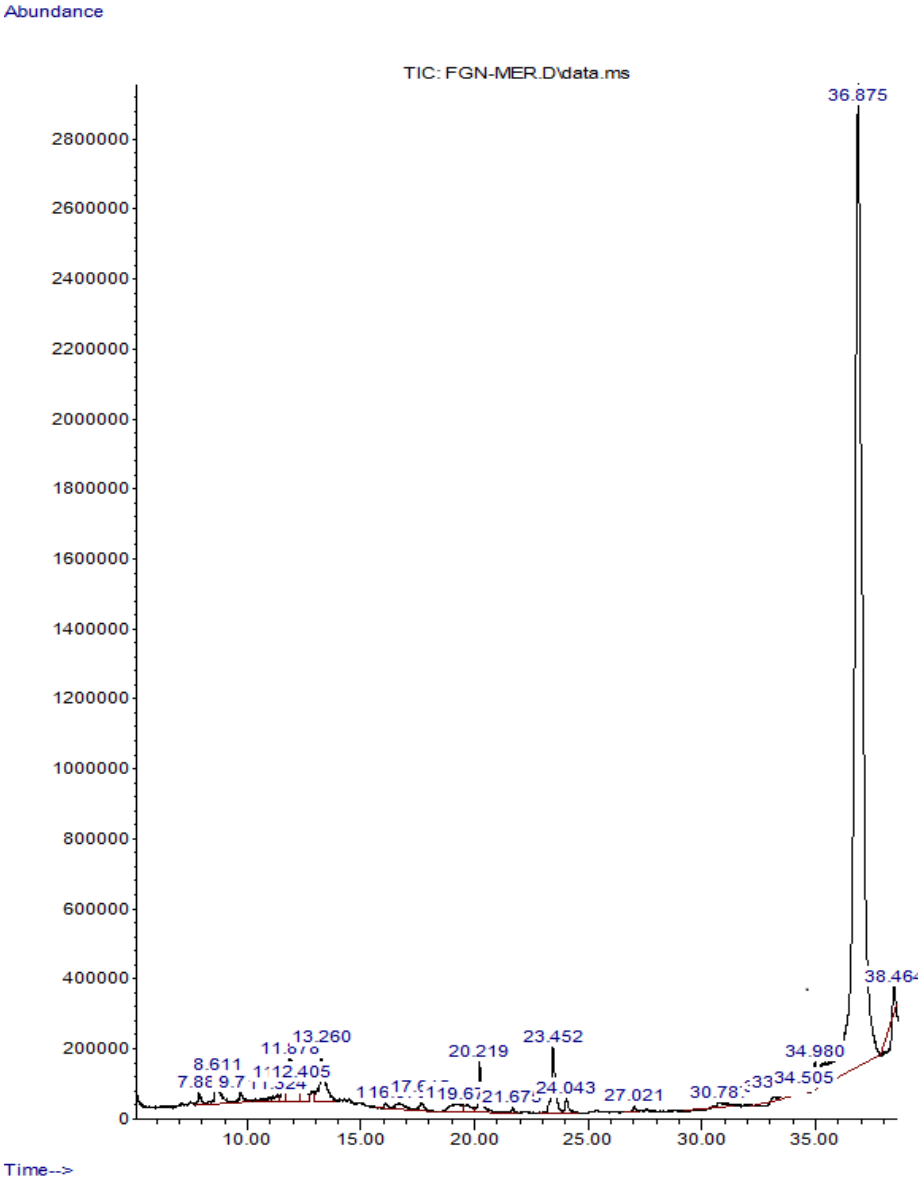

Fig. 1: GC-MS chromatogram for the whole methanol root extract of *Tephrosia vogelii*

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

Peak	Retention Time	% Peak Area	Compound	Ref	CAS	Qual
1	7.8827	0.4084	1,7-Dimethyl-4-(1-methylethyl) cyclodecane	74583	000645-10-3	49
2	8.6108	1.4874	5-Tetradecene, (E)-	61866	041446-66-6	93
3	9.7133	0.427	Cyclohexane, 1,1'-(1-methyl-1,2-ethanediyl) bis-	72750	041851-34-7	53
4	11.3243	0.4539	Eicosylpentafluoropropionate	255065	1000351-80-8	55
5	11.5744	0.6312	Dodecanoic acid, methyl ester	78067	000111-82-0	50
6	11.8782	3.6657	2,4-Di-tert-butylphenol	70634	000096-76-4	94
7	12.4047	0.9913	1H-Indene, 2,3,3a,4,7,7a-hexahydro-2,2,4,4,7,7-hexamethyl-	70784	061142-60-7	50
8	13.2602	2.826	Cetene	87833	000629-73-2	98
9	16.0711	0.1589	Tridecanoic acid, 12-methyl-, methyl ester	104333	005129-58-8	64
10	16.6735	0.6055	delta.-Lindane	148334	000319-86-8	72
11	17.645	0.5065	1-Tridecene	49686	002437-56-1	93
12	19.1525	1.0763	Dimethyl(ethenyl)silyloxycyclopentane	40659	1000278-80-8	43
13	19.675	0.5349	.beta.-d-Mannofuranoside, methyl	60078	026295-70-5	59
14	20.2193	1.9583	Hexadecanoic acid, methyl ester	130822	000112-39-0	98
15	21.6748	0.1398	1-Docosene	167463	001599-67-3	76
16	23.452	2.6794	10-Octadecenoic acid, methyl ester	155731	013481-95-3	99
17	24.0427	0.5587	Methyl stearate	157879	000112-61-8	98
18	27.0209	0.1007	Palmitoleic acid	115311	000373-49-9	58
19	30.7874	0.3117	9-(2',2'-Dimethylpropanoilhydrazono)-3,6-dichloro-2,7-bis-[2-(diethylamino)-ethoxy]fluorine	272195	1000111-04-6	14
20	33.2063	-0.1876	Benzene, 1-isothiocyanato-3-(trifluoromethyl)-	67718	001840-19-3	38
21	33.538	0.2506	Oleic Acid	142071	000112-80-1	53
22	34.5052	0.6814	Methyl 2-hydroxy-16-methyl-heptadecanoate	173327	1000336-40-8	55
23	34.98	0.717	Squalene	243221	000111-02-4	87
24	36.875	79.6913	Pyrazole-3-carboxylic acid, 1-(3-chloro-2-cyanophenyl)-5-methyl-, ethyl ester	148574	1000270-58-2	49
25	38.4637	-0.6743	Indole, 5-methyl-2-(4-pyridyl)-	72593	107919-92-6	76

Comment [BD4]: Is quality the same as the similarity index?
We recommend using a similarity index of more than 90

Comment [BD5]: Why negative values? Is it a writing error?

Comment [BD6]: Why negative values? Is it a writing error?

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

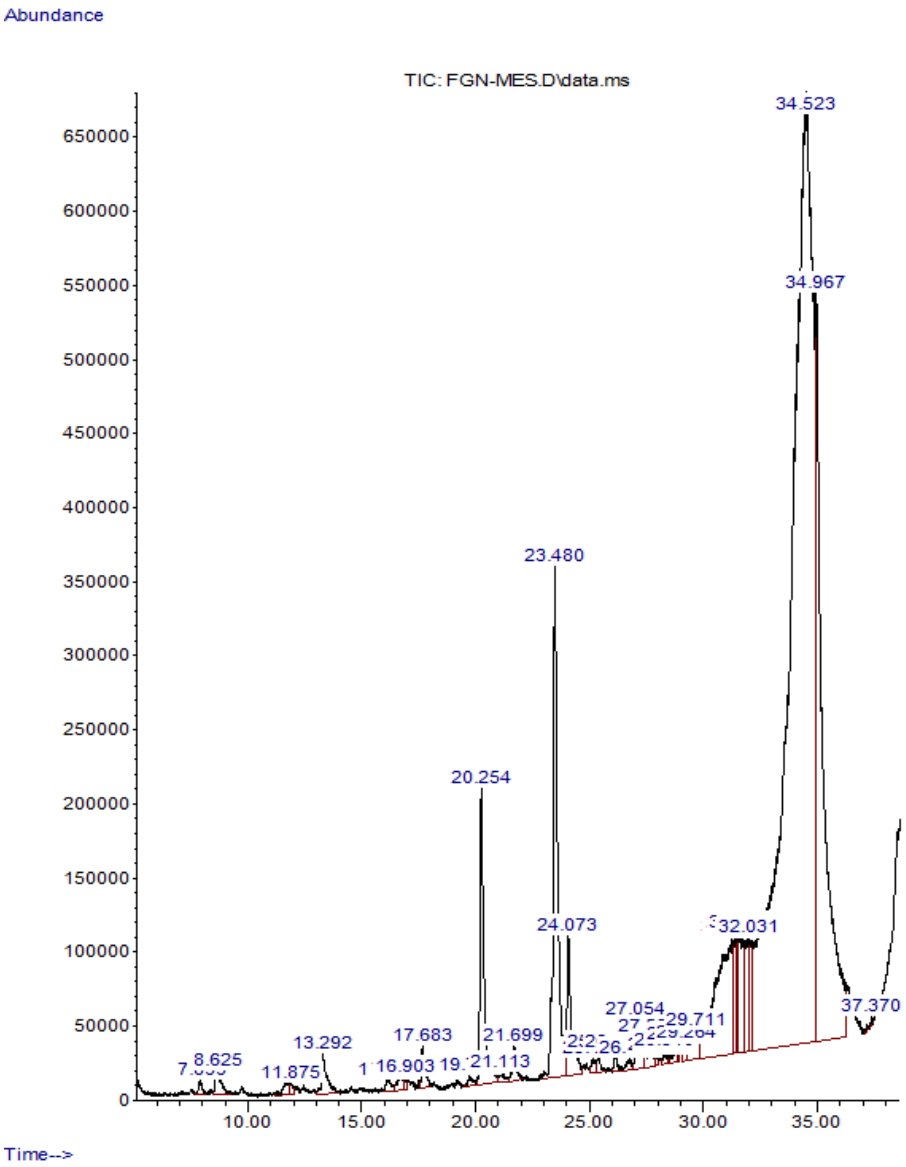

Fig 2: GC-MS chromatogram for the whole methanol stem extract of *Tephrosia vogelii*

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

Peak	Retention Time	% Peak Area	Compound	Ref	CAS	Qua
1	7.8993	0.1228	Cyclooctanecarboxylic acid, 1-ethyl-, methyl ester	63357	007393-18-2	4
2	8.6247	0.3545	1-Dodecanol	53012	000112-53-8	91
3	11.7589	0.1897	Dodecanoic acid, methyl ester	78067	000111-82-0	38
4	11.8754	0.1073	Nonanoic acid, 9-oxo-, methyl ester	52517	001931-63-1	35
5	13.2919	0.5976	1-Octadecene	113634	000112-88-9	90
6	16.1298	0.1613	2-Acetoxytetradecane	117420	1000245-61-6	46
7	16.7454	0.2138	delta.-Lindane	148334	000319-86-8	53
8	16.9029	0.0421	delta.-Lindane	148334	000319-86-8	49
9	17.6829	0.3998	1-Nonadecene	126870	018435-45-5	91
10	19.7418	0.1219	9-Hexadecenoic acid, methyl ester, (Z)-	128698	001120-25-8	58
11	20.2536	3.079	Hexadecanoic acid, methyl ester	130813	000112-39-0	99
12	21.1133	0.1449	Cyclododecanemethanol	63535	001892-12-2	38
13	21.6988	0.3449	Trichloroacetic acid, tetradecyl ester	211617	074339-52-9	81
14	23.4805	5.9298	11-Octadecenoic acid, methyl ester	155737	052380-33-3	99
15	24.0726	1.566	Methyl stearate	157879	000112-61-8	99
16	25.1501	0.1522	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	153891	000112-63-0	92
17	25.395	0.1453	Dichloroacetic acid, heptadecyl ester	217449	1000282-98-2	90
18	26.1229	0.1654	Adipic acid, isobutyl 2-methylpent-3-yl ester	146065	1000353-55-7	46
19	26.7367	0.1218	Heptadecanolide	128637	005637-97-8	49
20	27.0539	0.5638	cis-Methyl 11-eicosenoate	182558	002390-09-2	80
21	27.5939	0.3357	Eicosanoic acid, methyl ester	184598	001120-28-1	49
22	28.0024	0.0695	7-Hexadecenal, (Z)-	100566	056797-40-1	60
23	28.3096	0.0946	Propyl tetradecyl ether	117560	1000406-27-8	49
24	28.7935	0.1613	2- Chloropropionic acid, hexadecyl ester	190083	086711-81-1	64
25	28.8589	0.0676	i-Propyl 9-tetradecenoate	128647	1000336-60-7	52
26	29.2636	0.1606	1-Cyclohexylnonene	72732	114614-84-5	53
27	29.7105	0.5476	Indole, 6-methyl-2-(4-pyridyl)-	72594	107919-93-7	42
28	31.2562	5.4049	3,4-Dimethoxycinnamic acid	71974	002316-26-9	55
29	31.4014	0.8464	2-Vinylbenzophenone	72691	052095-42-8	45
30	31.477	0.5393	Oleic Acid	142070	000112-80-1	50
31	31.5704	1.63	6-Octadecenoic acid	142075	1000336-66-8	55
32	31.9379	1.0793	Octadec-9-enoic acid	142076	1000190-13-7	60
33	32.0309	0.9241	Butyl 9-tetradecenoate	142081	1000336-51-4	47
34	34.5228	58.869	.beta.-Sitosterol	245059	000083-46-5	90
35	34.967	14.722	Bicyclo[4.2.0]oct-2-ene, 3,7-dimethyl-7-(4-methyl-3-pentenyl)-8-(2,6,10-trimethyl-1,5,9-undecatrienyl)-, [1.alpha.,6.alpha.,7.beta.,8.alpha.(1E,5E)]-	242333	113681-03-1	60
36	37.37	0.0234	Oleic Acid	142070	000112-80-1	70

Comment [BD7]: Is quality the same as the similarity index?
We recommend using a similarity index above 90

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. [14] although slightly lower while the yield of the stem does not agree with the 0.40% reported by Tole and Neme [15]. This significant difference in yield may be due to difference in geographical area and climatic conditions.

Comment [BD8]: Why do roots have a greater extraction yield than stems?

Comment [BD9]: The method states that the extraction uses methanol and petroleum ether, while in the results and discussion there is only methanol. What are the results of the extraction using petroleum ether?

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

4.3 Nutritive composition of the extracts

Comment [BD10]: In the introduction, Tephrosia vogelii was cultivated on a commercial scale to kill fish and, to a lesser extent, as part of a bone-strengthening drug, so it is best to relate the discussion to the uses mentioned in the introduction.

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.[17] This study revealed that the methanol stem extract and methanol roots extracts had a moisture content of 6.75and 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*. [18] 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*. [19]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*.[20]. 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,[21] 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*. [20] 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*. [18] 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 6a: Compounds detected with their biological/medicinal activity in methanol root extract of *Tephoresiavogelii*

S/N	Compounds	Molecular formula	Molecular weight (g/mol)	Family of compounds	Medicinal/Biological activity
1	1,7-Dimethyl-4-(1-methylethyl) cyclodecane	$C_{15}H_30$	210.40	Sesquiterpene	Antibacterial and antioxidant activities (Siqueira *et a*l.,2015)
2	5-Tetradecene, (E)-	$C_{14}H_{28}$	196.37	Unsaturated aliphatic hydrocarbon	Antibacterial, ant tuberculosis activities (kuppuswamy*et al*.,2013)
3	Cyclohexane, 1,1'-(1-methyl-1,2-ethanediyl)bis-	$C_{15}H_{28}$	208.38	Hydrocarbon	Not reported
4	Eicosylpentafluoropropionate	$C_{23}H_{41}F_5O_2$	444.56	Polyunsaturated fatty acid	Antimicrobial activity (Kumar & Sharma.,2021)
5	Dodecanoic acid, methyl ester	$C_{13}H_{26}O_2$	214.34	Fatty acid methyl esters	Antibacterial, antiviral, antifungal activities (ozcelik*et al*.,2005)
6	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206.32	Phenol	Antioxidant, antibacterial and antifungal activities (Konthame*t al*.,2015)
7	1H-Indene, 2,3,3a,4,7,7a-hexahydro-2,2,4,4,7,7-hexamethyl-	$C_{15}H_{26}$	206.37	No record	Not reported
8	Cetene	$C_{16}H_{32}$	224.42	Unsaturated aliphatic hydrocarbon	Antimicrobial and antioxidant effect, also had highest value of antifungal activity (Edet *et al*.,2023)
9	Tridecanoic acid, 12-methyl-, methyl ester	$C_{15}H_{30}O_2$	242.39	Fatty acid methyl esters	Antifungal and antibacterial activities (Elaiyaraja *et al*.,2018)
10	.delta.-Lindane	$C_6H_6Cl_6$	290.83	Polychlorinated biphenyls	Insecticide and ovicidal activities (Brown., 2008)
11	1-Tridecene	$C_{13}H_{26}$	182.34	Unsaturated aliphatic hydrocarbon	Antibacterial activity (Kumar *et al*.,2011)
12	Dimethyl(ethenyl)silyloxycyclopentane	$C_9H_{18}OSi$	170.11	Alkane	Not reported
13	.beta.-d-Mannofuranoside, methyl	$C_7H_{14}O_6$	194.18	Monoterpenes	Antibacterial activity (Manilal *et al*., 2014)
14	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45	Fatty acid methyl esters	Antioxidant, decrease blood cholesterol, anti-inflammatory activities (Hema.,2011)

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

S/N	Compounds	Molecular formula	Molecular weight (g/mol)	Family of compounds
15	1-Docosene	$C_{22}H_{44}$	308.58	Straight chain Hydrocarbon	Antibacterial, antifungal and anti-inflammatory activities (Subban *et al*.,2011)
16	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.48	Fatty acid ester	Antioxidant and antimicrobial activities (Elaiyaraja *et al*.,2018)
17	Methyl stearate	$C_{19}H_{38}O_2$	298.50	Fatty acid methyl esters	Anti-diarrheal, cytotoxic and anti-proliferative activities (Ayoola *et al*.,2020)
18	Palmitoleic acid	$C_{16}H_{30}O_2$	254.41	Fatty acid	Anti-inflammatory activity (Odiase-omoighe& Agoreyo.,2022)
19	9-(2',2'-Dimethylpropanoilhydrazono)-3,6-dichloro-2,7-bis-[2-(diethylamino)-ethoxy]fluorene	$C_{30}H_{42}Cl_2N_4O_3$	577.58	Polycyclic aromatic hydrocarbon	Antimicrobial, antioxidant and cytotoxic effect (Naine*et al*., 2015)
20	Benzene, 1-isothiocyanato-3-(trifluoromethyl)-	$C_8H_4F_3NS$	203.19	Isothiocyanates	Not reported
21	Oleic Acid	$C_{18}H_{34}O_2$	282.46	Long chain Fatty acid	Anti-cancer, anti-tumor, anti-inflammatory activities (subban*et al.,* 2011)
22	Methyl 2-hydroxy-16-methyl-heptadecanoate	$C_{19}H_{38}O_3$	214.50	Hydro fatty acids	Not reported
23	Squalene	$C_{30}H_{50}$	410.73	Triterpenoid	Antitumor, antioxidant, antistatic, antibacterial and anticancer activities (Amarowicz.,2009) and (Lozano.,2018)
24	Pyrazole-3-carboxylic acid, 1-(3-chloro-2-cyanophenyl)-5-methyl-, ethyl ester	$C_7H_{10}N_2O_2$	154.16	Pyrazoles and monocarboxylic acid	Anticancer, antibacterial, antifungal, antioxidant and anti-inflammatory (Kumar *et al*.,2013)
25	Indole, 5-methyl-2-(4-pyridyl)-	$C_{14}H_{14}N_2$	208.26	Alkaloids	Antitumor, antibacterial, antiviral, antifungal and anti-plasmodial activities (Umer *et al*.,2022)

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

S/N	Compounds	Molecular formula	Molecular weight	Family of compounds	Medicinal/Biological activity
1	Cyclooctanecarboxylic acid, 1-ethyl-, methyl ester	$C_{12}H_{22}O_2$	198.30	Carboxylic acid esters	Not reported
2	1-Dodecanol	$C_{12}H_{26}O$	186.33	Fatty alcohol	Antibacterial activity (Farina *et al.*,2014)
3	Dodecanoic acid, methyl ester	$C_{13}H_{26}O_2$	214.34	Fatty acid esters	Antibacterial, antiviral and antifungal activities (Ozcelik B *et al*.,2005)
4	Nonanoic acid, 9-oxo-, methyl ester	$C_{10}H_{18}O_3$	186.24	Fatty aldehyde	Antifungal, antioxidant, antimicrobial and larvicidal (sen*et al*.,2017)
5	1-Octadecene	$C_{18}H_{36}$	252.28	Long chain hydrocarbon (alkene)	Antibacterial, antioxidant and anticancer (Lee *et al*.,2007)
6	2-Acetoxytetradecane	$C_{16}H_{32}O_2$	256.42	No record	Antibacterial activity (Kabouchz*et al*.,2013)
7	Delta -Lindane	$C_6H_6Cl_6$	290.83	Polychlorinated biphenyls	Insecticide and ovicidal activities (Brown 2008)
8	Delta -Lindane	$C_6H_6C_{l6}$	290.83	Polychlorinated biphenyls	Insecticide and ovicidal activities (Brown 2008)
9	1-Nonadecene	$C_{19}H_{38}$	266.50	Unbranched hydrocarbon (alkene)	Antituberculosis, anticancer, antioxidant, and antimicrobial activities (Rukachaisirikue*t al*.,2004)
10	9-Hexadecenoic acid, methyl ester, (Z)-	$C_{17}H_{32}O_2$	268.43	Fatty acid esters	Not reported
11	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45	Fatty acid esters	Antioxidant decrease blood cholesterol and anti-inflammatory activities (Hema 2011)
12	Cyclododecanemethanol	$C_{12}H_{26}O$	198.34	No record	Not reported
13	Trichloroacetic acid, tetradecyl ester	$C_{16}H_{29}Cl_3O_2$	359.75	Fatty acid ester	Precipitation agent , an ingredient agent, herbicide (Mary & Giri., 2018)
14	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.49	Fatty acid esters	Anti-cholesterolemic and anticancerogenic (Asghar *et al*.,2011)
15	Methyl stearate	$C_{19}H_{38}O_2$	298.50	Fatty acid methyl esters	Anti-diarrheal, cytotoxic and antiproliferative activities (Ayoola *et al*.,2020)
16	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294.47	Fatty acid methyl esters	Insecticidal (Christiana et al.,2019) anti-inflammatory and anticancer activities (Adeyemi *et al*.,2017)
17	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*.,2020)
18	Adipic acid, isobutyl 2-methylpent-3-yl ester	$C_{16}H_{30}O_4$	286.41	Ester	Not reported

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

S/N	Compounds	Molecular formula	Molecular weight	Family of compounds	Medicinal/Biological activity
19	Heptadecanolide	$C_{17}H_{32}O_2$	268.43	Fatty acid	Flavoring agent, as perfume (Yakubu *et al*.,2017)
20	cis-Methyl 11-eicosenoate	$C_{21}H_{40}O_2$	324.54	Unsaturated fatty acid ester	Antioxidant, pesticide, flavor, antifibrinolytic, inhibitor, antialopecic activities (Ayoola *et al*.,2020)
21	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	326.55	Fatty acid methyl ester	Alpha-glucosidase inhibitors activity (Elaiyaraja *et al*.,2018)
22	7-Hexadecenal, (Z)-	$C_{16}H_{30}O$	238.40	Fatty aldehyde	Antiviral activity and organic fertilizer (Devakumar *et al* 2017)
23	Propyl tetradecyl ether	$C_{17}H_{36}O$	256.46	No record	Not reported
24	2- Chloropropionic acid, hexadecyl ester	$C_{19}H_{37}ClO_2$	332.95	Fatty acid ester	Acidifier, acidulant arachidonic acid inhibitor, uric acid production inhibitor and antifungal activities (Zainurin*et al* 2020)

Table 8a: Compounds detected with their biological/medicinal activity in methanol stem extract of *Tephoresiavogelii*

S/N	Compounds	Molecular formula	Molecular weight	Family of compounds	Medicinal/Biological activity
25	1-Propyl, 9-tetradecenoate	$C_{17}H_{32}O$	268.43	Fatty acid ester	Perfuming agent, binding agent, polar emollient used in cosmetics, antimicrobial, antioxidant and stimulants (Kadhim *et al.*,2016)
26	1-Cyclohexylnonene	$C_{15}H_{28}$	208.38	Unsaturated hydrocarbon	Antimicrobial activity (Waheed *et a*l.,2019)
27	Indole, 6-methyl-2-(4-pyridyl)-	$C_{14}H_{12}N_2$	208.26	Alkaloids	Antitumor, antibacterial, antifungal, antiviral and antiplasmodial activities (Umer *et a*l.,2023)
28	3,4-Dimethoxycinnamic acid	$C_{11}H_{12}O_4$	208.21	Phenol	Reduce risk of type 2 diabetes as well as colorectal cancer, antioxidant and anti-inflammatory activities (Farrell *et al*.,2012)
29	2-Vinylbenzophenone	$C_{15}H_{12}O$	208.26	Benzophenone (an aromatic ketone)	Antifungal, anti-HIV, antimicrobial, antioxidant, antiviral and cytotoxic activities (Shi-biaowu*et al*.,2014)
30	Oleic Acid	$C_{18}H_{34}O_2$	282.26	Long chain fatty acid	Anticancer, antitumor and anti-inflammatory activities (Subban *et al.*,2011)
31	6-Octadecenoic acid	$C_{18}H_{34}O_2$	282.26	Fatty acid	Antiandrogenic, anticancer and anti-inflammatory activities (Lutfi *et al.*,2021)
32	Octadec-9-enoic acid	$C_{18}H_{34}O_2$	282.26	Monounsaturated fatty acid	Antimicrobial, antioxidant, anticancer anemiagenic and anti-androgenic activities (Elaiyaraja *et al*.,2018)
33	Butyl 9-tetradecenoate	$C_{18}H_{34}O_2$	282.46	Fatty acid ester	Not reported
34	.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 2020)
35	Bicyclo[4.2.0]oct-2-ene, 3,7-dimethyl-7-(4-methyl-3-pentenyl)-8-(2,6,10-trimethyl-1,5,9-undecatrienyl)-, [1.alpha.,6.alpha.,7.beta.,8.alpha.(1E,5E)]-	$C_{30}H_{48}$	408.70	No record	Not reported
36	Oleic Acid	$C_{18}H_{34}O_2$	282.46	Long chain fatty acid	Anticancer, antitumor and anti-inflammatory activities (Subban *et al.*,2011)

The active principles in Table 5 and 6 detected 61 bioactive phytochemical compounds in the two extracts of *Tephrosiavogelii*. The major compounds detected in the MRE and MSE of *Tephrosia vogelii*are Pyrazole-3-carboxylic acid, 1-(3-chloro-2-cyanophenyl)-5-methyl-ethyl ester (79.69%) and 11-Octadecenoic acid, methyl ester (5.93%) respectively.

Table 7 and 8 (a-b) captures the bioactive compounds detected in MRE and MSE, molecular formula, molecular weight, family of compounds and their biological/medicinal activity. In the MRE, 25 compounds were detected (Table 5) while 36 compounds were detected in the MSE (Table 6). The family of compounds detected in the extracts are fatty acids esters, fatty acids, hydrocarbons, terpenoids, phenol, alkaloids, pyrazoles, lsothiaocyanotes, polychlorinated biphenyls, steroids, esters, benzophenones, fatty alcohols, fatty aldehydes, Heterocyclic, Pyrenes, naphthoquinone, phthalate esters and organobromide. Fatty acids have many unique and important biological properties such as antifungal, anti-inflammation, anticancer and antibacterial activity.[57] Heterocyclic Compounds have antifungal, anti-inflammatory, antioxidant, anticancer, herbicidal, antiallergic and antibacterial activities.[58] 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.[59] Benzophenone have antifungal, anti-HIV, antimicrobial, antioxidant, antiviral and cytotoxic activities.[54] Naphthoquinone exhibit Cytotoxic, antibacterial, antifungal, antiviral, insecticidal, anti-inflammatory and antipyretic properties.[60] Pyrazoles show anticancer, antibacterial, antifungal, antioxidant and anti-inflammatory activities.[37] Alkaloids shows several Pharmacological activities which include antitumor, antibacterial, antifungal, antiviral and antiplasmodial activities.[38] Terpenoids possessing lactone moieties are known for their cytotoxic, anti-inflammatory, antimicrobial, anticancer, and antimalarial activities.[61]Most of the pyrene are used to conduct research. Pyrene is used to make dyes, plastics and pesticides. It has also been used to make another pyrene called Benzo(a)pyrene.[62] Phthalic acid esters or phthalate esters have been widely used in numerous consumer products, including cosmetics, food packaging, building materials, medical supplies, home furnishings and also possess antimicrobial and insecticidal activity.[63]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. [29] 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.[64]

Esters such as Heptadecyl heptafluorobutyrate shows biological activity of antioxidant, antibacterial, antifungal, hepatopreservative, anticancer, anti-inflammatory agent.[65]Hydrocarbons such as Cetene shows antimicrobial and antioxidant effect, also had highest value of antifungal activity.[26] Fatty alcohols such as 1-dodecanol shows antibacterial activity and also used as chemical to remove flower buds and suckers from tobacco plants.[39] Polychlorinated biphenyls are used as coolants and lubricants in electrical equipment and are also used in hundreds of industrial and commercial applications including electrical, heat transfer, and hydraulic equipment; as plasticizers in paints, plastics, and rubber products; in pigments, dyes, and carbonless copy paper; and many other industrial applications such as insecticides.[66] Isothiocyanates possess strong anti-oxidant, anti-inflammatory activity, anti-microbial, neuroprotective, cardioprotective activity and potent anticancer activity against various forms of cancer and tumors.[67] Fatty aldehydes such as Nonanoic acid, 9-oxo-, methyl ester exhibit antifungal, antioxidant, antimicrobial and larvicidal activity.[40] Organobromides such as Tetrapentacontane, 1,54-dibromo- possess antioxidant activity.[68] 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.

4.6CONCLUSION

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 much more possible therapeutic characteristics of the plant than already put into use.

REFERENCES

1. Ekpendu TOE, Obande OD, Anyogo PU, Attah AD. Nigerian ethnomedicine and medicinal plant flora-The Benue experience. J. Pharmaceut. Res. Dev. 1998;3, 37-46.

2. Burkill HM. The Useful Plants of West Tropical Africa (2nd ed., Vol. 3). Royal Botanical Gardens: Kew, 857; 1995.

3. Sharma R, Khanna P. Production of rotenoids from Tephrosia spp. In vivo and in vitro tissue cultures. Indian J. Exp. Biol.,*1975*; 13:84-85.

4. 4. Barnes DK, Freyre RH, Higgins JJ, Martin JA. Rotenoid content and growth characteristics of Tephrosia vogelii as affected by latitude and within-row spacing. Crop Sci., 1967; 7:93- 95.

5. Dewick PM. Medicinal natural products: a biosynthetic approach (3rd ed.). West Sussex: John Wiley & Sons, 2009. http://dx.doi.org/10.1002/9780470742761 » http://dx.doi.org/10.1002/9780470742761

6. Gurib-Fakim, A. Medicinal plants: traditions of yesterday and drugs tomorrow, Mol. Asp. Med.27 1–93, Med. Sci., 2016; 4 (2016) 1–14.

7. Ha AW, Kang HJ, Kim SL, Kim MH, Kim WK. Acute and subacute toxicity evaluation of Abbas Al-Mulla. Biological Importance of Heterocyclic Compounds. Der Pharma Chemical, 2018; 9*(13), 141-147.

8. Dubey MS. Phytochemical status of some selected medicinal plants (Eclipta alba, Cathranthus roseus and Swertia chirata). Asian J Plant Sci Res., 2014; 4:28–34.

9. Soni A, Sosa S. Phytochemical analysis and free radical scavenging potential of herbal and medicinal plant extracts. Journal of Pharmacognosy and Phytochemistry,2013; 2(4/), 22–24.

10. AOAC International. Official Methods of Analysis of AOAC International, 16th ed., 2nd rev. Method 954.01. The Association, Gaithersburg, MD; 1996.

11. AOAC. Official Methods of Analysis of the Association of Analytical Chemists international. 18th ed. Washington DC, USA: Official methods: 8; 2005.

12. Konappa N, Udayashankar AC, Krishnamurthy S, Pradeep CK, Chowdappa S, Jogaiah S. GC–MS Analysis of phytoconstituents from Amomum nilgiricum and molecular docking interactions of bioactive Serverogenin acetate with target proteins. Scientific Reports, 2020; 10, 1-23.

13. Shalini K, Ilango K. Preliminary phytochemical studies, GC-MS analysis and in vitro antioxidant activity of selected medicinal plants and its polyherbal formulation. PharmacognMag., 2021; 13, 648-59.

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14. Mlozi SH, Mmongoyo JA, Musac, O. The in- vivo toxicity evaluation of leaf and root methanolic extracts of Tephrosia Vogelii Hook. F using animal model. Clinical phytoscience,2020; 6:73.

15. Tegene TT, Belay AN. Chemical Studies of Tephrosia vogelii and Commiphoraschimperi Occurring in Ethiopia. Modern Chemistry, 2019; 7(3), 45-53.

16. Kabera JN, Semana E, Mussa AR, He X. Plant secondary metabolites: Biosynthesis, classification, function and pharmacological properties. J. Pharm. Pharmacol., 2014; 2, 377–392

17. Robert LB, Bradly L. Moisture and total solid content determination. In Introduction to the chemical analysis of food. New Delhi (India), 2002.

18. Arukwe U, Amadi BA, Duru MKC. *Chemical composition of Persea americana leaf, fruit and seed*. IJRRAS, 2012; 11(2): 346-39.

19. Gumte SV, Taur AT, Sawate AR, Kshirsagar RB. Effect of fortification of mango (Mangifera indica) kernel flour on nutritional, phytochemical and textural properties of biscuits.Journal of Pharmacognosy and Phytochemistry, 2018; 7(3): 1630-1637

20. Justina YT, Olukemi AO, Ajayi OO, Adegoke GO. "Nutritional and Antinutritional Compositions of Processed Avocado (Persea americana Mill) seeds". Asian Journal of Plant Science and Research, 2016; 6(2): 6-12.

21. Kittiphoom S. Utilization of mango seed. International Food Research Journal, 2012; 19(4): 1325-1335.

22. Siqueira CAT, Serain AF, Pascoal ACRF, Andreazza NL, de Lourenco CC, Gois Ruiz ALT, de Carvalho JE, de Souza ACO, Mesquita JT, Tempone AG, Salvadora MJ. Bioactivity and chemical composition of the essential oil from the leaves of Guatteria australis A. St.-Hil. Nat Prod Res., 2015.

23. Kuppuswamy MK, Jonnalagadda B,Arockiasamy S. GC-MS Analysis of Chloroform extract of Croton bonplandianum. International Journal of Pharmacology and Biological Sciences, 2013; 4(4):613-617.

24. Özçelik B, Aslan M, Orhan I, Karaoglu T. Microbiol. Res., *2005;* 160- 159.

25. Kontham KV, Leena D, Ganesan S, Sulochana P, Pandey A, Kesavan MN. 2, 4-Di-tert-butyl phenol as the antifungal, antioxidant bioactive purified from a newly isolated Lactococcus sp. Int. J. Food Microbiology, 2015; 211: 44-50.

26. Edet UO, Mbim EN, Ezeani E. Antimicrobial analysis of honey against Staphylococcus aureus isolates from wound, ADMET properties of its bioactive compounds and in-silico evaluation against dihydropteroate synthase. BMC Complement Med Ther, 2023; 23, 39 https://doi.org/10.1186/s12906-023-03841-z

27. Elaiyaraja A, Chandramohan G. Comparative Phytochemical Profile of *Crinum Defixum* Ker-Gawler Leaves Using GC-MS. Journal of Drug Delivery and Therapeutics, 2018; 8*(4), 365-380.

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28. Brown VJ. Life after lindane in California. Environmental Health Perspectives,2008; 116(3), A128.

29. Kumar V, Bhatnagar AK, Srivastava JN. Antibacterial activity of Crude extracts of Spirulina platensis and its structural elucidation of Bioactive compound.Journal of Medicinal Plants Research, 2011;5(32):7043-7048.

30. Manilal A, Merdekios B, Velappan JK, Paul JPV, Idhayadhulla A, Muthukumar C, Melkie M. An in vitro efficacy validation of mangrove associates. Journal of Coast Life Medicine, 2014;2(7), 560–565.

31. Hema R, Kumaravel S, Alagusundaram. GC/MS Determination of Bioactive Components of *Murrayakoenigii.*J. Am. J .Sci., 2011; 7(1):80-83

32. Subban M, Ramasamy V, Annamalai P. Evaluation of Phytochemical Constituents from the Leaves of MemecylonumbellatumBurm.f. Research Journal of Pharmaceutical, Biological and Chemical Sciences,2011; 2(4), 1145.

33. Ayoola AA, Ekunseitan DA, Muhammad SB, Oguntoye MA, and Adejola YA. The Pacific Journal of Science and Technology. Volume 21, Number 1, May 2020 (Spring)

34. Odiase-Omoighe JO,Agoreyo BO. Nig. J. Biotech. Spec. Edtn: 39-50 (May, 2022).

35. Naine SJ, Devi CS, Mohanasrinivasan V. Antimicrobial, antioxidant and cytotoxic activity of marine Streptomyces parvulus VITJS11 crude extract. Braz. Arch. Biol. Technol., 2015; 58, 198–207.

36. Lozano A, Gorinstein S, Espitia-Rangel E, Davila-Ortiz G, Martinez-Ayala AL. Plant sources, extraction methods and uses of squalene. International Journal of Agronomy, 2018; 1-13.

37. Kumar V, Kaur K, Kumar Gupta G, Sharma AK. Pyrazole Containing Natural Products: Synthetic Preview and Biological Significance. Eur.J. Med. Chem.,2013; 69:735–753.

38. Umer SM, Solangi M, Khan KM, Saleem RSZ. Indole-Containing Natural Products Isolations, Reappraisals, Syntheses, and Biological Activities. *Molecules*,2022; 27, 7586.

39. Farina M, PreetiB, Neelam P. Bio Med Research International,2014; ID 497606, 11 p

40. Sen S, Yalçin M, TasçiogluC,Özbayram AK. Larvicidal activities of some bark and wood extracts against wood-damaging insects. Maderas. Cienciatecnología,2017;. 19(3):273–284.

41. Lee YS, Kang MH, Cho SY, Jeong CS. Effects of constituents of Amomum xanthioides on gastritis in rats and on growth of gastric cancer Cells. Arch Pharm Res., 2007; 30:436-43.

42. Kabouche Z, Lakhal H,Ghorab H, Chibani S, Kabouche A, Semra Z, Smati F,Abuhamdah S. Chemical composition and biological activities of the essential oil of *Salvia officinalis* from Batna (Algeria). Der Pharmacia Lettre, 2013; 5 (3):310-314

43. Rukachaisirikul T, Siriwattanakit P, Sukcharoenphol K, Wongvein C, Ruttanaweang P,Wongwattanavuch P. Chemical constituents and bioactivity of Piper sarmentosum. Journal of Ethnopharmacology, 2004; 93, 173-6.

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44. Mary APF, Giri RS. GC-MS analysis of bioactive compounds of Achyranthes aspera. *World J Pharm Res.,2018;* 7(1):1045–1056

45. Asghar SF, Rehman H, HappyU, Choudahry MI, Atta-ur-Rahman. Gas Chromatography-mass spectrometry (GC-MS) Analysis of petroleum ether extract (oil) and bioassays of crude extract of Iris germanica. Int. J. Genet. Mol. Biol., 2011; 3(7): 95-100.

46. Adeyemi MA, Ekunseitan DA, Abiola SS, Dipeolu MA, Egbeyale LT, Sogunle OM.Phytochemical analysis and GC-MS determination of *Lagenaria breviflora R. Fruit. International Journal of Pharmaceutical and Phytochemical Research,2017; *9*(7), 1045–1050.*

47. Reddy RVN, Khalivulla SI, Reddy BAK, Reddy MVB, Gunasekar D, Deville A, Bodo B. Flavonoids from Tephrosia calophylla. Nat Prod Commun., 2009;4:59-62.

48. Yakubu EO, Otitoju O, Onwuka J. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Aqueous Extract of Daniellia Oliveri Stem Bark. Pharmaceutical Analysis and Acta,2017; 8, 568. https://doi.org/10.4172/2153-2435.1000568

49. Devakumar J, Keerthana V, Sudha SS.Asian Journal of Pharmaceutical and Clinical Research,2017;10*(1), 364-369.

50. Zainurin NAA, Hashim YZ, Mohamed Azmin NF, Al-Khatib MFR. Food Research, 2020; 4 (Suppl. 1), 63 – 73.

51. Kadhim MJ, Mohammed GJ, Hameed IH. In Vitro antibacterial, antifungal and phytochemical Analysis of methanolic fruit extract of *Cassia fistula.* Oriental Journal of Chemistry, 2016;32(2), 10-30.

52. Waheed A, Chohan MM, Ahmed D, Ullah N. The First Report on The In Vitro Antimicrobial Activities of Extracts of Leaves of Ehretia serrata. Saudi Journal of Biological Sciences, 2019; 26, 1253–1261. https://doi.org/10.1016/j.sjbs.2018.05.025

53. Farrell, T.L. (2012) Absorption of Dimethoxycinnamic Acid Derivatives in Vitro and Pharmacokinetic Profile in Human Plasma following Coffee Consumption. *Molecular Nutrition & Food Research,* 56(9), 1413–1423.

54. Shi-Biao Wu, Chunlin Long and Edward J. Kennelly. (2023). Structural diversity and bioactivities of natural benzophenones. *Natural Product Reports*, Issue 8, P, 1293- 1458.

55. Lotfi F, Akbarzadeh-Khiavi M, Lotfi Z. Micronutrient therapy and effective immune response: a promising approach for management of COVID-19. Infection, 2021; 49, 1133–1147. https://doi.org/10.1007/s15010-021-01644-3

56. Khaled R.Beta-Sitosterol Medical properties: A Review Article. IJSIT, 2020; 9(4), 208-212.

57. Elyasi H, Sepahvand A, Rahimi H, Nafari A, Azizi S, Khadem E, Bahmani M. Fatty Acids and Herbal Medicine. Current Traditional Medicine, 2019; 5(3).

58. Abbas Al-Mulla. Biological Importance of Heterocyclic Compounds. Der Pharma Chemica, 2017;9(13):141-147

59. Enas JK, Duha AA. Phytochemical characterization using GC-MS Analysis of methanolic extract of *Moringa olifera* (Family Moringaceae) plant cultivated in Iraq. Chemical Materials Research, 2014; 6(5), 9-26.

60. Babula P, Adam V, Havel L, Kizek R. "Naphthoquinones and their Pharmacological Properties". Ceská a Slovenská Farmacie (in Czech), 2007; 56 (3), 114–120. PMID 17867522.

61. Surowiak AK, Sowała M, Talma M. Cytotoxicity, early safety screening, and antimicrobial potential of minor oxime constituents of essential oils and aromatic extracts. Sci Rep., 2022; 12, 5319. https://doi.org/10.1038/s41598-022-09210-z

62. Faust RA. Toxicity Summary for Pyrene. Oak Ridge National Laboratory, Chemical Hazard Evaluation Group. Oak Ridge, TN; 1993.

63. Huang C, Huang L, Wang Y, Li X, Ren L, Gu X, Kang L, Guo L, Liu M, Zhou X, Luo J, Huang Z, Tu S, Zhao Y, Chen L, Xu D, Li Y, Li C, Peng L, Li Y, Xie W, Cui D, Shang L, Fan G, Xu J, Wang G, Wang Y, Zhong J, Wang C, Wang J, Zhang D, Cao B. 6-month consequences of COVID-19 in patients discharged from hospital: a cohort study. Lancet. 2021 Jan 16;397(10270):220-232. Doi: 10.1016/S0140-6736(20)32656-8. Epub2021; Jan 8. PMID: 33428867; PMCID: PMC7833295

64. Snehal SP, Jignasha KS. Systematic review of plant steroids as potential antiinflammatory agents: current status and future perspectives. J Phytopharmacol., 2015; 4(2):121–125

65. Veeraraghavan B, Bakthavatchalam YD, Sahni RD. Oral Antibiotics in Clinical Development for Community-Acquired Urinary Tract Infections. Infect Dis Ther. 2021 Dec;10(4):1815-1835. Doi: 10.1007/s40121-021-00509-4. Epub., 2021; Aug 6. PMID: 34357517; PMCID: PMC8572892.

66. Judson R, Houck K, Martin M, Knudsen T, Thomas RS, Sipes N, Shah I, Wambaugh J, Crofton K. In Vitro and Modelling Approaches to Risk Assessment from the U.S. Environmental Protection Agency ToxCastProgramme. Basic Clin PharmacolToxicol, 2014; 115: 69-76. https://doi.org/10.1111/bcpt.12239

67. Kala C, Ali SS, Ahmad, N., Jamal Gilani, S., and Ali Khan, N. (2018). Isothiocyanates: a review. *Research Journal of Pharmacognosy*, 5(2), 71-89. Doi: 10.22127/rjp.2018.58511

68. Addai ZR, Abood MS,Hlail SH. GC-MS Profiling, Antioxidants and Antimicrobial Activity of Prickly Pear (Opuntiaficus-indica) Pulp Extract. Pharmacognosy Journal, 2022;14(2):262-267.