

GC-MS Profiling and Secondary Metabolite Analysis of Selected Plant Extracts for Potential Anti-Hyperglycemic Activity

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Abstract

This study investigates the phytochemical composition and potential therapeutic applications of three medicinal plants *Balanites aegyptiaca*, *Garcinia kola*, and *Ziziphus mauritiana* commonly used in traditional medicine in Sokoto State, Nigeria, for the treatment of diabetes and other ailments. The research was conducted at Usmanu Danfodiyo University, Sokoto, where plant samples were collected, identified, and subjected to phytochemical analysis using methanol and aqueous extraction methods. Gas Chromatography-Mass Spectrometry (GC-MS) was employed to identify and quantify the secondary metabolites present in the extracts. The results revealed the presence of numerous bioactive compounds, including phenols, quinoline derivatives, and triazolo pyrimidines, which are known for their antimicrobial, antioxidant, anti-inflammatory, and antidiabetic properties. *Balanites aegyptiaca* exhibited significant amounts of Phenol,2-methoxy (77.57%), while *Ziziphus mauritiana* and *Garcinia kola* showed compounds such as E-8-Methyl-9-tetradecen-1-ol acetate and [1,2,4] Triazolo[1,5-a] pyrimidin-5-ol,7-methyl-6-nitro, respectively. The study highlights the potential of these plants in treating various diseases, including diabetes, cancer, and microbial infections, and underscores the importance of further pharmacological research to validate their traditional uses. The findings suggest that these plants could serve as valuable sources of natural compounds for developing new therapeutic agents.

Keywords: GC-MS Profiling, Secondary Metabolite, Anti-Hyperglycemic Activity, Medicinal Plants.

Introduction

Since ancient times, humans have been intimately connected with plants, utilizing them in various ways throughout history. Many medicinal plants were discovered through the reciprocal relationship between humans and their natural environment (Anichini et al., 2020). According to the World Health Organization (WHO), 255 medications are considered essential, with 11% of them derived from plants. Additionally, several synthetic drugs are developed from natural precursors (Barman et al., 2024). Diabetes mellitus, a widespread endocrine disorder, leads to elevated blood sugar levels due to either insufficient insulin production by beta cells (Type 1) or the body's inability to effectively use the insulin

produced (Type 2). This condition arises from issues related to insulin resistance, insulin production, or both, disrupting the metabolism of proteins, fats, and carbohydrates (Barman et al., 2024). Chronic hyperglycemia, a hallmark of diabetes, has been associated with long-term damage to organs, leading to dysfunction and failure, particularly affecting the kidneys, eyes, nerves, heart, and blood vessels. Despite significant advancements in diabetes management and the availability of various oral medications, the scientific community continues to explore new treatments due to the limitations of existing therapies (Reuben et al., 2021). Antioxidants are believed to play a crucial role in managing diabetes, as oxidative stress and the resulting generation of free radicals have been linked to the development of type 2 diabetes.

The onset of diabetes is closely associated with oxidative stress, mainly through oxidation, nonenzymatic protein glycation, and oxidative degradation of glycated proteins. Reactive oxygen species (ROS) are continuously formed during energy production by cells using the essential cellular metabolism ingredient oxygen. Reactive oxygen species (ROS) are capable of oxidizing cellular proteins, nucleic acids, and lipids, which increases in both types of diabetes. Elevation of ROS such as mitochondrial superoxide in endothelial cells and endoplasmic reticulum stress, followed by reduced antioxidant defence mechanisms, provokes cellular and enzyme damage and lipid peroxidation, which subsequently lead to the development and progression of insulin resistance by disrupting insulin receptor signal transduction and insulin deficiency by attacking insulin-producing cells (Khouya et al., 2022).

Sokoto State, like much of Nigeria, boasts significant environmental and ecological diversity. Traditional healers in the region have long utilized a variety of medicinal plants, many of which have been reported for their efficacy and safety (Aisha et al., 2022). Indigenous knowledge systems and practices play a crucial role in human and animal healthcare (Zamzami et al., 2023). To further validate and enhance the effectiveness of these medicinal plants, extensive phytochemical and pharmacological research is essential. It is widely recognized that traditional medicine is often the first recourse for patients due to its cultural significance and economic accessibility.

Publications of phytochemical validation of medicinal plants for traditional remedies by tribal people in the Sokoto South senatorial districts (Shagari and Tambuwal) vicinity are scanty and until recently, researchers have not conducted a comprehensive study of plants used by the ethnic people from an ethnomedicinal and phytopharmacological point of view. Hence, the present study was designed with the following objectives: (a) to conduct GC-MS profiling of selected medicinal plants; and (b) to carry out Secondary Metabolite Analysis of Selected Plant Extracts for Potential Anti-Hyperglycemic Activity.

Materials and Methods

Study Area

The research was conducted at the department of Biochemistry Usmanu Danfodiyo University, Sokoto for the phytochemical analysis of the selected plant. Data were collected

from two local government areas of Sokoto State, (i.e. Shagari and Tambuwal). Sokoto is located between the coordinates; latitude 11°30'' to 13°50'' North and Longitude 4° to 6° East 253.44 above sea level. It has an annual average temperature of 28.3°C and a maximum day time temperature under 40°C. the rainy season is from June to October (Aisha *et al.*, 2023).

Plant Sample Collection and Identification

The samples of the selected medicinal plants were collected from the Shagari and Tambuwal Communities and their environs from 6th August 2024 to 16th September 2024, and were identified with voucher number given to each plant at the herbarium of the Department of plant science Usmanu Danfodiyo University Sokoto. The fresh sample was cleaned using tap water to eliminate impurities or soil debris. The sample was crushed to a fine powder with an electric blender after shade drying at room temperature and kept in a zip lock bag in a freezer (–20°C) for further analysis (Mainasara *et al.*, 2021).

Table 1. Taxonomic Identification and Herbarium Voucher Details of Selected

	Botanical Names	Local Names	Voucher Number
3	<i>Balanites aegyptiaca</i>	Aduwa	UDUH/ANS/0024
4	<i>Garcinia kola</i>	Namijin goro	UDUH/ANS/0123
6	<i>Ziziphus mauritiana</i>	Magarya	UDUH/ANS/0017

Determination of Secondary Metabolites in the Selected Plants

Extraction Procedure

The plant parts were extracted with water, and methanol, using activity-guided fractionation. 1.5 kg of the powder of each plant parts were soaked in 3 litres of water for 24 hours and filtered. In this case, 400g of each of the extracts were reconstituted into 500ml of each of the organic solvents, hexane, ethyl acetate, chloroform, and water were added successively based on their polarity. These were mixed continuously for 10 minutes and the two layers were separated with a separating funnel. The fractions obtained were dried in a drying cabinet at 50°C and weighed (Zilani *et al.*, 2020). Ten grams (10g) were measured from each. The extracts were stored in labelled glass bottles with lids under room condition until required for bioassay. The percentage yields of the extracts were determined as: Equation (3.1) given by (Barman *et al.*, 2024).

% Yield = $\frac{\text{weight of dry extract}}{\text{Weight of the sample extracted}} \times 100$

Methanolic Extract

For the collection of methanolic extract, 50g of sample powder was packed in thimble and extracted in the Soxhlet apparatus using 250 ml of methanol. The temperature was kept between 60 to 80°C. The samples in the thimble of Soxhlet apparatus were kept boiling for approximately 4 hrs till the solution became clear and the dark colour extract at the bottom

of the apparatus. These were collected in petri dishes and left to dry for 24 hrs. The dried extract having a sticky appearance was stored in 25ml in McCartney bottles at the temperature of about 4°C in the refrigerator for further use. The whole process was repeated 3 times for the collection of a substantial amount of extracts for the study (Brisky *et al.*, 2022).



Plate 1: Methanol extract of the selected plant samples

Aqueous Extract

For the collection of aqueous extracts, 40 of the sample powder was measured and mixed with 480ml of distilled water. This was left for 2 days in a sterile environment. The liquid extract was then filtered through Whatman filtered paper No.40. The filtrate was kept in the water bath at 80 to 90°C till the extract dried out. The dried extract was then stored at 4°C in McCartney bottles ((Brisky *et al.*, 2022).



Plate 2: Aqueous extract of the selected plant samples

Storage of Extracts

The extracts were collected in 25ml McCartney bottles. Each bottle was filled with extract. The bottles were then stored in a fridge at 4°C in the laboratory until they were used for qualitative and GCMS analysis (Debnath *et al.*, 2020).

Identification of Compounds in the Selected Plants using GC-MS Analysis

Methanolic extract

For the collection of methanolic extract, 50g of sample powder was packed in a thimble and extracted in the Soxhlet apparatus using 250 ml of methanol. The temperature was kept between 60 to 80°C. The samples in the thimble of Soxhlet apparatus were kept boiling for approximately 4 hours till the solution became clear and the dark colour extract at the bottom of the apparatus. These were collected in petri dishes and left to dry for 24 hrs. The dried extract having a sticky appearance was stored in 25ml in McCartney bottles at a temperature of about 4°C in the refrigerator for further use. The whole process was repeated 3 times for the collection of a substantial number of extracts for the study (Zamzani *et al.*, 2023; Barman *et al.*, 2024).

Storage of extracts

The extracts were collected in 25ml of McCartney bottles. In each bottle, extract was collected till the bottle were filled. They were stored in a fridge at 4°C in the laboratory until it was used for qualitative and GCMS analysis.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC-MS analysis was carried out on a Perkin Elmer Turbo Mass Spectrophotometer which includes a Perkin Elmer autosampler XLGC. The column used in GC was Perkin Elmer Elite - 5 capillary columns measuring 30 m × 0.25 mm with a film thickness of 0.25 mm composed of 95% dimethylpolysiloxane. The helium (99.999%) was used as carrier gas at a flow rate of 0.5 ml/min. The sample injection volume utilized was 1 µl. The inlet temperature was maintained at 250°C. The oven temperature was programmed 110°C (isothermal for 2 min) with an increase of 10°C/ min to 200°C then 5 °C/min to 280°C, ending with a 5 min isothermal at 280°C. Total run time was 30 min. The MS transfer line was maintained at a temperature of 200 °C. The source temperature was maintained at 180 °C. GCMS was analyzed using electron impact ionization at 70eV, and data was evaluated using Total Ion Count (TIC) for compound identification and quantification. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS library. Measurement of peak areas and data processing were carried out by Turbo-Mass OCPTVS-Demo SPL software (Elizabeth *et al.*, 2013; Debnath *et al.*, 2020; Dey *et al.*, 2022; Nazmun *et al.*, 2024).

Results

The result of *Balanites aegyptiaca* revealed 12 peaks [Table 4], with 12 compounds identified representing 100% of the entire extract. The major among them were Phenol,2-methoxy with (77.57%), Dodecane,1,1,-oxybis with (2.97%),3-Methyl-4-(methoxycarbonyl)hexa-,4-dienoic acid with(2.63%),Ethyl3-{[3-ethoxy-3-oxoprop-1-en-1-yl]oxy}prop-2-enoate with (2.13%),Benzene,2-chloro-1-ethyl-5-methoxy-3-3-methyl with (1.97%), Dodecane,1,1,-oxybis and 6-Ethoxy-9-thia-bicyclo(3.3.1) non-2-ene both with (1.96%), each while Dodecanol,2-octyl had (1.95%),3-Methyl-4-(methoxycarbonyl)hexa-2,4-dienoic acid with (1.82%),Tetradecane,1-fluoro and (1.74%),4-Bromobutanoic acid, hexadecyl ester with (1.73%),DL-3-Camphorcarboxylic acid (1.58%).

The results of *Z. Mauritiana* revealed 40 peaks [Table 5], with 40 compounds identified representing 100% of the entire extract. The major among them were E-8-Methyl-9-tetradecen-1-ol acetate with (22.34%), Dibenzo[g,c][1,2]diazocine,5-oxid having (4.21%),2-Methyl-Z-4-tetradecene (3.26%),[1,2,4] Triazole [1,5,-a]pyrimidin-5-ol,7-methyl-6-nitro with (2.93%), E-8-Methyl-9-tetradecen-1-ol acetate (2.88%), Cyclohexadecane with (2.59%), Quinoline,2-chloro-6-methoxy-4-methyl having (2.42%), Cyclopentadecane and (2.41%),2-butenedioic acid,2,3-bis(phenylamino)-, diethyl ester (2.39%),Ethanone,1-cyclododecyl with (2.15%), N-(2-Acetylcyclopentylidene) cyclohexylamine and (2.11%),3,3-Diisopropoxy-1,1,1,5,5,5-hexamethyltrisiloxane and (1.99%),Cyclododecanone,2-methylene and (1.98%), Methyl-Z-4-tetradecene with (1.95%),Ethanone,1-cyclododecyl and (1.92%), Benzene,1,1¹-oxybis [3-phenoxy and(1.89%),3-Hydroxy-4-methoxybenzaldehyde, O-methoxycarbonyl with (1.88%),Silane,dimethyl(2-fluorophenyl)oxy)dodecyloxy with (1.87%), Tricyclo[4.2.1.0(2,5)]nona-3,7-diene,9-methoxy-1-phenyl with (1.85%), Tricyclo[4.2.1.0(2,5)]nona-3,7-diene,9-methoxy-1-phenyl,Pentatriacontane,13-docosenylidene and 4-phenyl-3,4-dihydroisoquinoline both with (1.79%) each, 2-Methyl-Z-4-tetradecene with (1.78%), 4¹-(10-Amino-9-phenanthrylazo) acetanilide with (1.77%), Pentadecafluorooctanoic acid, pentadecyl ester with (1.76%), Methyl 6,8-dodecadienyl ether and Amino-1,1-dicarboxylic acid,N-3-fluorophenyl both with (1.75%) each, [1,2,4]Triazolo[1,5-a]pyrimidin-5-ol,7-methyl-6-nitro with (1.65%), 1,2,4-Triazol-3-amine,5-(1,3,5-trimethyl-4-oxazolyl) amino and 1,2,5-Oxadiazol-3-amine,4-(3-methoxyphenoxy) both with (1.63%) each, [1,2,4]Triazolo[1,5-a]pyrimidin-5-ol,7-methyl-6-nitro with (1.62%), Cyclododecane, ethyl with (1.60%), E-8-Methyl-9-tetradecen-1-ol acetate with (1.59%),9-Undecen-2-one,6,10-dimethyl with (1.58%),2-Methyl-Z-4-tetradecene with (1.56%),2-Methyl-Z-4-tetradecene with (1.54%), Fumaric acid,2-decyl pentadecyl ester with (1.52%), 15-Oxapentacyclo[10.2.1.1(5,8).0(2,11).0(4,9)] hexadeca-2,4(9),6,10,1,3-pentaene with (1.50%).

The result of *Garcinia kola* revealed 40 peaks (Table 6) with 40 compounds identified representing 61.2% of the entire extract. The major among them were Benzamide,3,4-dimethoxy-N-hexadecyl with (2.77%),2-Methyl-Z-4-tetradecene (2.39%), [1,2,4] Triazolo[1,5-a] pyrimidin-5-ol,7-methyl-6-nitro (2.37%), Phenanthrene,9,10-dihydro-1-methyl (2.26%), Fluorene,4-[1,2-dihydroxyethyl] (2.06%),5,8,11,14-Eicosatetraynoic acid

with (1.99%), 2H-1,4-Benzodiazepin-2-one, 7-amino-1,3,4,5-tetrahydro-5-phenyl with (1.97%), [1,2,4] Triazolo[1,5-a] pyrimidin-5-ol, 7-methyl-6-nitro with (1.91%), Boroxin, isopropylidipropyl with (1.89%), [1,2,4] Triazolo [1,5-a] pyrimidin-5-ol, 7-methyl-6-nitro (1.81%), Oxirane, 2,2-diphenyl with (1.75%), Hexanamide, 6-bromo-N-ethylhexyl (1.68%), 1,1,6,6-Tetramethylspiro [4.4] nonan (1.66%), Fluorene, 4-[1,2-dihydroxyethyl] (1.63%), Methyl-2-(5-amino-1H-1,2,4-triazol-3-yl) cyclohexane-1-carboxylate (1.59%), [1,2,4] Triazolo[1,5-a] pyrimidin-5-ol, 7-methyl-6-nitro (1.55%), Cyclohexane, 1-(cyclohexylmethyl)-2-ethyl-cis (1.48%), 1,2,5-Oxadiborolane, 2,3,3,4,5-pentaethyl (1.47%), Spiro[2,4,5,6,7,7a-hexahydro-2-oxo-4,4,7a-trimethylbenzofuran]-7,2¹-(oxirane) 1.46%, Cyclohexane, 1-(cyclohexylmethyl)-2-ethyl-cis (1.45%), Benzamide, 2,3,4-trimethoxy-N-methyl and Phenanthrene, 9,10-dihydro-1-methyl both with (1.32%) each Tricyclo[4.2.1.0(2,5)]nona-3,7-diene, 9-methoxy-1-phenyl and 1,4-Dicyano-2-(5-hexenyl) benzene both had (1.31%) each, (4H), 3,2-Dioxaborin, 4-ethyl-4,6-diethyl-5-(1-methylethyl) (1.30%), Cyclohexane, 1-(cyclohexylmethyl)-2-ethyl-cis and (1.29%), 4,7-Dimethoxy-5-[2-nitroethenyl]-2H-1,3-benzodioxole and 3-Buten-2-one, 4-(3-hydroxy-6,6-dimethyl-2-methylenecyclohexyl) both had (1.26%) each, 2,6-pyridine diamidoxime and (1.23%), 2-(3-Hydroxy-propyl)-cyclohexanol (1.22%), Cyclopentadecane and D,L-3-Camphorcarboxylic acid both had (1.21%), Cyclohexane, 1-(cyclohexylmethyl)-2-ethyl-cis with (1.20%), 2H-1,4-Benzodiazepin-2-one, 7-amino-1,3,4,5-tetrahydro-5-phen (1.19%), Phosphetane, 1-chloro-2,2,3,4,4-pentamethyl-1-oxide (1.11%), [1,2,4] Triazolo[1,5-a] pyrimidin-5-ol, 7-methyl-6-nitro had (1.09%), Cyclohexane, 1-(cyclohexylmethyl)-2-ethyl-, cis and 2-Methyl-Z-4-tetradecene both had (1.06%) each, Fluorene, 4-[1,2-dihydroxyethyl] obtained (1.05%).

Table 2. Showing the phytochemical compounds identified from *Balanites aegyptiaca* plant extract using methanol

Peak No	RT (min)	Name of identified compounds	Area (%)	Quality
1	7.073	Phenol, 2-methoxy	77.57	76
2	14.391	4-Bromobutanoic acid, hexadecyl ester	1.73	22
3	14.597	3-Methyl-4-(methoxycarbonyl) hexa-2,4-dienoic acid	2.63	43
4	15.684	Dodecane, 1,1-oxybis	1.96	43
5	16.079	D, L-3-Camphorcarboxylic acid	1.58	62
6	16.297	Dodecane, 1,1-oxybis	2.97	38
7	16.697	Benzene, 2-chloro-1-ethyl-5-methoxy-3-methyl	1.97	25
8	17.767	Tetradecane, 1-fluoro	1.74	50
9	17.973	6-Ethoxy-9-thia-bicyclo (3.3.1) non-2-ene	1.96	64
10	18.019	3-Methyl-4-(methoxycarbonyl) hexa-2,4-dienoic acid	1.82	43
11	19.890	Dodecanol, 2-octyl	1.95	50
12	19.947	Ethyl 3-[[3-ethoxy-3-oxoprop-1-en-1-yl] oxy] prop-2-enoate	2.13	46
		Total compounds	12	

Table 3. Showing Phytochemical compounds identified from *Ziziphus mauritiana* plant extract using methanol.

Peak No	RT (min)	Name of identified compounds	Area (%)	Quality
1	6.020	E-8-Methyl-9-tetradecen-1-ol acetate	22.34	91
2	14.197	2-Methyl-Z-4-tetradecene	1.54	43
3	14.380	Fumaric acid,2-decyl pentadecyl ester	1.52	45
4	14.546	E-8-Methyl-9-tetradecen-1-ol acetate	2.88	46
5	15.078	Pentadecafluorooctanoic acid,pentadecyl ester	1.76	38
6	15.221	Dibenzo[c,g][1,2]diazocine,5-oxid	4.21	56
7	15.398	[1,2,4] Triazolo[1,5-a] pyrimidin-5-ol,7-methyl-6-nitro	1.65	35
8	15.673	Methyl-Z-4-tetradecene	1.95	46
9	15.970	Methyl 6,8-dodecadienyl ether	1.75	25
10	16.514	2-Methyl-Z-4-tetradecene	1.56	47
11	17.069	Cyclododecanone,2-methylene	1.98	90
12	17.155	Benzene,1,1 ¹ -oxybis [3-phenoxy	1.89	25
13	17.326	Tricyclo[4.2.1.0(2,5)]nona-3,7-diene,9-methoxy-1-phenyl	1.79	49
14	17.601	E-8-Methyl-9-tetradecen-1-ol acetate	1.87	45
15	18.643	Cyclopentadecane	2.41	52
16	18.889	Ethanone,1-cyclododecyl	1.92	40
17	18.929	[1,2,4] Triazole [1,5,-a]pyrimidin-5-ol,7-methyl-6-nitro	2.93	30
18	19.037	2-Methyl-Z-4-tetradecene	1.78	45
19	19.444	9-Undecen-2-one,6,10-dimethyl	1.58	55
20	19.570	[1,2,4]Triazolo[1,5-a]pyrimidin-5-ol,7-methyl-6-nitro	1.62	22
21	19.615	Pentatriacontane,13-docosenylidene	1.79	27
22	20.399	Cyclohexadecane	2.59	35
23	20.542	15-Oxapentacyclo [10.2.1.1(5,8).0(2,11).0(4,9)] hexadeca-2,4(9),6,10,1,3-pentaene	1.50	25
24	20.886	3-Hydroxy-4-methoxybenzaldehyde, O-methoxycarbonyl	1.88	43
25	21.389	Silane,dimethyl(2-fluorophenoxy)dodecyloxy	1.87	38
26	21.452	Cyclododecane,ethyl	1.60	74
27	21.710	2-Methyl-Z-4-tetradecene	3.26	83
28	22.110	E-8-Methyl-9-tetradecen-1-ol acetate	1.59	45
29	22.150	Tricyclo[4.2.1.0(2,5)]nona-3,7-diene,9-methoxy-1-phenyl	1.53	59
30	22.213	2-butenedioic acid,2,3-bis(phenylamino)-, diethyl ester	2.39	30
31	22.265	Tricyclo[4.2.1.0(2,5)]nona-3,7-diene,9-methoxy-1-phenyl	1.85	55
32	22.694	4 ¹ -(10-Amino-9-phenanthrylazo) acetanilide	1.77	14
33	22.917	1,2,4-Triazol-3-amine,5-(1,3,5-trimethyl-4-oyrazolyl) amino	1.63	35
34	23.295	4-phenyl-3,4-dihydroisoquinoline	1.79	35
35	23.461	N-(2-Acetylcyclopentylidene) cyclohexylamine	2.11	42
36	23.501	Ethanone,1-cyclododecyl	2.15	25
37	23.724	1,2,5-Oxadiazol-3-amine,4-(3-methoxyphenoxy)	1.63	35
38	24.101	Quinoline,2-chloro-6-methoxy-4-methyl	2.42	38

39	24.302	3,3-Diisopropoxy-1,1,1,5,5,5-hexamethyltrisiloxane	1.99	47
40	26.230	Amino-1,1-dicarboxylic acid, N-3-fluorophenyl	1.75	25
		Total compounds	40	

Table 4. Showing Phytochemical compounds identified from *Garcinia kola* plant extracts using methanol.

Peak No	RT (min)	Name of identified compounds	Area (%)	Quality
1	16.823	Fluorene,4-[1,2-dihydroxyethyl]	1.63	64
2	17.029	Spiro[2,4,5,6,7,7a-hexahydro-2-oxo-4,4,7a-trimethylbenzofuran]-7,2 ¹ -(oxirane)	1.46	44
3	17.527	Cyclopentadecane	1.21	68
4	17.716	2H-1,4-Benzodiazepin-2-one,7-amino-1,3,4,5-tetrahydro-5-phenyl	1.97	25
5	18.019	2-Methyl-Z-4-tetradecene	2.39	66
6	18.185	5,8,11,14-Eicosatetraynoic acid	1.99	30
7	18.253	Cyclohexane,1-(cyclohexylmethyl)-2-ethyl-, cis	1.06	51
8	18.397	Tricyclo[4.2.1.0(2,5)]nona-3,7-diene,9-methoxy-1-phenyl	1.31	25
9	18.500	[1,2,4] Triazolo [1,5-a] pyrimidin-5-ol,7-methyl-6-nitro	1.81	11
10	18.585	Phosphetane,1-chloro-2,2,3,4,4-pentamethyl-,1-oxide	1.11	15
11	18.608	Cyclohexane,1-(cyclohexylmethyl)-2-ethyl-cis	1.20	25
12	18.688	1,4-Dicyano-2-(5-hexenyl) benzene	1.31	18
13	19.009	[1,2,4] Triazolo[1,5-a] pyrimidin-5-ol,7-methyl-6-nitro	1.55	55
14	19.117	[1,2,4] Triazolo[1,5-a] pyrimidin-5-ol,7-methyl-6-nitro	1.91	27
15	19.209	Cyclohexane,1-(cyclohexylmethyl)-2-ethyl-cis	1.29	44
16	19.243	(4H),3,2-Dioxaborin,4-ethyl-4,6-diethyl-5-(1-methylethyl)	1.30	25
17	19.312	2,6-pyridine diamidoxime	1.23	35
18	19.381	Cyclohexane,1-(cyclohexylmethyl)-2-ethyl-cis	1.45	46
19	19.449	Methyl2-(5-amino-1H-1,2,4-triazol-3-yl) cyclohexane-1-carboxylate	1.59	25
20	19.501	Fluorene,4-[1,2-dihydroxyethyl]	1.05	25
21	19.581	2-(3-Hydroxy-propyl)-cyclohexanol	1.22	41
22	19.747	Tricyclo[4.2.1.0(2,5)]nona-3,7-diene,9-methoxy-1-phenyl	1.06	60
23	19.861	1,2,5-Oxadiborolane,2,3,3,4,5-pentaethyl	1.47	86
24	20.004	2H-1,4-Benzodiazepin-2-one,7-amino-1,3,4,5-tetrahydro-5-phenyl	1.19	22
25	20.107	Benzamide,2,3,4-trimethoxy-N-methyl	1.32	64
26	20.136	Phenanthrene,9,10-dihydro-1-methyl	1.32	38
27	20.153	1,1,6,6-Tetramethylspiro [4.4] nonan	1.66	30
28	20.233	Hexanamide,6-bromo-N-ethylhexyl	1.68	14
29	20.376	2-Methyl-Z-4-tetradecene	1.06	38
30	20.405	Cyclohexane,1-(cyclohexylmethyl)-2-ethyl-cis	1.48	35
31	20.462	Oxirane,2,2-diphenyl	1.75	22

32	20.542	[1,2,4] Triazolo[1,5-a] pyrimidin-5-ol,7-methyl-6-nitro	2.37	27
33	20.634	4,7-Dimethoxy-5-[2-nitroethenyl]-2H-1,3-benzodioxole	1.26	25
34	20.737	Phenanthrene,9,10-dihydro-1-methyl	2.26	25
35	20.851	D,L-3-Camphorcarboxylic acid	1.21	46
36	20.931	Fluorene,4-[1,2-dihydroxyethyl]	2.06	60
37	21.017	3-Buten-2-one,4-(3-hydroxy-6,6-dimethyl-2-2-methylenecyclohexyl)	1.26	38
38	21.057	Boroxin, isopropylidipropyl	1.89	25
39	21.074	Benzamide,3,4-dimethoxy-N-hexadecyl	2.77	27
40	21.132	[1,2,4] Triazolo[1,5-a] pyrimidin-5-ol,7-methyl-6-nitro	1.09	42
Total compounds			40	

Discussion

The result extract of *Z. mauritiana* leaves using methanol extract revealed that 40 phytochemical compounds were identified in 100% peak area. As per the known biological activity action of the identified compounds [1,2,4] Triazolo[1,5-a] pyrimidine-5-ol,7-methyl-6-nitro is identified in the extract of *Ziziphus mauritiana* three times but in different quantities with (2.93%), (1.65%) and (1.62%) respectively. The [1,2,4] triazolo[1,5-a] pyrimidines (TPs) are present in diverse important structures in agriculture and medicinal chemistry, such as antidiabetic, antibacterial, antifungal, antiparasitic, herbicidal, antimalarial, antiviral, cardiovascular vasodilators, anti-inflammatory, analgesic, and anti-cancer. This is similar to the study of Ohnishi *et al.*, 1981; Zhou *et al.*, 2004; and Watanabe *et al.* (2012); Dey *et al.*, (2022). which revealed that Some synthetics of [1,2,4] triazolo[1,5-a] pyrimidines have been commercially employed in medical practice and agro chemistry. Trepidil is a platelet-derived growth factor antagonist and a phosphodiesterase inhibitor marketed in several countries and used in ischemic coronary heart, liver, and kidney diseases. This also corresponds with the findings of (Lamberth, 2018) which revealed that Some [1,2,4] triazole pyrimidines containing sulfonamide functions are commercial herbicides among them, flumetsulam has been used to control weeds and grasses in soybean, pea, and corn leaves, but it has the disadvantage of its low rate of degradation, leaving traces of residues in the soil.

The presence of methoxylated rings leads to rapid soil degradation due to its conversion to hydroxyl and, in this context, the commercial herbicide metosulam stands out. While diclosulam has been using in the control of broad-leaved weeds in soybeans, pyroxsulam has been applying in the same control, but in cereals. This corresponds to the findings of Serey *et al.* (2007); Montel *et al.* (2008) which revealed that the 7-amino derivatives such as BAS600F (7) represent a class of active fungicides against a broad range of plant pathogens that act as promoters of tubulin polymerization with a paclitaxel like mode of action. This is similar to the previous findings of Zamzani *et al.* (2023) which revealed the mechanism of action of TPs and analogues in the cardiovascular system. In this context, the 2-(benzylthio)-1,2,4-triazolo[1,5-a] pyrimidines 13a and 13b as cyclic adenosine 3',5'-

monophosphate (cAMP) phosphodiesterase (PDE) inhibitors, where 13a is as potent as theophylline as a cardiovascular vasodilator.

The specificity of the action of these PDE inhibitors was attributed to the selective binding at a given cAMP PDE site in the cardiovascular system. This is similar to the findings of Uryu *et al.* (2002) which revealed that, Alzheimer's disease (AD) is among the most severe irreversible neurodegenerative disorders of the Central Nervous System (CNS), characterized by memory loss. In this context, some synthetic [1,2,4] triazolo [1,5-a] pyrimidines and [1,2,4] triazolo [1,5-a] pyrimidin-5-ones acting by different mechanisms of action were considered as a potential candidate therapeutic for AD. However, an intervention strategy in AD has been the amyloid hypothesis, in which the abnormal accumulation of β -amyloid peptide ($A\beta$), especially $A\beta_{40}$ and $A\beta_{42}$, plays a fundamental role in the pathogenesis of the disease.

The $A\beta$ activates microglia that can induce inflammation in the brain leading to cell loss and cognitive decline. Thus, compounds that inhibit $A\beta$ -induced activation of microglia have been considered in the therapy for AD. This also correspond to the finding of Patil *et al.* (2014) who reported that, infection by *Mycobacterium tuberculosis* (MTB), the causative bacterial agent of tuberculosis (TB), represent an escalating threat for global health with the increased prevalence of multi- and extensively drug-resistant tuberculosis. Thus, some triazolo pyrimidines and analogs have been investigated against *M. tuberculosis*. This corresponds to the findings of El-Gendy *et al.* (2008) who reported that, some [1,2,4] triazolo[1,5-a] pyrimidines, as well as their dihydro analogs and [1,2,4] triazolo[1,5-a] pyrimidinones showed activity against other bacteria and some fungi. This corresponds to the findings of Reis *et al.* (2017) who revealed that, many synthetic [1,2,4] triazole [1,5-a] pyrimidines and analogs have shown activity against parasitic diseases, such as malaria, leishmaniasis, Chagas disease, and African human trypanosomiasis. This is similar to the findings of Steinmetz and Prota, (2018) who reported that [1,2,4] triazolo[1,5-a] pyrimidines, a subtype of purine bioisosteric analogs, have been extensively reported to possess promising activities against a panel of cancer cell lines via different mechanisms. Moreover, Quinoline,2-chloro-6-methoxy-4-methyl is identified in the extract of *Ziziphus mauritiana* with (2.42%).

Quinoline is a heterocyclic aromatic organic compound having molecular formula C_9H_7N , characterized by a double-ring structure that contains a benzene ring fused to pyridine at two adjacent carbon atoms. Quinoline,2-chloro-6-methoxy-4-methyl compounds derivatives are widely used as "parental" compounds to synthesize molecules with medical benefits, especially with anti-malarial and anti-microbial activities. Several quinolines and its derivatives are known to possess antimicrobial, antitumor, antidiabetic, anticancer, antifungal, analgesic and anti-inflammatory activities. This is similar to the findings of Ryckebusch *et al.* (2008) who reported that applications of quinoline derivatives are fast spreading from anticancer drugs to almost every branch of medicinal chemistry, quinoline derivatives represent a large number of antiproliferative agents exhibiting cytotoxicity through DNA intercalation, causing interference in the replication process, Actinomycin D,

doxorubicin, mitoxantrone and streptonigrin are quinoline analogues possessing antibacterial or anti-cancer activity through DNA intercalation. This also corresponds to the study of Ghorab and Mansour, (2015) who reported that, a series of 3-quinoline derivatives. compounds were synthesized via a reaction of 3 amino quinoline with ethyl cyanoacetate followed by a reaction with substituted aldehydes.

The structures of the newly synthesized compounds were confirmed by elemental analyses and spectroscopic techniques. All the newly synthesized compounds were evaluated for their cytotoxic activity against a human tumour breast cancer cell line (MCF7). It was found that the compounds 2-Cyano-3-(4-hydroxy-3-methoxyphenyl) N-(quinolin-3-yl) acrylamide (11), 3-Oxo-N-(quinolin-3-yl)3H-benzol[f]chromene-2-carboxamide (12), 2-Cyano-3-(4-fluorophenyl)-N-(quinolin-3-yl) acrylamide (13) and 2-Cyano-5-(4-(dimethylamino) phenyl)- N-(quinolin-3-yl) penta-2,4dienamide (14) exhibited higher activity with IC_{50} values of 29.8, 39.0, 40.0, 40.4 $\mu\text{mol L}^{-1}$, respectively. This is also similar to the findings of Ryckebusch *et al.* (2003); Sagheer *et al.* (2013) who reported that, N-alkylated quinoline derivatives exhibited a wide spectrum of pharmacological activities, such as antiplasmodial, cytotoxic, antibacterial, antiproliferative, antimalarial, and anticancer activity. This corresponds to findings of Cheng *et al.* (2008); Castellano *et al.* (2008) who reported that, 5,8-quinoline derivatives possess a wide spectrum of biological properties that include anti-tumor, anti-inflammatory and anti-bacterial activities.

The extract of *Ziziphus mauritiana* using methanol extract revealed the presence of 1,2,5-Oxadiazol-3-amine,4-(3-methoxyphenoxy) compounds. 1,2,5-Oxadiazol-3-amine,4-(3-methoxy phenoxy) derivatives contain several synthesized compounds with biological activities with their analgesic, antimicrobial, and anti-inflammatory activities. This is similar to the findings of Boshelli, *et al.* (1993) who revealed that substances containing 1,2,5-Oxadiazol-3-amine,4-(3-methoxy phenoxy) have occupied a unique position in the design and synthesis of biologically active agents with remarkable analgesic, anti-inflammatory and antimicrobial activities.

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The results of extract of *Garcinia kola* using methanol extract revealed 40 phytochemical compounds identified in 61.2% peak area. The extract of *Garcinia Kola* revealed the presence of [1,2,4] Triazolo [1,5-a] pyrimidin-5-ol,7-methyl-6-nitro compound four times with different quantity (2.37%), (1.91%), (1.55%) and (1.09%) respectively. The [1,2,4] triazolo[1,5-a] pyrimidines (TPs) are present in diverse important structures in agriculture

and medicinal chemistry, such as antibacterial, antifungal, antiviral, antidiabetic, antiparasitic, herbicidal, antimalarial, antiviral, cardiovascular vasodilators, anti-inflammatory, analgesic, and anticancer. This is similar to the study of Ohnishi *et al.* (1981); Zhou *et al.* (2004); Watanabe *et al.* (2012) which revealed that Some synthetics of [1,2,4] triazolo[1,5-a] pyrimidines have been commercially employed in medical practice and agro chemistry. Trepidil is a platelet-derived growth factor antagonist and a phosphodiesterase inhibitor marketed in several countries used in ischemic coronary heart, liver, and kidney diseases. (Zhou *et al.*, 2004; Watanabe *et al.*, 2012).

Filbuvir is a potent and orally available drug for the treatment of hepatitis C acting by inhibition of the HCV virus NS5B polymerase enzyme (Singer *et al.*, 2014) This also corresponds with the findings of (Lamberth, 2018) which revealed that Some [1,2,4] triazolo pyrimidines containing sulfonamide functions are commercial herbicides Among them, flumetsulam has been used to control weeds and grasses in soybean, pea, and corn leaves, but it has the disadvantage of its low rate of degradation, leaving traces of residues in the soil. The presence of methoxylated rings leads to rapid soil degradation due to its conversion to hydroxyl and, in this context, the commercial herbicide metosulam stands out. while diclosulam has been used in the control of broad-leaved weeds in soybeans, pyroxsulam has been applied in the same control, but in cereals. This also correspond to the findings of Serey *et al.* (2007); Montel *et al.*, (2008) which revealed that the 7-amino derivatives such as BAS600F (7) represent a class of active fungicides against a broad range of plant pathogens that act as promoters of tubulin polymerization with a paclitaxel like mode of action. This is similar to the previous findings of Achika *et al.* (2023) which revealed the mechanism of action of TPs and analogs in the cardiovascular system. In this context, the 2-(benzylthio)-1,2,4-triazolo[1,5-a] pyrimidines 13a and 13b as cyclic adenosine 3',5'-monophosphate (cAMP) phosphodiesterase (PDE) inhibitors, where 13a is as potent as theophylline as a cardiovascular vasodilator.

The specificity of the action of these PDE inhibitors was attributed to the selective binding at a given cAMP PDE site in the cardiovascular system. This corresponds to the findings of Uryu *et al.* (2002) which revealed that, Alzheimer's disease (AD) is among the most severe irreversible neurodegenerative disorders of the Central Nervous System (CNS), characterized by memory loss. In this context, some synthetic [1,2,4] triazolo [1,5-a] pyrimidines and [1,2,4] triazolo [1,5-a] pyrimidin-5-ones acting by different mechanisms of action were considered as a potential candidate therapeutic for AD. However, an intervention strategy in AD has been the amyloid hypothesis, in which the abnormal accumulation of β -amyloid peptide ($A\beta$), especially $A\beta_{40}$ and $A\beta_{42}$, plays a fundamental role in the pathogenesis of the disease. The $A\beta$ activates microglia that can induce inflammation in the brain leading to cell loss and cognitive decline. Thus, compounds that inhibit $A\beta$ -induced activation of microglia have been considered in the therapy for AD. This also correspond to the findings of Patil *et al.* (2014) who reported that, infections by Mycobacterium tuberculosis (MTB), the causative bacterial agent of tuberculosis (TB), represent an escalating threat for global health with the increased prevalence of multi- and

extensively drug-resistant to tuberculosis. Thus, some triazolo pyrimidines and analogs have been investigated against *M. tuberculosis*. This also corresponds to the findings of El-Gendy *et al.* (2008) who reported that, some [1,2,4] triazolo[1,5-a] pyrimidines, as well as their dihydro analogs and [1,2,4] triazolo[1,5-a] pyrimidinones showed activity against other bacteria and some fungi. This corresponds to the findings of Reis *et al.* (2017) who revealed that, many synthetic [1,2,4] triazole [1,5-a] pyrimidines and analogs have shown activity against parasitic diseases, such as malaria, leishmaniasis, Chagas disease, and African human trypanosomiasis. This is similar to the findings of Steinmetz and Prota, (2018) who reported that [1,2,4] triazolo[1,5-a] pyrimidines, a subtype of purine bioisosteric analogs, have been extensively reported to possess promising activities against a panel of cancer cell lines acting by different mechanisms. The results extract of *Garcinia kola* revealed the presence of 2H-1,4-Benzodiazepin-2-one, 7-amino-1,3,4,5-tetrahydro-5-phenyl compound two times with different quantity (1.97%), (1.19%) respectively.

The biological activity of this compound is widely used in the treatment of various diseases, such as neurological pathologies, cancer, allergy, bacteria, and fungal infections. This corresponds to the findings of Meerpoel *et al.* (2005); Bose, *et al.* (2012); Yousefi, *et al.* (2013) who reported that Benzodiazepins are important compound that are widely used in the treatment of a series of neurological pathologies. However, these compounds and derivatives have also demonstrated potential pharmacological activity and can act, for instance, against cancer, allergy, bacterial and fungal infection. However, the results extract of *Balanites aegyptiaca* using methanol extract revealed that 12 phytochemical compounds identified in 100% peak area.

The extract of *Balanites aegyptiaca* revealed the presence of Phenol, 2-methoxy with (77.57%). Pheno, 2-methoxy are important compound that is widely used in treatment of antimicrobial and antifungal activities. This corresponds to the findings of Mitova *et al.* (2003) who reported that Phenolics compounds usually possess antimicrobial and antifungal activities. The extract of *Balanites aegyptiaca* revealed the presence of Dodecanol, 2-octyl compound. Dodecanol, 2-octyl is importance compound that is widely used in antioxidant, antibacterial and insecticidal activities. This is similar to the findings of Jawonisi, and Adoga, (2013) who reported that Dodecanol compounds showed antioxidant, antibacterial and insecticidal activities.

Conclusion

This study successfully investigated the phytochemical composition and potential therapeutic applications of three medicinal plants *Balanites aegyptiaca*, *Garcinia kola*, and *Ziziphus mauritiana* commonly used in traditional medicine in Sokoto State, Nigeria, for the treatment of diabetes and other ailments. Through GC-MS profiling, a wide array of bioactive compounds was identified, including phenols, quinoline derivatives, and triazolo pyrimidines, which are known for their antimicrobial, antioxidant, anti-inflammatory, and antidiabetic properties. The findings revealed that *Balanites aegyptiaca* contained significant amounts of Phenol, 2-methoxy (77.57%), while *Ziziphus mauritiana* and *Garcinia*

kola exhibited compounds such as E-8-Methyl-9-tetradecen-1-ol acetate and [1,2,4] Triazolo [1,5-a] pyrimidin-5-ol,7-methyl-6-nitro, respectively. These compounds have demonstrated diverse biological activities, including antidiabetic, anticancer, antimicrobial, and anti-inflammatory effects, supporting the traditional use of these plants in managing various diseases. The results underscore the potential of these plants as valuable sources of natural compounds for developing new therapeutic agents, particularly for diabetes and related metabolic disorders. The presence of compounds like [1,2,4] Triazolo [1,5-a] pyrimidines and quinoline derivatives, which have been extensively studied for their pharmacological properties, further highlights the importance of these plants in modern drug discovery. However, further pharmacological and clinical studies are necessary to validate the efficacy, safety, and mechanisms of action of these bioactive compounds. This research contributes to the growing body of knowledge supporting the use of medicinal plants in traditional medicine and emphasizes the need for continued exploration of natural products in the development of novel therapeutic agents.

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