Trop J Nat Prod Res, November 2025; 9(11): 5779 - 5794 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Phytochemical Study and Evaluation of Antioxidant and Alpha-amylase Inhibitory Activities of *Acacia nilotica* (L.) Stem-Eark Extracts

Seide M. Akoro[1]*, Mutiat O. Omotayo[1], Sunday O. Ajibade[2], Afeez A. Ogunbo[1], Rebecca T. Oriowo[1]

[1]*Department of Chemical Sciences, College of Basic Sciences, Lagos State University of Science and Technology (Formerly Lagos State Polytechnic), Ikorodu, Lagos State, Nigeria*
[1]*Department of Chemical Sciences, Faculty of Natural Sciences, Redeemers University, Ede, Osun State, Nigeria*

ARTICLE INFO

Article history:
Received 28 April 2025
Revised 25 August 2025
Accepted 08 September 2025
Published online 01 December 2025

ABSTRACT

Historically, plants have helped man in the healing and treatment of various diseases. *Acacia nilotica is a* plant used in *the* treatment of inflammatory conditions, *Diabetes mellitus*, and certain types of cancer. In this work, we investigated the antioxidant and alpha-amylase inhibitory activities of the crude methanol extract, fractions, and flavonoid extract of *A. nilotica* stem bark, as well as determined the flavonoid and volatile compound content of the crude extracts. The powdered stem bark was soaked in methanol for 72 h, and the resulting extract was partitioned using n-hexane and ethyl acetate to yield the fractions. The quantity of flavonoids of the plant was determined, and the crude extracts and fractions were screened for secondary metabolites. The reducing power and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay protocols were explored for antioxidant studies. Alpha-amylase inhibitory activity was determined preliminarily using the starch–iodine test. High-Performance Liquid Chromatography (HPLC) determined the flavonoid constituents, while Gas Chromatography-Mass Spectrometry (GC-MS) determined the volatile compound constituents. All the extracts contain alkaloids, tannins, saponins, cardiac glycosides, terpenoids, steroids, and anthraquinones. Reducing power and DPPH activities, which increase with concentration, were observed in the crude methanol extract (ANSM). The results are significantly similar ($p>0.05$) to ethyl acetate (ANSME), residual methanol fractions (ANSMR), and flavonoid extract (ANBF). ANSM, ANSMR and ANBF indicated positive alpha-amylase inhibitory activity. HPLC identified quercetin and other polyphenols, while GC-MS identified several volatile compounds with medicinal relevance. These results support the traditional uses of *A. nilotica* and may be a possible source of pharmacologically active compounds.

Keywords: *Acacia nilotica* stem bark, Alpha-amylase activity, Gas Chromatography-Mass Spectrometry, High-Performance Liquid Chromatography.

Introduction

Medicinal plants have remained a cornerstone of traditional healthcare systems worldwide.[1] Various plant-based remedies are employed in the management and treatment of a wide range of ailments and diseases.[2] The therapeutic effects of medicinal plants have been mainly attributed to their rich content of secondary metabolites such as tannins, terpenoids, alkaloids, and flavonoids, which differ among plant species.[1, 3] The accumulated knowledge of the medicinal uses of plants, their bioactive compounds, and their traditional applications has long served as a foundation for modern drug discovery.[3, 4] *Acacia nilotica* (family: Leguminosae) is a common medicinal plant widely distributed in Nigeria and other tropical and subtropical regions of Africa.[5] Locally, it is known as "*Bonni*" among the *"Yorubas"* of South-West Nigeria.[5] The plant is referred to as a multipurpose plant, which is traditionally used for the treatment of various diseases.[5, 6] Several studies have reported its antifungal, antimicrobial, and antiviral properties, including inhibitory activity against HCV and HIV-1.[7]

*Corresponding author. Email: akoro.sm@lasustech.edu.ng
Tel.: +08023216177

Citation: Akoro SM, Omotayo MO, Ajibade SO, Ogunbo AA, Oriowo RT. Phytochemical Study and Evaluation of Antioxidant and Alpha-amylase Inhibitory Activities of *Acacia nilotica* (L.) Stem-Bark Extracts. Trop J Nat Prod Res. 2025; 9(11): 5779 – 5794 https://doi.org/10.26538/tjnpr/v9i11.69

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Other pharmacological effects attributed to *A. nilotica* include antihypertensive, antimutagenic, antioxidant, anti-inflammatory, and antiplatelet aggregation activities.[8, 9] The stem bark has been traditionally employed as an aphrodisiac, diuretic, expectorant, and treatment for colds, bronchitis, diarrhoea, bleeding piles, wound ulcers, skin diseases, leprosy, and seminal weakness.[10-13]

Existing literature documented various bioactivities and phytochemical profiles of different parts of *A. nilotica*.[9,13-15] The phenolic and tannin contents in the different plant parts were reported.[14] The chemopreventive activity of polyphenols from the bark,[9] and the neuroprotective effects of the methanol extract were reported.[15] Other studies have examined the antidiarrheal, antispasmodic, and antifungal activity of the methanol flower extract and profiled phytochemicals via GC-MS.[13] Additional work reported the GC-MS profiles of the ethanol, chloroform, and aqueous extracts from the leaves, fruit, and stem bark.[13, 16, 17] A comparative study on the solvent-specific composition of the leaf extract with the GC-MS profile and the antimicrobial properties of the aqueous bark extract has been documented.[18]

Diabetes mellitus continues to be a global problem.[19] In 2021, approximately 537 million adults were living with diabetes worldwide.[20] The figure is projected to rise to 643 million by 2030 and 783 million by 2045.[20] High blood glucose can lead to serious, life-threatening and undesirable complications if not properly managed.[21] These complications include hypertension, dyslipidaemia, microalbuminuria, diabetic nephropathy, and a range of cardiovascular diseases such as coronary heart disease, cerebrovascular accident, heart failure and coronary artery disease.[21,22] Oxidative stress is associated with several complications of *D. mellitus*.[23] Several medicinal plants have demonstrated antioxidative potential since they exhibit the power to remove free radicals, thereby reducing oxidative stress and its risks in diabetes. One of the key strategies in managing diabetes, which brings the control easily back to the individual, is controlling postprandial blood sugar levels.[25-27] Liquid-liquid partitioning is a versatile method in organic chemistry used for the separation and purification of crude extracts and organic mixtures.[28]

In this method, the separation of organic mixtures is based on the solubility of the contents in different solvents.[28] In carrying out liquid-liquid fractionation, the solvents are added to the crude extract solution in increasing order of polarity, separating the contents based on how well they dissolve in the different solvents.[28-30] The fractionation and separation of components in plant extracts, as well as the isolation of compounds, have been achieved through various chromatographic methods, including HPLC and GC-MS.[31-33] One of the standard methods used to separate non-volatile organic mixtures, such as flavonoids, is HPLC. [31, 34] In contrast, GC-MS is commonly used for the identification of volatile organic components in crude organic mixtures.[16-18] Some research has been conducted on the phytochemicals and bioactivity of *A. nilotica*. Still, none of these studies investigated the antioxidant potentials and alpha-amylase inhibitory activities of the stem bark's methanol extract and its fractions and the crude flavonoid extract in a comparative study. This study assessed and compared the antioxidant and alpha-amylase inhibitory activity of *A. nilotica* stem bark crude methanol extract, fractions, as well as the crude flavonoid extract. Additionally, the study reported the flavonoid constituents and volatile phytochemical compounds of the methanol extract using HPLC and GC-MS, respectively.

Materials and Methods

Chemicals and reagents

All the solvents and chemicals used in this study are analytical grade. Pancreatic alpha-amylase (EC 3.2.1.1) was obtained from Sigma-Aldrich, and potato starch was from North Carolina, USA.

Sample collection, drying and extraction

The fresh plant sample, *Acacia nilotica* stem bark, was purchased from the Mushin Market (6°31′44.07″N, 3°21′8.49″E), Lagos State, Nigeria, in April 2019, and then authenticated at the Herbarium of the University of Lagos, where a voucher number was assigned (LUH 3259). The plant samples were washed and cut into pieces before air-drying for approximately five weeks, before being ground with a manual grinder to a coarse powder. The ground sample (700 g) was extracted by macerating in methanol (1.5 L) in a glass container. The extract obtained was filtered into a clean beaker after 72 h. More solvents were added to the "marc" until it faded. The extract was concentrated in a rotary evaporator and then placed in an air oven to further dry at 40°C. The resulting methanol extract (ANSM) was kept appropriately at 4°C until needed for subsequent analysis.

Partitioning of Crude Methanol Extract

A portion of ANSM (50 g) was dissolved in a water-methanol mixture (3:1). The ANSM extract solution was poured into a separating funnel, followed by the addition of 100 mL of n-hexane. The mixture was shaken and allowed to stand until a clear separation was achieved. This was partitioned to give the n-hexane extract (ANSMH). The procedure was repeated two more times. The remaining phase was further partitioned with 100 mL ethyl acetate (repeated two more times) to obtain the ethyl acetate fraction (ANSME). The final aqueous residue was labelled ANSMR (residual methanol extract). All fractions were dried and stored appropriately.[35]

Qualitative phytochemical screening

All extracts - ANSM, ANSMH, ANSME, ANSMR - were subjected to standard phytochemical screening for the detection of secondary metabolites, including alkaloids, tannins, saponins, flavonoids, steroids, cardiac glycosides, terpenoids, and anthraquinones, following the protocols described by Sofowora.[36]

Flavonoid Extraction and Quantification

The flavonoid content was determined using the method of Ezeonu and Ejikeme.[37] The coarsely powdered sample (5.0 g) was soaked in 100 mL aqueous methanol (80%) in a 250 mL beaker. This mixture was left at room temperature for 24 h, and the supernatant was removed. The residue was then re-extracted three times using the same solvent. Filtration was carried out using a Whatman filter paper (No. 42, 125 mm). The filtrates were pooled together and dried by evaporating in a water bath. The crude flavonoid extract was allowed to cool in a desiccator, after which the weight was determined until a constant mass was obtained. The amount of flavonoids was calculated and presented as a percentage of the total amount of flavonoids. The experiment was repeated three times.

Flavonoid Profile Analyses using High-Performance Liquid Chromatography (HPLC)

The component flavonoids in the flavonoid mixture were determined using HPLC. The extract (10 mg) was dissolved in HPLC-grade methanol (5 mL), filtered, and the filtrate was cleaned up before injection for analysis. The sample (10 µL) was injected into the mobile phase and run on an HPLC machine (Agilent 1260; Agilent Technologies, Germany) using a Zorbax Eclipse column (150 × 4.6 mm) and a flow rate of 7 mL/min. The mobile phase consists of acetonitrile containing 0.1% formic acid.[38]

Antioxidant assays

Antioxidant activity was evaluated using the reducing power and DPPH radical scavenging assays as described by Hatano *et al*. and Omotayo *et al*. [39, 40]

Reducing Power Assay

The extracts (10, 100, 1000 µg/L each) in 1 mL of distilled water were mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was then placed in an incubator set at 50°C for 20 mins. The mixture was removed from the incubator, and 10% trichloroacetic acid (2.5mL) was added, followed by centrifugation (3000 rpm, 10 mins). This was followed by the addition of water (2.5 mL) and Iron (III) chloride (0.1%, 0.5 mL) to 2.5 mL of the upper layer of the solution. Absorbance was then determined at 700 nm using a UV spectrophotometer (Spectrum Lab 752S, Shenyang Ebetter Optics Technology Co., Ltd., China). An increase in absorbance indicates an increase in reducing power.[39, 40]

DPPH Radical Scavenging Assay

The free radical scavenging activity of the extracts was assessed using the DPPH method.[39] Each extract (10, 100, and 1000 µg/mL) was shaken with methanolic DPPH solution (1 mM, 1 mL), and then left for 30 mins at room temperature. Absorbance of the solution was recorded at 517 nm using a UV spectrophotometer (Spectrum Lab 752S, Shenyang Ebetter Optics Technology Co., Ltd., China). The control was prepared at various concentrations without adding the extract, while ascorbic acid was the known standard. The radical scavenging activity was expressed as inhibition of DPPH using the formula:

$$\% \text{ Inhibition} = \frac{\text{control absorbance - sample absorbance}}{\text{control absorbance}} \times 100$$

Alpha-amylase inhibitory assay

The alpha-amylase inhibitory activity was carried out using the starch-iodide assay method as previously described.[23, 41] The extract solution (250 µL) was prepared by dissolving in DMSO to make different concentrations (10 µg/mL, 100 µg/mL, and 1000 µg/mL) and the control was without extract (0 µg/mL). The enzyme solution - pancreatic alpha-amylase (EC 3.2.1.1; IU/mL, 250 µL), which was prepared by dissolving in 0.02 M Sodium phosphate buffer (pH 6.9), containing 0.006 M sodium chloride, was added to the extract and then incubated at 37°C for 10 mins. This process was followed by the addition of potato starch solution (1%, 250 µL) to each of the reaction mixtures and further incubated for another 10 mins. The enzymatic reaction was terminated by the addition of 1M HCl (250 µL), followed by 100 µL of 5 mM Iodine reagent. The experiment was repeated for all studied concentrations. The colour change was recorded.

GC-MS analysis

The GC-MS analysis of the crude methanol extract was done on an Agilent gas chromatograph (Agilent 7820A; Agilent Technologies,

USA) coupled with a 5975C inert mass spectrometer (MS), equipped with a triple-axis detector which was fitted with an HP-5 capillary column (30 m × 0.32 mm diameter × 0.25 µm film thickness) coated with 5% Phenyl Methyl Siloxane (Agilent Technologies). The electron ionisation by the MS was in electron-impact mode with an ionisation energy of 70 eV. Helium gas (flow rate: 1.573 mL/min, baseline pressure: 1.9514 psi, mean velocity: 46 cm/s) was the carrier gas and the sample (1 µL) was injected into the machine in a splitless mode (260°C). The purge flow was 21.5 mL/min (starting from 0.50 min). The gas saver mode was activated, and the total gas flow rate was 23.355 mL/min. The oven temperature was initially maintained at 60°C for 1 min, and then increased to 110°C (3 min), then at 8°C/min to 260°C (5 min), and at 10°C/min to 300°C (12 min). The total runtime, including the solvent delay of 3 min, was 56.25 min. The Mass spectra data were acquired at 70 eV; range of m/z 30–550; scan rate of 2.62 s/scan. The data was processed using Agilent's MSD Chemstation software, and the acquired spectra were compared with the NIST 14 Mass Spectral Library database to identify the compounds.

Statistical analysis

Microsoft Excel 2016 was used to carry out the statistical analysis. The results are presented as mean ± SD. All the graphs were drawn with Microsoft Excel 2016. The mean values were compared using the Student's t-test. Differences in means are considered statistically significant at $p \leq 0.05$.

Results and Discussion

Extraction of Plant Material

The crude methanol extract, ANSM, was obtained from plant material by maceration (Table 1). The partitioning of ANSM in two solvents, n-hexane and ethyl acetate, gave the respective fractions - ANSMH and ANSME - and the residual methanol fraction, ANSMR. Table 1 shows the colour, yields and percentage of the extract and fractions.

Extract	Colour	Mass Obtained	Percentage Yield (%)
ANSM	Black solid	62.3	8.90
Fractions and Crude Flavonoid			
ANSMH	Yellow oil	2.85	5.7
ANSME	Black solid	12.75	25.5
ANSMR	Black solid	28.50	57.0
ANBF	Reddish brown solid	0.75±0.02[a]	15.0 ± 0.4[a]

Key: a: Mean of three experiments;
ANSM- *A. nilotica* Stem-bark methanol extract; ANSMH- *A. nilotica* Stem-bark hexane fraction; ANSME- *A. nilotica* Stem-bark ethyl acetate fraction; ANSMR- *A. nilotica* residual Stem-bark methanol fraction; ANBF- *A. nilotica* Stem-bark crude flavonoid extract

Phytochemical screening results

The preliminary phytochemical screening of the crude extract and fractions indicated alkaloids, tannins, saponins, steroids, cardiac glycosides, terpenoids, and anthraquinones in all. However, flavonoids were not detected in the ethyl acetate fractions (Table 2). In medicinal plants, phytochemical contents have been associated with their healing properties.[42,43] Recent research suggests that phytochemical intake may help prevent diseases such as diabetes, cancer, obesity, and several cardiovascular diseases. [42,43] Alkaloids and flavonoids are mentioned for their healing properties, as they have been reported to exhibit antidiabetic, antihypertensive, and antioxidant properties.[44] Many flavonoids and phenolic compounds have been reported to reduce blood glucose due to their ability to stimulate the rebuilding of beta cells, which improves insulin activity.[41]

Table 1: Extracts and their percentage yield

Table 2: Phytochemical Content of Plant Extract and Fractions

Secondary Metabolites	ANSM	ANSMH	ANSME	ANSMR
Alkaloids	+	+	+	+
Flavonoids	+	+	-	+
Tannins	+	+	+	+
Saponins	+	+	+	+
Cardiac glycosides	+	+	+	+
Terpenoids	+	+	+	+
Steroids	+	+	+	+
Anthraquinones	+	+	+	+

Key: (+) detected, (-) not detected; ANSM- *A. nilotica* Stem-bark methanol extract; ANSMH- *A. nilotica* Stem-bark hexane fraction; ANSME- *A. nilotica* Stem-bark ethyl acetate fraction; ANSMR- *A. nilotica* residual Stem-bark methanol fraction

High-Performance Liquid Chromatography results

HPLC analysis of the crude flavonoid extract (ANBF) from the *A. nilotica* stem-bark identified the phenolic compounds, p-coumaric acid, catechin, ferulic acid, rutin, gallic acid, kaempferol and quercetin (Table 3, Figures 1 and 2). Apigenin was not detected, and the most abundant flavonoid is quercetin (246.54 ppm). This result is consistent with the findings of early work on the *A. nilotica* plant parts.[45]

Interestingly, the absence of apigenin from our result from the stem-bark methanol extract aligns with the ethanol extract profile reported by Alain *et al.*[46] However, in their report, it is important to note the absence of kaempferol and the identification of ferulic acid as the most abundant phenolic compound.[46] Such discrepancies in phytochemical contents may result from environmental variables such as geographical location, climatic conditions, time, and season of plant collection.[47]

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

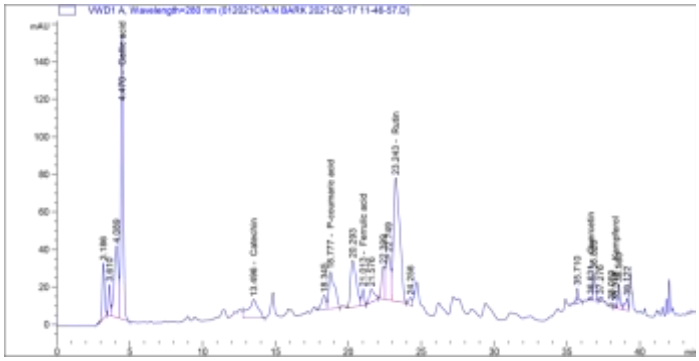


Figure 1: HPLC profile of *A. nilotica* stem-bark

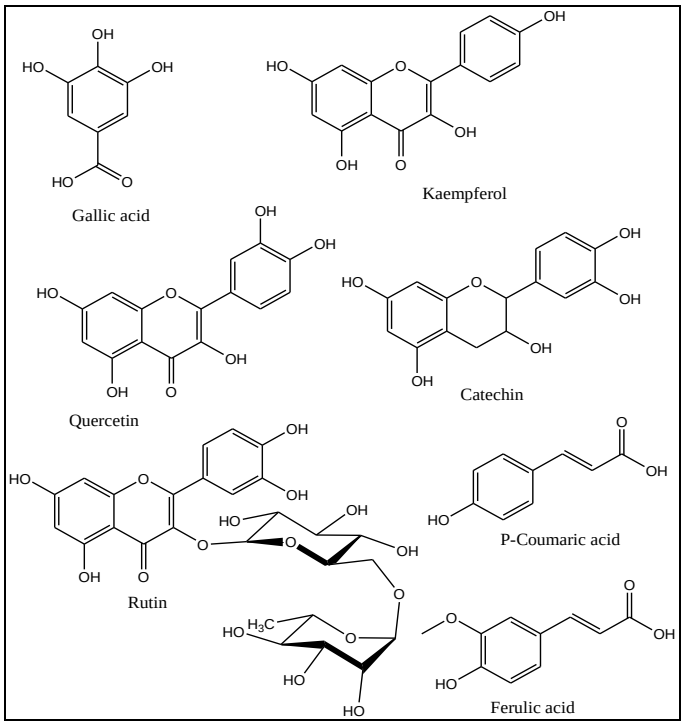


Figure 2: Structures of flavonoid constituents of *A. nilotica* stem bark from HPLC

Quercetin is a well-known bioactive phenolic compound with demonstrated antioxidant, anticancer, anti-inflammatory, cardiovascular protection, antimicrobial, and antiviral properties.[48] It has also been studied for its potential benefits in managing age-related diseases and COVID-19 treatment.[48, 49] Other major flavonoids detected from *A. nilotica* stem bark are rutin (152.31 ppm), gallic acid (18.64 ppm), and catechin (10.99 ppm). Rutin (quercetin-3-rutinoside) is widely recognised for its pharmacological and nutraceutical applications.[50] Reported pharmacological applications include cardioprotective, antidiabetic, antioxidant, anti-allergic, anticancer, and anti-inflammatory activities.[50] Other studies have shown its ability to reduce blood clots, cholesterol, and arthritis.[51] Gallic acid exhibits anti-inflammatory and anti-neoplastic activities,[52] catechin shows antiaging activity.[53] The minor components of *A. nilotica* flavonoids are *p*-coumaric acid, ferulic acid and kaempferol with area percentages of 0.43%, 0.27% and 1.12% respectively. *P-Coumaric* acid is noted for its anti-cancer potential,[54,55] and ferulic acid demonstrates broad therapeutic effects, including antidiabetic, antioxidant, anticancer, and antimicrobial properties.[56,57] Kaempferol exhibits anticancer and anti-inflammatory activities.[58]

Table 3: Flavonoid content of *A. nilotica* stem bark from HPLC

Flavonoid	Amount (ppm)	Percentage
Gallic Acid	18.64	4.31
Catechin	10.99	2.55
p-Coumaric acid	1.86	0.43
Ferrulic Acid	1.15	0.27
Rutin	152.31	35.2
Quercetin	246.54	56.99
Kaempferol	1.12	0.26
Apigenin	ND	-

Antioxidant activities results

The antioxidant activities of the different extracts and fractions of *A. nilotica* stem bark were assessed using the reducing power and DPPH radical scavenging assays. These two methods are among the various techniques used to determine the free radical scavenging activities of plant extracts.[40, 59]

Reducing power assay

The assay results indicate an increase in absorbance that is concentration dependent, suggesting an increase in reducing power. From the results, all the extracts and fractions showed a dose-dependent increase in absorbance except the *n*-hexane fraction (ANSMH), which showed no significant variation in absorbance across concentrations (Figure 3). The crude methanol extract, (ANSM), exhibited reducing power, which was significantly different ($P < 0.05$) from that of ANSMH but not different from that of ANSME, ANSMR, and ANBF ($p > 0.05$). These results suggest that the reducing power properties of *A. nilotica* stem-bark crude extracts may be due to the phytochemical content of ANSME, ANSMR, and ANBF, indicating a synergistic interaction among the contents of the crude and flavonoid extracts (Figure 3). The reducing power of ANSM, ANSME, ANSMR, and ANBF is not significantly different ($p>0.05$) from that of the ascorbic acid standard (AA).

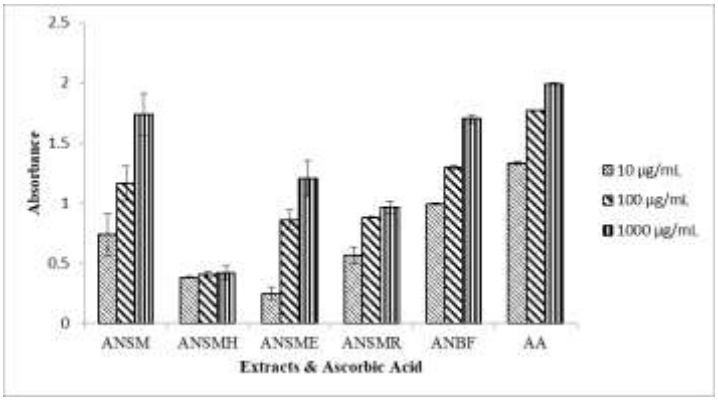


Figure 3: Reducing power activity of extracts and fractions at different concentrations compared with the ascorbic acid standard**.** Keys: ANSM- *A. nilotica* Stem-bark methanol extract; ANSMH- *A. nilotica* Stem-bark hexane fraction; ANSME- *A. nilotica* Stem-bark ethyl acetate fraction; ANSMR- *A. nilotica* residual Stem-bark methanol fraction; ANBF- *A. nilotica* Stem-bark crude flavonoid extract

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

DPPH Radical Scavenging Assay results

The DPPH radical scavenging assay is a widely accepted method for evaluating the antioxidant activity of plant extracts and phytochemicals.[59] In the assay, the violet-coloured solution of the DPPH is reduced to a yellow-coloured product.[59, 60] This observed change results from the fact that the DPPH radicals, when receiving an electron from an antioxidant compound, are reduced to hydrazine, causing a colour change from the violet colour of the DPPH radical to the yellow of the hydrazine.[60]

In this work, a concentration-dependent increase in DPPH radical scavenging activity was observed for all samples, including the ascorbic acid standard, AA, except in ANSMH, which demonstrated a decline in activity, indicating minimal or no radical scavenging effect (Figure 4). A closer look at the results revealed that the concentration-dependent DPPH radical scavenging activity of the crude methanol extract (ANSM) is significant and similar to what was observed for ANSME, ANSMR, and ANBF ($p>0.05$). Similarly, ANSM exhibited DPPH scavenging activity that was not substantially different ($p > 0.05$) from the ascorbic acid standard (Figure 4). The observed vigorous activity of the ANBF in this study aligns with previous reports on the potent antioxidant properties of flavonoids.[49-58] Antioxidants play a crucial role in mitigating the impact of free radicals on human health, which is the underlying reason for several degenerative diseases.[59]

Alpha-amylase inhibitory activities

The preliminary alpha-amylase inhibitory activities of the extracts and fractions were determined using the starch iodide assay as previously described.[25] In this study, ANSM, ANBF and ANSMR showed a positive alpha-amylase inhibitory activity, evidenced by the development of a blue-black colouration. In contrast, the *n*-hexane (ANSMH) and ethyl acetate (ANSME) fractions exhibited no inhibitory activity, as an orange colour formation signifies a complete starch hydrolysis (Table 4). This result suggests the presence of possible phytochemicals with the potential of inhibiting the activity of alpha-amylase enzyme in the crude extracts and the residual methanol fraction, as shown by the formation of a blue-black colour.[37] In enzyme-inhibition assays involving starch digestion, the formation of coloured complexes can indicate a positive or negative alpha-amylase inhibition.[37] Alpha-amylase enzyme inhibition is positive when a blue-black colouration is formed, hence suggesting the presence of undigested starch. The formation of an orange colour signifies complete starch digestion, suggesting the extracts did not inhibit the action of the enzyme.[25,41] The alpha-amylase inhibitory potential of *A. nilotica* stem bark ethanol extract was previously reported.[46]

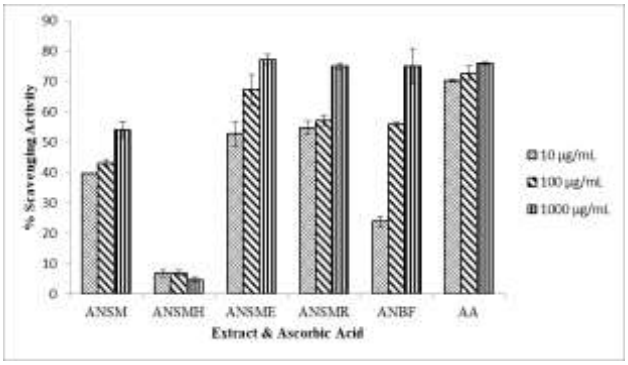


Figure 4: DPPH radical scavenging activity of different extracts and fractions at different concentrations compared with the ascorbic acid standard

Keys: ANSM- *A. nilotica* Stem-bark methanol extract; ANSMH- *A. nilotica* Stem-bark hexane fraction; ANSME- *A. nilotica* Stem-bark ethyl acetate fraction; ANSMR- *A. nilotica* residual Stem-bark methanol fraction; ANBF- *A. nilotica* Stem-bark crude flavonoid extract

Several studies have documented the antihyperglycemic effects of flavonoids due to their ability to inhibit alpha-amylase; hence, they can cause a reduction in blood sugar concentration and a protective effect against the development of diabetes.[61-63] It has been reported that the interactions of flavonoids and alpha-amylase affect the catalytic potential of the enzyme, and this is associated with the force that binds the two and the structure of the flavonoid. [61,62] Research has shown that for a flavonoid to inhibit alpha-amylase, the structure must have a double bond between C2 and C3 and the A5 and B3 positions must have OH groups.[62,64] These features are observed in quercetin and rutin, which are the most abundant flavonoids in the *A. nilotica* stem bark, giving a credible explanation for the results obtained from this work (Figure 2, Table 3). A combination of quercetin and rutin was reported to enhance alpha-amylase inhibition.[64,65]

Table 4: Colour of the complex formed in the starch iodide assay and Inference

Conc. (µg/mL)	Extract	Colour Observed	Remark	Inference
0	ANSM	Orange	Starch completely digested	No Inhibition
10		Blue-black	Starch present	inhibition
100		Blue-black	Starch present	inhibition
1000		Blue-black	Starch present	inhibition
0	ANSMH	Orange	Starch completely digested	no inhibition
10		Orange	Starch completely digested	no inhibition
100		Orange	Starch is completely digested.	no inhibition
1000		Orange	Starch completely digested	no inhibition
0	ANSME	Orange	Starch completely digested	no inhibition

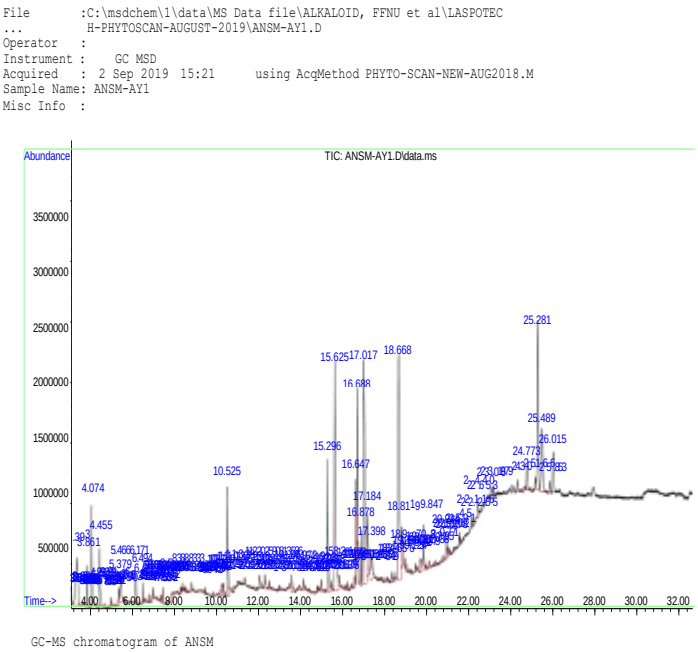
ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

10		Orange	Starch completely digested	no inhibition
100		Orange	Starch completely digested	no inhibition
1000		Orange	Starch completely digested	no inhibition
0	ANSMR	Orange	Starch completely digested	No Inhibition
10		Blue-black	Starch present	inhibition
100		Blue-black	Starch present	inhibition
1000		Blue-black	Starch present	inhibition
0	ANBF	Orange	Starch completely digested	No Inhibition
10		Blue-black	Starch present	inhibition
100		Blue-black	Starch present	inhibition
1000		Blue-black	Starch present	inhibition

Key: ANSM- *A. nilotica* Stem-bark methanol extract; ANSMH- *A. nilotica* Stem-bark hexane fraction; ANSME- *A. nilotica* Stem-bark ethyl acetate fraction; ANSMR- *A. nilotica* residual Stem-bark methanol fraction; ANBF- *A. nilotica* Stem-bark crude flavonoid extract

GC-MS Analysis

Gas Chromatography-Mass Spectrometry is a widely used analytical tool for identifying volatile phytochemicals in plant extracts.[66,67] The GC-MS chromatogram of ANSM detected 152 peaks, and ninety-five (95) compounds were identified (Table 5, Figure 5).



GC-MS chromatogram of ANSM

Figure 5: GC Chromatogram of *A. nilotica* stem bark methanol extract (ANSM)

Twenty-one of these compounds have an area percentage above 1% (one per cent), accounting for approximately 76% of the total compounds eluted (Table 6). These twenty-one compounds consist of organosilicon compounds (32.583%), fatty acid amides (8.4462%), fatty acids (12.5052%), triterpene (4.2735%), fatty acid ester (5.7553%), phenolic and benzenetriol (3.8179%), triamine (1.0012%), and various heterocyclic compounds such as triazopyrimidine ester, pyrazole carboxylic acid, pyrrolyl ester and imidazothiazole (7.1295%). Among these are ten compounds with area percentages above 2%, which are considered the major constituents, contributing approximately 61% of the total compounds eluted (Tables 5 and 6). These compounds are 1,2-bis (trimethylsilyl) benzene (11.9601%); cyclotrisiloxane, hexamethyl- (8.6842%); 1,4-bis(trimethylsilyl) benzene (7.8423%); 9-octadecenamide, (Z)-(7.3745%); oleic acid (6.2392%); n-hexadecanoic acid (5.1681%); lup-20(29)-en-3-one (4.2735%); 6-octadecenoic acid, methyl ester, (Z)-(3.9885%); [1,2,4] triazolo[1,5-a]pyrimidine-6-carboxylic acid, 7-amino-, ethyl ester (3.2874%); and bisphenol C (2.2469%). Multiple peaks are observed for some of the compounds (Table 5), which may be attributed to column impairment.[68] The most abundant compound, 1,2-bis (trimethylsilyl) benzene, is an organosilicon compound that has been reported to possess antioxidant and antimicrobial activities.[69,70]

Other organosilicon compounds identified as major in the *A. nilotica* stem bark - cyclotrisiloxane, hexamethyl- and 1,4-bis(trimethylsilyl) benzene - have been cited in the literature for their anticancer, antiviral, antimicrobial, antifungal, antiparasitic, anti-inflammatory, antihistamine, antidiabetic, antidiarrheal, and neurotropic effects.[71] Another notable compound is 9-octadecenamide, also known as oleamide, a fatty acid amide with diverse medicinal properties, including its use in the treatment of mood and sleep disorders.[72,73]

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 5: Compounds detected from the *A. nilotica* stem-bark methanol extract from GC-MS

Peak	RT	Compounds	Area Pct	Molar mass (g/mol)	Molecular formula
1	3.3928	Cyclotrisiloxane, hexamethyl-	1.9535	222.5	$C_6H_{18}O_3Si_3$
2	3.4967	9H-Fluorene-2-carboxylic acid, 9-oxo, (2-hydroxyethyl)(methyl)amide	0.0239	281.3	$C_{17}H_{15}NO_3$
3	3.6873	Tricyclo[6.3.0.0(2,6)]undecan-10-one, 3-[(2-methoxyethoxy)methoxy]-2-methyl-	0.1657	282.4	$C_{16}H_{26}O_4$
4	3.8606	Benzeneacetaldehyde, 2-methoxy-.alpha.,5-dimethyl-	0.4353	178.2	$C_{11}H_{14}O_2$
5	4.0743	Benzyl alcohol, TBDMS derivative	1.2458	222.4	$C_{13}H_{22}OSi$
6	4.1609	1-(4-Nitrophenyl)piperazine	0.1241	207.2	$C_{10}H_{13}N_3O_2$
7	4.3572	Benzofuran-2-one, 2,3-dihydro-3,3-dimethyl-4-nitro-	0.0209	207.2	$C_{10}H_9NO_4$
8	4.4554	2-Propen-1-one, 1-(4-aminophenyl)-3-phenyl-	0.792	223.3	$C_{15}H_{13}NO$
9	4.6807	2-Benzothiazolamine, 6-nitro-	0.1124	195.2	$C_7H_5N_3O_2S$
10	4.8655	Dibutylphosphinothioamide, N-methyl-	0.0542	219.3	C10H22NPS
11	4.9925	Trisiloxane, 1,1,3,3,5,5-hexamethyl-	0.2117	208.5	$C_6H_{20}O_2Si_3$
12	5.1889	Butane-1,3-dione, 1-(pyrrol-2-yl)-	0.0275	151.2	$C_8H_9NO_2$
13	5.4661	Cyclotetrasiloxane, octamethyl-	1.2224	296.6	$C_8H_{24}O_4Si_4$
14	5.5932	Benzene, ethoxy-	0.1233	122.2	$C_8H_{10}O$
15	5.83	Pyrimidine, 5-methyl-	0.1725	94.1	$C_5H_6N_2$
16	6.1707	Arsenous acid, tris(trimethylsilyl) ester	0.3951	342.5	$C_9H_{27}AsO_3Si_3$
17	6.2689	1-[(1H-Pyrrol-2-ylcarbonyl)oxy]-2,5-pyrrolidinedione	0.0133	208.2	$C_9H_8N_2O_4$
18	6.4133	3-Methylpyridazine	0.0337	94.1	$C_5H_6N_2$
19	6.4941	6-Chloro-2-methyl-4-phenyl-quinoline-3-carboxylic acid	0.2132	297.7	$C_{17}H_{12}ClNO_2$
20	6.5923	Phenol, 2-methyl-	0.1557	108.1	C_7H_8O
21	6.7771	N-(1-Pyrenyl)-maleimide	0.1213	297.3	$C_{20}H_{11}NO_2$
22	6.985	1,3-Diphenyl-5-methylthio-1,2,4-triazole	0.7995	267.4	$C_{15}H_{13}N_3S$
23	7.3604	Methane, bis(4-methylphenoxysulfonyl)-	0.1749	356.4	$C_{15}H_{16}O_6S_2$
24	7.447	Cyclopentasiloxane, decamethyl-	0.1597	370.8	$C_{10}H_{30}O_5Si_5$
25	7.7416	2,3-Dihydroxy-6-nitroquinoxaline	0.0841	207.1	$C_8H_5N_3O_4$
26	7.9091	4-Dodecanol, acetate	0.4716	228.4	$C_{14}H_{28}O_2$
27	8.117	1-.beta.-d-Ribofuranosyl-3-[5-tetraazolyl]-1,2,4-triazole	0.8726	269.1	$C_8H_{12}N_4O_5$
28	8.3826	2-Isopropyl-4,5-dimethylthiazole	0.8669	155.3	$C_8H_{13}NS$

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

29	8.5559	2,4,6-Pyrimidinetriamine, 5-t-butoxycarbonylamino-	0.7319	240.3	$C_9H_{16}N_6O_2$
30	8.6483	Methenamine	0.509	140.2	$C_6H_{12}N_4$
31	8.8331	Acetic acid, [[2-(3,4-dihydro-2H-pyrrol-5-yl)-1-methylethenyl]thio]-, ethyl ester	1.0639	225.3	$C_{11}H_{15}NO_2S$
32	9.0526	meta-Methoxybenzenethiol	0.386	140.2	C_7H_8OS
33	9.197	Benzenethiol, 4-methoxy-	0.488	140.2	C_7H_8OS
34	9.3356	2-Amino-5,6-dihydro-4H-cyclopentathiazole	0.4773	140.2	$C_6H_8N_2S$
35	9.48	Cyclohexasiloxane, dodecamethyl-	0.3935	444.9	$C_{12}H_{36}O_6Si_6$
36	9.5666	2-Methyl-3-methoxy-4H-pyran-4-one	0.4083	140.1	$C_7H_8O_3$
37	9.8553	meta-Methoxybenzenethiol	0.0604	140.2	C_7H_8OS
38	10.0633	Patulin	0.2748	154.1	$C_7H_6O_4$
39	10.3289	p-Phenylenediamine, N,N,N'-trimethyl-N'-[2-(N-methylanilino)ethyl]-	1.0012	283.4	$C_{18}H_{25}N_3$
40	10.5253	Bisphenol C	2.2469	256.3	$C_{17}H_{20}O_2$
41	10.8314	3-Amino-4-methoxybenzamide	0.3602	166.2	$C_8H_{10}N_2O_2$
42	11.1375	Pyrazole-5-carboxylic acid, 3-methyl-	1.75	126.1	$C_5H_6N_2O_2$
43	11.328	1,2,4-Benzenetriol	1.571	126.1	$C_6H_6O_3$
44	11.6746	Pyrazine, (methylthio)-	0.1774	126.2	$C_5H_6N_2S$
45	11.7959	2',4'-Dihydroxyacetophenone oxime	0.4753	167.2	$C_8H_9N0_3$
46	11.9287	3-Imino-6-phenylimino-cyclohexa-1,4-diene	0.2945	182.2	$C_{12}H_{10}N_2$
47	12.05	1-(6-Hydroxyimidazo[2,1-b]thiazol-5-yl)ethanone	0.8067	182.2	$C_7H_6N_2O_2S$
48	12.2983	Acetic acid, chloro-, octadecyl ester	0.8559	347.0	$C_{20}H_{39}ClO_2$
49	12.5178	2,2'-Dimethylbiphenyl	0.5673	182.3	$C_{14}H_{14}$
50	12.6622	1,1'-Biphenyl, 2-ethyl-	0.2848	182.3	$C_{14}H_{14}$
51	12.8239	4-Hydroxyimino-6-oxo-4,5,6,7-tetrahydrobenzofurazan	0.1744	167.1	$C_6H_5N_3O_3$
52	12.9856	Benzene, 1-methyl-4-(phenylmethyl)-	0.2798	182.3	$C_{14}H_{14}$
53	13.1299	2,5-Dimethoxybenzoic acid	0.3297	182.2	$C_9H_{10}O_4$
54	13.3898	Dehydrochamazulene	0.3305	182.3	$C_{14}H_{14}$
55	13.4765	1-(6-Hydroxyimidazo[2,1-b]thiazol-5-yl)ethanone	0.2215	182.2	$C_7H_6N_2O_2S$
56	13.5862	2-(1-Aminoethyl)-4-amino-6-dimethylamino-S-Triazine	0.5273	182.2	$C_7H_{14}N_6$
57	13.823	4,4'-Dimethylbiphenyl	0.1221	182.3	$C_{14}H_{14}$
58	13.9443	Benzene, 1-methyl-3-(phenylmethyl)-	0.1197	182.3	$C_{14}H_{14}$
59	14.1464	1-Octadecene	0.4508	252.5	$C_{18}H_{36}$

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

60	14.4236	2,2'-Dimethylbiphenyl	0.145	182.3	$C_{14}H_{14}$
61	14.5622	2,2'-Dimethylbiphenyl	0.0245	182.3	$C_{14}H_{14}$
62	14.7875	Benzene, 1-methyl-3-(phenylmethyl)-	0.2012	182.3	$C_{14}H_{14}$
63	14.9723	Thiophenol, TMS derivative	0.1736	182.4	$C_9H_{14}SSi$
64	15.1282	benzaldehyde, 2-hydroxy-3,4-dimethoxy-	0.0913	182.2	$C_9H_{10}O_4$
65	15.2957	Hexadecanoic acid, methyl ester	1.7668	270.5	$C_{17}H_{34}O_2$
		Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-			
66	15.469	hydroxy-, methyl ester	0.19	292.4	$C_{18}H_{28}O_3$
67	15.6249	n-Hexadecanoic acid	5.1681	256.4	$C_{16}H_{32}O_2$
68	16.0985	Methyl 9-tetradecenoate	0.0686	240.4	$C_{15}H_{28}O_2$
		Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-		577.2	$C_{16}H_{48}O_7Si_8$
69	16.2371	hexadecamethyl-	0.1169		
70	16.4277	Quinoline, 2-chloro-6-methoxy-4-methyl-	0.1846	207.7	$C_{11}H_{10}ClNO$
71	16.6875	6-Octadecenoic acid, methyl ester, (Z)-	3.9885	296.5	$C_{19}H_{36}O_2$
72	16.8781	Methyl stearate	0.6164	298.5	$C_{19}H_{38}O_2$
73	17.0167	Oleic Acid	6.2392	282.5	$C_{18}H_{34}O_2$
74	17.1842	Octadecanoic acid	1.0979	284.5	$C_{18}H_{36}O_2$
75	17.3979	Behenic amide	1.0717	339.6	$C_{22}H_{45}NO$
76	17.8484	2-Methyl-Z,Z-3,13-octadecadienol	0.1536	280.5	$C_{19}H_{36}O$
		Pyridine, 1,2,3,6-tetrahydro-1-methyl-4-[4-			
77	17.9581	chlorophenyl]-	0.0403	207.7	$C_{12}H_{14}ClN$
78	18.0851	i-Propyl 9-tetradecenoate	0.2361	268.4	$C_{17}H_{32}O_2$
79	18.3451	Methyl 9-tetradecenoate	0.2048	240.4	$C_{15}H_{28}O_2$
80	18.4663	Silicic acid, diethyl bis(trimethylsilyl) ester	0.1527	296.6	$C_{10}H_{28}O_4Si_3$
81	18.6685	9-Octadecenamide, (Z)-	5.5518	281.5	$C_{18}H_{35}NO$
82	18.8186	9-Octadecenamide, (Z)-	1.8227	281.5	$C_{18}H_{35}NO$
83	19.1536	Cyclobarbital	0.0443	236.3	$C_{12}H_{16}N_2O_3$
84	19.246	2'-Hydroxypropiophenone, TMS derivative	0.0611	222.1	$C_{12}H_{18}O_2Si$
		1-(4-Chlorophenoxy)-1-(1H-imidazol-1-yl)-3,3-			
85	19.4655	dimethylbutan-2-one	0.375	292.8	$C_{15}H_{17}ClN_2O_2$
86	19.708	1,2,5-Oxadiazol-3-amine, 4-(3-methoxyphenoxy)-	0.4047	207.2	$C_9H_9N_3O_3$
87	19.8466	Bis(2-ethylhexyl) phthalate	0.6824	390.6	$C_{24}H_{38}O_4$
88	20.1065	Silicic acid, diethyl bis(trimethylsilyl) ester	0.1931	296.6	$C_{10}H_{28}O_4Si_3$
89	20.2625	1,2,5-Oxadiazol-3-amine, 4-(3-methoxyphenoxy)-	0.0184	207.2	$C_9H_9N_3O_3$
90	20.3953	1H-imidazole-2-methanol, 1-decyl-	0.1556	238.4	$C_{14}H_{26}N_2O$

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

91	20.7707	Thymol, TBDMS derivative	0.5189	222.4	$C_{13}H_{22}OSi$
92	20.9439	Tetracosanoic acid, methyl ester	0.4657	382.7	$C_{25}H_{50}O_2$
93	21.0883	4H-1,2,4-triazole-3,5-diamine, N3-(4-fluorophenyl)-N5-methyl-	0.0968	207.2	$C_9H_{10}FN_5$
94	21.3366	1,2-Bis(trimethylsilyl)benzene	0.2511	222.5	$C_{12}H_{22}Si_2$
95	21.4464	Silicic acid, diethyl bis(trimethylsilyl) ester	0.0066	296.6	$C_{10}H_{28}O_4Si_3$
96	21.5446	Silicic acid, diethyl bis(trimethylsilyl) ester	0.3695	296.6	$C_{10}H_{28}O_4Si_3$
97	21.8218	1,4-Bis(trimethylsilyl)benzene	0.328	222.5	$C_{12}H_{22}Si_2$
98	22.1163	1,2-Bis(trimethylsilyl)benzene	0.6227	222.5	$C_{12}H_{22}Si_2$
99	22.4397	1,2-Bis(trimethylsilyl)benzene	0.8381	222.5	$C_{12}H_{22}Si_2$
100	22.6534	1,4-Bis(trimethylsilyl)benzene	0.5425	222.5	$C_{12}H_{22}Si_2$
101	22.9595	1,2-Bis(trimethylsilyl)benzene	1.8906	222.5	$C_{12}H_{22}Si_2$
102	23.0693	Cyclotrisiloxane, hexamethyl-	1.2295	222.5	$C_6H_{18}O_3Si_3$
103	23.179	1,4-Bis(trimethylsilyl)benzene	0.985	222.5	$C_{12}H_{22}Si_2$
104	23.3522	Cyclotrisiloxane, hexamethyl-	1.3341	222.5	$C_6H_{18}O_3Si_3$
105	23.4851	1,2-Bis(trimethylsilyl)benzene	1.0867	222.5	$C_{12}H_{22}Si_2$
106	23.6526	1,2-Bis(trimethylsilyl)benzene	1.7092	222.5	$C_{12}H_{22}Si_2$
107	23.9586	1,2-Bis(trimethylsilyl)benzene	1.5552	222.5	$C_{12}H_{22}Si_2$
108	24.0741	Cyclotrisiloxane, hexamethyl-	1.754	222.5	$C_6H_{18}O_3Si_3$
109	24.3052	Cyclotrisiloxane, hexamethyl-	1.6078	222.5	$C_6H_{18}O_3Si_3$
110	24.4842	2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene	0.9527	282.3	$C_{17}H_{14}O_4$
111	24.6517	Thymol, TMS derivative	0.8027	222.4	$C_{13}H_{22}OSi$
112	24.773	1,2-Bis(trimethylsilyl)benzene	1.824	222.5	$C_{12}H_{22}Si_2$
113	24.9404	1,2-Bis(trimethylsilyl)benzene	0.8643	222.5	$C_{12}H_{22}Si_2$
114	25.1657	1,4-Bis(trimethylsilyl)benzene	1.2224	222.5	$C_{12}H_{22}Si_2$
115	25.2812	Lup-20(29)-en-3-one	4.2735	424.7	$C_{30}H_{48}O$
116	25.4891	[1,2,4]Triazolo[1,5-a]pyrimidine-6-carboxylic acid, 7-amino-, ethyl ester	3.2874	207.2	$C_8H_9N_5O_2$
117	25.8529	1,4-Bis(trimethylsilyl)benzene	0.8174	222.5	$C_{12}H_{22}Si_2$
118	26.0147	1,4-Bis(trimethylsilyl)benzene	1.598	222.5	$C_{12}H_{22}Si_2$
119	26.1821	Cyclotrisiloxane, hexamethyl-	0.5435	222.5	$C_6H_{18}O_3Si_3$
120	26.3323	1,2-Bis(trimethylsilyl)benzene	0.3474	222.5	$C_{12}H_{22}Si_2$
121	26.5229	Cyclotrisiloxane, hexamethyl-	0.1904	222.5	$C_6H_{18}O_3Si_3$
122	26.8059	1,2-Bis(trimethylsilyl)benzene	0.4223	222.5	$C_{12}H_{22}Si_2$
123	26.9503	4-tert-Butylphenol, TMS derivative	0.1075	222.4	$C_{13}H_{22}OSi$

124	27.1524	1,4-Bis(trimethylsilyl)benzene	0.1091	222.5	$C_{12}H_{22}Si_2$
125	27.3834	1,4-Bis(trimethylsilyl)benzene	0.0645	222.5	$C_{12}H_{22}Si_2$
126	27.5047	N-Methyl-1-adamantaneacetamide	0.0548	207.3	$C_{13}H_{21}NO$
127	27.7184	Tetrasiloxane, decamethyl-	0.1788	310.7	$C_{10}H_{30}O_3Si_4$
128	27.9205	1,2-Bis(trimethylsilyl)benzene	0.4854	222.5	$C_{12}H_{22}Si_2$
129	28.2843	Thymol, TMS derivative	0.0493	222.4	$C_{13}H_{22}OSi$
130	28.4807	1,2-Bis(trimethylsilyl)benzene	0.0631	222.5	$C_{12}H_{22}Si_2$
131	28.654	4-tert-Amylphenol, TMS derivative	0.0454	236.4	$C_{14}H_{24}OSi$
132	28.8388	Thymol, TMS derivative	0.0356	222.4	$C_{13}H_{22}OSi$
133	28.9543	Thymol, TMS derivative	0.0783	222.4	$C_{13}H_{22}OSi$
134	29.0698	Thymol, TMS derivative	0.0571	222.4	$C_{13}H_{22}OSi$
135	29.243	1,4-Bis(trimethylsilyl)benzene	0.0612	222.5	$C_{12}H_{22}Si_2$
136	29.3412	4-tert-Butylphenol, TMS derivative	0.0506	222.4	$C_{13}H_{22}OSi$
137	29.4972	Thymol, TMS derivative	0.0622	222.4	$C_{13}H_{22}OSi$
138	29.7282	1,4-Bis(trimethylsilyl)benzene	0.1587	222.5	$C_{12}H_{22}Si_2$
139	29.9072	4-tert-Butylphenol, TMS derivative	0.0713	222.4	$C_{13}H_{22}OSi$
140	30.2075	Cyclotrisiloxane, hexamethyl-	0.0714	222.5	$C_6H_{18}O_3Si_3$
141	30.4847	Methyltris(trimethylsiloxy)silane	0.7012	310.7	$C_{10}H_{30}O_3Si_4$
142	30.6927	Thymol, TMS derivative	0.2955	222.4	$C_{13}H_{22}OSi$
143	30.8082	1,4-Bis(trimethylsilyl)benzene	0.2794	222.5	$C_{12}H_{22}Si_2$
144	30.9179	1,4-Bis(trimethylsilyl)benzene	0.8336	222.5	$C_{12}H_{22}Si_2$
145	31.2471	Thymol, TMS derivative	0.1726	222.4	$C_{13}H_{22}OSi$
146	31.3337	1,4-Bis(trimethylsilyl)benzene	0.0689	222.5	$C_{12}H_{22}Si_2$
147	31.4088	Thymol, TMS derivative	0.0749	222.4	$C_{13}H_{22}OSi$
148	31.582	1,4-Bis(trimethylsilyl)benzene	0.366	222.5	$C_{12}H_{22}Si_2$
149	31.6745	1,4-Bis(trimethylsilyl)benzene	0.1086	222.5	$C_{12}H_{22}Si_2$
150	31.7322	1,4-Bis(trimethylsilyl)benzene	0.299	222.5	$C_{12}H_{22}Si_2$
151	31.9574	4-tert-Butylphenol, TMS derivative	0.1876	222.4	$C_{13}H_{22}OSi$
152	32.0672	4-(4-Hydroxyphenyl)-4-methyl-2-pentanone, TMS derivative	0.4429	264.4	$C_{15}H_{24}O_2Si$

Key: RT: Retention time; Area Pct: Area percentage

It has also been documented for its anticancer, antiseizure, immunomodulatory, neuroprotective, and hypolipidemic effects.[74,75] Recent studies further suggested its potential as an enzyme inhibitor against α-glucosidase and acetylcholinesterase.[75]Oleic acid is a fatty acid with anticancer and anti-inflammatory properties, and its potential modulating effect on various health conditions, including insulin sensitivity, blood pressure, gastrointestinal function, and certain types of cancer, has been reported.[76] The possibility of oleic acid inhibiting the development of rheumatoid arthritis was reported,[77] and a recent report also suggested its potential to inhibit *D. mellitus* and Alzheimer's disease.[78] Hexadecanoic acid (palmitic acid) has been well-reported for its antioxidant, anti-inflammatory, cytotoxic, and antimicrobial activities.[79-82] Studies demonstrates its usefulness in treating *D. mellitus* and Alzheimer's disease.[79] Several derivatives of lup-20(29)-en-3-one have been reported for their anticancer and anti-inflammatory properties.[84,84] The fatty acid ester, 6- 6-Octadecenoic acid, methyl

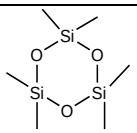
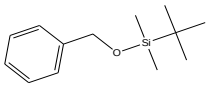
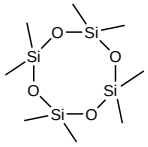
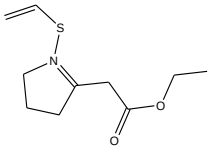
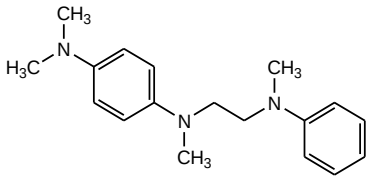
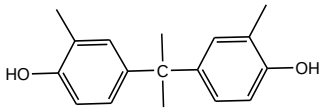
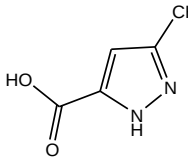
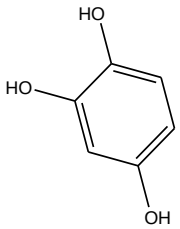
ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

ester, (Z)-, is associated with potent antioxidant, antiviral, anticancer, and anti-inflammatory activities.[85.] Research has shown that compounds containing the 1,2,4-triazolo [1,5-a]pyrimidine nucleus display diverse biological activities, including antitumour, anti-inflammatory, and antimicrobial activities.[86,87] Bisphenol C has recently been identified as a possible source of a drug against *Nipah henipavirus* infection.[88] The GC-MS profiling of *A. nilotica* methanol leaf extract and the aqueous stem-bark extracts was reported. [17, 18]

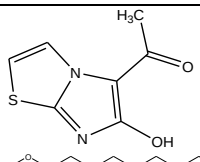
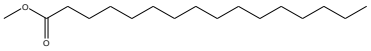
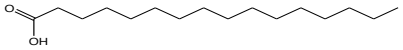
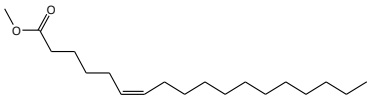
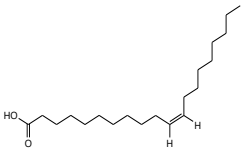
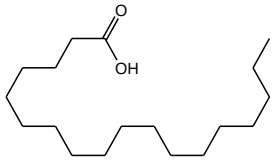
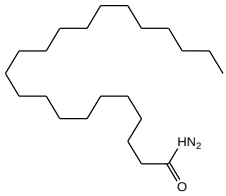
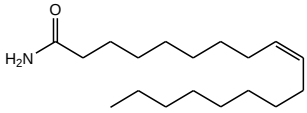
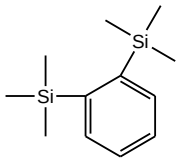
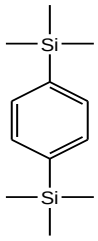
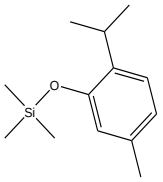
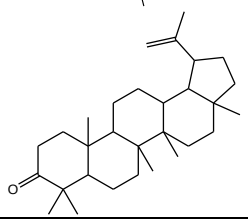
Conclusion

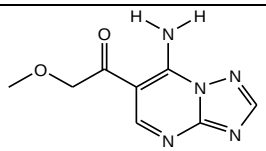
The results of this study confirmed *A. nilotica* as a plant with diverse phytochemical compounds, exhibiting notable medicinal and therapeutic properties. The flavonoid and volatile compound contents support the observed antioxidant activity and the ability to inhibit alpha-amylase enzyme exhibited by the methanol crude extract and fractions. These findings provide scientific validation for the folkloric uses of *A. nilotica* in managing oxidative stress-related conditions and their associated problems, including inflammatory conditions and *D. mellitus*. This plant may be a source of new drugs for managing oxidative stress and its related complications. This plant may also be a natural source of some heterocyclic compounds, eluted as part of the volatile constituents of the *A. nilotica* stem-bark methanol extract.

Table 6: Compounds of ANSM with area percentage above one, their classifications and structures

S/N	Compounds	Area Pct	Type of compound	Structure
1	Cyclotrisiloxane, hexamethyl-	8.6842	Organosilicon	
2	Benzyl alcohol, TBDMS derivative	1.2458	Organosilicon	
3	Cyclotetrasiloxane, octamethyl-	1.2224	Organosilicon	
4	Acetic acid, [[2-(3,4-dihydro-2H-pyrrol-5-yl)-1-methylethenyl]thio]-, ethyl ester	1.0639	Pyrrolyl ester	
5	P-Phenylenediamine, N,N,N'-trimethyl-N'-[2-(N-methylanilino) ethyl]-	1.0012	Triamine	
6	Bisphenol C	2.2469	Phenolic	
7	Pyrazole-5-carboxylic acid, 3-methyl-	1.75	Pyrazole carboxylic acid	
8	1,2,4-Benzenetriol	1.571	Benzenetriol	

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

9	1-(6-Hydroxyimidazo[2,1-b]thiazol-5-yl)ethanone	1.0282	Imidazothiazole	
10	Hexadecanoic acid, methyl ester	1.7668	Fatty acid ester	
11	n-Hexadecanoic acid	5.1681	Fatty acid	
12	6-Octadecenoic acid, methyl ester, (Z)-	3.9885	Fatty acid ester	
13	Oleic Acid	6.2392	Fatty acid	
14	Octadecanoic acid	1.0979	Fatty acid	
15	Behenic amide	1.0717	Fatty acid amide	
16	9-Octadecenamide, (Z)-	7.3745	Fatty acid amide	
17	1,2-Bis(trimethylsilyl)benzene	11.9601	Organosilicon	
18	1,4-Bis(trimethylsilyl)benzene	7.8423	Organosilicon	
19	Thymol, TMS derivative	1.6282	Organosilicon	
20	Lup-20(29)-en-3-one	4.2735	Triterpene	

21	[1,2,4]Triazolo[1,5-a]pyrimidine-6-carboxylic acid, 7-amino-, ethyl ester	3.2874	Triazopyrimidine ester	

Key: Area PCT: Area percentage

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

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

Acknowledgements

The authors thank the laboratory personnel of the Department of Chemical Sciences at the Lagos State University of Science and Technology for providing the laboratory facilities for this work. The authors thank Jamiu Oni for his assistance with this project.

References

1. Riaz M, Khalid R, Afzal M, Anjum F, Fatima H, Zia S, Rasool G, Egbuna C, Mtewa AG, Uche CZ, Aslam MA. Phytobioactive compounds as therapeutic agents for human diseases: A review. Food Sci Nutr. 2023; 11 (6):2500-2529. https://doi.org/10.1002/ fsn3.3308.
2. Abubakar IB, Kankara SS, Malami I, Danjuma JB, Muhammad YZ, Yahaya H, Singh D, Usman UJ, Ukwuani-Kwaja AN, Muhammad A, Ahmed SJ, Folami SO, Falana MB, Nurudeen QO. Traditional medicinal plants used for treating emerging and re-emerging viral diseases in northern Nigeria. Eur J Integr Med. 2022; 49:102094. https://doi.org/10.1016/j.eujim.2021.102094.
3. Iheanacho CM, Akubuiro PC, Oseghale IO, Imieje VO, Erharuyi O, Ogbeide KO, Jideonwo AN, Falodun A. Evaluation of the Antioxidant Activity of the Stem Bark Extracts of *Anacardium occidentale* (Linn) Anacardiaceae. Trop J Phytochem Pharm. Sci. 2023; 2 (2):65-69. https://doi.org/10.26538/tjpps/v2i2.4.
4. Hosseinzadeh S, Jafarikukhdan A, Hosseini A, Armand R. The application of Medicinal Plants in Traditional and Modern Medicine: A Review of *Thymus vulgaris*. Int. J. Clin. Transfus. Med. 2015; 66: 635-642. https://doi.org/10.4236/ijcm.2015.69084.
5. Singh A. Exotic flora of the Chandauli district, Uttar Pradesh, India: an overview. Indian J. For. 2012; 35 (1): 79-84. https://doi.org/10.54207/bsmps1000-2012-RSIG24.
6. Rather LJ, Islam S, Mohammad F. *Acacia nilotica* (L.): A review of its traditional uses, phytochemistry, and pharmacology. Sustain. Chem. Pharm. 2015; 2:12-30. https://doi.org/10.1016/j.scp.2015. 08.002.
7. Sultana B, Anwar F, Przybylski R. Antioxidant activity of phenolic components present in barks of *Azadirachta indica*, *Terminalia arjuna*, *Acacia nilotica* and *Eugenia jambolana* Lam. trees. Food Chem. 2007; 104 (3): 1106-1114. https:/doi.org/10.1016/j.food chem.2007.01.019.
8. Singh BN, Singh BR, Singh RL, Prakash D, Sarma BK, Singh HB. Antioxidant and anti-quorum-sensing activities of green pod of *Acacia nilotica* L. Food Chem Toxicol. 2009; 47 (4):778-786. https://doi.org/10.1016/j.fct.2009.01.009.
9. Singh BN, Singh BR, Sarma BK, Singh HB. Potential chemoprevention of N-nitrosodiethylamine-induced hepatocarcinogenesis by polyphenolics from *Acacia nilotica* bark. Chem Biol Interact. 2009; 181(1):20-28. https://doi.org./10.1016/j.cbi.2009.05.007.
10. Del WE. In vitro evaluation of peroxyl radical scavenging capacity of water extract reactions of *Acacia nilotica* (L.). Afr. J. Biotechnol. 2009; 8 (7): 1270-1272.
11. Misar A, Bhagat R, Mujumdar AM. Antidiarrhoeal activity of *Acacia nilotica* Willd. Bark methanol extract. Hindustan Antibiot Bull. 2007; 49- 50 (1-4):14-20.
12. Akintunde TA, Babayi HM, Alfa S. Effect of Aqueous Extract of *Acacia nilotica* on Microbial and Castor Oil Induced Diarrhoea Nig J. Biotech. 2015; 29: 34-37. https://doi.org/10.4314/ njb.v29il.5.
13. Rehman NU, Ansari MN, Ahmad W, Amir M. GC-MS Analysis and In Vivo and Ex Vivo Antidiarrheal and Antispasmodic Effects of the Methanolic Extract of *Acacia nilotica*. Molecules. 2022; 27 (7):2107. https://doi.org/10.3390/molecules27072107
14. Wassel GM, Abd-El-Wahab, SM, Aboutabl EA, Ammar NM, Afifi MS. Study of phenolic constituents and tannins isolated from *Acacia nilotica* L. Willd and *Acacia farnesiana* L. Willd growing in Egypt. Herba Hungarica. 1990: 29 (1-2): 43-50.
15. Rauf A, Ibrahim M, Alomar TS, AlMasoud N, Khalil AA, Khan M, Khalid A, Jan MS, Formanowicz D, Quradha MM. Hypoglycemic, anti-inflammatory, and neuroprotective potentials of crude methanolic extract from *Acacia nilotica* L. – results of an in vitro study. Food Sci. nutr. 2024; 12 (5): 3483-3491. https://doi.org/10.1002/fsn3.4017.
16. Bai S, Bharti P, Seasotiya L, Malik A, Dalal S. GC-MS analysis of chloroform extract of *Acacia nilotica* L. leaves. J Pharmacogn Phytochem. 2014: 2: 79-82.
17. Gupta AK, Bhat JL. GC-MS analysis of the methanol extract of *Acacia nilotica* (L.) Leaves. Int J Pharm Chem. 2016; 6 (2):50-53.
18. Janani M, Parimala T, Punithavathi VR, Mukesh J, Arulanandam A, Devipriya A. GC-MS Profiling and antibacterial activity of *Acacia nilotica* bark pertained aqueous extract. Res J Pharm Technol. 2024; 17 (12): 5931-5936. https://doi.org/10.52711/ 0974-360X.2024.00899.
19. Al-Lawati JA. *Diabetes Mellitus*: A Local and Global Public Health Emergency! Oman Med J. 2017; 32 (3):177-179. https://doi.org/10.5001/omj.2017.34.
20. Farag HFM, Elrewany E, Abdel-Aziz BF, Sultan EA. Prevalence and predictors of undiagnosed type 2 diabetes and pre-diabetes among adult Egyptians: a community-based survey. BMC Public Health. 2023; 23: 949. https://doi.org/10.1186/s12889-023-15819-0.
21. Tomic D, Shaw JE, Magliano DJ. The burden and risks of emerging complications of *Diabetes mellitus*. Nat Rev Endocrinol. 2022; 18 (9):525-539. https://doi.org/10.1038/s41574-022-00690-7.
22. Leon BM, Maddox TM. Diabetes and cardiovascular disease: Epidemiology, biological mechanisms, treatment recommendations and future research. World J Diabetes. 2015; 6 (13):1246-1258. https://doi.org/10.4239/wjd.v6.i13.1246
23. Pasupuleti VR, Arigela CS, Gan SH, Salam SKN, Krishnan KT, Rahman NA, Jeffree MS. A Review on Oxidative Stress, Diabetic Complications, and the Roles of Honey Polyphenols. Oxid Med Cell Longev. 2020; 2020:8878172. https://doi.org/10.1155/ 2020/ 8878172.
24. Okolie NP, Falodun A, Davids O. Evaluation of the antioxidant activity of root extract of pepper fruit (*Dennetia tripetala*), and its potential for the inhibition of lipid peroxidation. Afr J Tradit Complement Altern Med. 2014; 11 (3):221-227. https://doi.org/10.4314/ajtcam.v11i3.31
25. Akoro SM, Omotayo MA, Ajibaye S, Dada F. Comparative Alpha-amylase Inhibitory Properties of the Leaf Extracts of *Petiveria alliacea* L. Chem. Sci. Int. J. 2017;19 (1):1-9. https://doi.org/10.9734/CSJI/2017/32528
26. Gafar MK, Okoye IF, Olatunji LK, Ahmad J, Umar AO, Ibrahim OS, Salim F, Ahmad R. The Volatile Constituents from *Lepidium sativum* and *Tridax procumbens* Shoots Exhibit Antimicrobial

Effect and Inhibitory Activities Against α-Amylase Enzyme and 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Radical. Trop J Nat Prod Res. 2024;8(2):6433-6439. https://doi.org/10.26538/tjnpr/v8i2.35
27. Rijai AJ, Fidrianny I, S Sukrasno. Phytochemical and Screening of α-Glucosidase and α-Amylase Inhibitory Activities of Five Lit Sea Plants from East Borneo, Indonesia. Trop J Nat Prod Res. 2023;7(8):3666-3670. https://doi.org/10.26538/tjnpr/v7i8.15
28. Abu F, Mat Taib CN, Mohd Moklas MA, Mohd Akhir S. Antioxidant Properties of Crude Extract, Partition Extract, and Fermented Medium of *Dendrobium sabin* Flower. Evid Based Complement Alternat Med. 2017; 2017:2907219.
29. Ndayako HH, Muhammed HM, Salihu IM, Gabi UA, Yahaya I, Hamza UI, Aliyu AD. In vitro activity of methanol extracts of the root of *Sarcocephalus latifolius* (African peach) on *Plasmodium falciparum*. Sci. World J. 2024; 19 (2): 533-538. https://doi.org /10.4314/swj.v19i2.31.
30. Kolawole OT, Adeyeba OA, Awodele O, Wakeel OK, Ayankunle AA. Crude and Partition Extracts of *Newbouldia laevis* Leaves Attenuate Excitotoxin-induced Stereotypy in Mice. Euro. J. Med. Plants. 2020;31(18):29-36.
31. Ng KS, Mohd Zin Z, MohdMaidin N, Mamat H, Juhari NH, Zainol MK. High-performance liquid chromatography (HPLC) analysis for flavonoids profiling of *Napier grass* herbal tea. Food Res. 2021; 5(1): 65-71. https://doi.org/10.26656/fr.2017.5(1).311.
32. Mattila P, Astola J, Kumpulainen J. Determination of flavonoids in plant material by HPLC with diode-array and electro-array detections. J Agric Food Chem. 2000; 48(12):5834-5841. https://doi.org/10.1021/jf000661f.
33. Bustamante-Pesantes KE, Miranda-Martínez M, Gutiérrez-Gaitén YI, Guaranda IA, Pesantes-Domínguez O, Fernández MC. Chemical Composition, Acute Oral Toxicity and Analgesic Activity of Hydroalcoholic Extracts of *Mimusops coriacea* (A.DC) Miq (Sapotaceae).Trop J Nat Prod Res. 2023;7 (4): 2688-2695. http://doi.org/10.26538/tjnpr/v7i4.3
34. Mizzi L, Chatzitzika C, Gatt R, Valdramidis V. HPLC Analysis of Phenolic Compounds and Flavonoids with Overlapping Peaks. Food Technol Biotechnol. 2020;58 (1):12-19. https://doi.org/ 10.17113/ftb.58.01.20.6395.
35. Elnour AAM, Mirghani MES, Kabbashi NA, Md Alam Z, Musa KH. Study of Antioxidant and Anti-Inflammatory Crude Methanol Extract and Fractions of *Acacia seyal* Gum. Am J Pharmacol Pharmacother. 2018; 5 (1:3): 1-11. https://doi.org/10.21767/2393-8862.100012.
36. Sofowora A. Medicinal plants and traditional medicine in Africa. (3rd ed.). Ibadan, Nigeria: Spectrum Books Ltd; 2008. 134-156 p.
37. Ezeonu CS, Ejikeme CM. Qualitative and Quantitative Determination of Phytochemical Contents of Indigenous Nigerian Softwoods. New J. Sci. 2016; (1): 5601327. https://doi.org/ 10.1155/2016/5601327
38. Farid M, Abdelgayed SS, Soliman MH, El-Fadhany M, Hussein RH. Polyphenolic and Flavonoids Content, HPLC Profiling and Antioxidant Activity of Some Medicinal Plants with Pancreatic Histological Study in Alloxan-induced Diabetic Rats Model**.** J Microbiol Biotech Food Sci. 2020; 9 (4): 746-750. https://doi.org/10.15414/jmbfs.2020.9.4.746-750
39. Hatano T, Kagawa H, Yasuhara T, Okuda T. Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effects. Chem Pharm Bull (Tokyo). 1988; 36 (6):2090-2097. https://doi.org/10.1248/cpb.36.2090
40. Omotayo MA, Akoro SM, Avungbeto MO, Uwakwe H. Evaluation of Free Radical Scavenging and Antibacterial Activity of *Acalypha wilkesiana* and *Terminalia catappa* Methanolic Leaf Extracts. Microbiol. Res. J. Int. 2017; 19 (3):1-9. https://doi.org/10.9734/Mrji/2017/32761
41. Sudha P, Zinjarde SS, Bhargava S, Kumar AR. Potent α-amylase inhibitory activity of Indian Ayurvedic medicinal plants. BMC Complement Altern Med. 2011; 11:5. https://doi.org/10.1186/ 1472-6882-11-5
42. George BP, Chandran R, Abrahamse H. Role of Phytochemicals in Cancer Chemoprevention: Insights. Antioxidants (Basel). 2021; 10 (9):1455. https://doi.org/10.3390/antiox10091455
43. Kumar A, P N, Kumar M, Jose A, Tomer V, Oz E, Proestos C, Zeng M, Elobeid T, K S, Oz F. Major Phytochemicals: Recent Advances in Health Benefits and Extraction Method. Molecules. 2023; 28 (2):887. https://doi.org/10.3390/molecules28020887
44. Singh S, Bansal A, Singh V, Chopra T, Poddar J. Flavonoids, alkaloids and terpenoids: a new hope for the treatment of *Diabetes mellitus*. J Diabetes Metab Disord. 2022; 21(1):941-950. https://doi.org/10.1007/s40200-021-00943-8
45. Sadiq MB, Hanpithakpong W, Tarning J, Anal AK. Screening of phytochemicals and in vitro evaluation of antibacterial and antioxidant activities of leaves, pods and bark extracts of *Acacia nilotica* (L.) Del. Ind. Crops Prod. 2015; *77*: 873-882. https://doi.org/10.1016/j.indcrop.2015.09.067
46. Alain KY, Tamfu AN, Kucukaydin S, Ceylan O, Cokou Pascal AD, Félicien A, Koko Dominique SC, Duru ME, Dinica RM. Phenolic profiles, antioxidant, antiquorum sensing, antibiofilm and enzyme inhibitory activities of selected *Acacia* species collected from Benin. LWT-Food Sci Technol. 2022; 171: 114162. https://doi.org/10.1016/j.lwt.2022.114162
47. Ramasar R, Naidoo Y, Dewir YH, El-Banna AN. Seasonal Change in Phytochemical Composition and Biological Activities of *Carissa macrocarpa* (Eckl.) A. DC. Leaf Extract. Horticulturae. 2022; 8(9):780. https://doi.org//10.3390/horticulturae8090780
48. Aghababaei F, Hadidi M. Recent Advances in Potential Health Benefits of Quercetin. Pharma. (Basel). 2023; 16 (7):1020. https://doi.org/10.3390/ph16071020
49. Deepika Maurya PK. Health Benefits of Quercetin in Age-Related Diseases. Molecules. 2022; 27 (8):2498. https://doi.org/10.3390/ molecules27082498
50. Semwal R, Joshi SK, Semwal RB, Semwal DK. Health benefits and limitations of rutin - A natural flavonoid with high nutraceutical value, Phytochem Lett. 2021; 46:119-128. https://doi.org/10.1016/j.phytol.2021.10.006
51. Bazyar H, Zare Javid A, Ahangarpour A, Zaman F, Hosseini SA, Zohoori V, Aghamohammadi V, Yazdanfar S, Ghasemi Deh Cheshmeh M. The effects of rutin supplement on blood pressure markers, some serum antioxidant enzymes, and quality of life in patients with type 2 *Diabetes mellitus* compared with placebo. Front Nutr. 2023; 10:1214420. https://doi.org/10.3389/fnut.2023. 1214420
52. Hadidi M, Tarahi M, Christodoulou MC, Aghababaei F. The Potential Health Benefits of Gallic Acid: Therapeutic and Food Applications. Antioxidants (Basel). 2024; 13 (8): 1001. https://doi.org/10.3390/antiox13081001
53. Bae J, Kim N, Shin Y, Kim SY, Kim YJ. Activity of catechins and their applications. Biomed Dermatol. 2020; 4 (1): 1-10. https://doi.org/10.1186/s41702-020-0057-8
54. Hu X, Yang Z, Liu W, Pan Z, Zhang X, Li M, Liu X, Zheng Q, Li D. The Anti-tumour Effects of *p*-Coumaric Acid on Melanoma A375 and B16 Cells. Front Oncol. 2020; 10:558414. https://doi.org/10.3389/fonc.2020.558414
55. Tehami W, Nani A, Khan NA, Hichami A. New Insights into the Anticancer Effects of *p*-Coumaric Acid: Focus on Colorectal Cancer. Dose Response. 2023; 21 (1): 15593258221150704. https://doi.org/10.1177/15593258221150704
56. Zduńska K, Dana A, Kolodziejczak A, Rotsztejn H. Antioxidant Properties of Ferulic Acid and Its Possible Application. Skin Pharmacol Physiol. 2018; 31 (6): 332-336. https://doi.org/10. 1159/000491755
57. Neto-Neves EM, da Silva Maia Bezerra Filho C, Dejani NN, de Sousa DP. Ferulic Acid and Cardiovascular Health: Therapeutic and Preventive Potential. Mini Rev. Med. Chem. 2021; 21:1625-1637. https://doi.org/10.2174/1389557521666210105122841
58. de Morais EF, de Oliveira LQR, Farias Morais HGd, Souto Medeiros MRd, Freitas RdA, Rodini CO, Coletta RD. The Anticancer Potential of Kaempferol: A Systematic Review Based on In Vitro Studies. Cancers. 2024; 16 (3):585. https://doi.org/0.3390/cancers16030585
59. Bhaskar M and Aruma K. Assessment of the Antioxidant Capacity and Cytotoxic Activity of *Ipomoea pes-tigridis*. Trop J Nat Prod Res. 2024;8(8): 7965-7969. https://doi.org/10.26538/tjnpr/v8i8.5

60. Gulcin İ, Alwasel SH. DPPH Radical Scavenging Assay. *Processes*. 2023; 11 (8): 2248. https://doi.org/10.3390/pr11082248
61. Tadera K, Minami Y, Takamatsu K, Matsuoka T. Inhibition of alpha-glucosidase and alpha-amylase by flavonoids. J Nutr Sci Vitaminol (Tokyo). 2006; 52 (2):149-153. https://doi.org/10.3177/jnsv.52.149
62. Yuan E, Liu B, Wei Q, Yang J, Chen L, Li Q. Structure activity relationships of flavonoids as potent alpha-amylase inhibitors. Nat Prod Commun. 2014; 9 (8):1173-1176.
63. Lim J, Ferruzzi MG, Hamaker BR. Structural requirements of flavonoids for the selective inhibition of α-amylase versus α-glucosidase. Food Chem. 2022; 370:130981. https://doi.org/10.1016/ j.foodchem.2021.130981
64. Oboh G, Ademosun AO, Ayeni PO, Omojokun OS, Bello F. Comparative effect of quercetin and rutin on α-amylase, α-glucosidase, and some pro-oxidant-induced lipid peroxidation in rat pancreas. Comp Clin Path, 2015; 24 (5): 1103-1110. https://doi.org/10.1007/s00580-014-2040-5
69. Olukanni OD, Abiola T, Olukanni AT, Ojo AV. Chemical Composition, *In Silico* and *In Vitro* Antimutagenic Activities of Ethanolic and Aqueous Extracts of Tigernut (*Cyperus esculentus*). Prev Nutr Food Sci. 2022; 27(2):198-211. https://doi.org/10.3746/pnf.2022.27.2.198
70. Moronkola DO, Faruq UZ, Adigun OA, Ajiboye CO. Essential oil compositions of leaf, stem-bark, stem, root, flower, and fruit with seed of *Blighia unijugata* Baker (Sapindaceae). Afr. J. Pharm. Pharmacol. 2017; 11 (7): 108-119. https://doi.org/10.5897/ajpp2016.4721
71. Panayides J, Riley DL, Hasenmaile F, van Otterlo WAL. The role of silicon in drug discovery: a review. RSC Med, Chem. 2024: 15: 3286-3344
72. Cheng MC, Ker YB, Yu TH, Lin LY, Peng RY, Peng CH. Chemical synthesis of 9(Z)-octadecenamide and its hypolipidemic effect: a bioactive agent found in the essential oil of mountain celery seeds. J Agric Food Chem. 2010; 58 (3):1502-1508. https://doi.org/10.1021/jf903573g
73. Chen J, Garcia EJ, Merritt CR, Zamora JC, Bolinger AA, Pazdrak K, Stafford SJ, Mifflin RC, Wold EA, Wild CT, Chen H, Anastasio NC, Cunningham KA, Zhou J. Discovery of Novel Oleamide Analogues as Brain-Penetrant Positive Allosteric Serotonin 5-HT_{2C} Receptor and Dual 5-HT_{2C}/5-HT_{2A} Receptor Modulators. J Med Chem. 2023; 66 (14):9992-10009. https:// doi.org/10.1021/acs.jmedchem.3c00908.
74. Nam HY, Na EJ, Lee E, Kwon Y, Kim HJ. Antiepileptic and Neuroprotective Effects of Oleamide in Rat Striatum on Kainate-Induced Behavioral Seizure and Excitotoxic Damage via Calpain Inhibition. Front Pharmacol. 2017; 8: 817. https://doi.org/10.3389/fphar.2017.00817.
75. Wattanalaorsomboon S, Mansalai P, Payaka A, Baiya S, Sansenya S. The inhibition effect of oleamide for acetylcholinesterase and α-glucosidase from edible wild mushroom by in vitro, in silico and fluorescence analysis. Int J Biol Macromol. 2025; 308 (Pt 4):142681. https://doi.org/10.1016/j.ijbiomac.2025.142681.
76. Sales-Campos H, Souza PR, Peghini BC, da Silva JS, Cardoso CR. An overview of the modulatory effects of oleic acid in health and disease. Mini Rev Med Chem. 2013; 13 (2):201-210.
77. Karacor K, Cam M. Effects of Oleic Acid. Med Sci Discov. 2015; 2: 125-132. https://doi.org/10.36472/msd.v2i1.53
78. Piccinin E, Cariello M, De Santis S, Ducheix S, Sabbà C, Ntambi JM, Moschetta A. Role of Oleic Acid in the Gut-Liver Axis: From Diet to the Regulation of Its Synthesis via Stearoyl-CoA Desaturase 1 (SCD1). Nutrients. 2019;11(10):2283. https://doi.org/10.3390/nu11102283.
65. Qin Y, Chen X, Xu F, Zhu K, Wang P, Zhang Y, Zhang Y. Pectin enhances the inhibition of α-amylase via the mixture of rutin and quercetin. Int. J. Biol. Macromol, 2024; 285: 138251. https://doi.org/10.1016/j.ijbiomac.2024.138251.
66. Akoro S, Ogundare OC, Oyedola A. Comparative GC-MS Analysis, Antioxidant and cytotoxic activities of *Garcinia kola* Heckel seed and stem-bark n-hexane extract. J. Med Herbs. 2023: 14 (2): 35-43.
67. Akoro SM, Ogundare OC, Ajibade SO, Awofeso DO. Phytochemical Investigation of *Aristolochia ringens* (Vahl.) n-hexane root extract using GC-MS and FTIR. Trop J Phytochem Pharm. Sci. 2025; 4(3) 111-122. https://doi.org/10.26538/tjpps/v4i3.2
68. Ogunlesi M, Okiei W, Osibote E. Analysis of the essential oil from the leaves of *Sesamum radiatum*, a potential medication for male infertility factor, by gas chromatography-mass spectrometry. Afr. J. Biotechnol. 2010; 9:1060-1067. https://doi.org/10.5897/AJBO9.941
79. Wechakorn K, Payaka A, Masoongnoen J, Wattanalaorsomboon S, Sansenya S. Inhibition potential of n-hexadecanoic and oleic acids from edible insects against α-glucosidase, α-amylase, tyrosinase, and acetylcholinesterase: in vitro and in silico studies. J Sci Food Agric. 2025;105 (7):3701-3711. https://doi.org/10.1002/jsfa.14121
80. Aparna V, Dileep KV, Mandal PK, Karthe P, Sadasivan C, Haridas M. Anti-inflammatory property of n-hexadecanoic acid: structural evidence and kinetic assessment. Chem. Biol. Drug Des. 2012; 80(3):434-439. https://doi.org/10.1111/j.1747-0285.2012. 01418. x.
81. Lokesh R, Kannabiran K. Cytotoxic Potential of N-hexadecanoic Acid Extracted from *Kigelia pinnata* Leaves. Asian J. Cell Biol. 2017; 12: 20-27. https://doi.org/10.3923/ajcb.2017.20.27.
82. Purushothaman R, Vishnuram G, Ramanathan T. Antiinflammatory efficacy of n-Hexadecanoic acid from a mangrove plant *Excoecaria agallocha* L. Through in silico, in vitro and in vivo. Pharmacol. Res. - Nat. Prod. 2025; 7: 100203. https://doi.org/10.1016/j.prenap.2025.100203
83. Hata K, Hori K, Ogasawara H, Takahashi S. Anti-leukaemia activities of Lup-28-al-20(29)-en-3-one, a lupane triterpene. Toxicol Lett. 2003; 143: 1-7. https://doi.org/10.1016/S0378-4274(03)00092-4
84. Liu K, Zhang X, Xie L, Deng M, Chen H, Song J, Long J, Li X, Luo J. Lupeol and its derivatives as anticancer and anti-inflammatory agents: Molecular mechanisms and therapeutic efficacy. Pharmacol Res. 2021; 164:105373. https://doi.org/10.1016/j.phrs.2020.105373.
85. Singh R and Chaturvedi P. Phytochemical characterization of rhizome, fruit, leaf and callus of *Rheum emodi* Wall using GC-MS. Pharmacogn J. 2019; 11 (3): 617-623.
86. Amin NH, El-Saadi MT, Ibrahim AA, Abdel-Rahman HM. Design, synthesis and mechanistic study of new 1,2,4-triazole derivatives as antimicrobial agents. Bioorg. Chem. 2021; 111: 104841. https://doi.org/10.1016/j.bioorg.2021.104841
87. Guan Q, Xing S, Wang L, Zhu J, Guo C, Xu C, Zhao Q, Wu Y, Chen Y, Sun H. Triazoles in Medicinal Chemistry: Physicochemical Properties, Bioisosterism, and Application. J Med Chem. 2024; 67 (10):7788-7824. https://doi.org/10.1021/acs.jmedchem.4c00652
88. Hasan M, Tariquzzaman M, Islam MR, Susmi TF, Rahman MS, Rahi MS. Plant-derived Bisphenol C is a drug candidate against *Nipah henipavirus* infection: an in-vitro and in-silico study of *Pouzolzia zeylanica* (L.) Benn. In Silico Pharmacol. 2025; 13 (1):43. https://doi.org/10.1007/s40203-025-00328-2