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Original ResearchArticle

In vitro Investigation and GC-MS Analysis of the Chemical Constituents in the Fraction of Hexane Leaf Extract of *Tapinanthus bangwensis* (Engl. and K. Krause) Loranthaceae

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ABSRTACT

The exploration of medicinal plants as potential alternative therapeutic model comes from the quality content of its chemical compounds that exacerbate remarkable activity, nevertheless, there is the need for further exploit. This study investigated the *in vitro* antioxidant and antidiabetic potential of the fraction (HEX-ETACF or CF 2) of hexane leaf extract of *Tapinanthus bangwensis*, but importantly analyzed the likely chemical compounds present in the sample. The study used GC-MS (gas chromatography-mass spectroscopy) machine and spectrophotometer to elucidate the possible compounds and estimate the antioxidant and antidiabetic property respectively. The fraction had significant amount of alkaloids content, while phenolics content appeared higher than the flavonoids content The antioxidant results had the activity of lipid peroxy, 2,2-diphenyl-1-hydrazyl and nitrite radicals significantly inhibited compared to the ferric radicals. Furthermore, the result showed that the fraction inhibited α-amylase's metabolic activity higher than α-glucosidase. The Fourier transition infrared (FTIR) chromatogram had ten peaks, indicated the fraction contains complex molecules that bears carbonyl, methyl, carboxyl and aromatic groups respectively. The GC-MS chromatogram revealed twenty-one peaks, such that butyl-9-octadecenoic acid (8.01%) and nonahexacontanoic acid (0.05%) had the highest and lowest percentage areas (% area). Another GC-MS chromatogram had twenty-seven peaks, with squalene (peak 23; 37.29%) and 1,2-benzenedicarboxylic acid, buty-2-ethylhexyl ester (peak 27; 19.87%) been significantly abundant, suggested the fraction was rich in phenolics and phthalates. Our findings revealed that the compounds demonstrate beneficial effects as antioxidant and antidiabetic agents.

Keywords: Gas chromatography-mass spectroscopy, Fourier transition infrared, Antioxidant, Anti-diabetic. *Tapinanthus bangwensis.*

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Introduction

Currently, research tries to move away from the use of normal conventional treatment model to an alternative pathway, often referred to as traditionasal medicine (herbal therapy), thereby resonating global interest in ethno-medicine.[1] Considering the high economic cost connected to the use of synthetic drugs, and since about 70-80% rural dwellers cannot afford it, an alternative treatment which involves herbal medication was necessary, taking into account its potency, affordability and availability.[2,3] The efficacy of herbal plants is attached to the huge localization of phytoconstituents (or secondary metabolites), that have shown unique and interesting bioactivities.[4] In comparison with pharmacopeia documentation, statistical data confirm that drugs from natural sources account for approximately 20-25%, with several of them without any form of structural modification.[5]

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Research data agree that disease progression in organisms occur when free radicals attack organelles, leading to oxidative stress, nevertheless, natural products which contain high level of antioxidants (phytochemicals), have been used to address health related issues arising from ROS attack. As a result, serious concern is being placed on phytomedicine.[6,7] Phytomedicine, is a long aged global practice that has gained recognition as a therapeutic way of treating specific physiopathological situations.[8,9] There are over seven hundred documented species of mistletoe, found on different host plants as parasites, however, *Viscum album* (European mistletoe), *Loranthus micranthus* and *Tapinanthus spp*, are the commonly investigated species. The leaf part of the plants has shown potential in the treatment of epilepsy, diabetes, hypertension, cancer, infertility, arthritis, pathogenic attack, and a host of other medicinal benefits.[10-12]
Moreover, reported studies suggest that the plant's host may contribute to the ameliorating, elemental, nutritional and chemical content. In the light of the aforementioned, *T. bangwensis* may be a more likely choice.[13,14] Already published articles revealed that *T. bangwensis* leaf contained chemical compounds such as eudesmic acid, methyl-3,4,5-trimethoxybenzoate and friedelin,[15] as well as avicularin, fisetin-7-glucoside, isovitexin, isoscutellarein-7-xyloside and 8-hydroxyluteolin-8- glucoside.[16] However, in our thinking, there is need for further characterization, to identify other interesting phytoconstituents. This study investigated the possible chemical constituents, the radical scavenging and antidiabetic property (*In vitro*) of the fraction (HEX-ETACF or CF 2) of hexane leaf extract of *T. bangwensis*. African mistletoe is a parasitic plant, that is fondly found in tropical regions, and bears *Afomo onisana*, *Awuresie* and *Kauci*

(Kanchi) in Yoruba, Igbo and Hausa dialects, but in English, it is called "bird lime", "life-giving tree" or "healing tree "respectively.

Materials and Methods

Plant Collection
In March 2022, fresh leaves of the plant were bought from Mushin located at 6º32'6. 84"N and 3º20'56. 28"E of Lagos. Mr. Adeleke, at the University of Lagos (Pharmacognosy Department), authenticated and provided the specimen number (LUH 4232). In the University's herbarium, a sample was deposited.

Extraction of the plant sample
The leaves were pulverized to a powdered mass, after two weeks of air-drying. The initial extraction was done by cold maceration, and later by column chromatography.[17] Briefly, 1500g of the sample was soaked in 5000mL hexane solvent for 72hrs, with moderate stirring at intervals. On exposing the volatile filtrate under dry atmospheric condition, a constant weight (62.04g) of solid hexane extract was recovered.
In the next phase, a transparent column glass was packed with silica gel solution, followed by introducing some quantity of sand on the gel, before adding the analyte. The eluents (Hexane and ethyl acetate) in various combinations were then used to wash the column, and eighty-eight fractions were recovered. Finally, the retention factor of the different spots was used for the pooling exercise, which eventually produced three fractions.

Quantitative determination of some phytochemicals
The approach described by Ihegboro *et al*., 2019 was used to quantify the concentration of phenolics, flavonoids and alkaloids content in the fraction (HEX-ETACF).

Quantification of the Antioxidant capacity of the fraction
FRAP (Ferric Reducing capacity) Assay
The method put up by Nayan et al., 2013 was followed. A varying concentrations were prepared for both ascorbic acid and sample (20 - 100 μg/mL), from where 1.0mL was collected, dissolved in 1.0mL deionized water and buffer of phosphate (2.5mL, pH 6.6), as well as 1% potassium ferricyanide (2.5mL). The overall content was heated at 50°C for 20 minutes, after which 10% trichloroacetic acid (2.5mL) (TCA) was included. The content was spin at 3000 revolutions per min for approximately 10 minutes. Finally, absorbance of the final solution containing 0.1% ferric chloride (0.5mL), 2.5mL of water and 2.5mL supernatant, read at 700nm by spectrophotometer (CE4001UV/VIS, England).

2,2-Diphenyl-1-picrylhydrazyl) Test
The experimental protocol described by Zhu et al., 2006 was used. Briefly, 20 - 100µg/mL of the sample were prepared, 2.0mL was dropped into 0.1 mM DPPH solution (2.mL). The resulting solution agitated vigorously and left in a non-illuminated place as long as 30 minutes. The absorbance was measured at 517nm against the control. A similar procedure was followed when the positive control (Ascorbic acid) was being prepared. From the triplicate data, the inhibition activity and half-maximal inhibitory concentration (IC_{50}) were determined.

$$\%\ \text{inhibition activity} = \frac{\text{Absorbance of control} - \text{Absorbance of sampl}}{\text{Absorbance of control}} \text{ x } 100$$

Nitrile radical Inhibition Assay.
The method stated by Fadzai *et al*., 2014 was good enough. Briefly, we prepared a stock solution (100 mg/mL) of the samples. In a serial dilution process, 20 -100 µg/mL were prepared. A 50μL of buffer solution of sodium nitroprusside (10mM) in 0.1M phosphate, was incorporated in 50μL of each prepared concentration in 96-well plate and allowed to stand for 180 minutes. In conclusion, 100 μL of Griess reagent – made by adding same quantity of 0.1% naphthyl ethylenediamine dihydrochloride and 2% sulphanilamide in 5% phosphoric acid and was added. The absorbance was then taken at 512nm, and both the inhibition concentration and % inhibition calculated.

$$\%\ \text{inhibition activity} = \frac{\text{Absorbance of control} - \text{Absorbance of sampl}}{\text{Absorbance of control}} \text{ x } 100$$

Thiobarbituric reactive species (TBARS)
Briefly, a solution consisting of 0.67% thiobarbituric acid (1.0mL), 0.1 M buffer phosphate (0.58mL, pH = 7.4), liver homogenate (0.2mL) and 1.0 mL of 0.67% thiobarbituric acid were prepared, from where 1.0mL was obtained and boiled for 20 mins. It was the removed and placed in an ice blocked container for cooling, before proceeding to be spin at 2000 rpm for 10 mins. In the final analysis, absorbance was taken against with blank at 535 nm. The triplicate data were used to calculate the percentage inhibition, while the IC_{50} was extrapolated from the graph. The above procedure was that described by Ihegboro *et al*., 2022.

In Vitro Anti-Diabetic Assays
A protocol described by Goboza *et al*., 2020 was followed for these analyses.

α-Amylase Inhibitory Test.
Firstly, a stock solution (1-300 mg/ml) of acarbose and the sample are prepared separately. A volume of 250 μl from the stock solution was introduced into 250 μl of 0.02M buffer solution of sodium phosphate buffer at pH 6.9 (having 0.5 mg/mL α-amylase), and then pre-incubated at 25°C for 10 mins. The next step involved the addition of 250 μl of 1% starch solution in the buffer solution, then incubated at 25°C for another 10 minutes. Furthermore, an inhibitor known as dinitrosalicylic acid (500 μl) was introduced to stop the reaction, thereafter, it was heated at 95°C for 5 minutes. The cooled content was added 5.0mL water, and absorbance measured of 540 nm.

$$\%\ \text{inhibition activity} = \frac{\text{Absorbance of control} - \text{Absorbance of sampl}}{\text{Absorbance of control}} \text{ x } 100$$

α-Glucosidase Inhibitory Test.
Shortly, α-glucosidase (1 U/ml) was pre-incubated with 250 μl extract for 10 minutes. 250 μl of 3mM Pnitrophenyl glucopyranoside substrate solution (pNPG,) in 20mM phosphate buffer (pH6.9) containing 2mg/ml BSA, was added for the reaction to start. The content was heated at 37°C for 20 minutes, halted with 1.0 M sodium carbonate (1.0mL), and absorbance at 405nm was taken.

$$\%\ \text{inhibition activity} = \frac{\text{Absorbance of control} - \text{Absorbance of sampl}}{\text{Absorbance of control}} \text{ x } 100$$

Identification of functional groups using FTIR Agilent
The FTIR analysis was conducted using the Satapute *et al*., 2019 protocol. In this process, a translucent sample disc, was prepared by encapsulating 1s0mg of the sample in potassium bromide pellet (100mg). The product obtained was then run on spectrum FTIR machine (PerkinElmer), within wavenumbers between 400 - 4000 cm^{-1}, as well as 4.0 cm^{-1} resolutions and 4 scan numbers. The information generated were then converted into FTIR chromatogram via OriginPro2019b.Win software. The compounds were identified through the database of NIST (National institute of Standard and Technology)

Gas Chromatography-Mass Spectroscopy Analysis
The analysis was done on 7890B machine (Agilent Technologies, USA), following the methods articulated by Konappa *et al*., 2020; Tsegu *et al*., 2022 A 1.0µL of the sample was introduced in an already prepared GC-MS machine, via the inlet using a springe. A carrier gas (helium gas, 99.9%) was maintained at constant of 1.0 mL/min (low rate), found in the vacuum heated at 300°C. The eluted compounds were then detected and transcribed as spectral lines. The database of NIST (Gaithersburg, USA) was used to identify the compounds, in relation to their retention time and the % area.

Statistical Analyses
A one-way ANOVA test, using version 23.0 of SPSS was conducted. All the triplicate data were converted to Mean ± SD, and the significant limit set at $p < 0.05$. Also, the Tukey's post hoc test package was used.

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Results and Discussion

Extraction yield of crude hexane extract and the fractions
The percentage yield of crude hexane leaf extract was 4.13% (62.04g). The detailed information of the chromatographic extraction is captured in Table 1.

Phytochemical analysis
The Figure 1, indicated that alkaloids were substantially present, while phenolics content was significantly abundant compared to flavonoids content. In comparison to reported work, our result was a direct opposite.[16]

Analyses of the fraction's Antioxidant property
Between the concentration of 20-60 µg/mL, both the fraction (HEX-ETACF or CF 2) and ascorbic acid, had a similar pattern of inhibition, but as concentration inclined (80-100 µg/mL), there was a significant increase in the inhibition of ferric radicals by ascorbic acid compared to the fraction (Figure 2). A careful observation of Figure 3, indicated that across the concentration gradients (20-100 µg/mL), ascorbic acid had a strong inhibition against DPPH radicals than the fraction. Moreover, there was concentration-dependent inhibition observed.
From the nitric oxide assay (Figure 4), the fraction and ascorbic acid inhibited nitrile radicals in a closely similar pattern at 80-100 µg/mL. On a final note, the fraction showed increase in lipid peroxy radicals inhibition compared to the ascorbic acid at the 20 and 40 µg/mL concentration, but at mid-concentration (50µg/mL), the two samples exhibited similar inhibitory activity. Moreover, the inhibitory effect increased with ascorbic acid compared to the fraction (Figure 5).
Considering the half-maximal inhibition concentration (IC_{50}), there was significant inhibition of DPPH radicals, a similar inhibitory effect was exhibited against nitrile and lipid peroxy radicals, while the inhibition of FRAP radicals was the lowest (Figure 6).
It is noteworthy, that natural antioxidants exacerbate their effects by supplying hydrogen (or electrons) to the orbital of the free radical molecule with the aim of stabilizing the structural configuration.[27] In the light of our result, the fraction showed the capacity to inhibit free radicals, however, the effect was strong against DPPH, NO and LP radicals rather than Fe^{3+} radicals, and this had been previously reported in a study conducted by [28, 29]

Effect of the fraction on digestive enzymes of carbohydrate.
As indicated in Figure 7, 8 and 9, acarbose had a significant inhibition against the digestive activity of α-glucosidase and α-amylase, when related to the fraction. However, both samples had selective affinity for α-amylase compared to α-glucosidase.
The global empirical data on cases of diabetes mellitus, indicated that synthetic drugs lack the temerity to provide optimal hypoglycemic result, which has been hedged on efficacy and toxicity problem, however, naturally-rich polyphenolic products from plant origin, presents to human, an alternative medication. This is because polyphenols are excellent sources of antioxidants against various pathological disorders, including hyperglycemic problem. As earlier stated, our finding revealed that our plant is more of α-amylase inhibitor than α-glucosidase, which had also been reported [29]. In an *in vitro* study, involving the molecular docking of 1,2-benzenedicarboxylic acid, buty-2-ethylhexyl ester), the metabolic activity of α-glucosidase, and α-amylase declined.[28] The positive reduction in the digesting power of these enzymes could likely be attributed to the huge amount of the phthalate and polyphenols.[30, 31, 22]

FTIR Analysis of the fraction of hexane leaf extract of T. bangwensis
In Figure 10, the chromatogram had ten peaks, suggesting that the fraction contained complex compounds. There were peaks around 2920. 369 cm^{-1} and 2851. 413 cm^{-1}, indicating the compound had long linear chains. Between the regions of 2000 cm^{-1} and 2500 cm^{-1}, no peak was found, connoting the lack of triple bond. Furthermore, absorption bands in the regions of 1500-2000 cm^{-1}, there was a peak with a wavenumber of 1733 cm^{-1}, indicating there are functional groups like carbonyl or carboxyl, from ketones, esters or carboxylic acids. Also, there were two peaks at 1459. 253cm^{-1} and 1244. 931 cm^{-1} regions, implying it may be aromatic compounds, while at 1379.115 cm^{-1}, carbonyl group (from carboxylic salt), methyl (bend) or phenol may be present (Table 2). The analysis of the FTIR chromatogram was extrapolated from an article published by Nanditato *et al*., 2019.

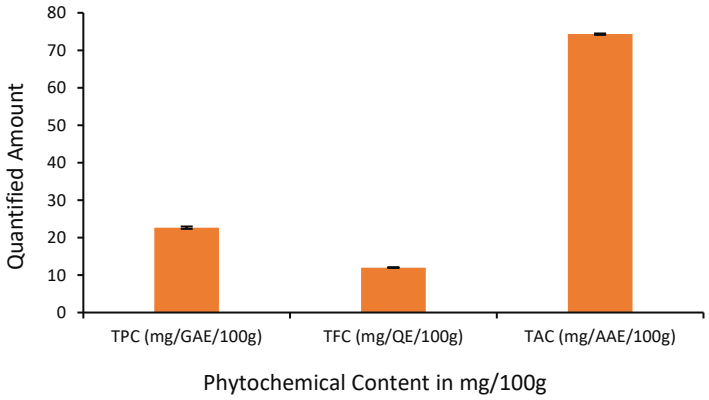
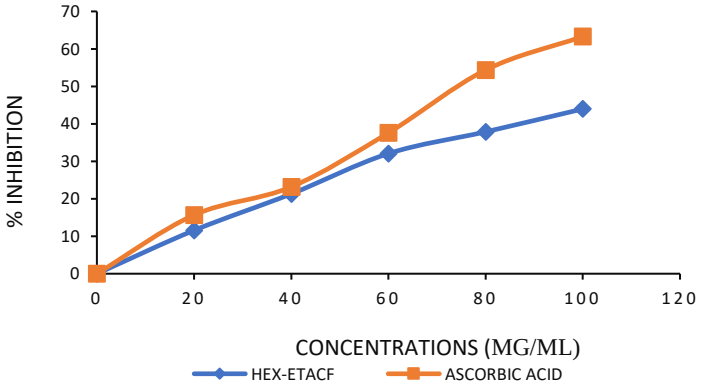


Figure 1: The concentration of some phytochemicals in the fraction HEX-ETACF or CF 2) of hexane leaf extract of *T. bangwensis*



Note: The MG/ML in all the figures stands for Microgram/Milliliter.
Figure 2: Antioxidant potential of the column fraction and ascorbic acid against FRAP radicals

Table 1: Extraction profile of crude hexane leaf extract of *T. bangwensis*

Samples	Eluting solvents	Collected Fractions	Nature of sample after dryness	Sample yield	Prep TLC Band
CF 1	100% HEX	25 (F_1-F_{25})	Yellowish oily extract	0.372g (0.74%)	-
CF 2	90% HEX : 10% ETAC	15 (F_{26} – F_{40})	Yellowish solid extract	4.106g (8.21%)	1
CF 3	85% HEX: 15% ETAC;			2.373g (4.75%)	6
	80% HEX : 20% ETAC	47 (F_{41} – F_{88})	Brownish solid extract		

Keys: CFs stands for column fractions; HEX represents Hexane while ETAC stands for Ethyl acetate

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GCMS Fatty acid profiling of fraction of hexane leaf extract of T. bangwensis

The GC-MS chromatogram had twenty-one peaks (figure 11), with highlights of fatty acids at different peak regions and % area as: 7,10-hexadecadienoic acid, methyl ester (P_2, 4. 1%), butyl-9-octadecenoate (P_4, 8. 01%), hexadecenoic acid, ethyl ester (P_7, 0. 93%), nonahexacontanoic acid (P_8, 0.05%), trichloroacetic acid, undec-10-enyl ester (P_9, 3. 33%), cis-13-octadecenoic acid (P_{10}, 5. 52%), cis-vacenic acid (P_{15}, 5.77%) and trans-13-octadecenoic acid (P_{19}, 6.13%). In comparison, the fraction was rich in unsaturated lipids compared to their counterpart (saturated lipids) (Table 3).

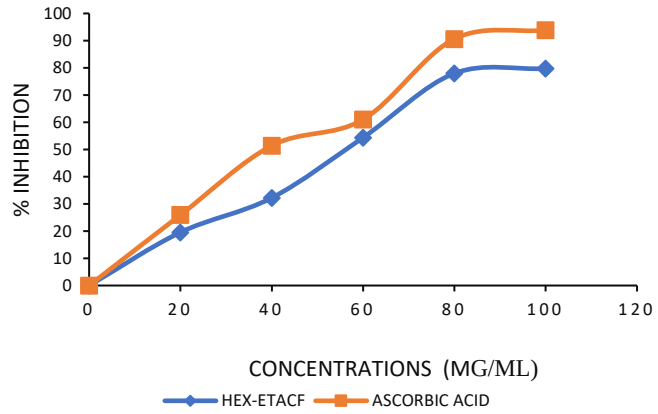

Figure 3: Antioxidant capacity of fraction and ascorbic acid against DPPH radicals

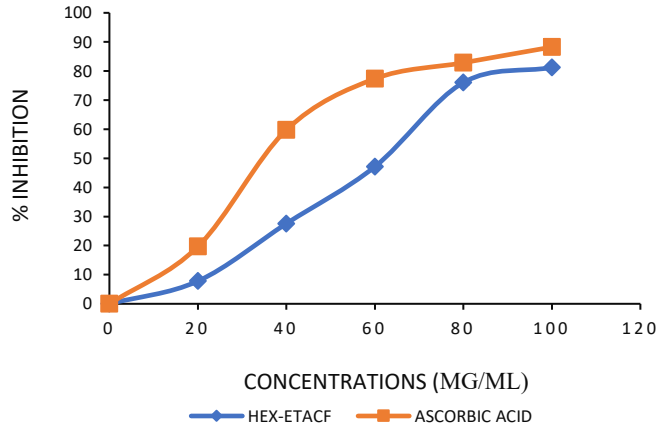

Figure 4: Free radical scavenging effect of fractions and ascorbic acid estimated using nitric oxide assay

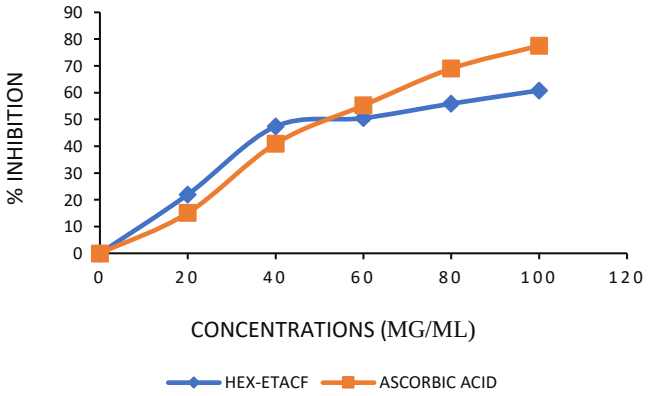

Figure 5: Lipid peroxidation activity of the fraction and ascorbic acid.

A literature check for the significance of these lipids suggest that 7,10-hexadecadienoic acid, methyl ester had antioxidant, anti-inflammatory and antimicrobial activity,[33] hexadecanoic acid, ethyl ester has antioxidant and hypocholesterolemic activity[34], cic-13-octadecenoic acid's bioactivity was unavailable,[35] while cis-vacenic acid has antioxidant activity.[36,37] It could be concluded that theses lipids may have contributed to the fraction's positive radical scavenging activity.

GCMS Chemical profiling of fraction of hexane leaf extract of T. bangwensis

In Figure 12, the GC-MS chromatogram had twenty-seven peaks, however, two peaks (P_{23}, 37. 29% and P_{27}, 19. 87%) were conspicuously the highest (Figure 13).

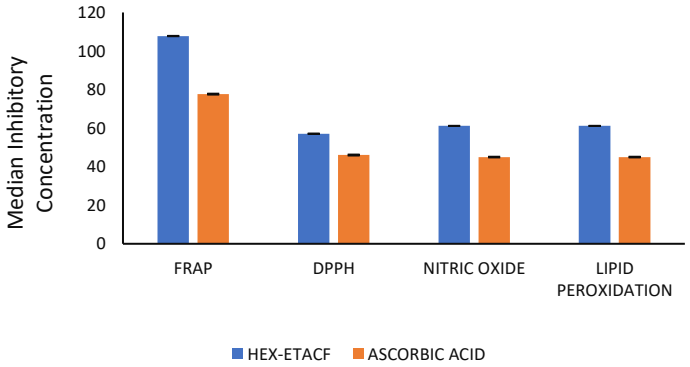

Figure 6: Average inhibition concentration of the fraction and ascorbic acid that inhibit free radical activity.

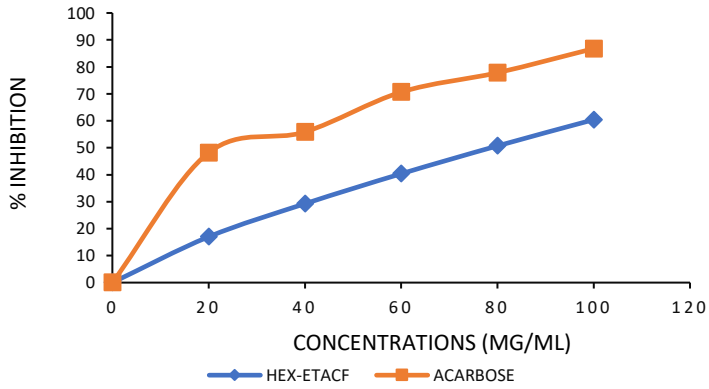

Figure 7: Inhibitory effects on the activity of alpha amylase by both the fraction and acarbose

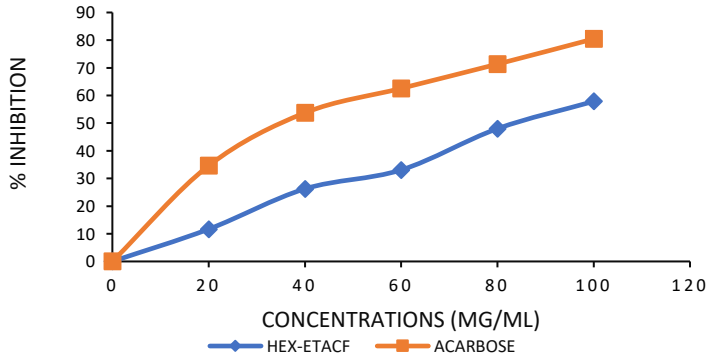

Figure 8: The suppressive effect of ascorbic acid and extract on alpha glucosidase activity

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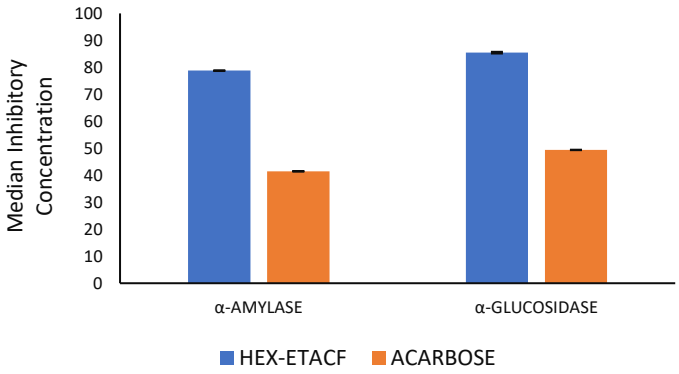

Figure 9: The average inhibition concentration that inhibited the activity of alpha amylase and glucosidase

The peak 23 had three hint compounds including 5, 9, 13-pentdecatrien-2-one, 6,10, 14-trimethyl -, (E, E); 2-methyl-3-(3-methyl-but-2-(enyl)-2-(4-methyl-pent-3-enyl)-oxetane and squalene, while in peak 27 region, we had phthalic acid, 2-ethylhexyl ioshexyl ester and 1,2-benzenedicarboxylic acid, butyl-2-ethylhexyl ester respectively (Table 4). Furthermore, heneicosane, hexanedioic acid, bis(2-ethylhexyl) ester and neophytadiene had % area between 3-6%, while sulfurous acid, 2-ethylhexyl hexyl ester; heptacosane; butanoic acid, 3-methyl-, 3,7-dimethyl-6-octenyl ester; 2-pentadieanone as well as carbonic acid, octadecy vinyl ester had % area below 2.0%.

Medicinal plants are enriched with diverse classes of secondary metabolites (or phytochemicals), that confer different physio-pharmacology via specific mechanisms targeting free radicals. They provide natural antioxidants, with documented levels of safety when juxtaposed with synthetic medicines.[38] Moreover, hydroxylated secondary metabolites (polyphenols) have significantly elucidated profound biological activity against free radicals, - causative agents for different ailments.[29] According to published studies, squalene – phenolic compound, belonging to the triterpene family has gastroprotective, hepatoprotective, antioxidant, antibacterial, anti-cancer, anti-atherosclerotic and anti-inflammatory agent.[39–41,29] In the work conducted by Swamy *et al*., 2017, neophytadiene (sequiterpenoid) was reported to stimulate antioxidant, anti-diabetic and anti-inflammatory activity,[29,42] while a terpene alcohol compound characterized as 3,7,11,15-tetramethyl-2-hexadecen-1-ol had anti-inflammatory and antimicrobial via antioxidant activity.[41] Also of significance, was sulfurous acid, 2-ethylhexyl hexyl ester, that has antioxidant characteristics.[43] In addition, 2-methyl-3-(3-methyl-but-2-enyl)-2-(4-methyl-pent-3-enyl)-oxetane and carbonic acid, octadecyl vinyl ester have anti-radical property.[44, 45] while hexanedioic acid, bis(2-ethylhexyl) ester and phthalic acid, 2-ethylhexyl isohexyl ester exhibit antidiabetic, anti-inflammatory, anticancer and diuretic potentials.[46, 47]

Table 2: FTIR result of the fraction (HEX-ETACF or CF 2) of hexane leaf extract of *T. bangwensis*

Peaks	Wavenumbers (cm^{-1})	Intensity	Interpretation
1	721.240	94.895	Phenyl compound
2	881.515	91.621	Peroxide (C-O-O) compound
3	978.426	88.268	Vinyl related compound (-CH=CH_2)
4	1174.111	83.617	Secondary amine (CN stretch), phenol (C=O stretch) compound
5	1244.931	86.848	Aromatic compound
6	1379.115	83.678	C=O (from COOH salt), methyl (Bend), phenol
7	1459.253	80.513	Aromatic ring
8	1733.212	69.401	C=O compound of ketone, aldehyde, ester or carboxyl
9	2851.413	62.397	Long chained linear aliphatic
10	2920.369	49.562	

Table 3: GC-MS fatty acid Analysis of the fraction of hexane leaf extract of *T. bangwensis*

Peaks	Retention time	Area (%)	Fatty acids identified	Bioactivities	References
2	9.783	4.1	7, 10-Hexadecadienoic acid, methyl ester	Antimicrobial, Antioxidant, Anti-inflammatory	[33]
4	14.249	8.01	Butyl-9-octadecenoate	Not found	Not reported
7	21.683	0.93	Hexadecanoic acid, ethyl ester	Antioxidant, Hypocholesterolemic	[34]
8	24.399	0.05	Nonahexacontanoic acid	Not found	Not reported
9	27.975	3.33	Trichloroacetic acid, undec-10-enyl ester	Not found	Not reported
10	30.185	5.52	Cis-13-octadecenoic acid	Not reported	[35]
11	30.499	3.27	Cis-13-octadecenoic acid	Not reported	[35]
15	34.774	5.77	Cis-vacenic acid	Antioxidant, Hypolipidemic and Antibacterial	[36, 37]
17	35.962	5.96	Trans-13-octadecenoic acid	Not found	Not reported
19	36.943	6.13	Trans-13-octadecenoic acid	Not found	Not reported
20	37.151	3.81	Trans-13-octadecenoic acid	Not found	Not reported
			Cis-vacenic acid.		
				Same as peak 15 above	

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	Same as peak 15 above.

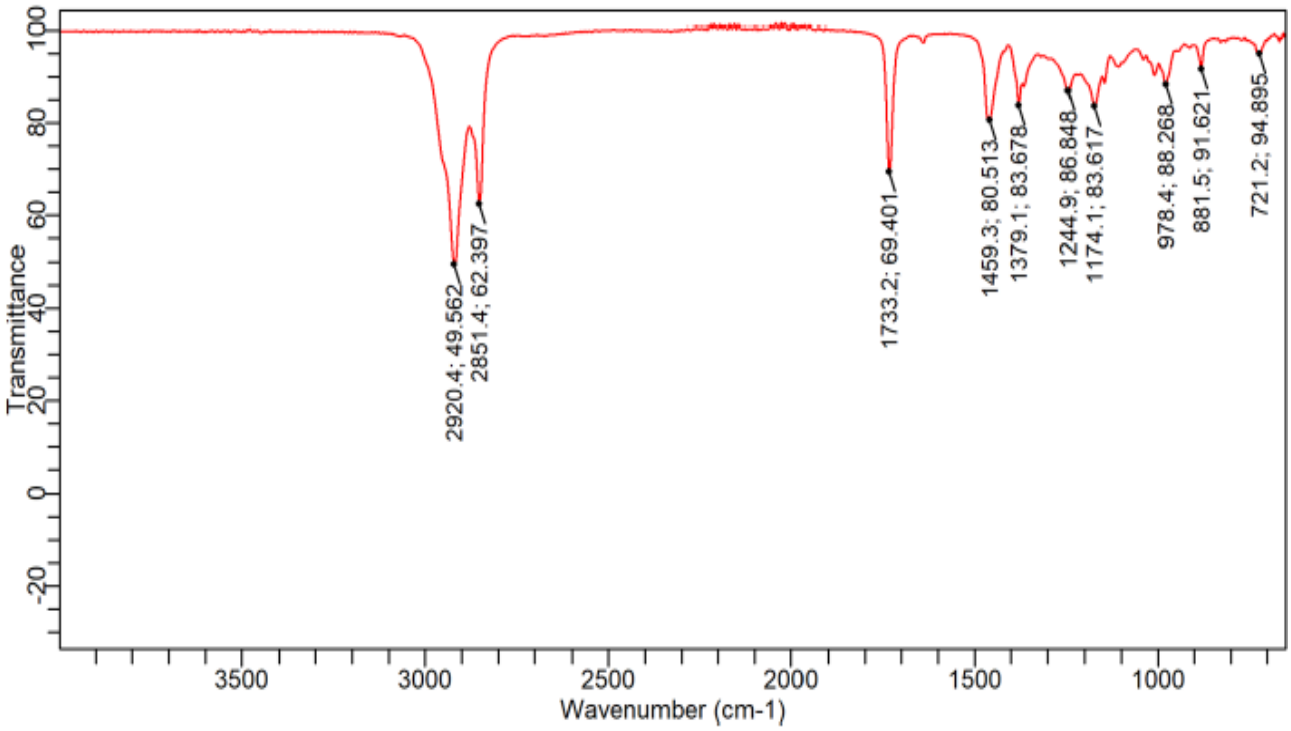

Figure 10: FTIR chromatogram of column fraction of hexane leaf extract of *T. bangwensis*

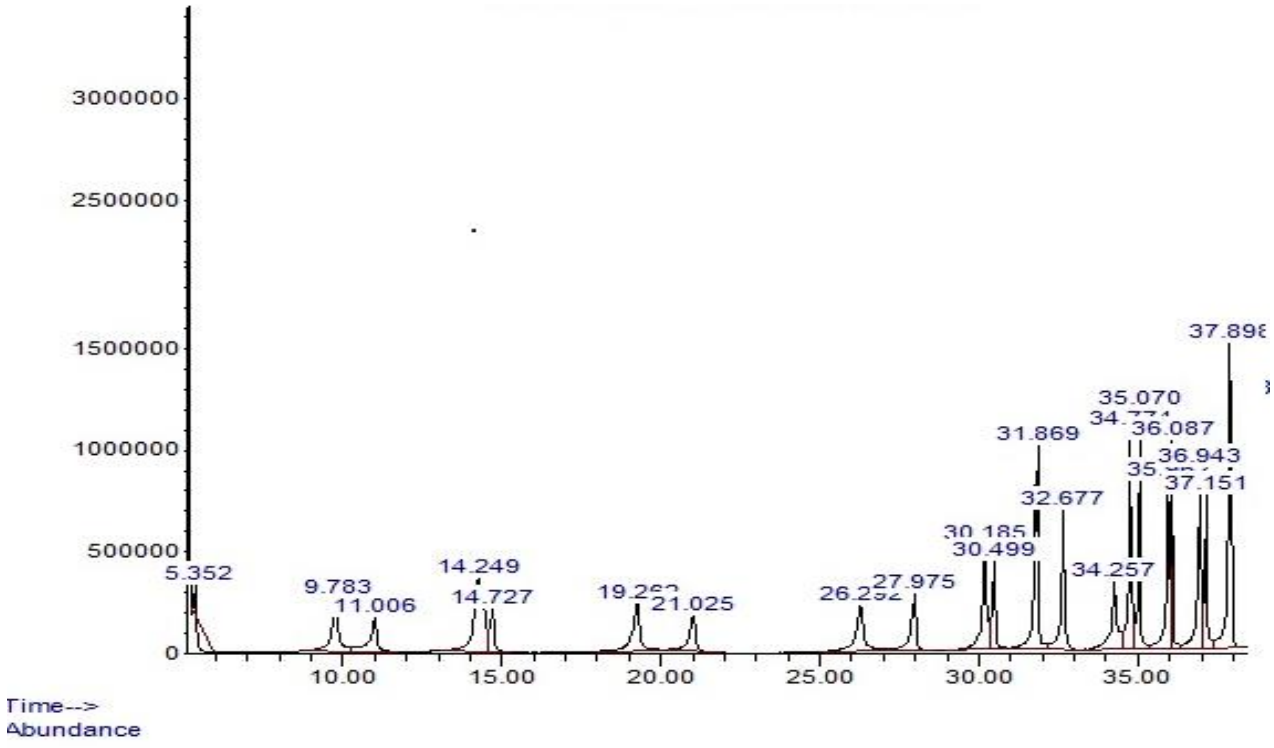

Figure 11: GC-MS chromatogram showing fatty acids in the fraction of hexane leaf extract of *T. bangwensis*

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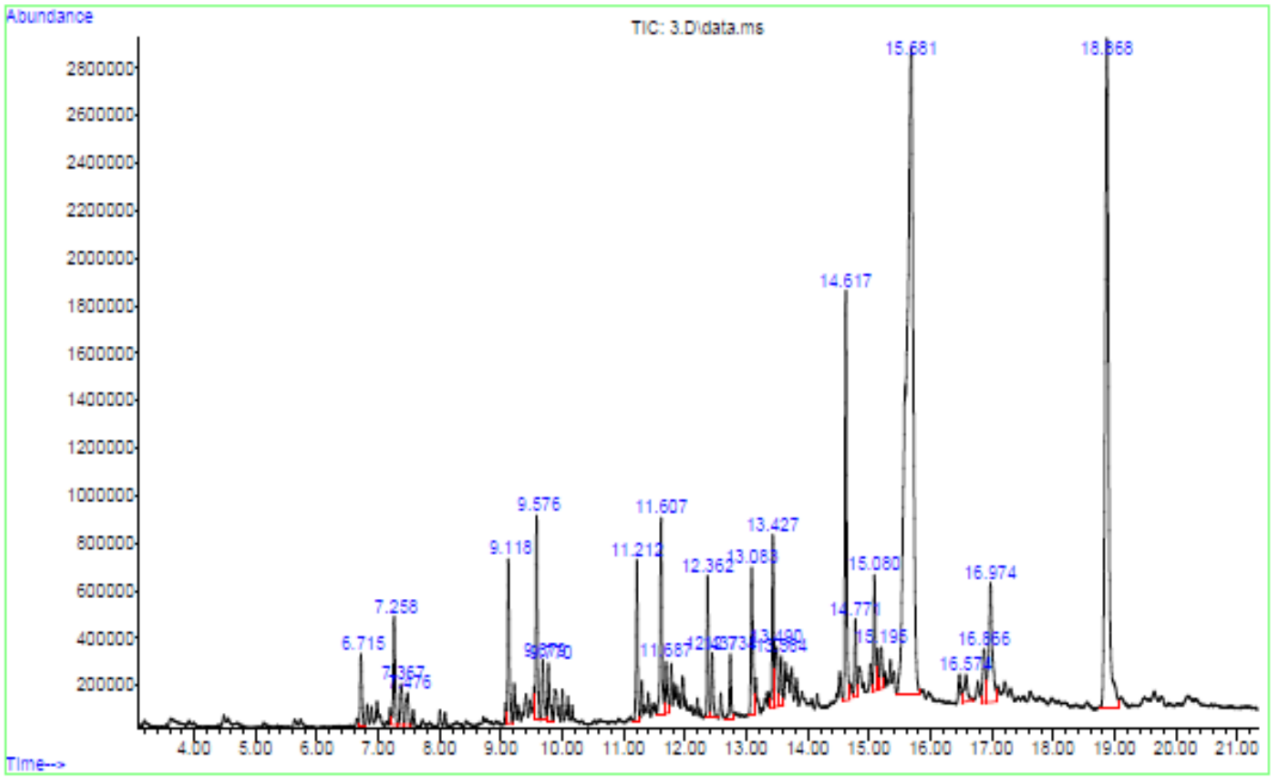

Figure 12: GCMS chromatogram indicating the chemical constituents in fraction of hexane leaf extract of *T. bangwensis*.

Table 4: GC-MS Analysis of the chemical constituents of fraction of hexane leaf extract of *T. bangwensis*

Peaks	Retention Time (min)	Area (%)	Compounds identified	Bioactivities	References
1	6.715	1.13	Sulfurous acid, 2-ethylhexyl hexyl ester	Antioxidant	[43]
7	9.670	0.98	Heptacosane	P-glycoprotein inhibitor, Antibacterial	[48]
10	11.607	3.05	Heneicosane	Antimicrobial	[49]
12	12.362	1.61	Butanoic acid, 3-methyl-, 3,7-dimethyl-6-octenyl ester	Catechol-O-methyl transferase inhibitor, inhibition of uric acid synthesis, inhibition of arachidonic acid, increase aromatic amino acid decarboxylase activity	[50]
13	12.457	1.02	2-pentadieanone	Wound healing, fibroblast proliferation, antibacterial, collagen deposition	[51]
14	12.734	0.93	Bicyclo[3,1,1] heptane, 2,6,6-trimethyl-, [1R-(1.alpha, 2.beta, 5.alpha]	Not found	Not reported
19	14.617	6.00	Neophytadiene	Anti-diabetic, Antioxidant, Anti-inflammatory	[29, 42]
			3,7,11,15-tetramethyl-2-hexadecen-1-ol	Antimicrobial and anti-inflammatory	[41]
23	15.681	37.29	Squalene	chemo protective; Antioxidant, Ant-atherosclerotic, Anti-diabetic; gastroprotective, anticancer and hepatoprotective Antioxidant Anti-inflammatory	[39 – 41, 29]
			2-methyl-3-(3-methyl-but-2-enyl)-2-(4-methyl-pent-3-enyl)-oxetane		[44]

			5, 9, 13-pentdecatrien-2-one, 6,10, 14-trimethyl -, (E, E)		52
24	16.574	0.79	Carbonic acid, octadecy vinyl ester	Antioxidant	47
			2H-pyran-2-one, tetrahydro-4-(2-methyl-1-propen-3-yl)	Not found	Not reported
26	16.974	3.52	Hexanedioic acid, Bis(2-ethylhexyl) ester	Antidiabetic, anti-inflammatory, anticancer	48
27	18.868	19.87	Phthalic acid, 2-ethylhexyl isohexyl ester	Antidiabetic, Anti-inflammatory, Diuretic	49
			1,2-Benzenedicarboxylic acid, butyl-2-ethylhexyl ester (or dibutyl. phthalate, DBP)	Anti-diabetic	31, 53

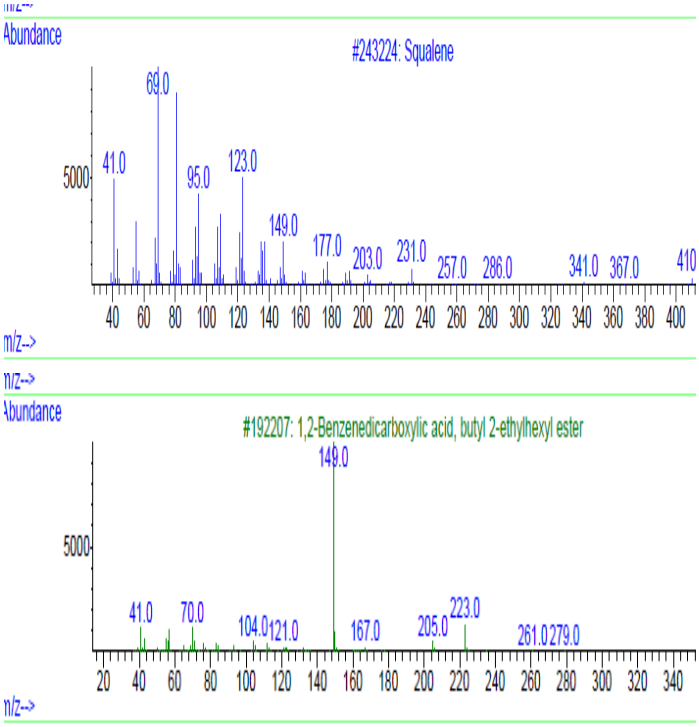

Figure 13: The fingerprints of the most prevalence phytochemicals in fraction of hexane leaf extract of *T. bangwensis*

Conclusion

This study investigated the possible presence of chemical compounds in the fraction of hexane leaf extract of *T. bangwensis*, including the *in vitro* antioxidant and antidiabetic potential. Our findings had the fraction inhibit LP, NO and DPPH radicals compared to Fe^{3+} radicals, while also inhibiting α-amylase's metabolic activity compared to α-glucosidase. The GC-MS result showed that squalene, 1,2-benzenedicarboxylic acid, buty-2-ethylhexyl ester and buty-9-octadecenoate were the predominant chemical compounds in the plant, and could have contributed to the antioxidant and inhibitory effect of the carbohydrate metabolic enzymes

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.

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