

UNVEILING THE PHYTOCHEMICAL PROFILING AND ANTIDIABETIC EFFECTS OF DIFFERENT EXTRACTS FROM *FICUS THONNINGII* (BLUME)

Abdullahi Shehu^{1*}, Imhalede Oladunni Pius¹, Abdulmumeen Amao Hamid², Olufunmilayo Adeola Ajayi³, Obuye Faith¹, Fatogun Oluwayomi Patrick¹, John Cecelia Uyomo¹, Yakubu Ejino Rofiyat¹

¹Department of Chemistry, Faculty of Sciences, Federal University Lokoja, Kogi State, Nigeria

²Department of Chemistry, Faculty of Physical Sciences, University of Ilorin, Ilorin, Nigeria

³Durable Crops Research Department, Cereals and Pulses Programme, Nigeria Stored Products Research Institute, PMB 1489, Ilorin, Nigeria

*Corresponding author: abdullahi.shehu@fulokoja.edu.ng

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Abstract

Ficus thonningii is used in traditional medicine for the treatment of diarrhoeal, gonorrhoeal, and diabetic conditions. This study evaluated the phytochemical and antidiabetic properties of the plant. The *n*-hexane, dichloromethane (DCM), and aqueous methanol extracts of *F. thonningii* leaves were screened for inhibitory activity against the carbohydrate hydrolysing enzymes α -amylase and α -glucosidase, and their metabolite profiles were resolved by gas chromatography mass spectrometry (GC-MS). The three extracts exhibited strong inhibition of α -amylase, with inhibition values of 96.02, 96.13, and 95.33 percent at 100 $\mu\text{g mL}^{-1}$, respectively, exceeding that of the standard drug acarbose (90.98 percent) at the same concentration. In contrast, the extracts showed low activity against α -glucosidase (10.95, 11.78, and 11.02 percent) relative to acarbose (71.29 percent). GC-MS profiling revealed forty seven, thirteen, and thirty eight compounds in the *n*-hexane, DCM, and aqueous methanol extracts, respectively, spanning a broad spectrum of bioactive metabolites that included steroids, fatty acids, esters, alkanes, alcohols, carboxylic acids, and alkaloids with reported antioxidant, antiproliferative, antibacterial, and antifungal properties. The findings demonstrate that *F. thonningii* harbours a rich diversity of promising antidiabetic metabolites and represents a credible source of lead compounds for the development of novel antidiabetic agents.

Keywords: *Ficus thonningii*, α -amylase, α -glucosidase, antidiabetic, GC-MS

1. Introduction

Diabetes mellitus ranks among the most formidable chronic diseases confronting global health, standing as the third leading cause of death after cancer and cardiovascular disease. The World Health Organisation has cautioned that an epidemic of diabetes mellitus is already in progress, and both primary diabetes and its complications impose a heavy burden on affected individuals and on health care systems worldwide (WHO, 1999). The search for effective antidiabetic agents has therefore intensified over recent decades. Natural products have served as a principal reservoir of therapeutic molecules for centuries, and a substantial proportion of the pharmaceuticals in current use are derived from natural sources. Plant derived medicines in particular have long been employed in the management of diabetes mellitus, and they continue to offer a fertile hunting ground for new chemical scaffolds (Alarcon-Aguilara et al., 1998).

Ficus thonningii (Blume), known as Odan among the Yoruba people of south western Nigeria, is an evergreen tree of the family Moraceae that is widely distributed across Nigeria, Zambia, and Angola. Members of the genus *Ficus*, collectively referred to as wild figs, are used extensively in traditional medicine for the treatment of a wide range of ailments. *F. thonningii* is applied against pain, diarrhoea, malaria fever, and microbial infections (Ezurike and Prieto, 2014), bronchitis, urinary tract infection, epilepsy, and diabetes (Cousins and Huffman, 2002; Etuk et al., 2010), as well as dysentery and wound infections (Usman et al., 2009). Earlier pharmacological investigations have documented antioxidant (Ahur et al., 2013), anti-inflammatory and antidiarrhoeal (Onkaeme and Udoh, 2000), analgesic (Otimenyin et al., 2004), antidiabetic (Olaokun et al., 2013; Musabayane et al., 2007), antimicrobial (Oyelana et al., 2011; Ndukwe et al., 2007), and antipsychotic (Maiha et al., 2013) activities for the plant. Phytochemical studies have shown that *F. thonningii* contains the flavonoid C glycosides orientin, vitexin, and isovitexin (Greenham et al., 2007), while GC-MS analysis of its essential oil has revealed volatile constituents such as α -pinene, β -pinene, β -caryophyllene, zingiberene, α and β -eudesmol, and isocaryophyllene (Ogunwade et al., 2008). Qualitative screening of the leaf extract has further reported alkaloids, tannins, anthocyanins, saponins, flavonoids, and carbohydrates (Ahur et al., 2013).

The conventional drug therapies for diabetes, namely oral hypoglycaemic agents and insulin injection, are not always satisfactory in maintaining glycaemic control and are frequently accompanied by adverse effects. Olaokun et al. (2013) reported the in vitro α -amylase and α -glucosidase activity of the acetone extract of *F. thonningii*; however, information on the chemical composition and the α -amylase and α -glucosidase inhibitory activities of the n-hexane, DCM, and aqueous methanol extracts of the plant remains scarce. It is therefore worthwhile to interrogate the chemical composition and the carbohydrate hydrolysing enzyme inhibitory activities of these leaf extracts. The objective of the present study was to identify the chemical composition and to evaluate the in vitro antidiabetic properties of the n-hexane, DCM, and aqueous methanol leaf extracts of *F. thonningii*.

2. Materials and Methods

2.1 Sample Preparation

The leaves of *F. thonningii* were collected from Lokoja, Kogi State, Nigeria. They were identified and authenticated by Mr Gbenga Akanni at the Herbarium of the Department of Plant Science, Federal University Lokoja, Nigeria, where voucher specimens (FULH 0228) were deposited.

2.2 Extraction and Fractionation of the Plant Material

Freshly harvested plant material was cut into pieces and dried at room temperature. The dried sample was pulverized with a wooden mortar and pestle into a coarse powder. The powdered material (500 g) was cold macerated successively with n-hexane (2 L) and dichloromethane (2 L) in separate extraction bottles for 60 h with periodic shaking. The mixtures were decanted and filtered through Whatman No. 1 filter paper, and the filtrates were concentrated to dryness at room temperature to yield the n-hexane extract (6.5 g) and the DCM extract (6.8 g). The DCM extract was dissolved in 100 mL and partitioned against 50 mL of distilled water in a separatory funnel. The organic and aqueous layers were collected separately and dried at room temperature to yield the DCM fraction (2.6 g) and the aqueous fraction (2.8 g).

2.3 GC-MS Analysis of the Extracts

The extract and fractions of *F. thonningii* leaves were analysed on a gas chromatography system coupled to an Agilent Technologies 5977A mass selective detector. Each sample was dissolved in a solvent at a ratio of 1:10 v/v, filtered through a 0.45 μ m nylon microfilter, and 2 μ L was injected at 250 °C in the injection port with a split ratio of 5:1. The oven was held initially at 110 °C for 2 min, ramped at 10 °C min⁻¹ to 200 °C, then at 5 °C min⁻¹ to 280 °C, and finally held for 9 min. The volatilized components were separated and transferred to the mass selective detector as ions of characteristic mass to charge ratio. The resulting mass spectra were identified by comparison with a qualified standard reference library, which returned compound names together with a percentage quality of match.

2.4 α -Amylase Inhibition Assay

The α -amylase inhibition assay was adopted from the procedure described by Nickavar and Yousefian (2011). Reagents comprised phosphate buffer (20 mM, pH 6.9), α -amylase (1 mg mL⁻¹) in phosphate buffer, 3,5-dinitrosalicylic acid, 0.5 percent starch solution, potassium sodium tartrate tetrahydrate, 2 M NaOH, and deionized water. The starch solution (0.5 percent w/v) was prepared by dissolving 0.25 g of starch in 50 mL of deionized water with stirring for 15 min. The enzyme solution was prepared by dissolving 1 mg of porcine pancreatic α -amylase in 110 mL of 20 mM potassium phosphate (pH 6.9) containing 6.7 mM NaCl. The colorimetric reagent was prepared from 20 mL of 3.5 to 9.6 mM 3,5-dinitrosalicylic acid, 8 mL of potassium sodium tartrate tetrahydrate, 5.31 mL of 2 M NaOH, and 12 mL of deionized water. For the reaction, 250 μ L of each extract concentration (100 to 500 μ g mL⁻¹) and 1 mL of the α -amylase solution were mixed and incubated at 25 °C for 30 min. Then 1 mL of starch solution was added to 1 mL of the mixture and incubated at 25 °C for 3 min, after which 1 mL of the colorimetric reagent was added and the tubes were placed in a water bath at 85 °C. After 15 min the mixture was cooled, diluted with 9 mL of distilled water, and the absorbance was read at 540 nm. Acarbose served as the positive control. The percentage inhibition was calculated as:

$$\text{Inhibition (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100 \quad (1)$$

where A_{sample} is the absorbance in the presence of the test sample or standard and A_{control} is the absorbance of the control.

2.5 α -Glucosidase Inhibition Assay

The α -glucosidase inhibitory activity was assayed following the method of Li et al. (2011) with slight modification. Reagents comprised 2.5 mM p-nitrophenyl glucopyranoside in 20 mM phosphate buffer (pH 6.8), 0.2 M Na₂CO₃, and α -glucosidase (0.2 U mL⁻¹). The enzyme solution contained 0.2 U of α -glucosidase per millilitre of phosphate buffer. The potassium phosphate buffer (pH 6.8) was prepared from 1.36 g of K₂HPO₄ and 2.72 g of KH₂PO₄ in 20 mL of distilled water, and the substrate (2.5 mM p-nitrophenyl glucopyranoside) was prepared from 7.53 mg in 10 mL of phosphate buffer. For the reaction, 8 μ L of each extract in DMSO at concentrations of 100 to 500 μ g mL⁻¹ was mixed with 112 μ L of phosphate buffer and 20 μ L of the enzyme solution and incubated at 37 °C for 15 min. A further 20 μ L of substrate was added and the reaction proceeded at 37 °C for 15 min before being terminated with 80 μ L of Na₂CO₃. Enzyme activity was quantified from the release of p-nitrophenol at 405 nm, with acarbose (0.1 mg mL⁻¹) as the positive control. The inhibitory percentage was computed using the same expression given in Equation 1.

2.6 Statistical Analysis

All qualitative tests were performed in triplicate. Statistical analysis was carried out in GraphPad Prism version 10.2.0, and results are reported as means $\pm$ standard deviation from triplicate experiments. Nonlinear regression was used to derive IC₅₀ values by relating percentage inhibition to concentration. A paired Student t test was used to compare two groups, while one way analysis of variance followed by the Dunnett post hoc test was used for multiple group comparisons. Statistical significance was accepted at $p < 0.05$.

3. Results and Discussion

3.1 GC-MS Analyses of the Extracts of *F. thonningii*

The GC-MS analysis of the n-hexane extract of *F. thonningii* resolved forty seven compounds, whose retention times, peak area percentages, molecular formulae, molecular weights, and reported bioactivities are summarized in Table 1. The chromatogram was dominated by cis-vaccenic acid, which accounted for 49.63 percent of the total peak area at a retention time of 19.602 min, and by a co eluting long chain constituent that represented 48.31 percent at 19.144 min. Together these two peaks constituted the overwhelming majority of the profile, while the remaining metabolites occurred as minor constituents. Notable minor components included cyclododecane, oxalic acid allyl hexadecyl ester, 2,5-cyclohexadiene-1,4-dione derivatives, benzene 1-(1,5-dimethyl-4-hexenyl)-4-methyl, 7,9-di-tert-butyl-1-oxaspiro-[4.5]deca-6,9-diene-2,8-dione, tridecan-1-ol, 17-pentatriacontene, and disparlure. Several of these constituents carry well documented pharmacological credentials. Cyclododecane possesses antibacterial activity (Vazquez and Rial, 2014), and benzene 1-(1,5-dimethyl-4-hexenyl)-4-methyl exhibits antimicrobial activity (Da Silva et al., 2015). Cis-vaccenic acid, the dominant metabolite, is a biologically important fatty acid noted for its antisickling property (Aimola et al., 2016), while 1-nonadecene is credited with antioxidant, antituberculosis, anticancer, and antimicrobial activities (Amudha et al., 2018; Rukachaisirikul et al., 2004; Lee et al., 2007). The isoprenoid ketone 2-pentadecanone 6,10,14-trimethyl serves hypocholesterolaemic, antioxidant, and lubricant roles (Kumar et al., 2010), whereas cetene and 2-dodecanone have been shown to induce mortality in various insects (Mitra et al., 2021; Wang et al., 2019). Trichloroacetic acid tetradecyl ester displays cytotoxic and antioxidant activities (Bourhia et al., 2020), and the biopesticidal compound disparlure is used in pest management to disrupt mating and to mass trap male moths (Douglas et al., 1990).

Table 1. Compounds identified in the n-hexane extract of *Ficus thonningii* by GC-MS and their reported biological activities

S/N	Compound name	RT (min)	Peak area (%)	Chemical formula	Molecular weight	Biological activity	Ref
1	2-Propen-1-one, 1-cycloxy-	15.389	0.02	C ₄ H ₉ O	138.21	NR	–
2	Cyclododecane	16.187	0.03	C ₁₂ H ₂₄	168.32	antibacterial	73

S/N	Compound name	RT (min)	Peak area (%)	Chemical formula	Molecular weight	Biological activity	Ref
3	Oxalic acid, allyl hexadecyl ester	16.396	0.01	C21H38O4	354.52	acaricide antiseptic pesticide	77
4	2,5-Cyclohexadiene-1,4-dione, 2,6-bis (1,1-dimethyl ethyl)-	18.204	0.10	C14H20O2	220.31	antifungal	39
5	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	18.593	0.31	C15H22	202.34	antimicrobial	15
6	Benzenethiol, 4-(1,1-dimethyl ethyl)-2-methyl-	18.748	0.03	C11H16S	180.31	NR	–
7	Tricyclo [4.1.0.0.(2,4)] heptane,3,3,7,7-tetramethyl-5-(2-methyl-1-propenyl)-	18.869	0.03	C15H24	204.35	NR	–
8	Acetophenone, 3'-allyl-4'-hydroxy-	19.444	48.31	C11H12O2	176.21	NR	–
9	Cis-vaccenic acid	19.602	49.63	C18H34O2	252.46	antisickle cell anaemia	7
10	Cetene	21.222	0.04	C16H32	224.43	insecticide	45
11	2,4-Hexadiene, 2,5-dimethyl	23.113	0.01	C8H14	110.20	NR	–
12	Oxalic acid, cyclobutyl pentadecyl ester	24.252	0.01	C21H38O4	354.52	NR	–
13	4H-1-benzopyran-2-carboxylic acid,8-amino-7-hydroxy-4-oxo-ethyl ester	24.803	0.02	C12H11NO5	249.22	NR	–
14	1-Nonadecene	25.775	0.06	C19H38	266.51	antioxidant antituberculosis, anticancer antimicrobial	9, 37, 64
15	2-Pentadecanone, 6,10,14-trimethyl-	26.956	0.03	C18H36O	268.48	hypcholesterolemic, antioxidant, lubricant	33
16	2-Dodecanone	27.568	0.03	C12H24O	184.32	insecticide	74
17	Docosylpentafluoropropanoate	28.512	0.03	C25H45F5O2	472.62	NR	–
18	7,9-Di-tert-butyl-1-oxaspiro-[4.5]deca-6,9-diene-2,8-dione	28.14	0.06	C17H24O3	276.37	α-amylase and Ache inhibition	4
19	Tetrachloroacetic acid, tetradecyl ester	29.911	0.10	C16H29Cl3O2	359.76	Antioxidant and cytotoxicity	13
20	Hexadecyl nonylether	30.076	0.06	C25H52O	368.68	NR	–
21	Tridecanoic acid, propyl ester	31.847	0.11	C16H32O2	256.42	NR	–
22	Heptane, 2,4-dimethyl-	32.123	0.06	C9H20	128.26	NR	–
23	Phytol	32.339	0.13	C20H40O	296.53	antitumour	31
24	3-Chloropropionic acid, heptadecyl ester	33.173	0.02	C20H39ClO2	346.98	antibiotic	30
25	1-Formyl-2,4,6-trimethyl-3-cis-(3-methylbut-2-enyl)-5-cyclohexene	33.210	0.01	C15H24O	220.35	NR	–
26	n-Tridecan-1-ol	33.255	0.02	C13H28O	200.36	hepatoprotective	11
27	Heptafluorobutyric acid, pentadecyl ester	33.699	0.05	C19H31F7O2		antimicrobial antioxidant anticancer	12, 67
28	Trans, trans-9,12-octadecadienoic acid, propyl ester	34.899	0.06	C21H38O2	322.53	NR	–
29	2-Decen-1-ol, (E)-	34.991	0.11	C10H20O	156.27	NR	–
30	Heptadecanoic acid, heptadecyl ester	35.486	0.02	C34H68O2	508.90	antimicrobial	26
31	Cyclohexane, 2-butyl-1,1,3-trimethyl-	35.649	0.06	C13H26	182.35	NR	–
32	Docosylpropylether	35.695	0.01	C25H52O	368.68	NR	–
33	3-Chloropropionic acid, pentadecyl ester	36.633	0.02	C18H35ClO2	318.92	NR	–
34	17-Pentatriacontene	37.179	0.02	C35H70	490.93	anti-inflammatory, antioxidant, anticancer, antiarthritic	34
35	Heptafluorobutyroxy tetradecane	37.240	0.004	C18H29F7O2	410.41	polymerization	41
36	9-Octadecenoic acid, (Z)-, 2,3-dihydroxy propyl ester	38.112	0.001	C21H40O4	356.54	nanocarrier	40
37	Disparlure	38.856	0.01	C19H38O	282.51	pesticide	17
38	4,8-Decadienal, 5,9-dimethyl-	38.887	0.01	C12H20O	180.29	NR	–
39	Propyl triacontyl ether	38.993	0.01	C33H68O	480.59	NR	–
40	Di-n-octyl phthalate	39.784	0.06	C24H38O4	390.28	antibacterial antifungal	68
41	3-Methyl-4-(phenylthiol)-2-prop-2-enyl-2,5,-dihydrothiophene-1,1-dioxide	39.882	0.005	C14H16O2S2	280.41	anticancer	19
42	3-Eicosene, (E)	39.983	0.01	C20H40	280.53	antioxidant	3
43	12-Methyl-Z, Z, -3,13-octadecadienol	40.034	0.01	C19H36O	280.50	insecticidal	44
44	1-Octanone, 1-(2-octylcyclopropyl)-	40.392	0.01	C19H36O	280.49	NR	–
45	Octacosyl propylether	40.418	0.004	C31H64O	452.84	NR	–
46	2,6,10-Dodecatrien-ol, 3,7,11-trimethyl-	41.190	0.004	C15H26O	222.37	anti-inflammatory antiallergic	32
47	12-Methyl-E, E-2,13-octadecadienol	41.756	0.002	C19H36O	280.49	anticholinergic	36

RT, retention time; NR, not reported. Reference numbers correspond to the numbered reference list.

The GC-MS profile of the DCM extract exhibited thirteen peaks, corresponding to thirteen phytoconstituents (Table 2). The extract was dominated by 12-methyl-E,E-2,13-octadecadien-1-ol (85.60 percent) and benzene 1-(1,5-dimethyl-4-hexenyl)-4-methyl (13.42 percent), with the remaining metabolites present only in trace amounts. The dominant constituent, 12-methyl-E,E-2,13-octadecadien-1-ol, has been reported to possess anticholinergic activity (Lakshmi et al., 2018). Among the minor constituents, 7-hexadecene displays anticancer properties and functions as a sex pheromone (El-fakir et al., 2021; Silva et al., 2018), dodecane is an oily hydrocarbon with antibacterial and antifungal activity (Padma et

al., 2019), and 6-octadecenoic acid, an aliphatic carboxylic acid found in plants such as *Coriandrum* and *Myrrhis odorata*, exhibits anticancer and insecticidal activity (Adegoke et al., 2019). Farnesol, formally 2,6,10-dodecatrien-1-ol 3,7,11-trimethyl, is produced by plants such as *Alpinia conchigera* and *Lonicera japonica* and possesses anti-inflammatory and antiallergic activity (Ku and Lin, 2015). The bioactivities of the remaining DCM constituents are listed in Table 2.

Table 2. Compounds identified in the DCM extract of *Ficus thonningii* by GC-MS and their reported biological activities

S/N	Compound name	RT (min)	Peak area (%)	Chemical formula	Molecular weight	Biological activity	Ref
1	12-Methyl-E, E-2,13-octadecadienol	19.567	85.60	C19H36O	280.49	anticholinergic	36
2	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	19.640	13.42	C15H22	202.34	antimicrobial	15
3	7-Hexadecene, (Z)	21.22	0.08	C16H32	224.25	anticancer aggregation sex pheromone	20, 69
4	1-Nonadecene	25.77	0.14	C19H38	266.51	antioxidant antituberculosis, anticancer antimicrobial	9, 37, 64
5	Dodecane	27.57	0.05	C12H26	170.33	antibacterial antifungal	57
6	7,9-Di-tert-butyl-1-oxaspiro-[4.5]deca-6,9-diene-2,8-dione	28.71	0.10	C17H24O3	276.37	α -amylase and Ache inhibition	4
7	Trifluoroacetoxyhexadecane	29.91	0.10	C18H32F3O2	338.45	NR	–
8	Tridecanoic acid, propyl ester	31.85	0.07	C16H32O2	256.42	NR	–
9	6-Octadecenoic acid	35.00	0.08	C18H34O2	282.46	anticancer insectifuge	2
10	Pentadecafluorooctanoic acid, dodecyl ester	37.18	0.06	C20H25F15O2	582.39	antibacterial	47
11	2,6,10-Dodecatrien-ol, 3,7,11-trimethyl-	37.82	0.011	C15H26O	222.37	anti-inflammatory antiallergic	32
12	Bis(2-ethylhexyl) phthalate	39.79	0.08	C24H38O4	390.56	antioxidant antiviral antitumor	21
13	Cycloeicosane	41.88	0.010	C20H40	280.51	cytotoxicity anticancer biopesticide	1, 65

RT, retention time; NR, not reported. Reference numbers correspond to the numbered reference list.

The GC-MS chemical profile of the aqueous methanol extract disclosed thirty eight phytochemicals (Table 3). The two most abundant components were a pentamethyl eicosapentane sesterterpene (51.87 percent) and isopropyl linoleate (46.13 percent), together with a suite of minor constituents that included 1-dodecanol, 2,5-cyclohexadiene-1,4-dione derivatives, a hexahydronaphthalene, decane, 9-eicosene, tert-hexadecanethiol, and 1-docosene. Several of these metabolites are pharmacologically significant. According to Li et al. (2015), the 2,5-cyclohexadiene-1,4-dione 2,6-bis(1,1-dimethylethyl) obtained from a *Trichoderma* strain strongly inhibits the phytopathogenic fungus *Cytospora chrysosperma*. 1-Dodecanol, a constituent of *Streptomyces viridodistatus*, is antibacterial against *Staphylococcus aureus* (Togashi et al., 2007); isopropyl linoleate is a fatty acid ester used in skin care that also exhibits antioxidant and anticancer activity (Rajendran et al., 2017); and decane, a saturated petroleum hydrocarbon, possesses antiparasitic and anthelmintic activity (Kumaresan et al., 2014). Tert-hexadecanethiol, a volatile organic compound reported in camel milk, is noted for its antitumor property (Raval et al., 2016), while 1-docosene, an unsaturated compound identified in seaweeds, has antibacterial activity (Sahar and Aida, 2018). Citral, formally 2,6-octadienal 3,7-dimethyl, is a monoterpene of plant essential oils with antibacterial activity (Dubey et al., 2014), and the sesterterpene 2,6,10,14,18-pentamethyl-2,6,10,14,18-eicosapentane exhibits antibacterial, antifungal, and antitumor activity (Baba et al., 2021). Bicyclo[7.7.0]hexadec-1(9)-ene, produced by *Chrysanthemum indicum*, has anticancer activity (Ramya et al., 2020). Collectively, these results confirm that the extracts of *F. thonningii* harbour bioactive compounds of considerable medicinal importance.

Table 3. Compounds identified in the aqueous methanol extract of *Ficus thonningii* by GC-MS and their reported biological activities

S/N	Compound name	RT (min)	Peak area (%)	Chemical formula	Molecular weight	Biological activity	Ref
1	2,2-Dicyclohexyl butane	15.399	0.012	C16H30	222.41	NR	–
2	1-Dodecanol	16.186	0.03	C12H26O	186.33	antibacterial	70
3	2,5-Cyclohexadiene-1,4-dione, 2,6-bis (1,1-dimethyl ethyl)-	18.216	0.06	C14H20O2	220.31	antifungal	39
4	3-Isobutyl-4,5-dimethyl-3H-isobenzofuran-1-one	18.465	0.14	C14H18O2	218.29	NR	–
5	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	18.593	0.39	C15H22	202.34	antimicrobial	15
6	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methyl ethyl)-	18.872	0.04	C15H24	204.35	antidiabetic antiemetic antidiuretic antihypertensive	42
7	Isopropyl linoleate	19.747	46.13	C21H38O2	522.53	antioxidant antimicrobial anticancer	60
8	Bicyclo [2.1.1] hexane-1-carboxylic acid, 5,5-dimethyl-	19.657	51.87	C9H14O2	154.21	NR	–
9	9-Octadecene, (E)	21.225	0.05	C18H36	252.48	antimicrobial	48
10	Decane	21.384	0.001	C10H22	142.38	antiparasitic anthelmintic	35
11	Dodecanoic acid, propyl ester	23.572	0.018	C15H30O2	242.40	NR	–
12	1-Decanol, 2-octyl-	24.243	0.018	C18H38O	20.40	NR	–
13	9-Eicosene, (E)	25.773	0.06	C20H40	280.53	antimicrobial	29
14	6-Undecanone	27.572	0.03	C11H22O	170.29	NR	–

S/N	Compound name	RT (min)	Peak area (%)	Chemical formula	Molecular weight	Biological activity	Ref
15	Tetradecanoic acid, propyl ester	27.897	0.019	C17H34O2	270.45	NR	–
16	2,3,5,6-(tetrafluorophenyl)-methyl, 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylate	28.499	0.03	C15H12Cl2F4O2	370.15	pesticide	58
17	7,9-Di-tert-butyl-1-oxaspiro-[4.5]deca-6,9-diene-2,8-dione	28.71	0.07	C17H24O3	276.37	α -amylase and Ache inhibition	4
18	1-Nonadecene	29.917	0.14	C19H38	266.51	antioxidant antituberculosis, anticancer antimicrobial	9, 37, 64
19	Tert-hexadecanethiol	30.083	0.019	C16H34S	258.51	Antitumour activity	63
20	Oxirane, [(dodecyloxy)]-methyl-	30.11	0.018	C15H30O2	242.40	NR	–
21	Hexadecanoic acid, propyl ester	31.852	0.21	C19H38O2	298.50	NR	–
22	Cis-vaccenic acid	32.038	0.009	C18H34O2	252.46	antisickle cell anaemia	7
23	1-Docosene	33.701	0.05	C22H44	308.58	antibacterial	66
24	Cis-9-octadecenoic acid, propyl ester	34.995	0.25	C21H40O2	324.54	NR	–
25	Octadecanoic acid, propyl ester	35.484	0.04	C21H42O2	326.56	antimicrobial	71
26	Heptyltetracosyl ether	35.668	0.004	C31H64O	452.84	NR	–
27	Nonadecyl pentafluoropropionate	37.187	0.018	C22H39F2O2	430.54	antioxidant	62
28	2,6-Octadienal, 3,7-dimethyl-, (Z)	37.791	0.011	C10H16O	152.23	antimicrobial	18
29	2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentane	37.823	0.009	C25H42	342.60	antibacterial antifungal antitumor	10
30	Aspidospermidin-17-ol, 1-acetyl-19,21-epoxy-15,16-dimethoxy-	38.978	0.02	C23H30N2O5	414.22	NR	–
31	Bis(2-ethylhexyl) phthalate	39.780	0.07	C24H38O4	390.56	antioxidant antiviral antitumor	21
32	Bicyclo [7.7.0] hexadec-1(9)-ene	39.976	0.02	C16H28	220.39	anticancer	61
33	Pentadecafluorooctanoic acid, octadecyl ester	40.416	0.014	C26H37F15O2	666.55	NR	–
34	Heptadecanoic acid, heptadecyl ester	40.677	0.012	C34H68O4	508.90	antimicrobial	25
35	Trans-13-octadecenoic acid	41.683	0.001	C18H34O2	282.46	Anti-inflammatory	28
36	Tetracosylheptafluoro butyrate	41.797	0.003	C20H49F2O2	550.68	NR	–
37	Cycloeicosane	41.88	0.010	C20H40	280.51	cytotoxicity anticancer biopesticide	1, 65
38	2,6,10-Dodecatrien-ol, 3,7,11-trimethyl-	37.82	0.011	C15H26O	222.37	anti-inflammatory antiallergic	32

RT, retention time; NR, not reported. Reference numbers correspond to the numbered reference list.

The complexity of the three profiles is compared in Figure 3, which contrasts the number of compounds resolved in each extract, and in Figure 4, which partitions the relative peak area of each extract into its two dominant peaks and the summed contribution of the minor constituents. The less polar solvents recovered the richest and most diversified metabolite complements, with the n-hexane extract yielding the greatest number of compounds. Figure 5 aggregates the reported bioactivities across all identified metabolites and shows that antibacterial, antimicrobial, anticancer, and antioxidant activities predominate, a distribution consistent with the ethnomedical reputation of the plant.

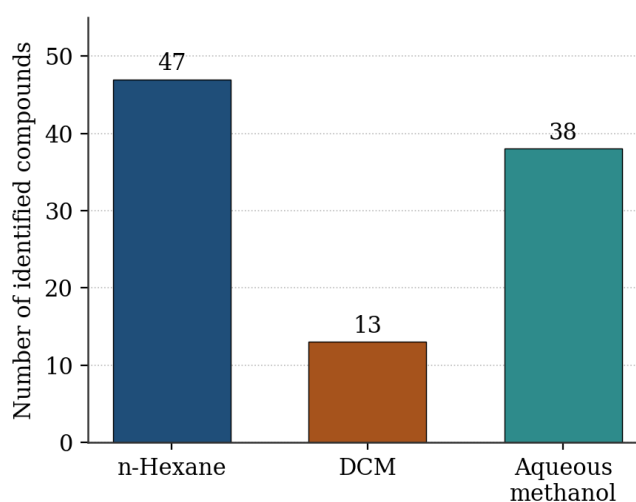


Figure 3. Number of compounds identified by GC-MS in the n-hexane, DCM, and aqueous methanol extracts of *Ficus thonningii* leaves.

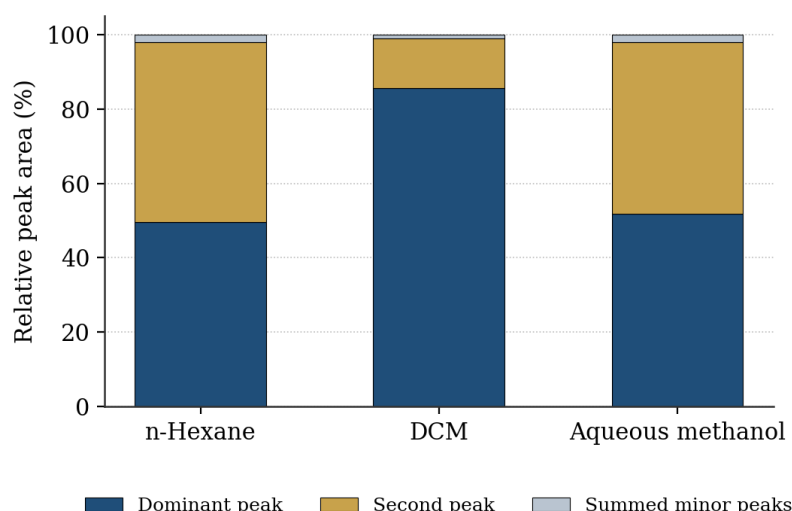


Figure 4. Relative peak area composition of each extract, showing the dominant peak, the second most abundant peak, and the summed contribution of the minor constituents.

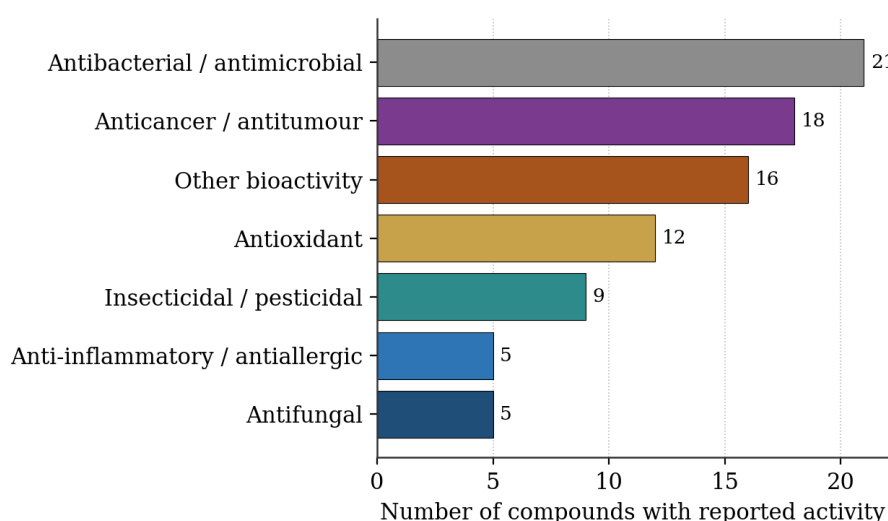


Figure 5. Distribution of reported biological activities across the compounds identified in the three extracts of *Ficus thonningii* leaves.

3.2 Antidiabetic Activity of *F. thonningii*

The α -amylase inhibitory activities of the three extracts across the concentration range are presented in Table 4 and Figure 1, with the maximum inhibition recorded at $100 \mu\text{g mL}^{-1}$. All three extracts inhibited α -amylase more potently than the standard drug acarbose, as reflected in their lower IC_{50} values (n-hexane 2.77 ± 0.00 , DCM 2.80 ± 0.00 , and aqueous methanol $2.83 \pm 0.00 \mu\text{g mL}^{-1}$) compared with acarbose ($2.98 \pm 0.00 \mu\text{g mL}^{-1}$). The n-hexane extract emerged as the most potent inhibitor, followed by the DCM extract, with the aqueous methanol extract the least active of the three. A clear trend was evident in which α -amylase inhibition increased as the polarity of the extracting solvent decreased, so that the least polar n-hexane extract furnished the strongest inhibition. In marked contrast, the extracts were weak inhibitors of α -glucosidase (n-hexane IC_{50} 15.11 ± 0.13 , DCM 15.54 ± 0.34 , and aqueous methanol $15.34 \pm 0.00 \mu\text{g mL}^{-1}$) relative to acarbose ($4.51 \pm 0.52 \mu\text{g mL}^{-1}$), as shown in Table 5 and Figure 2. Even so, the non polar n-hexane extract remained the most active of the plant extracts, a pattern that suggests the volatile and non polar constituents of this fraction possess the greatest binding affinity for the enzymes.

Table 4. α -Amylase inhibition activity (%) of *Ficus thonningii* leaf extracts and acarbose

Concentration ($\mu\text{g mL}^{-1}$)	n-Hexane	DCM	Methanol	Acarbose (std)
100	96.03 ± 0.01	96.14 ± 0.01	95.34 ± 0.01	90.99 ± 0.01
200	93.85 ± 0.01	93.27 ± 0.01	90.22 ± 0.01	91.28 ± 0.01
300	90.30 ± 0.01	87.38 ± 0.01	88.62 ± 0.01	93.93 ± 0.01
400	87.43 ± 0.01	90.24 ± 0.01	85.15 ± 0.01	96.28 ± 0.01

Concentration ($\mu\text{g mL}^{-1}$)	n-Hexane	DCM	Methanol	Acarbose (std)
500	82.31 ± 0.01	79.44 ± 0.01	81.49 ± 0.01	96.58 ± 0.01
IC ₅₀	2.77 ± 0.00	2.80 ± 0.00	2.83 ± 0.00	2.98 ± 0.00

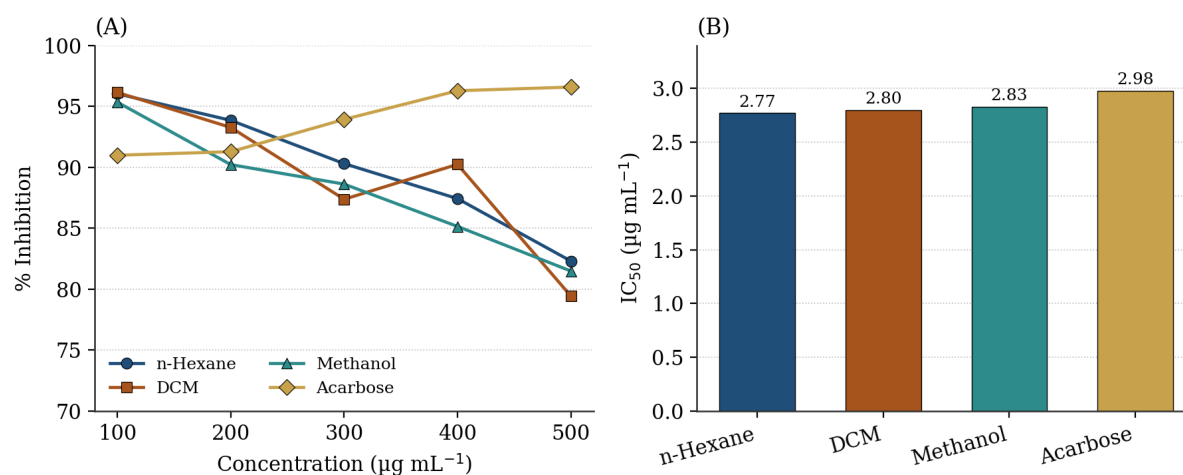


Figure 1. (A) α -amylase inhibition of the *Ficus thonningii* leaf extracts and acarbose across the concentration range and (B) the corresponding IC₅₀ values.

Table 5. α -Glucosidase inhibition activity (%) of *Ficus thonningii* leaf extracts and acarbose

Concentration ($\mu\text{g mL}^{-1}$)	n-Hexane	DCM	Methanol	Acarbose (std)
100	10.97 ± 0.01	11.79 ± 0.01	11.03 ± 0.01	71.30 ± 0.01
200	11.03 ± 0.01	11.79 ± 0.01	11.17 ± 0.01	73.87 ± 0.01
300	10.70 ± 0.01	11.79 ± 0.01	11.23 ± 0.01	74.72 ± 0.01
400	10.64 ± 0.01	11.56 ± 0.01	11.46 ± 0.01	75.58 ± 0.01
500	10.31 ± 0.01	11.56 ± 0.01	10.44 ± 0.01	79.01 ± 0.01
IC ₅₀	15.11 ± 0.13	15.54 ± 0.34	15.34 ± 0.00	4.51 ± 0.52

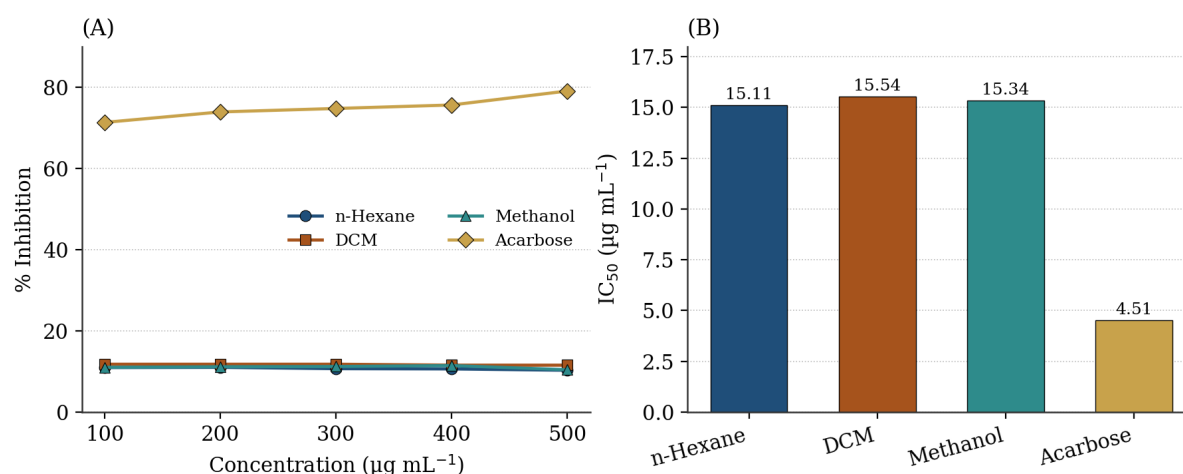


Figure 2. (A) α -glucosidase inhibition of the *Ficus thonningii* leaf extracts and acarbose across the concentration range and (B) the corresponding IC₅₀ values.

Diabetes mellitus is a metabolic disorder characterized by hyperglycaemia and by disturbances in the metabolism of carbohydrate, protein, and fat that arise from dysfunction of the pancreatic β cells and from deficiency in insulin secretion or insulin action (Nyholm et al., 2000). The disorder is broadly classified into type 1, or insulin dependent diabetes mellitus, in which the immune system destroys the pancreatic β cells so that insulin can no longer be produced, and type 2, or non insulin dependent diabetes mellitus, in which defects in insulin secretion and action predominate and which is frequently associated with obesity in adults (DeFranzo, 1997). A clinically attractive strategy for controlling postprandial hyperglycaemia is the inhibition of the carbohydrate hydrolysing enzymes α -amylase and α -glucosidase. In the digestive cascade, α -amylase catalyses the hydrolysis of dietary polysaccharides to disaccharides, while α -glucosidase completes the breakdown of disaccharides to absorbable monosaccharides; inhibition of these enzymes retards carbohydrate digestion and thereby moderates the rise in blood glucose (Nickavar and Yousefian, 2011). Inhibitors of α -amylase and α -glucosidase sourced from

natural products are generally regarded as safe, effective, well tolerated, and inexpensive, and they are increasingly deployed in the management of non insulin dependent diabetes mellitus (Yeh et al., 2003).

The three crude extracts inhibited α -amylase more potently than acarbose, an effect that may be traced in part to 7,9-di-tert-butyl-1-oxaspiro-[4.5]deca-6,9-diene-2,8-dione, a constituent detected in the GC-MS profiles that has been validated as a potent α -amylase inhibitor (Ahmad et al., 2023). The observation that the least polar extract exerted the strongest inhibition is mechanistically coherent, since the active site cleft of α -amylase accommodates lipophilic ligands, and the long chain fatty acids, esters, and terpenoids that dominate the n-hexane fraction are well suited to hydrophobic interactions with catalytic and substrate binding residues. The pronounced selectivity for α -amylase over α -glucosidase is itself pharmacologically meaningful, because a large fraction of the gastrointestinal discomfort associated with the clinical α -glucosidase inhibitors arises from excessive inhibition of intestinal α -glucosidase and the resulting fermentation of undigested carbohydrate. An agent that curbs luminal starch hydrolysis at the α -amylase step while sparing α -glucosidase could in principle blunt the postprandial glucose excursion with a more favourable tolerability profile. Comparable inhibition of these enzymes has been reported for other members of the genus, including *F. benghalensis* (Ponnusamy et al., 2011), *F. racemosa* (Ahmed and Urooj, 2010), and *F. deltoidea* (Forsi et al., 2011). The weak α -glucosidase activity observed here also accords with the earlier finding of Olaokun et al. (2013), who reported that *F. thonningii*, *F. capreifolia*, *F. cordata*, *F. religiosa*, and *F. glumosa* possess poor α -glucosidase inhibitory activity with EC_{50} values exceeding $1000 \mu\text{g mL}^{-1}$.

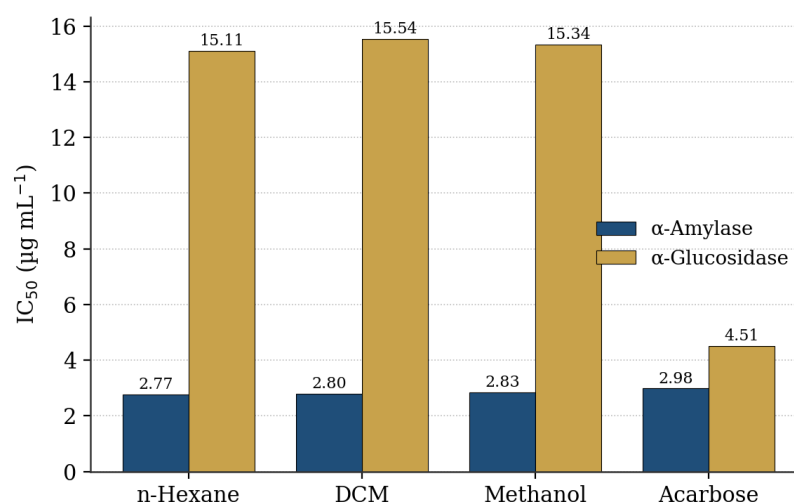


Figure 6. Comparison of the IC_{50} values of the three extracts and acarbose against α -amylase and α -glucosidase, highlighting the selective and potent inhibition of α -amylase by the plant extracts.

Taken together, the results reveal that the extracts of *F. thonningii* are weak inhibitors of α -glucosidase but potent and selective inhibitors of α -amylase, a combination that is indicative of a promising antidiabetic profile. Given the broad array of bioactive secondary metabolites disclosed by the GC-MS analyses, the α -amylase inhibition demonstrated by the extracts is most plausibly attributable to the concerted and possibly synergistic contribution of these phytoconstituents rather than to any single molecule. The dominance of fatty acids, terpenoids, and their esters in the active fractions is consistent with a mechanism in which lipophilic metabolites engage the enzyme through hydrophobic and hydrogen bonding interactions, and it signposts these compound classes as rational targets for the bioassay guided fractionation that would be required to isolate the principal active constituents.

4. Conclusion

This study documented the phytochemical composition and the antidiabetic potential of *F. thonningii* leaf extracts. The findings provide sound preclinical evidence for the value of the plant as a source of hypoglycaemic agents and as a candidate for the management of diabetic complications. GC-MS profiling substantiated the presence of a broad spectrum of phytochemicals with established bioactivity across the n-hexane, DCM, and aqueous methanol extracts, thereby reinforcing the pharmaceutical relevance of the plant and justifying further isolation and purification of its constituents. The potent and selective inhibition of α -amylase by the extracts indicates that *F. thonningii* may be tapped as a repository of natural antidiabetic molecules for the future development of drugs against diabetes mellitus. Subsequent work should pursue bioassay guided fractionation, structural elucidation of the active constituents, and in vivo validation to translate these in vitro observations into therapeutic leads.

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Authors Contributions

All authors contributed to the study conception and design. Sample preparation was carried out by I. O. P. and J. C. U.; methodology by A. S., A. A. H., and O. A. A.; and experimentation by O. F., I. O. P., Y. E. R., and F. O. P. The manuscript was written by A. S., and all authors read and approved the final manuscript.

Data Availability

All data supporting the findings of this study are available within the manuscript.

Funding

Not applicable.

Ethical Approval and Consent to Participate

The leaf samples used in the present study were collected from a local area of Lokoja with the permission and approval of the University, and the samples are documented. The collection of the plant therefore complies with the relevant local and national guidelines, and no further affirmation was required. The antidiabetic studies of the extracts were carried out in vitro.

Declaration of Competing Interest

The authors declare no competing interest.

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