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# Free radical scavenging potential of methanol extract of *Ficus thonningii* leave and liquid chromatography-mass spectrometer (LCMS) profiling

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Copyright: © 2022	Abstract
Muhammad <i>et al.</i> This	This study evaluated the in vitro and in vivo free radical scavenging potentials of
is an open-access	methanol extract of Ficus thonningii leaf and its liquid chromatography-mass
article published under	spectrometer (LCMS) profiling. The qualitative phytochemical screening was carried out
the terms of the	and the results show the presence of tannins, flavonoids, saponins glycoside and
Creative Commons	triterpenes. Antioxidant activities were carried out in vitro and in vivo and the reducing
Attribution License	power shows a value of 0.53 $\pm$ 0.007 with a significant (p < 0.05) difference compared
which permits	to the control and Diphenylpicrylhydrazyl (DPPH) value of 59.0 $\pm$ 0.007 with a
unrestricted use,	significantly different (p < 0.05) compared to the control. However, the two assays are
distribution, and	concentration-dependent. The <i>in vivo</i> analysis shows that lipid peroxidation, superoxide
reproduction in any	dismutase and catalase activities have values of 24.03± 8.04, 1.60± 0.03 and 0.003±
medium, provided the	0.001 respectively. All the values are significantly different when compared to the
original author and	control and are concentration-dependent. The LCMS results revealed the presence of
source are credited.	some important metabolites; phenolic glycosides and quassin, which are potential
	molecules against free radicals. Therefore, Ficus thonningii leaf contains vital
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#### Introduction

Since ancient times, medicinal plants, fruits and vegetables are known to be rich in biologically active compounds, which makes them a prospective source of therapeutic agents (Abalaka & Oyewole, 2011; Oyewole & Kalejaiye, 2012). In recent times, the

approach and development of new drugs from natural products is encouraging so as to treat chronic diseases. In both plants and or vegetables, phytochemicals are present and hence are used in ethno medicine for the cure and regulation of various ailments (Aja et al., 2010). Throughout human history, plants are known to be the main source of medicine. Many reports in the literature show that galactogogues such as foods, herbal medicines as well as pharmaceutical drugs can help in solving milk supply problems not only by increasing milk production but also by supporting the nursing mother's confidence (Westfall, 2003). The demand for milk can be attained either by introducing highyield cows or by enhancing milk production of our indigenous breeds, which is more economical due to the less risk of management in view of the socioeconomic status of our livestock farmers who are predominantly peasants. Nowadays, herbal medications are used for curing numerous serious disorders in man and livestock as well as in monitoring stress of diverse origins. It is now a known fact that the improvement of herbal galactogogues is vital for safe milk production (Prakash et al., 2013). Superoxide anion, hydroxyl radical and hydrogen peroxide are known as Reactive Oxygen Species (ROS), which are formed in vivo due to numerous metabolic activities in cells, and hence initiate oxidative stress (Lakshmi & Bindu, 2013). Cells have numerous antioxidant defence mechanisms that aid in avoiding the destructive effects of ROS (Amin & Razieh, 2007). Antioxidants are generated in vivo or may be chemically manufactured. Natural antioxidants are well-known to have a major role in curing human diseases (Pulido et al., 2000; Sumino et al., 2002). Cells have some antioxidant defence mechanisms that help to prevent the destructive effects of ROS. These include antioxidative enzymes, such as superoxide dismutase, and catalase, as well as glutathione peroxides and of small molecules such as glutathione, vitamins C and E (Fridovich, 1999). This study is aimed at assessing the in vitro and in vivo antioxidant potentials of methanol leaf extracts Ficus thonningii and its LCMS profile.

# **Materials and Methods**

#### Sample collection/ preparation

Leaf of *F. thonningii* were collected from Tarauni, Tarauni local government, Kano State, Nigeria. A botanist identified and authenticated it at the herbarium unit, Department of Plant Biology, Bayero University, Kano. The voucher number was deposited as BUKHAN 110. The leaf were shade dried at room temperature (25 °C) for two weeks and 500 g of the leaf were pulverized into a coarse powder using a clean laboratory mortar and pestle. The coarse powder was weighed, labelled and kept in an airtight container for further analysis.

### Extraction

Successive extraction was carried out as Sharif *et al.* (2017) described using three different solvents viz; n-hexane, ethyl acetate, and methanol. The mixture was labelled and kept for 48 hours with frequent intermittent agitation after which the mixture was filtered using filter paper (Whatman No.1). The filtrate was concentrated in a steam bath to evaporate and the crude extract was obtained (Iroha *et al.*, 2010).

# Phytochemical analysis

The crude extracts for the three different solvents were subjected to preliminary qualitative phytochemical screening according to Modupe *et al.* (2017). The crude extract was diluted to the concentration of 1 mg/cm<sup>3</sup>. The qualitative phytochemical analysis was conducted to determine the presence of secondary metabolites.

# Experimental animals

Twenty adult male Wistar albino rats of 3 to 4 months old weighing between 100-250 g were sourced from the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, ABU, Zaria, Kaduna State, Nigeria. The rats were kept in a well-ventilated aluminium cage at room temperature and under the natural light/darkness cycles of the Physiology Laboratory, Faculty of Basic Medical Science, Bayero University Kano, Nigeria. The animals were allowed access to feed and water ad libitum and allowed two weeks to acclimatize before the commencement of the experiment. The animals maintained in accordance with were the recommendation of the Guide for the care and use of laboratory animals (DHHS, 1985).

# Acute toxicity studies (determination of LD<sub>50</sub>)

An acute toxicity study of *F. thonningii* extract was carried out to determine the LD<sub>50</sub>. The procedure involves two phases. The acute toxicity (LD<sub>50</sub>) was estimated both orally and intraperitoneally in rats (n=16) in each case following Lorke's method (Lorke, 1983). The rats were weighed and grouped by randomization into four groups of three rats each. The first group was the control group. Dose levels of 10, 100, and 1000 mg/kg was used for the first phase. The number of deaths in each group within 24 hours was recorded. In the second phase, which was deduced from the first phase, four rats were grouped into four groups of one rat each and they were treated with doses of 1200 mg/kg, 1600 mg/kg, 2900 mg/kg, and 5000 mg/kg orally and intraperitoneally. They were also observed for 24 hours as in the first

phase, and final LD<sub>50</sub> value was determined from Lorke's formula as follows:

# $LD_{50} = \sqrt{a \times b}$

where a is the highest dose at which no death occurred in the second phase and b is the least dosage at which death occurred in the second phase. The extract was classified using the LD<sub>50</sub>.

# In vitro antioxidant assays

In vitro, an antioxidant assay was conducted using two different methods i.e. DPPH ( $\alpha$ ,  $\alpha$ -diphenyl- $\beta$ -picrylhydrazyl) and reducing power to assess the antioxidant activity of *F. thonningii*.

# Diphenylpicrylhydrazyl scavenging activity

The molecule 1, 1-diphenyl-2-picrylhydrazyl ( $\alpha$ ,  $\alpha$ diphenyl- $\beta$ -picrylhydrazyl; DPPH) is characterized as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole, so that the molecule does not dimerize, as would be the case with most other free radicals. The delocalisation of electrons also gives rise to the deep violet colour, characterized by an absorption band in ethanol solution centred at about 517 nm. When a solution of DPPH is mixed with that of a substrate (AH) that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of the violet colour.

In order to evaluate the antioxidant potential through free radical scavenging of the test samples, the change in optical density of DPPH radicals was monitored. As outlined by Manzocco *et al.* (1998), the sample extract (0.2 mL) was diluted with methanol and 2 mL of DPPH solution (0.5 mM) was added. After 30 minutes, the absorbency was then measured at 517 nm. The percentage of the DPPH radical scavenging was calculated using the equation given below:

% inhibition of DPPH radical =  $\frac{Abr - Aar}{Abr} \times 100$ where Abr is the absorbency before the reaction and Aar is the absorbency after the reaction has taken place.

# Reducing power

This method is based on the principle of increasing the absorbance of the reaction mixtures. An increase in the absorbance indicates an increase in the antioxidant activity. In this method, the antioxidant compound forms a coloured complex with potassium ferricyanide, trichloro acetic acid and ferric chloride, which is measured at 700 nm. An increase in absorbance of the reaction mixture indicates the reducing power of the samples (Jayaprakash *et al.*, 2001). In the method described by Oyaizu (1986), 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of  $K_3Fe$  (CN)<sub>6</sub> (1 % w/v) were added to 1.0 mL of sample dissolved in distilled water. The resulting mixture was incubated at 50 °C for 20 min, followed by the addition of 2.5 mL of Trichloro acetic acid (10 % w/v). The mixture was centrifuged at 3000 rpm for 10 minutes to collect the upper layer of the solution (2.5 mL), mixed with distilled water (2.5 mL) and 0.5 mL of FeCl<sub>3</sub> (0.1 %, w/v). The absorbance was then measured at 700 nm against a blank sample (Ascorbic acid).

# In vivo antioxidant effect of plants extracts

Twenty male albino Wistar rats were randomly divided into four groups of five animals each. Group 1 served as the control and receive 0.4 mL of distilled water. Group 2 received 100 mg/kg of the extract. Group 3 received 200 mg/kg of the extract, and group 4 received 400 mg/kg of the extract. The animals were orally dosed daily for 21 days and observed daily for changes and other signs of toxicity such as excessive salivation, change of eye colour, and death throughout the period of study. Twenty-four hours after the last treatment, blood was obtained through direct cardiac puncture and preserved in plain bottles, which was used to assay the plant extracts' in vivo antioxidant activity as described by Samuel et al. (2014). The blood was allowed to clot for 30 minutes and then centrifuged at 2500 rpm for 15 minutes and the serum was collected in plain bottles.

Determination of the lipid peroxidation (LPO) in serum Lipid peroxidation was estimated colourimetrically by measuring malondialdehyde (MDA) following the methods of Albro *et al.* (1986) and Das *et al.* (1990). About 0.1 ml of serum was treated with 2 ml of (1:1:1 ratio) TBA–TCA–HCL reagent (TBA 0.37 %, 0.25N HCL and 15 % TCA) and placed in the water bath for 15 minutes, cooled and centrifuged, and clear supernatant was transferred into fresh plain tubes. The absorbance was taken at a wavelength of 535 nm.

# Estimation of superoxide dismutase (SOD)

Superoxide dismutase (SOD) was determined by the method described by Fridovich (1989). Carbonate buffer (0.05 M) which comprises 114.3 g of Na<sub>2</sub>CO<sub>3</sub> and 4.2 g of NaHCO<sub>3</sub> was dissolved in distilled water and was made up to 1000 ml in a volumetric flask. The buffer was adjusted to pH 10.2. A fresh solution of 0.3 mM adrenaline was prepared. The serum of 0.1 ml was diluted in 0.9ml of distilled water to make 1:10 dilution of the microsome. An aliquot mixture of 0.2 ml of the diluted microsome was added to 2.5 ml of 0.05 M carbonate buffer. About 0.3 ml of 0.3 mM

adrenaline was added to initiate the reaction. The reference mixture contained 2.5 ml of 0.05 M carbonate buffer, 0.3 ml of 0.3 mM adrenaline as well as 0.2 ml of distilled water. The absorbance was measured from 30 seconds to 150 seconds at 480 nm. nute =  $\frac{(A2 - A1)}{A1}$ 

2.5

Where: A2 is the absorbance recorded at 150 secs and A1 is the absorbance recorded at 30 secs % Inhibition

$$= 100 - \frac{\text{Increase in absorbance for sample}}{\text{Increase in absorbance of blank}} \times 100$$

One unit of SOD activity is the quantity of SOD necessary to elicit 50% inhibition of the oxidation of adrenaline to adenochrome in 1 minute.

#### Estimation of catalase activity

Catalase (CAT) activity was measured using the methods of Abebi (1974). Exactly 10 µl of serum was added to a test tube containing 2.80 ml of 50 mM potassium phosphate buffer (pH 7.0). The reaction was initiated by adding 0.1 ml of freshly prepared 30 mM  $H_2O_2$  and the decomposition rate of  $H_2O_2$  was measured at 240 nm for 5 minutes on a spectrophotometer. A molar extinction coefficient (E) of 0.041 mM<sup>-1</sup> cm<sup>-1</sup> was used to calculate the Catalase activity. . .

Catalase Concentration = 
$$\frac{\text{Absorbance}}{\text{E}}$$

Catalase Activity **Catalase Concentration** Protein Concentration (mg/ml)

# Determination of protein

The total protein content of the serum was assayed using a commercially available total protein kit (Randox Laboratories, UK), using the direct Biuret method.

liquid chromatography-mass spectrometer analysis analyzed The samples were using liquid chromatography tandem (LC) mass spectrophotometer (MS) as described by Piovesana et al. (2018) with some modifications. The extracted samples were reconstituted in methanol and filtered through polytetrafluoroethylene (PTFE) membrane filter of 0.45 µm size. After filtration, the filtrate (10.0 µl) was injected into the LC system and allowed to separate on Sunfire C18 5.0 µm 4.6 mm x 150 mm column. The run was carried out at a flow rate of 1.0 mL/min, with Sample and Column temperature at 25 <sup>o</sup>C. The mobile phase consists of 0.1 % formic acid in water (solvent A) and 0.1 % formic acid in acetonitrile (solvent B) with a gradient as presented in Table 1. From the ratio of A/B 95:5, this ratio was maintained for a further 1 min, then A/B 5:95 for 13 min to 15 min. then A/B 95:5 to 17 min, 19 min and finally 20 min. The PDA detector was set at 210-400 nm with a resolution of 1.2 nm and a sampling rate of 10 points/sec. The mass spectra were acquired with a scan range from m/z 100 – 1250 after ensuring the following settings: ESI source in positive and negative ion modes; capillary voltage 0.8 kV (positive) and 0.8 kV (negative); probe temperature 600 °C; flow rate 10 mL/min; nebulizer gas, 45 psi. MS set in automatic mode applying fragmentation voltage of 125 V. The data was processed with Empower 3. The compounds were identified based on the fragmentation pattern, and Base m/z. using SBDS Data base as reported by Hanafi et al. (2018).

#### Statistical analysis

The experiment was laid in a completely randomized design (CRD). Descriptive statistics were used to calculate the means and standard deviations of the assays carried out. The data collected were subjected to analysis of variance (ANOVA) using JMP Software. Where significant differences exist, means were separated using Student's Newman Keuls test at a 5% probability level.

#### Results

Preliminary phytochemical screening for the three different fractions: the n-hexane, ethyl acetate and

**Table 1**: Percentage gradient of solvent used in liquid chromatography/mass
 spectrometry analysis

Time (Sec)	0.1% formic acid in water	0.1% formic acid in Acetonitrile
	(%A)	(%B)
0	95	5
1	95	5
13	5	95
15	5	95
17	95	5
19	95	5
20	95	5

methanol crude extracts of the thonningii leave were F. determined as presented in Table 2. It was observed that in the successive extraction, some phytochemicals were present in both three of the solvents used, whereas others showed presence in one or two of the solvents used. Steroids/triterpenes, cardiac

Phytoconstituents	n-Hexane	ethyl acetate	Methanol
Alkaloids	-	-	-
Steroids/ Triterpenes	+	+	+
Cardiac Glycosides	+	+	+
Anthraquinones	-	-	-
Tannins	-	+	+
Flavonoids	+	+	+
Saponin	-	-	+

**Table 2**: Qualitative phytochemical screening of *Ficus thonningii* leaf extracts

(+) indicates the presence and (-) indicates the absence

Table 3: Acute toxicity	test results of methanol Ficus	thonningii leaf extract	(orally and	Intra-peritoneally)
		5	· · ·	

Dose (mg/kg)	First Phase (Death/Survival)		
	Ficus thonningii		
10	0/3		
100	0/3		
1000	0/3		
Second Phase (Death/Survival)			
600	-		
1000	-		
1600	0/1		
2900	0/1		
5000	0/1		

glycoside and flavonoids are present in all three solvents used while tannins and saponin were only present in the methanol fraction. However, alkaloids and anthraquinones were absent in all the solvent fractions.

The result of the acute toxicity test of *F. thonningii* extract revealed the  $LD_{50}$  is above 5000 mg/kg through both oral and intra-peritoneal routes of administration (Table 3). In the first phase of the experiments, no death was recorded in the dosage of 10-1000 mg/kg. Additionally, in the second phase where the concentration was increased up to 5000 mg/kg no death was also recorded.

The antioxidant properties of *F. thonningii* leaf extracts using *in vitro* and *in vivo* bioassays were conducted. For the *in vitro* assay both the reducing power and DPPH scavenging activities of the fraction was conducted, while in the *in vivo* assays, determination of lipid peroxidation, as well as estimation of superoxide dismutase and catalase activity, were carried out as presented in Tables 4 and 5, respectively.

Table 5 compares the results obtained from the *in vivo* assays where parameters such as lipid peroxidation, superoxide dismutase and catalase activity were reported. The *F. thonningii* leaf extract when compared to the control did not produce a significant (p > 0.05) difference in lipid peroxidation and catalase activity, but superoxide dismutase

produced a significant difference (p < 0.05). *F. thonningii* leaf extract at 100, 200 and 400 mg/ml used in this study did not produce a significant (p > 0.05) effect on lipid peroxidation, superoxide dismutase and catalase activity. However, concentration and interaction effects were also not statistically significant (p>0.05) compared to the control.

Liquid Chromatography/ Mass Spectrometry (LC/MS) profile of *F. thonningii* leaf extract was conducted using LC Water e2695 separation module with W2998 PDA and coupled to ACQ-QDA MS. The tentative compounds identified are quassin and phenolic glycoside which was based on their mass to charge ration (m/z) and mass fragmentation pattern (Table 6). However, the total ion chromatogram showing different peaks with their MZ values were presented in Figure 1 and individual mass fragmentation of each tentative compound is presented in Figures 2 and 3.

# Discussion

The phytoconstituents evaluated include alkaloids, flavonoids, saponins, tannins, cardiac glycosides, anthraquinones, as well as steroids, which have been known to be excellent antioxidants (Ramanathan *et al.*, 2003; Wattarapenpaiboon & Wahlqvist, 2003). Barku & Abban (2013) reported that methanol extract of *Anogeissus leiocarpus* leaf was found to contain a greater number of metabolites than the ethyl acetate

Extracts	Reducing Power	α, α-diphenyl-β-picrylhydrazyl (DPPH)
Ficus thonningii	0.53 <sup>c</sup>	59.0 <sup>d</sup>
Control	0.43 <sup>e</sup>	88.2ª
SE±	0.007	0.007
Concentration (µg/ml)		
1000	0.72ª	86.7ª
500	0.68 <sup>b</sup>	85.8ª
250	0.58 <sup>c</sup>	<b>84.4</b> <sup>a</sup>
125	0.52 <sup>d</sup>	<b>79.4</b> <sup>b</sup>
62.5	0.49 <sup>e</sup>	69.2 <sup>c</sup>
31.25	0.37 <sup>f</sup>	55.6 <sup>d</sup>
15.625	0.27 <sup>g</sup>	43.4 <sup>e</sup>
SE±	0.008	1.342
Interaction		
Extract × Concentration	**	**

**Table 4**: In Vitro assays using reducing power and diphenylpicrylhydrazyl activity of Ficus thonningii

a, b, c, d, e, f, g means in the same row with different superscripts are significantly different (p < 0.05)

Table 5:	The effect of	Ficus thonning	ii extracts o	n lipid pero	kidation, sup	peroxide dism	utase and	catalase a	activity
in Wista	r rats using in	vivo assay							

Extracts	Lipid Peroxidation	Superoxide Dismutase	Catalase Activity
Ficus thonningii	24.03 <sup>c</sup>	1.60ª	0.003
Control	24.54 <sup>c</sup>	1.48 <sup>b</sup>	0.0025
SE±	9.29	0.03	0.0008
Concentration (mg/ml)			
100	32.41	1.57	0.004
200	34.12	1.55	0.002
400	38.78	1.54	0.003
SE±	8.04	0.03	0.0007
Interaction			
Extract × concentration	NS	NS	NS

 $^{\rm a,\ b,\ c}$  means in the same row with different superscripts are significantly different (p < 0.05) NS-not significant

**Table 6**: Liquid chromatography/mass spectrometry profile of *Ficus thonningii* methanol leaf extract

Peak	Tentative Compound	Molecular Mass	MZ(M+H)
1	Quassin	388	389
2	Phenolic Glycosides	366	367



Figure 1: Total ion chromatogram of Ficus thonningii methanol leaf extract showing peak with MZ values



Figure 2: Molecular fragmentation pattern of quassin obtained from liquid chromatography/mass spectrometry at positive mode

12.874 Peak 14 - QDa 1: MS Scan QDa 1: MS Scan CODA SM 5 MCQ 0.65 (1: QDa Positive(+) Scan (100.00-1250.00)Da, Centroid, CV=15)



Figure 3: Molecular fragmentation pattern of phenolic glycosides obtained from liquid chromatography/mass spectrometry at positive mode

and n-hexane extract this could be a result of the higher polarity of methanol than ethyl acetate and hexane. The result of the phytochemical screening shows that of all the three crude extracts, methanol extract showed the presence of more secondary metabolites compared to others. This necessitates the use of methanol crude extract to carry out the analysis of this study i.e. the acute toxicity, *in vitro*, *in vivo* and the LCMS analysis.

Based on the results obtained from the acute toxicity studies, it signifies that the methanol extract of F. thonningii leaf is safe for consumption. Aniagu et al. (2008) found that the LD50 of F. thonningii leaf extracts administered orally was shown to be above 3000 mg/kg body weight, which is in agreement with the results of the present study. This finding corresponds with Abubakar et al. (2008) and Ahur et al. (2010), which show the median lethal dose of ethyl acetate leaf extracts of F. thonningii administered orally above 5000 mg/kg body weight in adult Wistar rats. Therefore, based on the result obtained it can be concluded that the extract used is regarded as relatively safe upon administration within a short period when no mortality or any possible signs of toxicity is recorded at a dose of 5000 mg/kg body weight, of the experimental model. It can then be inferred that within the standard range of 500 – 5000 mg/kg body weight, the methanol extract of the plant can be described as non-toxic on the scale proposed by Lorke (1983).

Results from in vitro assay revealed variations in reducing power and DPPH activities and concentration-dependent. The high radical DPPH radical scavenging activity observed in this study is in agreement with the result of Kankara & Go (2016) which could be a result of high content of phenolic compounds in the extract. The Liquid Chromatography/ Mass Spectrometry (LC/MS) profile of F. thonningii leaf extract shows that the extract contains a higher level of quassin and phenolic glycosides which are prominent free radical scavengers. Therefore, free radical scavenging activity observed in this study could be a result of the presence of these prominent compounds, as reported by Umar & Garba (2020). They observed that the richness of polyphenols and flavonoid compounds shows the antioxidant potential of that plant. However, Sulaiman (2016) reported the high free radical scavenging activity of Guiera senegalensis, which could be linked to its high phenolic content. This is supported by the study conducted by Li et al. (2013). Similarly, many other medicinal plants, such as pigeon pea (Trollius

ledebouri Reichb), linseed oil (Linum usitatissimum), rooibos tea, contain C-glucosyl flavonoids, have antioxidant properties. Based on its ene-diol functionality, i.e. its dihydroxy substituents in the B ring and the double bond characteristic of the C-ring, Joubert & Ferreira (1996) found that it has free radical scavenging activity. Due to a missing OH on the C ring, vitexin and isovitexin have been reported to have antioxidant activity, flavonoids are powerful antioxidants that scavenge and reduce the production of free radicals (Grassi et al., 2010). Methylated flavonoids, quassinoids, and phenolic glycosides have been identified in F. thonningii methanol leaf extract. The antioxidant potential of Samadera indica may be due to the existence of triterpenoids; Quassinoids (Vidya et al., 2011). Quassin was found to be present in the African birch leaf in the present study, which collaborates with the report of Stephen et al. (2016) who reported the presence of this compound in the leaf, an indication that it has good antioxidant activity. Similarly, Oyelere et al. (2021) reported that flavonoid is present in trace amounts in 1% ethanol extract of F. thonningii leaf and 4% ethyl acetate extract of F. thonningii leaf. Phenol and tannin are present in moderate and high concentrations at 3% and 4% ethyl acetate extracts of F. thonningii leaf. Saponin is present in moderate to high concentrations at all selected extracts (except aqueous) of F. thonningii leaf. The medicinal properties of plants are a function of their phytoconstituents as plants rich in phytochemicals have been proven to exhibit varying pharmacological activities (Tunwagun et al., 2020).

In conclusion, *F. thonningii* methanol leaf extract has rich content of secondary metabolites when compared to its hexane and ethyl acetate extract. However, *in vitro* and *in vivo* antioxidant analysis revealed the potency of the extract against free radicals. Additionally, LC-MS analysis shows potential antioxidant compounds. Therefore, it can be concluded that the methanol fraction of *F. thonningii* leaf possesses free radical scavenging activity, which could be a result of the metabolites identified by LC-MS profilling. This plant could be useful in drug development or management of oxidative stress conditions.

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Nil

# **Conflict of Interest**

The authors declare that there is no conflict of interest.

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