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EVALUATION OF BIOACTIVE COMPOUNDS, IN-VITRO ANTIOXIDANT PROFILE AND ANTI-INFLAMMATORY PROPERTIES OF ETHANOLIC EXTRACTS OF *ISOBERLINIA TOMENTOSA*

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ABSTRACT

Oxidative stress and chronic inflammation are interlinked pathological processes implicated in the onset and progression of various chronic diseases. Isoberlinia tomentosa, a Fabaceae family species traditionally used in African medicine, has limited scientific validation despite its ethnomedicinal relevance. This study evaluated the phytochemical composition, in-vitro antioxidant profile, and in-vivo anti-inflammatory activity of the ethanolic fruit extract of I. tomentosa. Qualitative screening revealed significant levels of phenolic compounds, tannins, saponins, terpenoids, and alkaloids. Quantitative GC-FID analysis identified 22 bioactive compounds, including catechin, quercetin, kaempferol, rutin, and apigenin. Acute toxicity testing in Wistar rats indicated an LD_{50} greater than 5000 mg/kg, suggesting a favorable safety profile. Antioxidant assays (DPPH, ABTS, H_2O_2, OH•, NO• scavenging, FRAP, and TAC) demonstrated strong, dose-dependent radical-scavenging and reducing activities. In an oval albumin-induced paw edema model, the extract significantly reduced inflammation in a dose-dependent manner, with the 1000 mg/kg dose outperforming indomethacin ($p < 0.05$). These findings provide scientific evidence for the traditional use of I. tomentosa, highlighting its potent antioxidant and anti-inflammatory activities, high safety margin, and potential as a source of plant-based therapeutics for oxidative stress- and inflammation-related disorders.

Keywords: Anti-inflammatory, Antioxidant, GC-FID, Isoberlinia tomentosa, Oxidative stress, Phytochemicals

INTRODUCTION

Oxidative stress and inflammation are two interlinked pathological processes implicated in the development of numerous chronic diseases, including cardiovascular disorders, cancer, diabetes, neurodegenerative conditions, and autoimmune diseases (Wronka et al., 2022; Gambini, & Stromsnes, 2022; Krzemińska et al., 2022). Reactive Oxygen Species (ROS) and other free radicals produced during normal metabolic processes can lead to cellular damage if not effectively neutralized by endogenous antioxidant systems. Similarly, chronic inflammation, characterized by persistent activation of the immune response, contributes to tissue injury and disease progression (Gambini, & Stromsnes, 2022; Bezerra et al., 2023). As such, the search for effective natural agents with both antioxidant and anti-inflammatory effects has gained increasing attention in biomedical research. Inflammation is a dynamic physiological reaction to infection, tissue damage, or injury. This involves a complex chemistry of immune cells, chemical mediators, and signaling pathways aimed at repairing tissue homeostasis. Acute inflammation is protective and resolves quickly, chronic or uncontrolled inflammation can lead to many pathological conditions, including arthritis, asthma, cardiovascular diseases, metabolic disorders, neurodegenerative diseases, and cancer (Medzhitov, 2021; Soliman, & Barreda, 2022; Hannoodee, & Nasuruddin, 2024).

Oxidative stress rises from a discrepancy between the reactive oxygen species (ROS) production and the capacity of the body to cleanse these reactive intermediates and/or repair the resulting injury. ROS including hydrogen peroxide (H_2O_2), hydroxyl radicals (•OH), and superoxide anion (O_2^-) are produced as byproducts of normal cellular metabolism, especially in the mitochondria (Unsal et al., 2020; Afzal et al., 2023). In a physiological state, antioxidant defense systems such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) maintain redox homeostasis. Though, excessive ROS creation or impaired antioxidant protection can cause oxidative damage of proteins, lipids, and nucleic acids. This effect has a major role in the pathogenesis of different chronic and degenerative diseases, including cancer, cardiovascular disorders, diabetes, neurodegenerative diseases and accelerated aging (Sahoo et al., 2022; Afzal et al., 2023).

Medicinal plants have long served as cherished sources of bioactives for therapeutic use and in many parts of the world, especially in rural areas, traditional medicine remains a primary resource of medical remedy (Dehelean et al., 2021; Najmi et al., 2022. *Isoberlinia tomentosa*, a deciduous tree

belonging to the family of Fabaceae (ceasalpinoideae), is native to tropical Africa and commonly found in savannah regions. *Isoberlinia tomentosa* (Figure 1) exists as shrub or tree measuring about 10-20 m tall with a trunk of 40-50 cm in diameter, branches measure about 5 m upwards. The leaves are pinnate and paripinnate types of compound leaves with about 2-5 pairs of leaflets per leaf; arranged in opposite or sub-opposite; and petiotulated with short stalk. The fruits are described as two-valved pod which twist during dehiscence; obliquely oblong, compressed, becoming woody (Hadiza et al., 2020; Yilangai et al., 2023). *I. doka* has been scientifically reported including it phytochemicals presence, antioxidant activities, and enhance sexuality in male Wistar rats (Hadiza et al., 2020), but scanty reports about *I. tomentosa*. It is important to determine the phytochemical constituents of *I. tomentosa*, so as to know the type of biological activity which might be exhibited by the plant. The plant is traditionally used for treating conditions such as fever, wounds, arthritis, and gastrointestinal disturbances. Despite its local medicinal relevance, there is limited scientific evidence on the pharmacological properties of its plant parts. This study aims to evaluate the phytochemical composition, *in-vitro* antioxidant profile, and anti-inflammatory properties of the ethanolic fruit extract of *Isoberlinia tomentosa*. Establishing a scientific basis for its traditional use could pave the way for the identification of a novel plant-based therapies for the management of oxidative stress and inflammation-related problems.

The therapeutic relevance of plant-derived antioxidants has inspired extensive pharmacological research aimed at validating traditional medicinal uses of various plants. Plants contain naturally occurring bioactive compounds (phytochemicals) that possess strong antioxidant properties. These include phenolics, flavonoids, tannins, alkaloids, terpenoids, and saponins, which are secondary metabolites that help plants protect themselves against environmental stressors such as UV radiation, pathogens, and herbivores. These phytochemicals can scavenge free radicals, inhibit lipid peroxidation, chelate pro-oxidant metal ions, and modulate the activity of antioxidant enzymes in the human body. In traditional medicine systems, numerous plants have been used to treat inflammatory conditions, and modern research has validated many of these claims. Investigating plants such as *Isoberlinia tomentosa*, which are traditionally used for treating pain, fever, or swelling, can lead to the discovery of novel anti-inflammatory compounds. Herein, the present study investigates the bioactive components using GC-FID, *in vitro* antioxidant and anti-inflammatory properties ethanolic extracts. Many identified phytochemicals act synergistically, enhancing the overall anti-inflammatory potential of the plant extracts. The study offers the advantage of fewer side effects compared to synthetic anti-inflammatory drugs like NSAIDs and corticosteroids, which are often associated with gastrointestinal, renal, and cardiovascular risks.

Figure 1: *Isoberlinia tomentosa* Plant

MATERIALS AND METHODS

Collection and Identification of Plant Parts

The fresh parts of *I. tomentosa* plant were first identified on the field using their morphological features, and samples were collected from Igangan village (9.4140ºN latitude and 4.1058ºE longitude) of Oyo State, Nigeria, authenticated and deposited (voucher specimen: 1142093) at Forestry Institute of Nigeria (FRIN) in Ibadan, Oyo State.

Preparation of Plant Sample

The different plant parts (Fruit, Leaf, Stem stick, Stem back, root) were cut and washed in running clean water in order to remove all foreign matters. They were sliced into pieces before air-dried under the shade to obtain a constant weight, then pulverized into coarse powder with mortar and pestle before storage in cellophane bags at room temperature until further experiment.

Extraction and Fractionation of Plant Materials

The air-dried powders of the parts of *I. tomentosa* were first extracted with 70% ethanol (5 L) at room temperature by maceration for 48 h. Extracts were filtered differently using whatman filter paper No 1 and the filtrates were concentrated to dryness using a rotary evaporator at reduced pressure and temperature (60ºC).

Qualitative Phytochemical Analysis

The preliminary phytochemical analyses of *I. tomentosa* fruit extract was carried-out for the presence of different phytochemical compounds such as phenolic compounds, alkaloids, flavonoids, saponins, and tannins using standard procedures of analysis (Shaikh & Patil, 2020; Mohan et al., 2021). The qualitative results were presented as (+) for the presence and (-) for the absence of phytochemical.

Quantitative Phytochemicals Analysis Using GC-FID
Gas Chromatography (BUCK (M910) Scientific, USA) was used for the quantification of phytochemicals in the extracts. The specific gas chromatography was with a flame ionization detector, a RESTEK 15 m MKT-1 column (15 m× 20 m×0.15 um). The injection temperature was set at 280 °C while the injection velocity 30cm/s was used with 2 µL splitless injection of the samples. Helium (5.0pa) was used as carrier gas with a flow rate of 40 mL/min. Then, the initial oven temperature was at 200 °C, the oven was also heated to attain a temperature of 330 °C at the rate of 3 °C/min while the detector was also operated at 320 °C. Phytochemicals were therefore determined by the ratio between area and mass of internal standard and the area of identified phytochemicals. The concentrations of individual phytochemicals were thus expressed in µg/mL (Forghe, & Nna, 2020).

In-vitro Antioxidant Capacity Testing
The standard procedure of *in-vitro* antioxidant capacity test was utilized for determination of the antioxidant capacity of the *I. tomentosa* using ethanolic extract. The methods used by Oyewusi et al., 2024 with little adjustment was adopted to carry-out the free radical-scavenging action of DPPH (1,1-diphenyl-2- picrylhydrazyl), Hydrogen Peroxide scavenging potency, NO• radical inhibition, OH scavenging activities, total antioxidant capacity, in the crude extract. All the assays were carried out in triplicate.

Animals and Experimental Design
Wistar rats weighing between 180 - 200 g were purchased and housed for acclimatization under standard conditions (25 ± 2°C; 12 h light/dark cycles). The rats were fed with animal diet and tap water ad libitum for a week before treatment in polypropylene cages with three animals in a cage. All the experimental procedures conducted on the rats were performed in best practices according to the internationally accepted principles for laboratory animal use and care and Institutional animal care and use committee. The study got approval from the Research and Ethics committee of Micheal OKpara University of Agriculture, Umudike (REC approval number: UI-ACUREC/049-0521/26).

Acute Toxicity Studies
A total of 12 female Wistar rats were allocated in 2 phases randomly in order to estimate the oral median lethal dose (LD_{50}) of *Isoberlina tomentosa*. Observations were noted and recorded accordingly 1, 2, 4 and 24h after extracts administration. The physical observations were also noted included mobility, skin changes, aggressiveness, responses to sound or pain, as well as respiratory changes. The number of survivors after 24 h were noted and further observation continued once a day for 14 days. The LD_{50} was therefore determined using the Lorke's (1983) method (Namadina et al., 2020).
Phase I (Oral administration):

i. Group I (3 animals): 100 mg/kg of the *I. tomentosa* ethanolic fruit extract.
ii. Group II (3 animals): 500 mg/kg of the *I. tomentosa* ethanolic fruit extract.
iii. Group III (3 animals): 1000 mg/kg of the *I. tomentosa* ethanolic fruit extract.

The treated rats were fasted for an hour while under observations after the administration of the test extracts intermittently for 4 hours for over a period of 24 hours, and then daily for 14 days. Given that there was no death was recorded in phase I after 24 hours, then the need for phase II.
Phase II (Oral administration):

i. Group I (1 animal): 1600 mg/kg of the *I. tomentosa* ethanolic fruit extract.
ii. Group II (1 animal): 2900 mg/kg of the *I. tomentosa* ethanolic fruit extract.
iii. Group III (1 animal): 5000 mg/kg of the *I. tomentosa* ethanolic fruit extract.

After extracts administration in phase II, the same observation procedure was repeated as was done during the phase I. Gross behavioral changes including salivation, hair erection, sweating, tremors, loss of appetite, seizures, loose stools, death, and other toxic manifestations were recorded.

Anti-inflammatory Activities Using Oval Albumin-Induced Paw Edema Method in Rats
The acute inflammation was evaluated using oval albumin-induced paw edema assay as described by Omodamiro and Jimoh, 2014 with little adjustment. In this procedure, 24 albino rats were used and grouped into six containing four rats each. Groups D, E, and F served as control groups as shown in Table 1 (D was the negative control group, with 0.1ml oval albumin; E as the positive control group, with 50mg of indomethacin; F as normal control with 0.1ml/kg of distilled water). The plant extract was prepared by dissolving the *I. tomentosa* ethanolic fruit extract in sterile distilled water. The extract doses administered were based on the outcome of the acute toxicity (LD_{50}) studies, where 20%, 10%, 5% were considered as high, medium and low doses respectively.
The high, medium and low doses were administered to the animal groups A, B and C respectively (Table 1). After 30 minutes, 0.1 ml, oval albumin solution was administered through the sub-plantar tissue of the left hind paw of the rats. The circumference of the left hind paws of all the rats were measured and recorded immediately using a vernier caliper. The procedure was repeated in thrice at 60mins, 90mins, 120mins, 150mins and 180mins in 30mins intervals.

Table 1: Animal Treatment Model for Anti-inflammatory Test

Group	Treatment (dose)	Experimental groups (n=6)
A	1000mg (20%)	High dose +oval albumin
B	500mg (10%)	Medium dose + oval albumin
C	250mg (5%)	Low dose + oval albumin
D	0.1ml	Oval albumin (negative control), not treated
E	50mg	Indomethacin (positive control) + oval albumin
F	0.1ml/kg	Distilled water only (normal control)

The percentage of anti-inflammatory response was calculated for each extract using the relation (Perez, 1986). Also, the anti-inflammatory activity was determined as the reduction in paw size when the drug was present, relative to control (Parvin, 2020).

$$Inhibition\ (\%) = \frac{(C_t - C_o)\ control - (C_t - C_o)\ treated}{(C_t - C_o)\ control} X100$$

Where: C_t = final circumference; C_o = Initial circumference.

Statistical Analysis

All values were expressed as Mean ± Standard Error of the Mean (SEM). The data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's test using the Graph Pad Prism (statistical) software, version 8. The differences between means were then considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

Phytochemical Screening and GC-FID analysis

Phytochemical screening of *I. tomentosa* fruit extract shown that a substantial quantity of phenolic compounds, tannin, saponin, terpenoid, and alkaloids but without flavonoids, steroids, glycosides, amino acids, and phlobatanins were detected (Table 2).

Table 2: Preliminary Phytochemical Screening of Ethanolic Extract of Isoberlina tomentosa Fruit Extract

Phytochemicals	Ethanol Extract of *Isoberlina tomentosa*
Tannin	++
Saponin	++
Terpenoid	++
Glycosides	-
Steroids	-
Alkaloids	+
Flavonoids	-
Amino acids	-
Phenols	++
Phlobatannins	-

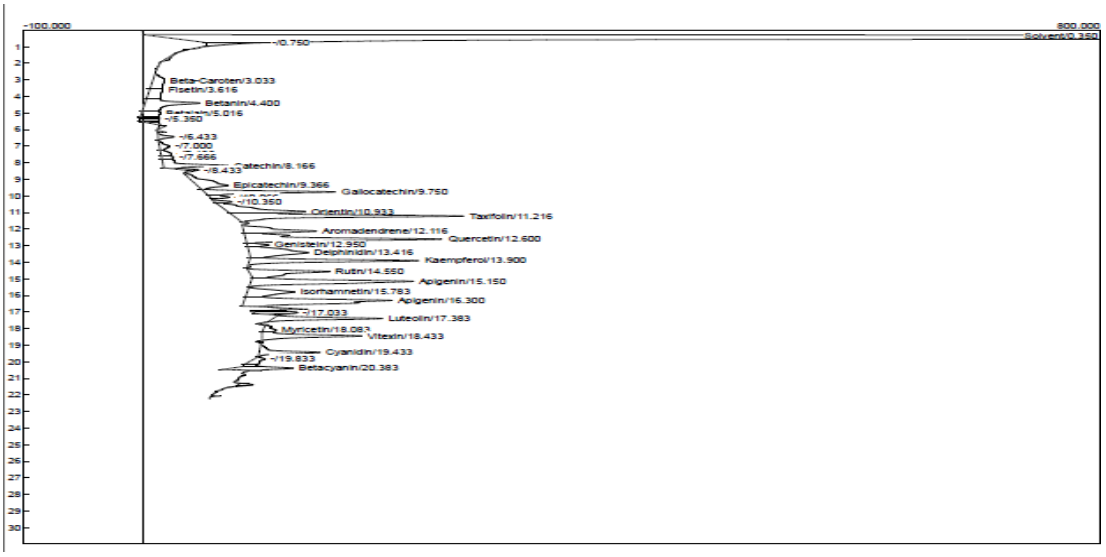
+ Moderately presence; ++ significantly present; - absent.

The GC-FID analysis of *I. tomentosa* ethanolic extract aimed to identify and quantify the bioactive compounds present in this plant species (Table 3 and Figure 2a-e).

Table 3: GC-FID Analysis of Different Parts of *Isoberlina tomentosa* Ethanolic Extract

S/N	Compound	RT	Peak Area				
			Fruit	Leaf	Stem Stick	Stem Bark	Root
1	Beta-Caroten	3.033	436.7920	255.0425	151.3610	232.5670	349.1770
2	Fisetin	3.616	361.0450	--	--	--	235.6285
3	Betanin	4.400	959.0340	431.5290	207.9390	279.9820	692.7630
4	Betalain	5.016	291.1100	--	--	--	165.0485
5	Catechin	8.166	688.8100	268.8085	352.3210	202.2730	426.3250
6	Epicatechin	9.366	695.2900	--	--	--	656.4880
7	Gallocatechin	9.750	1041.4755	808.0300	302.2870	391.6185	1004.5950
8	Orientin	10.933	1119.8920	668.2570	377.0090	582.6550	940.0905
9	Taxifolin	11.216	2141.6660	1108.7980	636.0280	946.1670	1851.4010
10	Aromadendrene	12.116	854.2250	663.2030	143.2940	190.3270	738.5950
11	Quercetin	12.600	2100.3680	1481.7715	495.7280	630.0140	1707.6170
12	Genistein	12.950	171.0630	--	--	--	--
13	Delphinidin	13.416	1192.1920	--	--	--	798.5550
14	Kaempferol	13.900	1493.1860	1214.1220	316.6380	375.4420	1214.1220
15	Rutin	14.550	1018.0185	823.6980	137.1390	137.1390	995.9745
16	Apigenin	15.150	2000.4480	1410.5710	461.4565	545.4980	1807.8220
17	Isorhamnetin	15.783	745.7050	--	--	--	536.6335
18	Apigenin	16.300	2266.9115	1328.6520	273.5065	273.5065	1661.4330
19	Luteolin	17.383	880.0725	695.8040	271.7130	371.4210	880.0725
20	Myricetin	18.083	181.3510	--	370.7000	--	--
21	Vitexin	18.433	961.6460	645.3150	--	628.8650	762.4385
22	Cyanidin	19.433	574.2540	364.5080	172.7695	143.3550	518.0730
23	Betacyanin	20.383	448.8850	307.2870	--	--	399.8405

(a)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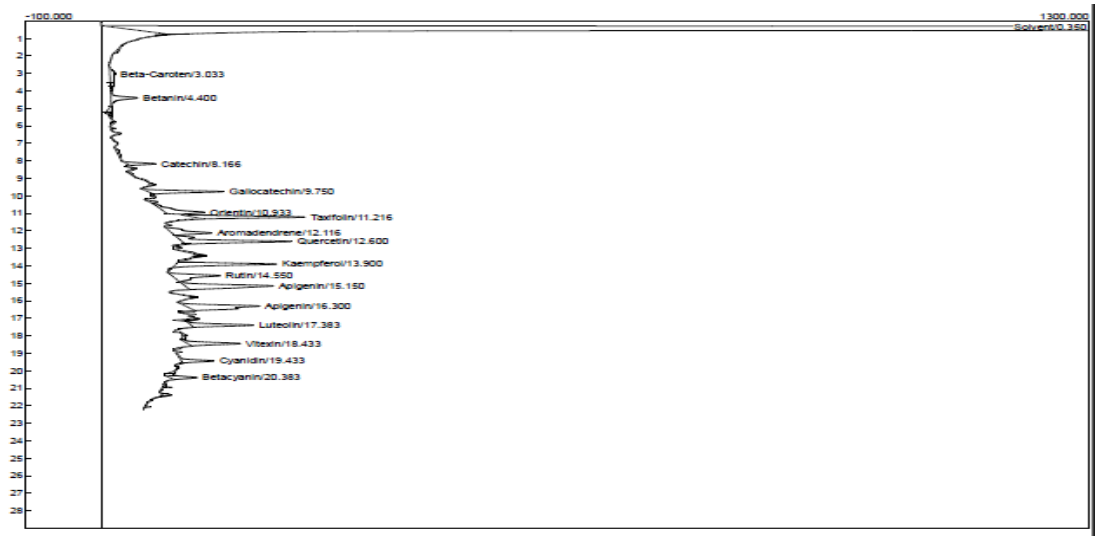
(b)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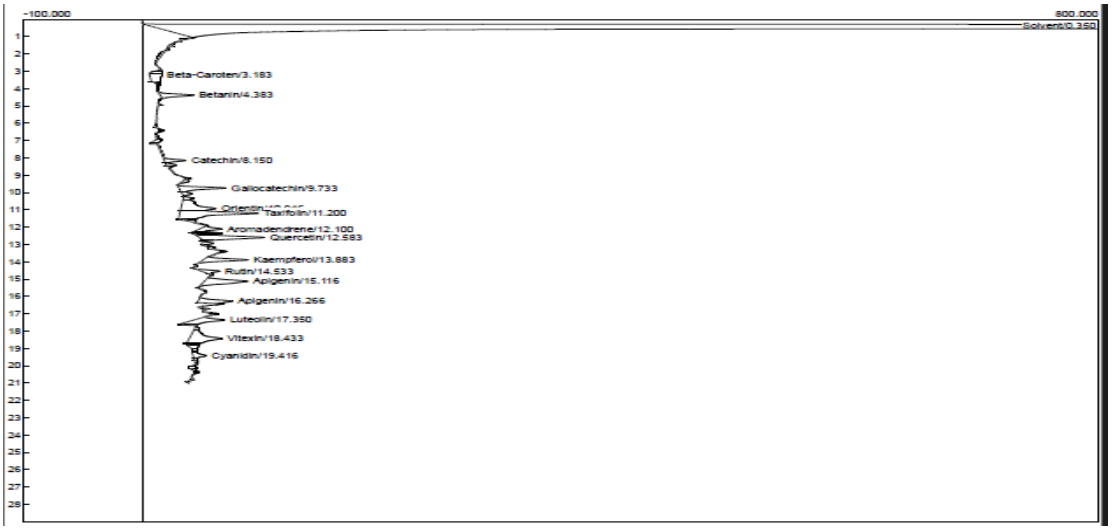
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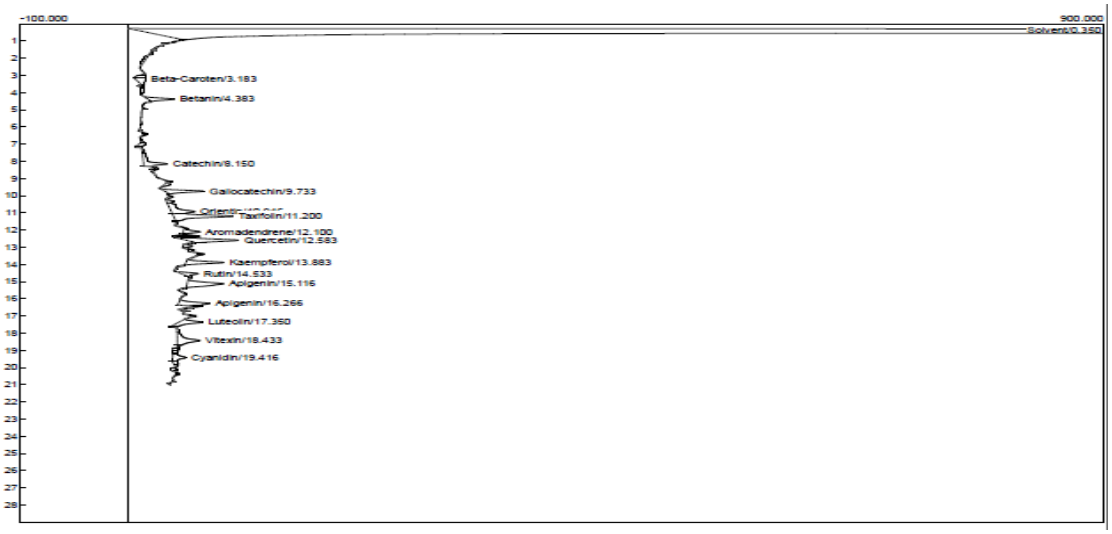
(d)



(e)

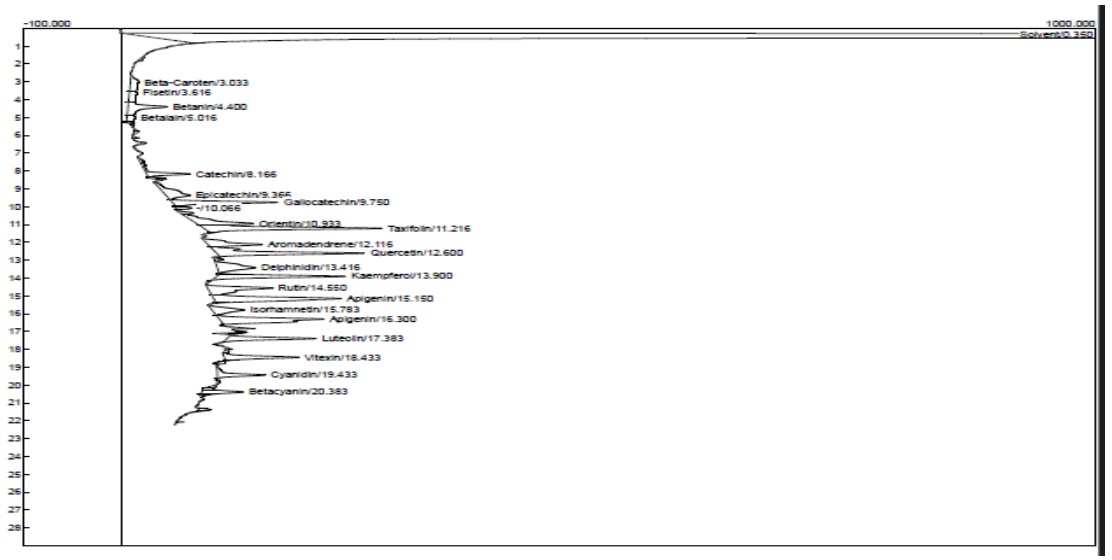


Figure 2: GC-FID result for ethanolic extract of *I. tomentosa* (a) Fruit (b) leaf extract (c) Stem stick (d) Stem bark, (e) Root

Determination of the Median Lethal Dose of the Ethanolic Fruit Extract of Isoberlina tomentosa in Wistar Rats

The results of the oral acute toxicity test for *I. tomentosa* ethanolic fruit extract using the Lorke's method guidelines are as shown in Table 4. Administration of *I. tomentosa* ethanolic fruit extract elicited various dose-dependent responses in the test animals: animals treated in phase I with doses between 100 – 1000 mg/kg showed mild to moderate effects such as hyperventilation, piloerection, and tremors. Animals treated in phase II of the study with doses between 1600 – 5000 mg/kg showed more pronounced effects including lethargy, urination, and diarrhea, which subsided by the second day. Notably however, no mortality was recorded even at the highest tested dose of 5000 mg/kg.

The study then estimated the LD_{50} of the *I. tomentosa* ethanolic fruit extract to be above 5000 mg/ kg given there was no death at any of the experimental doses tested as guided by the Lorke's method.

Here LD_{50} is determined from; $LD_{50} = \sqrt{(D_0 \times D_{100})}$;

Where; D_0 = Highest dose with no death

D_{100} = Lowest dose with death.

However, given that no mortality was seen at LD_{50} > 5000 mg/kg.

Table 4: Behavioral Patterns with *Isoberlina tomentosa* Ethanolic Fruit Extract Treatment

Treatment phase	Dose level (mg/kg)	Signs of toxicity observed in the treated Animals		
		Within 24 hours	**7 days**	**14 days**
Phase I	100	Piloerection, irritation	No observable change	No observable change
	500	Hyperventilation, tremors, piloerection		
	1000	Dyspnea, hyperventilation, tremors, piloerection		
Phase II	1600	Sedation, hyperventilation, tremors, urination		

	2900	Hyperventilation, tremors, piloerection, sedation, dyspnea, lethargy, diarrhea	No observable change	No observable change
	5000	Dyspnea, hyperventilation, lethargy piloerection, irritation, tremors, defecation, urination, diarrhea.		

In-vitro Antioxidant Capacity Screening

The Hydroxyl Radical (OH•) scavenging assay evaluates the ability of *I. tomentosa* extract to neutralize hydroxyl radicals (Figure 3A). Hydroxyl radicals are among the most reactive oxygen species (ROS), capable of causing severe damage to lipids, proteins, and DNA, leading to oxidative stress, aging, and diseases like cancer and neurodegenerative disorders. The absorbance values decrease as the extract concentration increases, confirming that higher concentrations are more effective at scavenging hydroxyl radicals. The DPPH (2,2-diphenyl-1-picrylhydrazyl) test was used to assess the free radical scavenging capacity using various concentrations (62.5 µg/ml to 1000 µg/ml). The absorbance values decrease as the concentration of the extract increases indicating that at higher concentrations, plant extract is more active in reducing DPPH radicals. The highest percentage inhibition was observed at 1000 µg/ml (≈ 85%), meaning the extract shows strong radical scavenging activity at high concentrations (Figure 3B). The ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay measures the ability of an antioxidant to neutralize ABTS radicals evaluating the total antioxidant capacity of plant extracts. This suggests strong antioxidant activity at higher concentrations (Figure 3C). This pattern is consistent with other assays (DPPH, ABTS), reinforcing the plant's strong radical scavenging ability. The H_2O_2 scavenging assay evaluates the ability of *I. tomentosa* extract to neutralize hydrogen peroxide, a reactive oxygen species (ROS) that can cause oxidative damage to cells. Figure 3D indicates that the higher concentrations have a stronger ability to neutralize H_2O_2. The inhibition decreases at lower concentrations but remains notable, confirming the plant extract's effectiveness in reducing oxidative damage. The Nitric Oxide (N.O) scavenging assay result shows the ability of *Isoberlina tomentosa* extract to neutralize nitric oxide radicals. Nitric oxide (NO) is a reactive nitrogen species (RNS) that plays essential roles in physiological processes but can become harmful at high concentrations, leading to inflammation, neurodegeneration, and cardiovascular diseases. The absorbance values decrease with increasing concentrations of the extract, indicating higher scavenging activity. The percentage inhibition increases in a dose-dependent manner, meaning higher concentrations of the plant extract are more effective at neutralizing NO radicals (Figure 3E). Figure F indicates the almost equal result of FRAP of the extract and the control indicateing greater antioxidant power, suggesting a higher content of reductants (such as polyphenols, Tanins, or other antioxidants) in the extract. The Total Antioxidant Capacity (TAC) assay measures the overall ability of a plant extract to neutralize free radicals by reducing molybdenum (VI) to molybdenum (V). This confirms that the plant extract contains compounds with strong reducing power (Figure 3G).

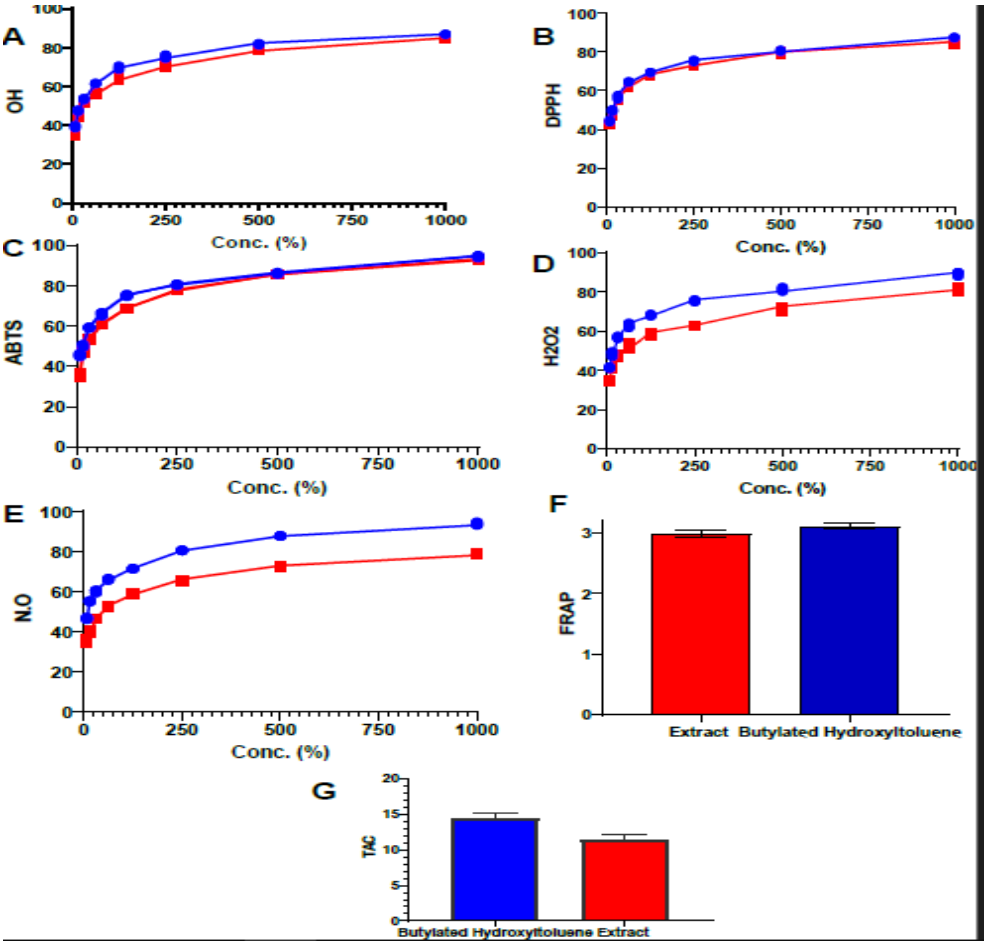


Figure 3: *In-vitro* Antioxidant Capacity Screening

In-Vivo Anti-Inflammatory Effects of the Isoberlina Tomentosa Ethanolic Fruit Extract Using Egg Albumin-Induced Paw Inflammation

The study demonstrates a substantial increase ($p < 0.0001$ and $p = 0.0106$) in the paw sizes at 60 minutes for the group D (negative control) and Group E (positive control) respectively, when compared to those in the normal control group (IT). Furthermore, compared to rats in Groups D, E and IT at 250, 500 and 1000 mg/kg respectively, showed a significant decrease ($p < 0.0001$) in the paw sizes. The study also indicated that there was a significant decrease ($p = 0.0106$) in the paw sizes for the Group IT(1000 mg/kg) when compared with those treated with Group E (Table 5,6).

The study's outcomes indicate that, after two hours, the rat paw sizes of the Group D were significantly larger ($p < 0.0001$) than those of the Group F (normal control). Rats treated with the positive control and extract at IT(250), IT(500), and IT(1000) respectively, showed significantly smaller paws ($p < 0.0001$) than those in the group D. Additionally, paw sizes of animals treated with 1000 mg/kg were significantly smaller ($p = 0.0106$) than those treated with the positive control (Table 5,6). After three hours, the paw sizes of the animals in the negative control group were significantly larger ($p < 0.0001$) than those of the normal control group. Additionally, the study showed that, in comparison to the negative control group, the paw sizes of the animals treated with the positive control and *I. tomentosa* ethanolic fruit extract at 250, 500, and 1000 mg/kg, respectively were significantly smaller ($p < 0.0001$). Comparing animals treated with 500 mg/kg and 1000 mg/kg of the *I. tomentosa* ethanolic fruit extract to those treated with the positive control, significant reductions ($p = 0.0331$ and $p = 0.0106$) in the rat paw sizes were observed (Table 5,6).

Table 5: The Anti-inflammatory Activity of Ethanolic Fruit Extract of *I. tomentosa* in Rats

Circumference in mm Groups	Initial	30 mins	60 mins	90 mins	120 mins	150 mins	180 mins
IT=(1000mg)	20.75±0.96	28.25±1.71	24.25±1.91	23.25±0.96	22.25±0.96	22±0.82	21.5±0.58
IT=(500mg)	23.25±2.36	29±1.82	28±2.31	27.5±2.08	26.5±2.08	26.25±2.22	25.5±2.08
IT=(250mg)	23.5±4.43	30.75±5.85	29.75±3.40	29.25±3.77	29.25±3.59	29.25±4.34	28.75±3.95
D=(0.1ml)Oval albumin	21.5±1	32±0.81	31.75±0.96	31.75±0.5	31±1.41	30.25±0.95	30.25±0.96
E=(50mg)IND	23±0.82	30.75±3.77	27.5±2.64	26±2.06	25.75±2.22	25.25±2.06	24.5±1.73
F=(distilled water 0.1ml/kg)	21.5±1	21±1	21.5±1	21.5±1	21.5±1	21.5±1	21.5±1

Values were expressed as Mean ± SEM; n=4 animals per group. Results were analyzed by One-way Analysis of Variance (ANOVA), followed by Dunnett' post hoc test.*p < 0.05, **p< 0.01. **IT =** *Isoberlina tomentosa* plant extract; **IND** = Indomethacin (standard drug).

Table 6: Percentage Anti-Inflammatory Response by the Ethanolic Fruit Extract of *Isoberlinia Tomentosa* as Compared with the Standard Drug (Indomethacin=Ind) Over Time

Group min/%	30min	60min	90min	120min	150min	180min
1000mg	47	63.40	74.40	84.20	85.20	91.30
500mg	45.20	53.70	56.40	65.80	65.70	74.30
250mg	31.00	39.00	42.00	39.50	34.30	40.00
IND(50mg)	26.20	56.10	67.80	71.10	74.30	82.90

Discussion

Qualitative and quantitative investigations of secondary metabolites are very critical to identify the presence of phytochemicals which are the major reason for the medicinal and physiological values on every plant (Oseni et al., 2024). The pharmacological potentials of medicinal plants occur because of the quality and quantity of different secondary metabolites such as flavonoids, alkaloids, glycosides, saponins, phenols, and steroids, etc. identified in them (Hadiza et al., 2020; Sulyman & Ibrahim, 2024). Therefore, this research shows the presence of phenolic compounds, tannin, saponin, terpenoid, flavonoids, and alkaloids. These phytochemicals suggest that *I. tomentosa* definitely have some pharmacological importance like anti-inflammatory due to the presence of Saponin, polyphenol, and Terpinoids, (Saleh et al., 2021; Culhuac et al., 2023); antineoplastic because of the presence of Saponin, Alkaloid, and Flavonoids (Khalid et al., 2021; Ali et al., 2024); therefore with the presence of these phytochemicals and bioactive compounds, there many pharmacological potentials of *I. tomentosa*. The GC-FID result also revealed 22, 15, 14, 15, and 21 bioactive compounds from the fruit, leaf, stem stick, stem bark and root respectively. This makes the selection of the fruit extract as the choice for further investigations. This result also align with the results of GC-FID/MS of other plants including *I. doka* (Hadiza et al., 2020), these active compounds make the potentials of many plants scientifically proven (Namadina et al., 2020; Adekilekun et al., 2024).

The test for acute toxicity of the *I. tomentosa* ethanolic fruit extract utilizing Lorke's method, with a maximum dose of 5000 mg/kg body weight indicated that the LD_{50} is greater than 5000 mg/kg, as no mortality was observed even at the highest dose. This finding aligns with previous studies on other *Isoberlinia* species, especially *I. Doka,* which have also reported high LD_{50} values *in-vivo*, suggesting a favourable safety margin for medicinal use (Hadiza et al., 2020). The observed mild to moderate symptoms such as hyperventilation and tremors are consistent with the effects noted in other studies involving herbal extracts, where similar dose-dependent responses were documented without fatal outcomes. These observations hint at mild toxic impacts, likely resulting from stimulation of both the autonomic and central nervous systems. These observations align with previous studies (Kathare *et al.,* 2021), which identified similar symptoms as indicators of toxicity in animals subjected to harmful substance levels. Based on the globally harmonized classification system (GHS) for acute toxicity of chemicals, the LD_{50} of the *Isoberlina tomentosa* ethanolic leaf

extract falls within GHS Category 5, suggesting a relatively low acute toxicity.

The high percentage inhibition at 1000 µg/ml and 500 µg/ml suggests that *I. tomentosa* contains potent antioxidant compounds capable of neutralizing free radicals indicating that the plant extract could be a potential natural antioxidant source, suitable for applications in pharmaceuticals, nutraceuticals, or food preservation. Also, Isoberlina tomentosa extract is rich in compounds capable of neutralizing ABTS radicals, making it a strong antioxidant source. The gradual decline in inhibition with lower concentrations aligns with typical antioxidant behavior, where higher doses provide better radical scavenging. The high TAC values also suggest that *Isoberlina tomentosa* is rich in bioactive compounds like polyphenols, and tannins, which contribute to its strong antioxidant potential, the result of the quantitative phytochemicals confirmed this. The scavenging capability of *Isoberlina tomentosa* to hydrogen peroxide suggests that it contains bioactive compounds capable of reducing oxidative stress thereby preventing diseases associated with oxidative stress, such as neurodegenerative disorders, cardiovascular diseases, and aging-related conditions. The strong NO scavenging activity of *Isoberlina tomentosa* suggests that it may be beneficial for reducing inflammation and oxidative stress-related diseases. The strong OH• scavenging ability of *Isoberlina tomentosa* suggests it is rich in bioactive compounds like polyphenols and flavonoids, which help protect against oxidative stress-related diseases. It is noted that the antioxidant activities throughout the tested methods show a dose-dependent trend confirming that higher concentrations offer stronger antioxidant protection.

The anti-inflammatory potentials of the extract was evidenced by a significant decrease in paw sizes in rats treated with varying doses of the extract as compared to the negative control group. The doses at 250, 500, and 1000 mg/kg significantly reduced inflammation, indicating a dose-dependent response. The findings are corroborated by existing works that highlights the anti-inflammatory properties of plants including *I. tomentosa* as shown by this research are attributed to their phytochemical constituents such as tanins, saponins and terpenoids, which are known to inhibit the assembly of pro-inflammatory cytokines (Matara et al*.,* 2021; Terefe et al*.,* 2022; Alkali et al., 2025). The extract displayed a superior anti-inflammatory effect at 1000 mg/kg compared to the positive control, signifying a more effective agent than conventional anti-inflammatory medications in certain contexts. This finding is significant as it positions *I. tomentosa* as a potential alternative or complementary treatment for inflammatory conditions (Omodamiro and Umekwe, 2013; Omodamiro and Jimoh, 2014; Akinloye et al. 2020; Eidangbe. 2025).

CONCLUSION

In conclusion, the study comprehensively evaluated the bioactive compounds, *in-vitro* antioxidant profile, and *in-vivo* anti-inflammatory properties of ethanolic fruit extract from *Isoberlinia tomentosa*, a plant traditionally use for many conditions. The phytochemical analysis shown significant presence of phenolic compounds, tannins, saponins, and alkaloids, which underline the pharmacological potential of the plant. Quantitative assessment using GC-FID identified 22 bioactive compounds, supporting the extract's therapeutic relevance. Acute toxicity studies indicated a high LD_{50} greater than 5000 mg/kg, suggesting a favorable safety profile and mild toxic effects, consistent with other herbal studies. The *in-vitro* antioxidant tests indicated that the extract has high antioxidant potentials with a dose-dependent trend making it a good plant with wide pharmacological benefits. Notably, the anti-inflammatory assessment demonstrated that the extract significantly reduced paw sizes in a dose-dependent fashion, outperforming the positive control, indomethacin. These results substantiate the anti-inflammatory activity linked to the phytochemical constituents, indicating that *Isoberlinia tomentosa* could serve as a viable alternative or adjunct to existing anti-inflammatory treatments, warranting further exploration for potential applications in complementary medicine. Consequently, this research lays a foundational framework for the scientific exploration of *Isoberlinia tomentosa*, amplifying its importance in drug development, safety validation, and conservation efforts within its native ecosystems.

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