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HPLC Profiling and Evaluation of Anti-inflammatory, Antioxidant and Analgesic Activities of *Funtumia africana* (Benth) Leaf Extract

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Different parts of *Funtumia africana* have been exploited by trado-medical practitioners to cure an array of diseases. The study is intended to determine to the flavonoid content and assess the antioxidant, analgesic, and anti-inflammatory activities of methanolic leaf extract of *F. africana* in Wistar rats. For the antioxidant activity, hydrogen peroxide scavenging, hydroxyl scavenging and copper ion reducing antioxidant capacity tests were determined. Anti-inflammatory activity and analgesic properties were evaluated utilizing the formalin induced paw edema test and the hot plate

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method respectively. Furthermore, high performance liquid chromatography technique (HPLC) was used for quantification of flavonoids. The *in vitro* antioxidant result exhibited potent copper ion reducing antioxidant activity of the extract with an IC_{50} value of 1.64 compared to the standard ascorbic acid. The result of the *in vivo* anti-inflammatory activity revealed that the plant extract at 1500 mg/kg dosage possessed significant anti-inflammatory activity ($p<0.05$) by reducing the paw edema from 2.53±0.12 to 2.26±0.06 with a 13.07% inhibition in the preventive acute inflammatory while for curative *F. africana* at 500 mg/kg dosage reduced the paw edema from 2.40±0.10 to 2.03±0.21 with a 15.41% inhibition. Also *F. africana* indicated noteworthy analgesic activity ($p<0.05$) by increasing the latency of thermal stimuli from 5 seconds to 11sec. HPLC fingerprinting gave 30 different flavonoids in different quantities. The outcomes of this investigation have shown that *F. Africana* methanol leaf extract possesses antioxidants, anti-inflammatory, and analgesic properties which could be attributed to flavonoid compounds such as quercetin, kaempferol, and apigenin present in the extract.

Keywords: Analgesics; anti-inflammation; antioxidant; flavonoids; Funtumia Africana; HPLC profile.

1. INTRODUCTION

Inflammation and pain causes discomfort in the body. Inflammation is a complex physiological response triggered by the body's immune system in response to tissue injury or infection while pain is a multifaceted sensory and emotional experience that serves as a protective mechanism [1].

Analgesics are drugs utilize to reduce or treat pain and they work by blocking the transmission of pain signals from the nerve to the brain [2]. These analgesics have their natural sources traced back to opiate and non-steroidal anti-inflammatory drugs (NSAIDs) but numerous of synthetic compounds have been utilized as anti-inflammatory agents and as analgesic however, these synthetic compounds have significant side effect such as addiction, respiratory depression, drowsiness, ulceration [3]

As a result, there is an increasing interest in identifying natural remedies with analgesic, anti-inflammatory, and antioxidant properties.

Funtumia africana (Benth) is a tropical plant found in the forest reserves of West Africa and is mostly found in the western and eastern part of Nigeria [4]. *Funtumia africana* is called bush rubber belonging to the family Apocynacea and it is widely studied for its medicinal properties which have been employed by herbalist to treat a range of ailments [5].

The purpose of this research is to ascertain the flavonoid contents and examine the antioxidant, analgesic and anti-inflammatory effect of leaves of *Funtumia africana* as potential natural remedy for pain and inflammation.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Material

Fresh leaves of *F. africana* were harvested in Amatolo Town, Wilberforce Island, located in the Niger Delta region of Nigeria. The plant's identification and authentication were performed by Prof. K. K. Ajibesin from the Department of Pharmacognosy and Herbal Medicine at Niger Delta University, Wilberforce Island, Nigeria. A sample from this collection was submitted to the herbarium at Niger Delta University, assigned the voucher number NDUP 112. The leaves of *F. africana* were air dried in the laboratory for three weeks and were pulverized using plant milling machine (Classic YC112M2-4, 5HP).

2.2 Sample Extraction

About 1.2 kg of the pulverized leaves was extracted for 72 hours by cold maceration in 5 L containing 50% methanol with frequent agitations. The extract was decanted, filtered through Whatman No 1 filter paper and concentrated using a water bath (B-480, Switzerland) to acquire the methanol extract.

2.3 Qualitative Phytochemical Screening

Phytochemical screening was conducted following established procedures, as outlined by Sofowora and Evan [6], to identify the secondary metabolites contained within the plant extract. Test for flavonoids, alkaloids, saponins, cardiac glycoside, anthraquinones, tannins and terpenoids was carried out.

2.4 Experimental Animals

Wistar rats weighing (80-165) g of both sexes were utilized for the *in vivo* anti-inflammatory and analgesic study. The animals were acquired from Pharmacology and Toxicity animal house, Niger Delta University, Wilberforce Island and were kept under natural light and allowed to acclimatize for two (2) weeks. Each rat was managed in accordance with the National Institute of Health's guidelines on the Care and Utilization of Laboratory Animals [7].

2.5 Anti-Inflammatory Study

2.5.1 Formalin induced oedema (Preventive measures)

The anti-inflammatory efficacy was evaluated employing the Hunskaar & Hole [8] Formalin-induced method, as documented by Young *et al.*, [9], with minor adjustments. A total of fifteen Wistar rats in five groups of three rats per group (n=3) were used. Each group was treated orally with distilled water, plant extract 500 mg/kg, 1000 mg/kg, 1500 mg/kg, and aspirin 300 mg/kg respectively. The initial paw circumference was measured and after one hour of administration of the various treatment agents, 10% formalin was injected into the left hind paw of the rats. The paw circumference (cm) was measured at 5 minutes, 30 minutes, 1 hour and 24 hour interval employing an improvised method utilizing a meter ruler and a thin thread.

2.5.2 Formalin induced oedema (Curative measures)

The curative acute anti-inflammatory activity was also evaluated utilizing the formalin induced technique. A total of fifteen Wistar rats in five groups of three rats per group (n=3) were used for the assay. The rat paw circumference was measured as initial paw size then 5% formalin was injected to induce acute inflammation at the left hind paw. The rat paw size was assessed again and after an hour, treatment was given orally according to the different dosage of the plant extract, the control, and the standard drug (ASA) as described in Table 2. At 30 minutes, 1 hour: 90 minutes, and 24 hour, the rat paw circumference was measured to ascertain the anti-inflammatory potency of the treatment.

The percentage inhibition of inflammation was calculated using equation 1

$$\%\ inhibition\ of\ inflammation = Vc - Vt / Vc \times 100 \quad (1)$$

Where, Vc signifies the mean paw size of the control group, while V_t indicates the mean paw size of the test group. The determination of both V_c and V_t involves assessing the alterations in paw size before and after the treatment.

2.5.3 Analgesic Study (Hot plate method)

The assessment of analgesic effects on the hot plate relies on the principle of thermal stimuli, where animals experience pain through the application of heat to their paws [10]. The analgesic property of the extract was evaluated utilizing the hot plate model as outlined by Fan *et al.,* [11] with minor modification. A total of 15 rats weighing 121-150 g were utilized and separated into five groups with three rats in each group and treatment was given orally. Group 1 as control was given 0.2 mL distilled water and group 2-4 was given (500, 1000 and 1500) mg/kg respectively while group 5 was given 50 mg/kg standard drug (tramadol).

Using hot plate (Stuart digital hot plate SD500) the rats were situated on the 55°C heated plate, and the reaction time, measured in seconds as the latency period, was assessed as the duration it took for the rats to respond to thermal pain through actions such as leaping or licking their paws. This reaction time in seconds was documented one hour after administering various treatment agents. A maximum reaction time of 45 seconds was established as a precautionary measure to safeguard the paw tissues from potential harm.

2.6 Antioxidant Study

2.6.1 Hydrogen peroxide scavenging assay

The capability of the methanolic *F. africana* extract to scavenge hydrogen peroxide (H_2O_2) was evaluated using the Ruch *et al.*, [12] method with slight modifications. A 0.1 mL aliquot of *F. africana* extract (25–400 µg/mL) was placed into eppendorf tubes and the volume was made up to 0.4 mL of 50 mM of hydrogen peroxide prepared in phosphate buffer (pH 7.4). 0.6 mL of the hydrogen peroxide solution (2 mM) was also added to the Eppendorf tubes and the mixture was subjected to vortex mixing. The absorbance of hydrogen peroxide was assessed at 230 nm after 10 minutes. The positive control used was ascorbic acid and the capability of the *F. africana*

extract to neutralize hydrogen peroxide was evaluated utilizing equation 2:

Hydrogen peroxide scavenging activity percentage = $[(A_0-A_1)/A_0] \times 100$ (2)

Where: A_0 = Control Absorbance (Ascorbic acid). A_1 = Sample Absorbance (*F. africana)*.

2.6.2 Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was measured by the deoxyribose method reported by Halliwell *et al.,* [13]. The generation of the hydroxyl radical occurred using the Fenton reaction and the sugar deoxyribose on exposure to the hydroxyl radicals produces a pink chromogen when subjected to heat in the presence of thiobarbituric acid (TBA) under acidic conditions.

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + HO\bullet$

Fenton reaction

The mixture of the reaction comprising 0.2 mL of the aqueous methanolic *F. africana* extract, 0.8 mL of phosphate buffer solution (50 $mmolL^{-1}$, pH 7.4), 0.2 mL of ethylenediaminetetraacetic acid (EDTA) (1.04 mmol L^{-1}), 0.2 mL of $FeCl_3$ (1 $mmolL^{-1}$), 0.2 mL of H_2O_2 (10 mmol L^{-1}), 0.2 mL of 2-deoxy-D-ribose (28 mmol L^{-1}) and 0.2 mL of ascorbic acid was subjected to incubation in a 37°C water bath for one hour. Thereafter, 1.5 mL of cold thiobarbituric acid (10 gL^{-1}) and 1.5 mL of HCl (25%) was introduced to the reaction mixture and warmed using a water bath at 100 ^oC for 15 mins and subsequently, cooled using water. The solution's absorbance was determined at 532 nm using a spectrophotometer and ascorbic acid was utilized as the positive control. The calculation of the hydroxyl radical scavenging activity percentage was performed utilizing equation 3

% hydroxyl radical scavenging activity $=[(A_0 - (A_1-A_2] \times 100/A_0$ (3)

Where: A_0corresponds to the control absorbance in the absence of a sample.

A_1 represents the absorbance following the addition of the *F. africana* extract and 2-deoxy-D-ribose.

A_2 is the aqueous *F. africana* extract absorbance without 2-deoxy-d-ribose.

2.6.3 Copper ion reducing antioxidant capacity (CUPRAC)

The CUPRAC assay was conducted following the technique stated by Apak *et al.,* [14], with minor adjustments. 100 µL of the aqueous *F. africana* extract was combined with 1 mL $CuCl_2$ solution (10 mM), 1 mL of neocuproine alcoholic solution (7.5 mM in absolute ethanol), 1 mL of $NH_4CH_3CO_2$buffer solution (pH 7.0), 4.1 mL of distilled water. Following a 30-minute incubation period, the spectrophotometer was used to measure the absorbance at 450 nm relative to the reagent blank. CUPRAC activity was assessed twice for each biological replicate. The data was stated as micro gram ascorbic acid equivalents per gram of sample fresh weight (µg/mL).

2.6.4 Analysis of Flavonoids by High-Performance Liquid Chromatography (HPLC)

The assessment of flavonoids in *F. africana* leaf extract was undertaken at Cation Analytical and Environmental Research Laboratory, situated in Lagos, Lagos State, using an Agilent 1200 Series HPLC system. This comprehensive system comprised an auto liquid sampler (ALS), a binary pump, a column compartment with thermostat control, a multiwavelength UV/VIS detector (MWD), and an online degasser. The analytical procedure involved the utilization of a C_{18} HPLC column (150×4.6mm I.D., 3.5 µm particle size) with a 20µL injection volume and a flow rate set at 1.0 mL/min. The analysis was conducted at a consistent temperature of 25°C, with a mobile phase consisting of phosphoric acid (0.1%) and acetonitrile. To determine the absorbance of the eluent, UV spectroscopy was applied at a wavelength of 280 nm.

2.6.5 Statistical analysis

The data was examined utilizing Statistical Package for Social Science Software (SPSS; version 20.0, USA). One-way analysis of variance (ANOVA) was used to determine the mean difference and statistically significant values at $P<0.05$. Values with the same superscripts were considered to have no statistically significant difference.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Analysis

The analysis of phytochemicals showed the existence of flavonoids, alkaloids, terpenoids, saponins, cardiac glycoside, and tannins.

3.2 Anti-Inflammatory Activity

3.2.1 Effect of F. africana leaf extract on formalin induced paw edema in rats (preventive acute inflammation)

The paw edema size was significantly reduced at 30 minutes for the 1000 mg/kg and 1500 mg/kg doses in contrast to the control group and the 500 mg/kg dose. At 1 hour, the 500 mg/kg, 1500 mg/kg, and 100 mg/kg doses significantly reduced the paw size compared to the standard drug (aspirin) as shown in Table 1. *Funtumia africana* did not have a significant effect on paw edema at the 500 mg/kg and 1000 mg/kg doses at 5 minutes compared to the control group. However, at 24 hours, there was a slight reduction in paw edema size, indicating a minimal effect of *F. africana* at the 500 mg/kg and 1500 mg/kg doses. These findings indicate that the extract's effectiveness is time-dependent, as observed in Table 1. Comparably, 1500 mg/kg has shown considerable effect in reducing inflammation at 30min and 1hour compared to the aspirin.

Based on the aforementioned findings, it is evident that the administration of the extract led to a substantial decrease in the size of the rat paw, indicating that *F. africana* possesses a mild anti-inflammatory effect in the prevention of inflammation.

The percentage inhibition of inflammation is a measure used to assess the effectiveness of anti-inflammatory drugs or compounds. It quantifies the reduction in inflammation caused by a particular treatment. Therefore, a higher percentage indicates a greater inhibition of inflammation and suggests that the test drug has a stronger anti-inflammatory effect.

At the 5-minute mark, the test doses of the extract, 500 mg/kg body weight and 1000 mg/kg body weight, exhibited inhibitions of -4.76% and -4.76% respectively, as shown in Table 1.

This suggests that at 5 minutes, these doses of 500 mg/kg body weight and 1000 mg/kg body weight of the extract either had no impact on reducing inflammation or potentially increased the inflammation levels when compared to the control or baseline measurement. However, noteworthy anti-inflammatory effects were observed for the same test doses between 30 minutes and 24 hours.

The extract, given at a dosage of 1500 mg/kg body weight, effectively prevented paw edema with inhibitions of 7.33%, 12.09%, 13.08%, and 6.45% at 5 minutes, 30 minutes, 1 hour, and 24 hours respectively (Table 1). The most potent anti-inflammatory efficacy of the extract was observed at the dose of 1500 mg/kg body weight, exhibiting a 13.08% inhibition at 1 hour, which was comparable to the standard (15.38%) at same time interval.

As for the standard drug aspirin, given at a dose of 300 mg/kg body weight, it demonstrated the most potent anti-inflammatory activity with a 17.10% inhibition at 24 hours.

From the result above, treatment with the extract considerably reduced the rat paw size which means that *F. africana* has slight anti-inflammatory activity in preventing inflammation.

Table 1. Preventive acute inflammation effect of *F. africana* and ascorbic acid on Wistar rats

Treatment Group	Dose (mg/kg)	Initial paw dimension (cm)	Paw size (cm) after Induction (% Inhibition)			
			5 min	30 min	1 hr	24 hrs
I (control)	Distilled water 0.2	2.13±0.06^{a}	2.73±0.12^{a}	2.73±0.06^{a}	2.60±0.10^{a}	3.10±0.10^{a}
II *F. africana*	500	2.13±0.06^{a}	2.86±0.06^{a} (-4.76%)	2.63±0.15^{a} (3.66%)	2.40±0.00^{b} (7.69%)	2.83±0.15^{b} (8.71%)
III *F. africana*	1000	2.17±0.06^{a}	2.86±0.12^{a} (-4.76%)	2.40±0.10^{b} (12.09%)	2.33±0.15^{b} (10.38%)	2.97±0.06^{a} (4.19%)
IV *F. africana*	1500	2.10±0.10^{a}	2.53±0.12^{b} (7.33%)	2.40±0.17^{b} (12.09%)	2.26±0.06^{b} (13.08%)	2.90±0.10^{b} (6.45%)
V (Aspirin)	300	2.10±0.00^{a}	2.43±0.06^{b} (10.99%)	2.23±0.06^{b} (18.32%)	2.20±0.00^{b} (15.38%)	2.57±0.12^{b} (17.10%)

The data (n=3) show means ± standard deviation. Means with differing superscript alphabets in the same column indicate significant differences ($p<0.05$) compared to the control group.

3.2.2 Effect of F. africana leaf extract on formalin induced paw edema in rats (curative acute inflammation)

At 1 hour, the doses of 500 mg/kg and 1000 mg/kg had a significant effect compared to the control group and standard drug. Furthermore, at 1 hour and 30 minutes, the doses of (500, 1000, 1500) mg/kg showed a notable decrease in paw edema size in relation to the standard drug (aspirin), as shown in Table 2. Similarly, at 24 hours, there was a significant reduction in paw size for the doses of (500, 1000, 1500) mg/kg compared to the standard drug and control group, as illustrated in Table 2.

The test doses of the extract, administered at 500 and 1000mg/kg body weight at 1hour, displayed percentage inhibitions of inflammation of 15.42% and 9.58% respectively (Table 2). In comparison, the standard drug achieved a percentage inhibition of 4.17% at same time interval. Furthermore, at 24h, the 500mg/kg body weight dose of the extract demonstrated a percentage inhibition of 12.15%, while the standard aspirin only achieved 8.10% inhibition. At 1h: 30mins a dosage of 1500mg/kg body weight of the extract also had 7.08% inhibition which was comparable to the standard aspirin (7.08%). The observed differences in percentage inhibition at different time intervals suggest that the extract doses (500 and 1000 mg/kg body weight) exhibit greater efficacy in reducing inflammation than the standard drug (aspirin). The findings showed that all the test dosages of the extract had anti-inflammatory effect at different time intervals.

The result indicates that *F. africana* extract exhibits noteworthy anti-inflammatory activity which can be ascribed to the existence of flavonoids. Studies have also indicated the importance of flavonoids such as quercitrin, kaemferol, quercetin and apigenin, identified in the plant extract by HPLC to inhibit prostaglandin synthesis, nitric oxide (NO), cytokines, thereby fighting the enzyme cyclooxygenase [COX], which generates inflammatory mediator, triggers pain and promote other aspects of inflammation [15,16,17].

3.3 Analgesic Study

3.3.1 Effect of methanolic extract of F. africana leaves on pain reaction time in Wistar rats

The findings demonstrated that there was no noteworthy variation in the response to thermal stimulus observed in rats that received distilled water treatment over the course of the 1-hour observation period. However, there was an increase in time at dose 500 mg/kg using the extract of *F. africana* in contrast to the control group. At dose 100 mg/kg a substantial increase was observed in the response time up to 10.33 ± 0.58^{b} of the rats compared to the control group at 5.67 ± 1.15 after 1hr of administration as seen in Table 3. Also at dose 1500 mg/kg the extract significantly increased the reaction time to 11.00 ± 1.0^{b} compared to dose 500 mg/kg and the

Table 2. Curative acute inflammation effect of *F. africana* and ascorbic acid on Wistar rats

Treatment group	Dose (mg/kg)	Initial paw dimension (cm)	Paw size (cm) after induction	Paw size (cm) after treatment (% Inhibition)		
				1 hr	1hr 30min	24 hrs
I (control)	Distilled water 0.2	1.97 ± 0.15^{a}	2.47 ± 0.15^{a}	2.40 ± 0.10^{a}	2.40 ± 0.10^{a}	2.47 ± 0.15^{a}
II *F. africana*	500	1.93 ± 0.12^{a}	2.40 ± 0.10^{a}	2.03 ± 0.21^{b} (15.41%)	2.23 ± 0.25^{a} (7.08%)	2.17 ± 0.31^{a} (12.15%)
III *F. africana*	1000	2.00 ± 0.00^{a}	2.43 ± 0.06^{a}	2.17 ± 0.06^{b} (9.58%)	2.27 ± 0.25^{a} (5.42%)	2.37 ± 0.06^{a} (4.05%)
IV *F. africana*	1500	2.07 ± 0.06^{a}	2.43 ± 0.15^{a}	2.30 ± 0.10^{a} (4.17%)	2.23 ± 0.06^{a} (7.08%)	2.30 ± 0.00^{a} (6.88%)
V Aspirin	300	2.13 ± 0.06^{a}	2.50 ± 0.10^{a}	2.30 ± 0.10^{a} (4.17%)	2.23 ± 0.06^{a} (7.08%)	2.27 ± 0.15^{a} (8.10%)

The data (n=3) show means ± standard deviation. Means with differing superscript alphabets in the same column indicate significant differences ($p<0.05$) compared to the control group.

control group. Sequel to the reaction time increment it can be observed that the analgesic activity exhibited by the plant extract is dose dependent.

In contrast to the control, tramadol produced the most significant analgesic effect after 1hr of administration followed by the plant extract at dose 1500 mg/kg by increasing the response to thermal stimulus time as seen in Table 3.

The analysis of phytochemicals shows the existence of tannins, flavonoids, saponins, alkaloids, identified from the leaves of *F. africana*. Hence the analgesic activity could be ascribed to these phytocompounds. A number of phytochemicals like alkaloids, flavonoids, and tannins are widely recognized for their capacity to suppress pain sensation [11]. Other research findings also indicated that the presence of flavonoids such as rutin, hesperidin, apigenin and quercetin as found in this plant extract inhibit prostaglandin synthesis and exerts the analgesic and anti-inflammatory effects [18, 19].

3.4 Antioxidant Activity

3.4.1 Hydrogen peroxide scavenging activity

The hydrogen peroxide test was conducted to assess the ability of *F. africana* to scavenge hydrogen peroxide. The *F. africana* extract demonstrated antioxidant capacity at all tested concentrations, with the effectiveness increasing in a manner that depends on the concentration. These findings were compared to the standard ascorbic acid, as displayed in Table 4 and Fig. 1. The findings revealed that the *F. africana* extract exhibited the minimum hydrogen peroxide scavenging activity of 25.59 ± 0.33 at 25 µg/mL, while its maximum activity reached 73.34 ± 0.17 at 100 µg/mL. In contrast, the standard ascorbic acid displayed a minimum activity of 25.95 ± 0.50 at 25 µg/mL and a maximum activity of 90.64 ± 0.17 at 100 µg/mL. Overall, these results imply that the *F. Africana* extract possesses the capability to scavenge hydrogen peroxide and reduce oxidants.

3.4.2 Hydroxyl radical scavenging activity

The aqueous methanol extract of *F. africana* has demonstrated significant potential in scavenging hydroxyl radicals across all tested concentrations, as observed in Table 4. The findings suggest that scavenging activity of the extract relies on concentration, with higher sample concentrations exhibiting increased activity. Specifically, the *F. africana* extract displayed a minimum activity of 12.27±0.27 at 25 µg/mL and a maximum activity of 52.13±0.27 at 100 µg/mL, as illustrated in Table 4 and Fig. 2. These findings highlight the extract's ability to effectively scavenge hydroxyl radicals.

Table 3. Analgesic effect of *F. africana* extract in Wistar rats using the hot plate method

Group/Treatment	Dose (mg/kg)	Time (sec) after 1hr of administration
I (Control) Distilled water	0.2	5.67±1.15^{a}
II *F. africana*	500	9.67±0.58^{b}
III *F. africana*	1000	10.33±0.58^{b}
IV *F. africana*	1500	11.00±1.0^{b}
V Tramadol	50	31.67±1.52^{b}

Values marked with distinct superscript letters exhibit significant differences ($p<0.05$) in comparison to the control group.

Table 4. Free radical scavenging activities (% inhibition) of *F. africana* extract and ascorbic acid (AA)

Concentration (µg/mL)	Hydrogen peroxide scavenging activity		Hydroxyl radical scavenging activity		Copper ion reducing activity	
	F. africana	**AA**	***F. africana***	**AA**	***F. africana***	**AA**
25	25.59±0.33^{a}	25.95±0.50^{a}	12.27±0.27^{a}	37.39±0.40^{a}	43.72±0.15^{a}	54.99±0.84^{a}
50	39.34±0.33^{b}	51.42±1.34^{b}	21.46±0.54^{b}	56.84±1.09^{b}	64.36±0.56^{b}	66.06±0.42^{b}
75	49.88±1.69^{c}	61.02±0.50^{c}	47.65±0.27^{c}	75.71±0.27^{c}	79.83±0.25^{c}	77.86±0.84^{c}
100	73.34±0.17^{d}	90.64±0.17^{d}	52.13±0.27^{d}	81.61±0.29^{d}	77.11±0.25^{d}	88.08±0.56^{d}

The data is displayed as mean ± standard deviation (n=2); Values with different superscript symbols in the same column are statistically significant ($p<0.05$)

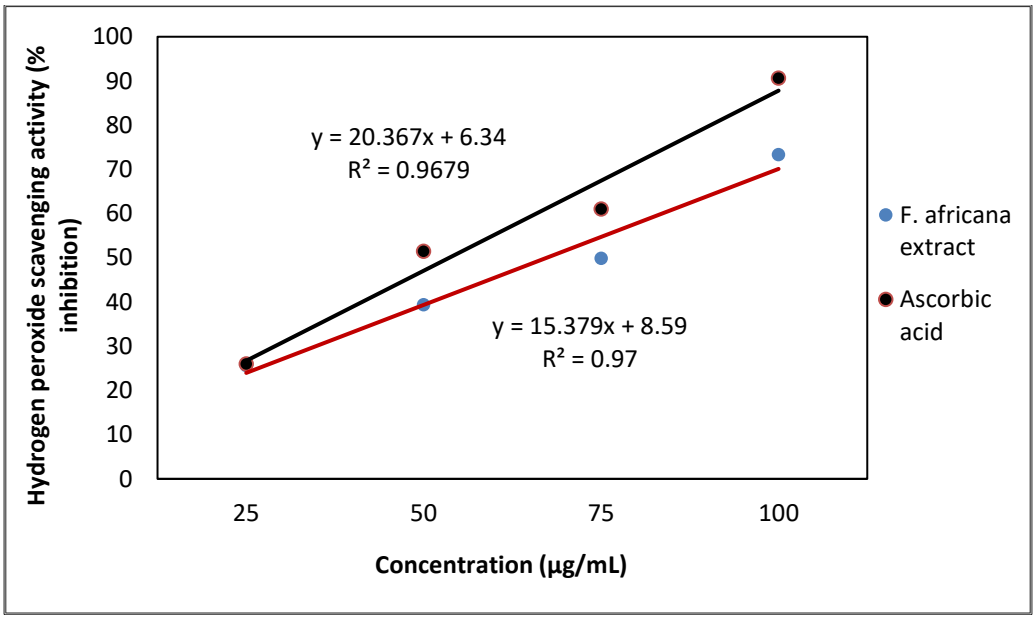

Fig. 1. Hydrogen peroxide scavenging activity of *F. africana* extract and ascorbic acid

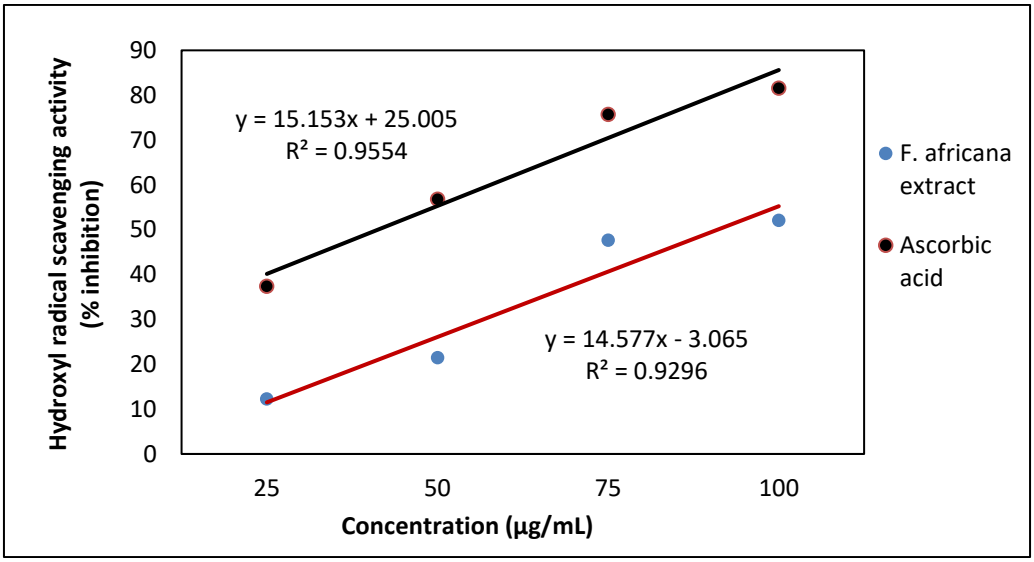

Fig. 2. Hydroxyl radical scavenging activity of *F. africana* extract and ascorbic acid

3.4.3 Copper ion reducing activity

The test relies on the capability of antioxidants to reduce copper ions from Cu(II) to Cu(I) resulting in the formation of a copper antioxidant complex that absorbs light at a specific wavelength [14]. *Funtumia africana* demonstrated antioxidant activity across all concentrations, with a slight reduction to 77.11 ± 0.25 at 100 µg/mL in contrast to the ascorbic acid at 88.08 ± 0.56 at the same concentration as presented in Table 4 and Fig. 3. However, at 75 µg/mL, *F. africana* exhibited higher antioxidant activity than the standard, with values of 79.83 ± 0.25 and 77.86 ± 0.84, respectively.

3.4.4 IC_{50} Values of F. africana for free radical scavenging activity

The IC_{50} represents the concentration of a substance required to suppress 50% of the target molecule's activity. It serves as a valuable tool for evaluating the efficacy of drugs, enzymes, or other agents that interact with a biological target. It is important to recognize that the antioxidant activity is more potent and effective when the

IC_{50} value is lower than that of the standard drug [20,21]. The IC_{50} values of *F. africana* for free radical scavenging activity as presented in Table 5 were determined using the linear equations on the graphs in Figs. 1 to 3.

The copper ion reducing assay indicated the strongest inhibition (IC_{50} 1.64 µg/mL) by the *F. africana* extract. However, the standard ascorbic acid exhibited higher antioxidant activity (IC_{50} 1.09 µg/mL). The results highlight the significant antioxidant potential of *F. africana* in scavenging free radicals, which can be attributed to the redox properties of flavonoids. Flavonoids such as quercetin, luteolin and +(-) catechin detected in *F. africana* possess multiple hydroxyl groups and conjugated double bonds in their chemical structures, enabling them to function as hydrogen donors, reducing agents, inhibitors of singlet and triplet oxygen, and even exhibit metal chelation properties [22,23].

3.4.5 HPLC analysis of flavonoids in F. africana leaf extract

The HPLC result revealed the presence of thirty (30) flavonoid compounds as displayed in Plates 1 and 2; while Table 6 and Fig. 4 show the major flavonoid constituents in the plant extract.

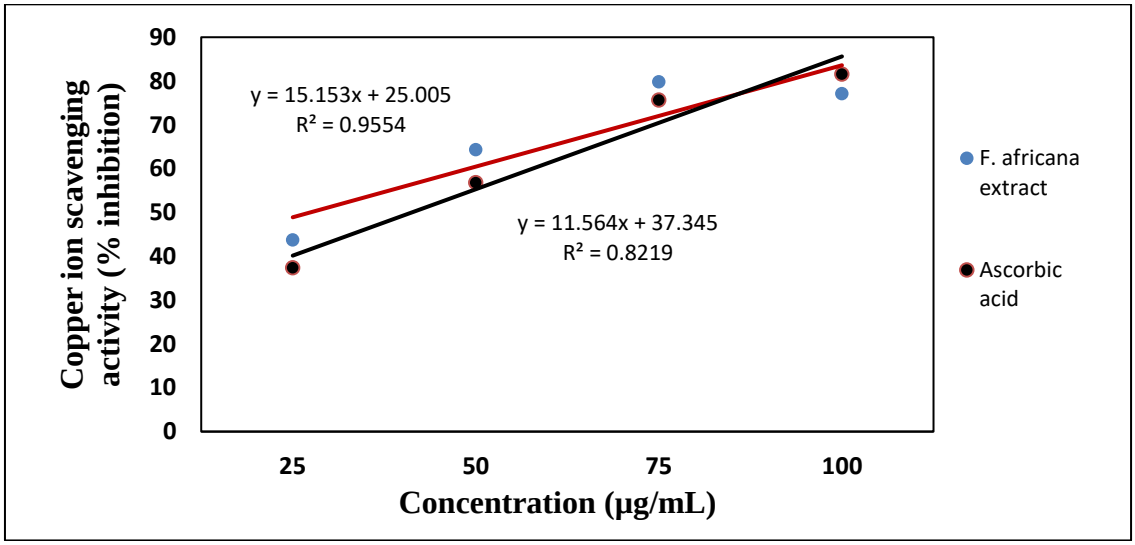

Fig. 3. Copper ion reducing activity of *F. africana* extract and ascorbic acid

Table 5. IC_{50} values of the free radical scavenging activities of F. *africana* extract

	IC_{50} Value (µg/mL)	
Parameters	***F. africana***	**Ascorbic acid**
Hydrogen peroxide scavenging activity	2.69	2.14
Hydroxyl radical scavenging activity	3.26	1.60
Copper ion reducing assay	1.64	1.09

Table 6. HPLC profile of major flavonoid compounds found in *F. africana* leaf extract

S/N	Retention Time (min)	Concentration (mg/100g)	Name of Flavonoids	Class of Flavonoids
1	13.827	37.54569	(+) – Catechin	Flavan-3-ol or Catechin
2	14.509	15.47290	Apigenin	Flavone
3	17.385	30.26511	Luteolin	Flavones
4	17.773	155.67414	Kaempferol	Flavonol
5	18.792	7.46610	(-) - Epicatechin	Flavan-3-ol or Catechin
6	21.209	35.66200	Quercetin	Flavonol
7	27.347	36.00304	Naringin	Flavanone-O-glycoside
8	28.025	3.78699	Hesperidin	Flavanone-O-glycoside
9	28.499	1.52725	Quercitrin	Flavanone-O-glycoside
10	29.008	2.44892	Rutin	Flavonols-O-glycoside

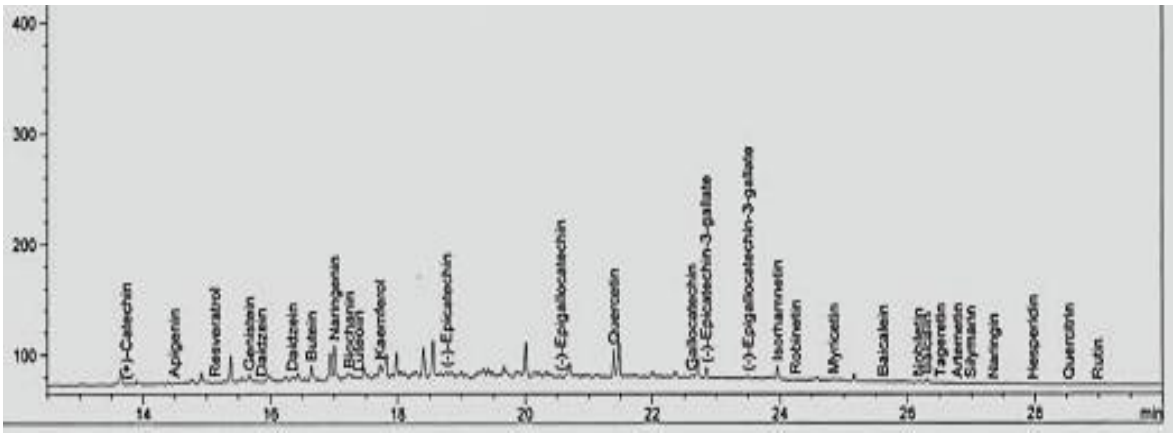

Plate 1. HPLC Chromatogram of *F. africana* leaf extract

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Analysis Method : C:\HPCHEM\1\METHODS\FLAV-S.PRE
Last changed    : 23/01/2023 1:09:40 AM
                  (modified after loading)
FLAVONOIDS ANALYSIS, UV @ 280nm
================================================================================
                        External Standard Report
================================================================================

Sorted By             :      Signal
Calib. Data Modified  :      23/01/2023 1:09:34 AM
Multiplier            :      1.0000
Dilution              :      1.0000

Signal 1: UVD1 A,

RetTime  Type     Area      Amt/Area    Amount    Grp   Name
 [min]          [pA*s]                [mg/100g]
-------|------|----------|----------|----------|--|------------------
 13.827 VV      318.41748 1.17913e-1    37.54569  1 (+)-Catechin
 14.509 VV      214.26875 7.22126e-2    15.47290  1 Apigenin
 15.117 VV       68.67956 1.41227e-7 9.69941e-6  1 Resveratrol
 15.611 VV       97.95615 1.55247e-7 1.52074e-5  1 Genistein
 15.860 VV       91.74443 1.55247e-7 1.42430e-5  1 Daidzein
 16.327 VV       54.48867 1.55330e-7 8.46371e-6  1 Daidzein
 16.643 VV       90.44008 1.55247e-7 1.40405e-5  1 Butein
 16.986 VV       70.79073 3.27938e-6 2.32149e-4  1 Naringenin
 17.094 VV      241.82393 1.61463e-7 3.90457e-5  1 Biochanin
 17.385 VV       87.16352 3.47222e-1    30.26511  1 Luteolin
 17.773 VV       83.44134    1.86567   155.67414  1 Kaemferol
 18.782 VV      252.61703 2.95550e-2     7.46610  1 (-)-Epicatechin
 20.575 VV       76.52641 3.36927e-8 2.57838e-6  1 (-)-Epigallocatechin
 21.209 VV       88.44176 4.03226e-1    35.66200  1 Quercetin
 22.577 VV       87.74737 1.55318e-7 1.36288e-5  1 Gallocatechin
 22.858 VV      142.25549 4.11184e-8 5.84932e-6  1 (-)-Epicatechin-3-gallate
 23.493 VV       70.01635 2.91240e-8 2.03915e-6  1 (-)-Epigallocatechin-3-gallate
 23.904 VV       95.06750 1.30753e-4 1.24304e-2  1 Isorhamnetin
 24.155 VV      147.78094 1.57942e-7 2.33408e-5  1 Robinetin
 24.784 VV       97.71014 2.01613e-7 1.96996e-5  1 Myricetin
 25.585 VV       63.87775 1.54561e-7 9.87303e-6  1 Baicalein
 26.175 VV       68.57063 1.54561e-7 1.05984e-5  1 Nobiletin
 26.335 VV       56.73971 1.55330e-7 8.81336e-6  1 Baicalin
 26.525 VV       91.78819 1.55330e-7 1.42574e-5  1 Tageretin
 26.801 VV       32.53189 8.02445e-2     2.61050  1 Artemetin
 26.988 VV       34.31936 1.54561e-7 5.30445e-6  1 Silymarin
 27.347 VV       44.85690 8.02620e-1    36.00304  1 Naringin
 28.025 VV      122.48642 3.09176e-2     3.78699  1 Hesperidin
 28.499 VV       50.43596 3.02810e-2     1.52725  1 Quercitrin
 29.008 VV       80.38351 3.04655e-2     2.44892  1 Rutin

Totals :                                328.47553
```

Plate 2. HPLC data for the flavonoid compounds found in *F. africana* leaf extract

Among the major compounds (Fig. 4), kaempferol displayed the highest concentration of 155 mg/100g. Other flavonoids that exhibited moderate concentrations are (+)-catechin (37.54 mg/100g), naringin (36.00 mg/100g), quercetin (35.66 mg/100g), luteolin (30.26 mg/100g), apigenin (15.47 mg/100g), (-)-epicatechin (7.46 mg/100g), hesperidin (3.78 mg/100g), quercitrin (1.52 mg/100g), and rutin (2.44 mg/100g). The remaining flavonoids were found to be present in lower concentrations. These compounds were also identified by HPLC profiling in *Triumfetta cordifolia* leaves [16].

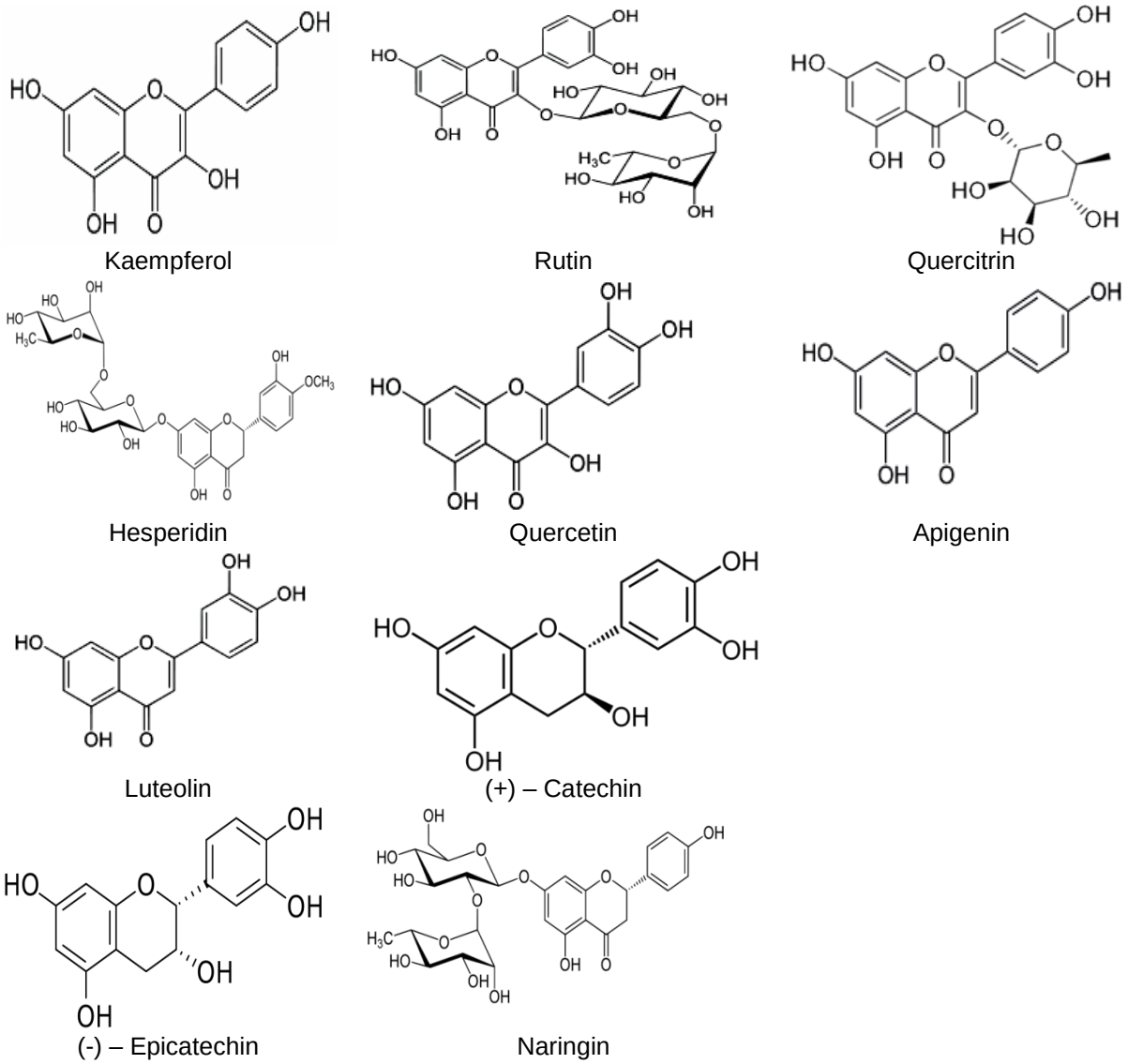

Fig. 4. Structures of some flavonoids present in *F. africana* leaf extract.

4. CONCLUSION

The present study denoted that methanol leaf extract of *F. africana* had significant anti-inflammatory, analgesic and antioxidant properties which can be ascribed to the presence of flavonoids found in the plant as revealed by the HPLC analysis. This research affirms the traditional utilization of the plant as a natural remedy on inflammation, oxidative stress and pain.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s)

COMPETING INTERESTS

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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