OPEN ACCESS

Scholars International Journal of Chemistry and Material Sciences

Abbreviated Key Title: Sch Int J Chem Mater Sci
ISSN 2616-8669 (Print) |ISSN 2617-6556 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: http://saudijournals.com

Original Research Article

HPLC Analysis and Anti-Inflammatory Effect of Methanol Extract of the Leaves of *Triumfetta cordifolia* A. Rich. (Malvaceae) Available in Bayelsa State, Nigeria

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DOI: 10.36348/sijcms.2023.v06i06.001 | **Received:** 30.04.2023 | **Accepted:** 08.06.2023 | **Published:** 04.07.2023

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Abstract

Triumfetta cordifolia is used in traditional medicine to treat inflammation in Bayelsa State, Nigeria, however, the aqueous methanol extract of *T. cordifolia* leaves have not been explored scientifically to confirm this ethno-medicinal claim using animal model. In this study, aqueous methanol extract of *T. cordifolia* leaves was tested for its anti-inflammatory properties and the profiling of flavonoid components using high performance liquid chromatography (HPLC). In vivo anti-inflammatory efficacy was performed utilizing a rat model of formalin-induced paw edema. The extract's anti-inflammatory effectiveness against formalin-induced paw edema revealed notable anti-inflammatory effects. The percentage inhibition of the extract at the dose of 500 mg/kg with 10.69% inhibition was comparable to the standard drug aspirin with 10.69% inhibition while the dose of 100 and 250 mg/kg has higher percentage inhibition (13.58%) in comparison with the standard drug aspirin (10.69%) at same time interval all in the curative measure. The major flavonoid compounds from the HPLC analysis include kaempferol, quercitrin, (+) - catechin, luteolin, quercetin, myricetin, hesperidin, narigin, apigenin and rutin. Thus, it can be suggested that the high content of flavonoids may be responsible for the anti-inflammatory activities exhibited by the methanol extract of *T. cordifolia*. Therefore, the results obtained in this study shows that the methanol leaf extract of *T. cordifloia* possess potent anti-inflammatory activity in acute inflammation.

Keywords: *Triumfetta cordifolia*, flavonoids, anti-inflammatory activity, HPLC.

INTRODUCTION

Plants have played a major role in human life for many centuries. Among plants that are most useful to man are medicinal plants. Medicinal plants are plants frequently employed for treating and preventing a wide range of illnesses and disorders [1]. The utilization of medicinal plants has been driven by the belief that they are effective with minimal side effects, in contrast to modern conventional medications derived from the same plant remedies [2].

In the event of an invasion by hostile agents like pathogenic bacteria, chemical substances, or parasites, or when the body undergoes tissue injury, inflammation is triggered [3]. Several biologically active substances, including alkaloids, terpenoids, curcumin, saponins, phenols, flavonoids, tannins, steroids, and glycosides, can be found in numerous plants. These secondary metabolites are able to inhibit cyclooxygenase (COX) and lipooxygenase (LOX) enzymes involved during inflammatory phase which thereby reduces inflammation [4]. From studies performed so far, flavonoids have been identified as the main inflammatory agents found in plants. These flavonoids have been demonstrated to suppress pro-inflammatory cytokine secretion such as interleukin-6 (IL-6) and boost interleukin-10 (IL- 10) secretion under varied situations [5] and some act as phospholipase inhibitors, which inhibit cyclooxygenase and lipoxygenase pathways [3].

Agents such as aspirin, indomethacin, and glucosaticoid medicines that block the enzyme COX, which is required for prostaglandin formation, are used to treat pain and inflammatory conditions. However, the

prolonged use of these drugs as inflammatory agents has been associated with adverse side effects, including water and salt retention, which makes them unsafe for long-term use [6]. Corticosteroids, a type of anti-inflammatory medicine, can lead to various side effects including immunological suppression, diabetes mellitus/glucose intolerance, hypertension, obesity, osteoporosis, immune suppression, glaucoma, and growth retardation in children [7]. Due to their immense potential in addressing a variety of ailments, the World Health Organization advises the incorporation of herbal medicines with established safety and efficacy into healthcare programs in developing countries [8]. Consequently, there is an increased demand for the creation of anti-inflammatory drugs that are both efficient and devoid of toxicity [4].

Triumfetta cordifolia is a frequently utilized plant in African traditional medicine, where different parts of the plant are employed to treat a diverse range of ailments. *Triumfetta cordifolia* plant extract is said to have exhibited pharmacological activity, including, anti-diarrhoeal, anti-ulcerogenic, anti-diabetic, anti-bacterial, anti-malarial, antifungal, antimicrobial, analgesic, anti-hyperlipemic, cytoxic [9]. The root of *T. cordifolia* is used to cure venereal infections, as well as liver and renal problems; while the fruit can also be macerated in local alcohol or water to treat labor that isn't quite ready to start [10]. In ethnomedicine, the sap obtained from the leafy twigs of *T. cordifolia* is frequently employed to alleviate a variety of conditions such as intestinal problems, hepatitis, rhinitis, diarrhea, diabetes, asthenia, dysentery, ulcerogenic conditions, inflammation, muscle pain, fever, backache, and mental disorders [9, 11].

Despite claims of *T. cordifolia* possessing anti-inflammatory properties, there is a scarcity of literature regarding its specific anti-inflammatory activity in Bayelsa State, Nigeria. For the first time, we examined the anti-inflammatory activity of *T. cordifolia* methanol leaf extract in rats using formalin-induced paw edema in an attempt to validate the presence of this activity.

EXPERIMENTAL METHOD

Plant Materials

Fresh *T. cordifolia* leaves were collected on 10th March 2022 along the Amassoma-Yenagoa road in Bayelsa State, Nigeria. The identification and authentication of the plant were carried out by Prof. K.K. Ajibesin from the Department of Pharmacognosy and Herbal Medicine, Faculty of Pharmacy, Niger Delta University, located in Bayelsa State. The leaves were air-dried under shade for three weeks and thereafter they were grounded to powdered form using plant milling machine. For preservation, the powdered sample was kept in an airtight container.

Extraction of the Plant Extract

The pulverized *T. cordifolia* leaves (420g) was macerated in 50% (v/v) methanol (3L) at room temperature for 72 hours with occasional stirring and shaking. The extract was then filtered into a clean conical flask. The marc was macerated for another 72 hours before being filtered and combined with the initial extract. Using rotary evaporator, the filtrate was concentrated in a vacuum to dryness at (30^{o}C). This produced a dark and sticky solid residue which was kept in the fridge for further analysis. The quantity of the extract obtained was 30.3 g.

Experimental Animals

All experiments were performed using Wistar rats of either sex weighing 76-154g. The animals used in the study were sourced from the Animal House at the Department of Pharmacology and Toxicology, Faculty of Pharmacy, Niger Delta University, located in Bayelsa State and were kept in laboratory cages made of clear plastic under standard environmental conditions. A standard laboratory diet was provided to the animals, and they were given unrestricted access to water. Before the experimentation, the animals were kept and familiarized with the laboratory room condition for at least 1 hour.

Ethical Consideration for the Use of Animals

The animal experimental protocols were in accordance with the university ethical policy concerning the use of animals in research and global standards for the use of animals as outlined in the Principle of Laboratory Animal Care [12].

In vivo Anti-inflammatory Activity

Formalin-induced inflammation test was performed to estimate the anti-inflammatory effect of the extract. It was carried out in two different ways.

Formalin-Induced Paw Oedema Assay (Preventive Measures)

The effect of methanol extract of *T. cordifiolia* on formalin-induced inflammation in rat paw using the preventive measure was conducted in accordance with [13] with slight modifications employing 5.0% formalin solution as the phlogistic agent. Rats were randomly assorted and bodily marked for indication. The rats were divided into five groups randomly, with each group comprising three rats. Group 1, serving as the control group, was administered an oral dose of 0.2 mL/kg of distilled water. Group 2, 3 and 4 were given 100, 250 & 500 mg/kg per b.w. of the *T. cordilolia* extract respectively and Group 5 was administered the standard drug-aspirin (300 mg/kg). All drugs were administered orally. Thirty (30) minutes after oral administration of the test materials each rat in each group had 0.1ml 5% formalin suspension injected into the dorsal surface of its left hind paw, leading to the formation of oedema. The rats were then returned to their individual cages so as to monitor their response to the treatments. The length of time they licked the

injected paw was interpreted as a sign of pain. Paw size of all rats were measured before and after inducing oedema.

Formalin-Induced Paw Oedema Assay (Curative measures)

A total of 15 rats were separated into five groups, each with three rats. All the rats' left hind paws were injected with 0.25 ml of 5% formalin. They were then treated with distilled water (0.2 ml/kg) in group 1 which served as control, group 5 which served as standard was treated with Aspirin (300 mg/kg bw), and 100, 250 and 500 mg/kg of *T. cordifolia* to the extract test groups (2, 3 & 4). The increased volume of the left hind paws was used to diagnose paw edema. The following times were used to evaluate paw size: right before formalin administration, 1 h, 1 h: 30 min, and 24 h after treatment. The paw size at each evaluation time was measured using an improvised technique of meter ruler and thin thread. The formula below was utilized to determine the % inhibition of inflammation (Equation 1):

$$\% \text{ inhibition of inflammation} = \frac{V_c - V_t}{V_c} \times 100$$

Eq 1

Where,

V_c = mean value of the paw size of control group, V_t = mean paw size test group.

V_c and V_t are obtained by taking the differences between the paw size before and after the treatment.

High Pressure Liquid Chromatography (HPLC) Analysis of Flavonoids

HPLC Procedure

The HPLC analysis of the methanol crude extract of *T. cordifolia* leaf was carried out at Cation Analytical and Environmental Research Laboratory in Lagos, Lagos State. It was carried out on an Agilent 1200 Series equipped with a binary pump, an auto liquid sampler (ALS), a thermostat column compartment, a multi wave length UV/VIS detector (MWD), and an online degasser. To obtain a composition profile of the studied matrices, a C_{18} HPLC column with dimensions of 150×4.6mm I.D. and particle size of 3.5 µm was employed. Flavonoid compounds from various classes were selected for separation. The mobile phase consisted of a gradient elution using a mixture of solvent A (Acetonitrile) and solvent B (0.1% Phosphoric acid in water). The flow rate was set at 1 mL/min, and 20 µL of samples and standards were injected. During the analysis, the column temperature remained constant at 25°C, and the spectra were recorded at a wavelength of 280 nm.

Preparation of Working Standards and Sample Solutions

To remove any traces of water, the sample was subjected to a series of oven drying at 105°C in the laboratory until a constant weight was achieved. Preparation of standard solution and sample solution was done using methanol of HPLC grade solvents.

Working Standard

10 mg of the reference compounds were mixed and dissolved in 10 mL of HPLC grade methanol in a 100 mL volumetric flask to obtain a stock solution of 1000µg/mL. This was then used for HPLC analysis.

Sample Solutions

1.0 g of the dry crude extract was weighed into 250 mL conical flask capacity with the addition of 100 mL of distilled water. The mixture was then sonicated for about 10mins to speed up the dissolution of extracts, after which 100 mL of the mixture of boiling methanol and water (70:30) was added to the sample in the conical flask. Approximately four hours were given for the entire mixture to macerate. A 0.45 um syringe filter was then used to filter the mixture before HPLC analysis.

Statistical Evaluation

SPSS version 21.0 was used to analyze the data. The results were reported as mean±standard deviation after descriptive statistics were applied to the data. One-way analysis of variance (ANOVA) was used to identify the statistically significant differences between groups. Significant values of $p<0.05$ were evaluated. The values with the same superscripts had no statistically significant differences between them.

RESULTS AND DISCUSSION

Anti-inflammatory Activity Evaluation

Formalin Induced Paw Oedema in Rats (Preventive measures)

The effect of *T. cordifolia* leave extract on the rat left hind paw after injection with formalin is given in Table 1 below. It is also presented in form of bar graph in Figure 1.

Table 1: Effect of *T. cordifolia* aqueous methanol leave extract on formalin induced paw edema in rats (Preventive measures)

Treatment group	Treatment given	Initial paw size	Paw size (cm)after induction (% Inhibition)		
			30 min	1 h	24 h
Group 1 (control)	Distilled water (0.2 mL/kg)	2.10±0.17[a]	2.37±0.12[a]	2.40±0.20[a]	2.37±0.23[a]
Group 2	*T. cordifolia* extract (100 mg/kg)	2.30±0.10[a]	2.30±0.10[a] (2.10%)	2.30±0.26[a] (4.17%)	2.43±0.21[a] (-2.53%)
Group 3	*T. cordifolia* extract	2.00±0.20[a]	2.30±0.00[a]	2.50±0.00[a]	2.27±0.06[a]

Treatment group	Treatment given	Initial paw size	Paw size (cm)after induction (% Inhibition)		
			30 min	1 h	24 h
	(250 mg/kg)		(2.10%)	(-4.17%)	(4.22%)
Group 4	*T. cordifolia* extract (500 mg/kg)	2.06±0.15[a]	2.17±0.58[b] (8.44%)	2.33±0.38[a] (2.92%)	2.20±0.26[a] (7.17%)
Group 5 (standard)	Aspirin (300 mg/kg)	2.03±0.21[a]	2.13±0.06[b] (10.13%)	2.10±0.10[a] (12.5%)	2.17±0.23[a] (8.44%)

Data are means ±standard deviation (n=3); means with different superscript alphabets on same column differ significantly (P<0.05) compared to control group. There were no statistically significant differences among values with the same superscripts.

The 500 mg/kg significantly reduced the paw edema size (P<0.05) relative to the normal distilled water (control). In the first 30 minutes, the paw edema was high in all the three doses as shown in Table-1. The dose 500 mg/kg showed a significant effect (P<0.05) against the normal distilled water (control), hence higher activity than the dose of 100 and 250 mg/kg at the first 30 minutes. Doses 100 and 250 mg/kg showed no significant effect (P>0.05) at thirty minutes, one hour and twenty four hours interval as compared to the normal distilled water (control) at same time intervals. Consequently, both the 100 mg/kg and 250 mg/kg doses did not exhibit any significant effect.

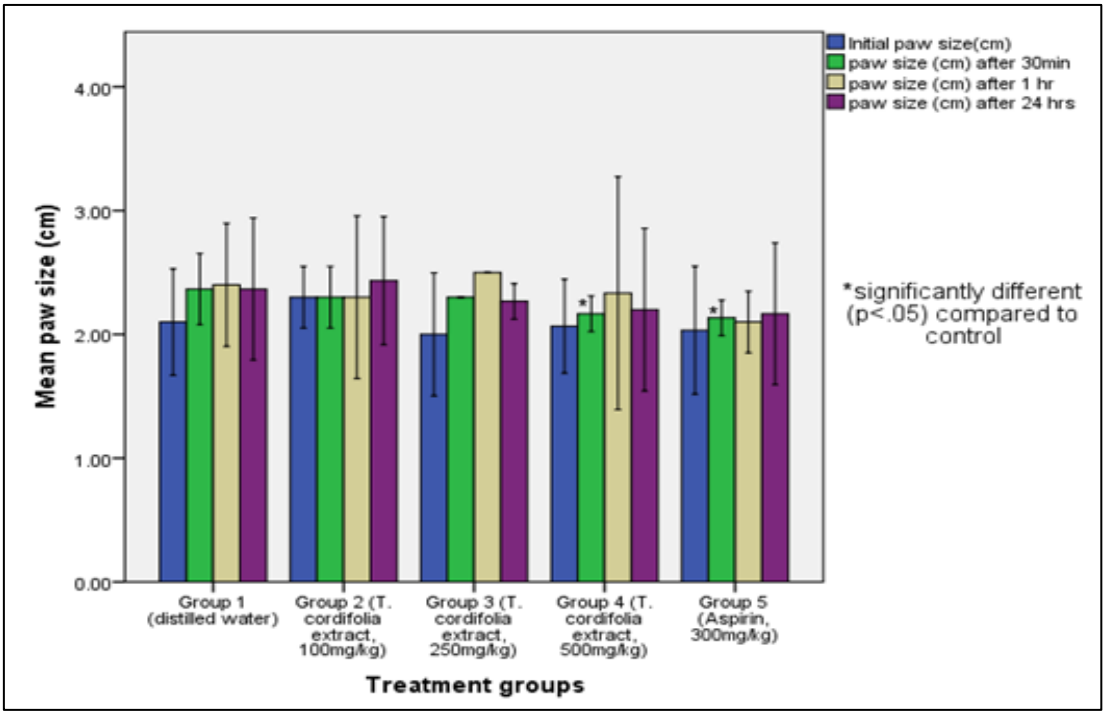


Fig. 1: Anti-inflammatory effects of *T. cordifolia* leave extracts (Preventive measures)

The % inhibition was calculated as an indicative of anti-inflammatory potency. *Triumfetta cordifolia* extract administered at a dose of 500 mg/kg, showed 8.44%, 2.92% and 7.17% inhibition at 30 min-24 h respectively (Table 1). At this dose, the extract showed considerable anti-inflammatory effect especially at 30 minutes and 24 h in different percentages of inhibition but the maximum percentage edema inhibition was observed at 30 minutes (8.44%). At the dosage level of 100 and 250 mg/kg *T. cordifolia* extract did not show significant anti-inflammatory effect at 30 min-24h (Table 1). The percentage inhibition of dose 100 mg/kg at the 24 hr (-2.53%) and 250 mg/kg *T. cordifolia* extract at 1hr (-4.17%) did not demonstrate anti-inflammatory activity as it has gave negative percentage inhibition. Among all the test doses, it was only 500 mg/kg (7.17% inhibition) that was comparable to the standard drug aspirin (8.44% inhibition) at 24 h. This dose also showed comparable effects to aspirin (300 mg/kg) in reducing inflammation at the 30 minutes and 24 hours (Table 1). At all time intervals, the extract administered at a dose level of 500 mg/kg body weight displayed the highest level of anti-inflammatory activity.

In the current study, employing preventive measures, it was revealed that the lower dose levels of 100 and 250 mg/kg body weight of the extract were not as effective as the higher dose level of 500 mg/kg body weight. The reason behind this could be attributed to the

swift metabolism and elimination of the active compound(s) present in insufficient amounts within the lower dosage levels of the extract [14]. Additionally, it is possible to infer that the extract displayed its strongest anti-inflammatory activity at a dosage of 500 mg/kg b.w. because it contained a significant concentration of the active principle(s).

From the results obtained from the preventive measures of acute inflammation demonstrated by formalin induced paw edema, it is possible to deduce that leaf of extract of *T. cordifolia* gave moderate anti-inflammatory effect.

Formalin Induced Paw Oedema in Rats (Curative measures)

The effect of *T. cordifolia* leave extract on the rat left hind paw after treatment with extract is given in Table 2 below. It is also presented in form of bar graph in Figure 2.

Table 2: Effect of *T. cordifolia* methanol leaf extract on formalin-induced paw edema in rats (Cuarative measures)

Treatment group	Treatment given	Initial paw size	Paw size after induction	Paw size (Mean±SD) after treatment (% inhibition)		
				1 h	1h: 30 min	24 h
Group 1 (control)	Distilled water (0.2 mL/kg)	2.13±0.06^{a}	2.47±0.58^{a}	2.53±0.15^{a}	2.43±0.06^{a}	2.53±0.06^{a}
Group 2	*T. cordifolia* extract (100 mg/kg)	2.06±0.12^{a}	2.43±0.21^{a}	2.20±0.10^{b} (13.04%)	2.10±0.10^{b} (13.58%)	2.30±0.26^{a} (9.09%)
Group 3	*T. cordifolia* extract (250 mg/kg)	2.10±0.00^{a}	2.30±0.10^{a}	2.33±0.06^{a} (7.91%)	2.10±0.00^{b} (13.58%)	2.27±0.23^{a} (10.28%)
Group 4	*T. cordifolia* extract (500 mg/kg)	2.17±0.06^{a}	2.33±0.06^{a}	2.27±0.06^{b} (10.28%)	2.17±0.12^{b} (10.69%)	2.37±0.15^{a} (6.32%)
Group 5 (standard)	Aspirin (300 mg/kg)	2.06±0.15^{a}	2.27±0.21^{a}	2.30±0.20^{b} (9.09%)	2.17±0.15^{b} (10.69%)	2.20±0.00^{b} (13.04%)

Data are means ± standard deviation (n=3); means with different superscript alphabets on the same column differ significantly (P<0.05) compared to the control group

Formalin induced paw edema in rats is one of the most suitable test procedure to screen the acute inflammation and it is believed to be a biphasic event.

The doses of 100 and 500 mg/kg of the extract had a statistically significant effect (P<0.05) when compared to normal distilled water (control) at 1 h and 1hour 30 minutes. Dose 100 mg/kg showed maximum suppression of the paw edema when compared to other two dosages of the leaf extract of *T. cordifolia* (Table-2 and Figure 2). At 1 hour and 1 hour 30 minutes dose of 100mg/kg exhibited a reduction more than aspirin (standard) at the same time interval (Table 2 and Fig 2). Dose 500 mg/kg also reduced the inflammation more than aspirin at 1 hour after treatment (Table 2). However, a dose of 250 mg/kg at 1hr does not have an effect on edema, but at 1 hour 30 minutes reduced edema even more significantly than aspirin. The presence of phytochemicals like flavonoids, which suppress cyclooxygenase synthesis, is believed to be responsible for the effect [15].

Triumfetta cordifolia extract at a dose of 100 mg/kg prevented paw edema with a percentage inhibition of 13.04%, 13.58% and 9.09% at 1h, 1h: 30 min and 24h respectively while the dose at 500 mg/kg showed percentage inhibition of 10.28%, 10.69% and 6.32% respectively at same time intervals (Table 2). *T. cordifolia* extract administered at a dose of 250 mg/kg, showed 7.91%, 13.58%, and 10.28% inhibition at 1h, 1h: 30 min and 24 h respectively. The maximum anti-inflammatory effect of the extract was recorded at the doses of 100 and 250 mg/kg with 13.58% inhibition at 1 h: 30 min which was higher than the standard drug aspirin with 10.69% inhibition at same time interval. The result indicates that all test doses of the extract showed significant anti-inflammatory activity in all-time points in various inhibition percentages. The difference between these three doses is due to more active metabolites that increase with concentration [13]. The standard drug aspirin at 300 mg/kg dose demonstrated the highest anti-inflammatory effect with 13.04% inhibition at the 24 h.

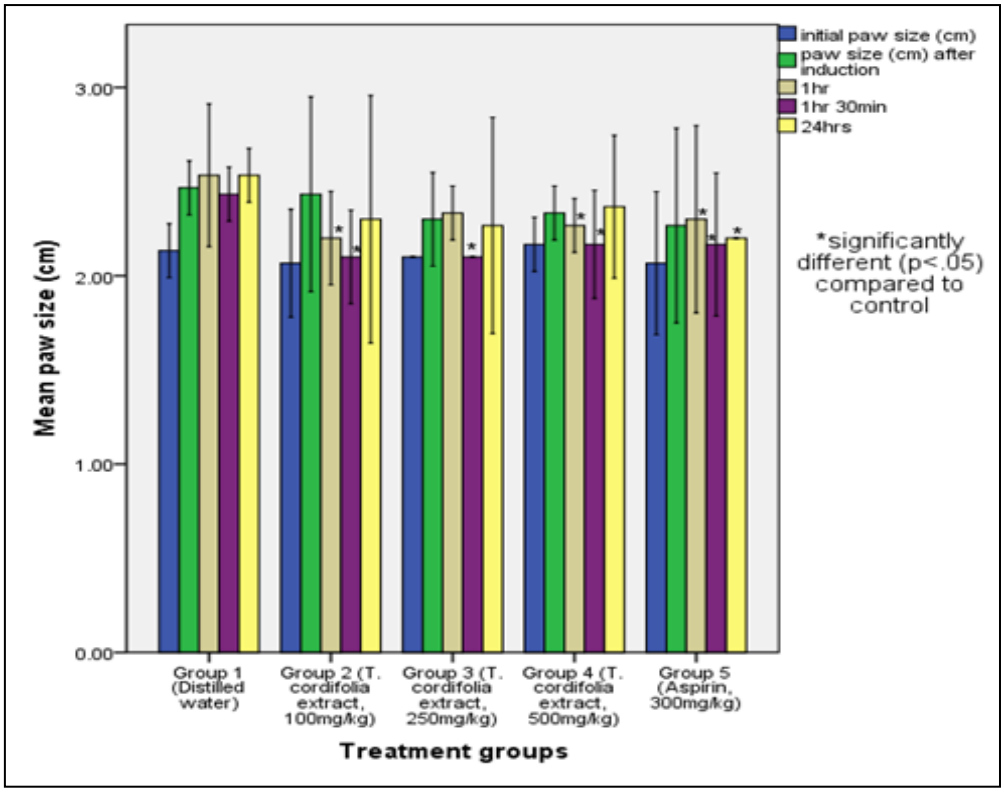


Fig. 2: Anti-inflammatory effects of *T. cordifolia* leaf extract (Curative measures)

From the results, it could be suggested that the decrease in paw size when treated with the aqueous methanol leaf extract of *T. cordifolia* means it may have better pharmacokinetics property than aspirin.

Studies have observed that among the various bioactive compounds derived from plants, flavonoids are the predominant ones known for their role as anti-inflammatory agents. Their effectiveness stems from their ability to inhibit the lipoxygenase and cyclooxygenase pathways [3, 15, 16]. Flavonoids from plants exhibit almost similar activities to the NSAIDS that are used for inflammatory diseases [17]. Despite *T. cordifolia* being known for its anti-inflammatory properties, there has been a lack of research exploring its potential as an inflammatory agent. However, the findings of this study demonstrate that the methanol leaf extract of this plant has the potential to be an effective anti-inflammatory agent.

HPLC Analysis of Flavonoids

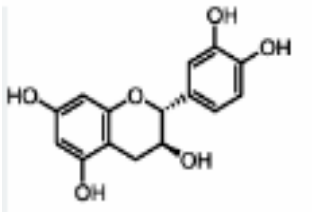
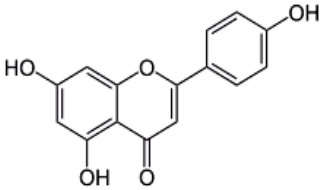
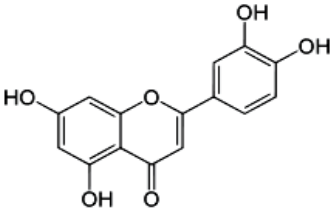
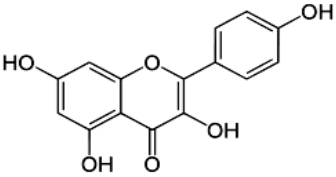
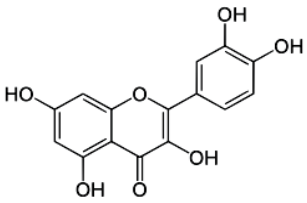
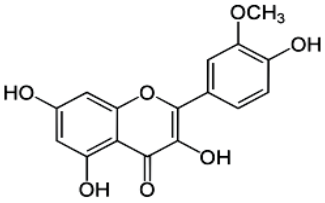
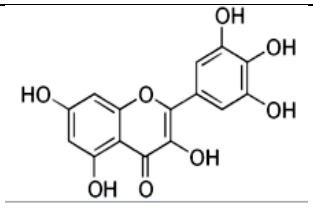
HPLC fingerprinting of the ethnomedicinal plants provided a quick analysis of the compounds present in crude drug [18]. It is by far the most popular technique for flavonoid analysis nowadays.

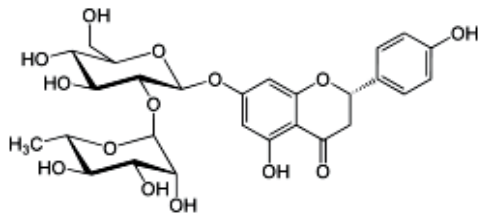
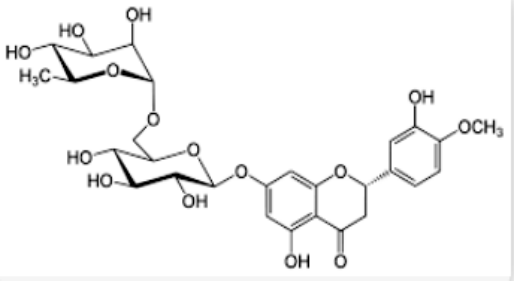
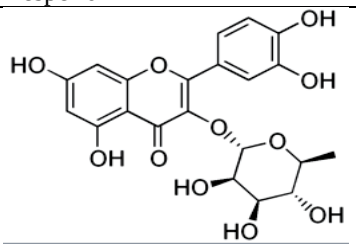
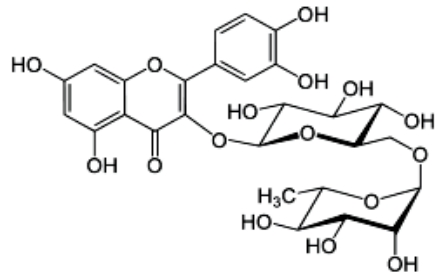
To ascribe the observed anti-inflammatory action to potential phytochemicals, an HPLC analysis of the crude methanolic extract of *T. cordifolia* leaf was carried out. The data obtained from the analysis shows that thirty (30) flavonoid compounds with distinct retention times were identified in the leaves of *T. cordifolia* and HPLC chromatogram. The retention times, name of flavonoid compounds and concentrations of various flavonoids identified in this study are depicted in the Figure 3 and Table-3.

From Table 3 and Figure 3, the HPLC analyses also showed that kaempferol (209.04789 mg/100g) was the major flavonoid compound, followed by quercitrin (63.64447 mg/100g), (+) catechin (48.04014 mg/100g), luteolin (43.25836 mg/100g), quercetin (41.48706 mg/100g), apigenin (28.58160 mg/100g), rutin (12.81232 mg/100g) and myricetin (9.34754 mg/100g). In addition, trace amounts of hesperidin (2.63595 mg/100mg) and narigin (1.82465 mg/100mg) were detected among the flavonoid substances. Silymarin, epicatechin, tangeretin, artemetin, baicalin, nobiletin, robinetin, baicalein, biochanin, (-) epigallocatechin-3-gallate, butein, gallocatechin, isorhamnetin, (-) epicatechin, (-) epicatechin-3-gallate, daidzein, naringenin, genistein, and resveratrol were also present, but in significantly lower concentrations.

One of the more interesting findings in this study regarding the phytochemical content of *T. cordifolia* leaves is that they are relatively high in the flavonols and flavones. The identified flavonoid compounds possess many biological properties. Findings from literature show that most of the observed flavonoids exhibit anti-inflammatory effects.

Table 3: Identification and quantification of the major flavonoid compounds in methanol leaf extract of *T. cordifolia* using HPLC technique

S/N	Retention Time (R_{time}) (Min)	Concentration (mg/100g)	Flavonoid Compounds	Class of Flavonoids
1	13.826	48.04014	 (+) – Catechin	Catechin
2	14.510	28.58160	 Apigenin	Flavones
3	17.383	43.25836	 Luteolin	Flavones
4	17.774	209.04789	 Kaempferol	Flavonols
5	21.153	41.48706	 Quercetin	Flavonols
6	23.915	1.00951	 Isorhamnetin	Flavonols
7	24.791	9.34754	 Myricetin	Flavonols

S/N	Retention Time (R_{time}) (Min)	Concentration (mg/100g)	Flavonoid Compounds	Class of Flavonoids
8	27.352	1.82465	 Naringin	Flavanone-O-glycoside
9	28.028	2.63595	 Hesperidin	Flavanone-O-glycoside
10	28.494	63.64447	 Quercitrin	Flavonol-O-glycoside
11	29.012	12.81232	 Rutin	Flavanol-O-glycoside

According to several researchers, kaempferol has been associated with significant benefits in reducing inflammation [19]. Kaempferol exerts its anti-inflammatory effects through a range of mechanisms, which involve inhibiting the release of IL-6, IL-1B, and tumor necrosis factor-alpha (TNF-alpha), as well as blocking the toll-like receptor 4 (TLR4) [21]. Furthermore, *in vitro* studies conducted by researchers have indicated that kaempferol exhibits a significant inhibitory effect on both COX-1 and COX-2 enzymes [22]. In other words, kaempferol suppresses cyclooxygenase enzymes and so avoids inflammation.

Studies have highlighted the crucial roles of quercetin and quercitrin in inflammation. Quercetin, in particular, has been utilized as a dietary supplement and shows potential benefits in combating various diseases [23]. Quercitrin, a glycoside of quercetin known for its antioxidant properties, demonstrates superior absorption compared to other quercetin forms [24]. One of the most notable aspects of quercetin is its capacity to control inflammation. Quercetin has demonstrated notable reductions in the levels of inflammatory mediators, including NO synthase, COX-2, and C-reactive protein (CRP), in preclinical in vitro experiments conducted on human hepatocyte-derived cell lines [25].

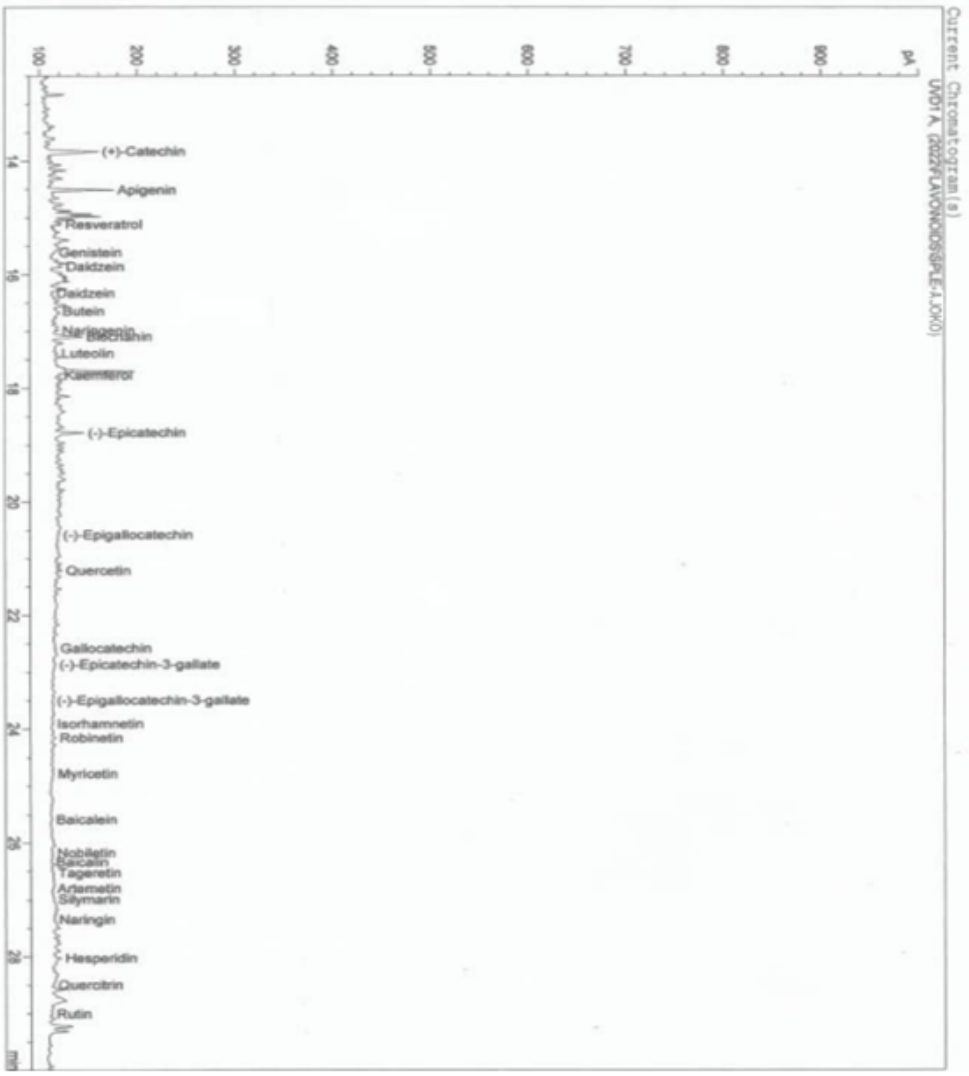


Fig. 3: HPLC Chromatogram of methanol leaf extract of *T. cordifolia*

In rats, quercetin at a dose equivalent to 80 mg exhibited significant anti-inflammatory effects, suppressing both acute and chronic inflammation, and demonstrated considerable efficacy in alleviating symptoms of adjuvant-induced arthritis [26, 27]. By inhibiting the inflammatory enzymes cyclooxygenase (COX) and lipooxygenase, quercetin reduces the levels of inflammatory mediators such as prostaglandins and leukotrienes [28, 29]. Studies have also indicated that catechin likely has a role to play in the activity of reducing inflammation [30].

In addition, rutin, a flavonol-O-glycoside, has demonstrated promising anti-inflammatory activity [31]. Apigenin is reputed to exhibit a broad spectrum of biological effects, encompassing antioxidant and anti-inflammatory actions among others [32].

The considerable abundance of flavonoids in the methanol crude extract of *T. cordifolia* may serve as the primary factor contributing to its anti-inflammatory activity.

CONCLUSION

The methanol extract of *T. cordifolia* leaves exhibited significant anti-inflammatory activity. The anti-inflammatory effect exhibited by the leaf extract could be ascribed to the presence of flavonoid compounds. From the results it can be concluded that this work is the first report dealing with the identification of flavonoid compounds in methanol extract of *T. cordifolia* leaves using HPLC technique. In the current study, HPLC analysis revealed the presence of thirty (30) flavonoid components in the methanol extract of *T. cordifolia*. The presence of these various flavonoid compounds validates the plant's usage in inflammatory treatment by traditional practitioners in Nigeria. The study also reveals that *T. cordifolia* has potential therapeutic importance and hence could be used in the treatment of inflammation.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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