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Anti-Inflammatory Potential of Ethanol Extract of *Feretia Apodanthera Delile* Root Bark and its Fractions and Identification of Their Bioactive Components

Oluwayinka Olufunmilayo Owolabi[1,2,3], Dorcas Bolanle James[2], William Chintem[2], Peculiar Okoro[3]

[1]Bioresources Development Centre, National Biotechnology Development Agency, Yar'adua way, Lugbe, Airport Road, Abuja FCT, Nigeria.

[2]Biochemistry Department, Faculty of Science, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

[3]Basic Research Department, National Research Institute for Chemical Technology, Zaria, Kaduna State, Nigeria.

Research Article

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***For Correspondence**

Oluwayinka Olufunmilayo Owolabi, 1Bioresources Development Centre, National Biotechnology Development Agency, Yar'adua way, Lugbe, Airport Road, Abuja FCT, Nigeria.

E-mail: oluwayinka.owolabi1@gmail.com

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ABSTRACT

Recent research using standard inflammation models have provided the evidence that *Feretia apodanthera* possess potent anti-inflammatory activities. *Feretia apodanthera del*. is used by traditional healers in some part of the world such as Nigeria to treat infective wounds and wound healing has been linked with acute inflammation via biochemical mediators. In this research the active components responsible for the anti-inflammatory effect of *Feretia apodanthera*, a plant from the family Rubiaceae, was determined. The root of the plant was collected from its natural habitat; the bark was scraped out, homogenized and a crude ethanol root bark extract was prepared. The median lethal dose (LD50) of the ethanol extract was determined and its fractions were obtained by subjecting the extract to thin layer and column chromatography. The anti-inflammatory activity of the fractions of the ethanol extract was determined using carrageenan induced right hind paw edema of albino rat model. The most potent ethanol fractions were analysed using FTIR and GCMS to determine their bioactive components. The lethal dose (LD50) of the ethanol extract was found to be greater than 5,000 mg/kg body weight showing relative safety; nine column chromatography fractions were obtained from the extract. Of all the fractions, 5, 8 and 9 were found to be most effective against carrageenan induced edema. The FTIR analysis in the most effective fractions showed the presence of functional groups possessed by ketones, carboxylic acids, alkanes, amines, ethers, ethenes and aromatic groups. The GCMS analysis of the fractions showed the presence of 9-Octadecenoic acid methyl esters and its derivatives as well as other compounds that are of anti-inflammatory effect such as corticosteroids and tetracosanol.

LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
COX	Cyclooxygenase
FTIR	Fourier transform infrared
GCMS	Gas chromatography mass spectrometry
iNOS	inducible nitric oxide synthase
IL	Interleukin
KBr	Potassium bromide

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LD50	Median lethal dose
NARICT	National Research Institute for Chemical Technology
NF-κβ	Nuclear factor kappa β
OECD	The Organization for Economic Cooperation and Development
RT	Retention Factor
SPSS	Statistical Package for the Social Sciences
STC	Shimadzu Training Centre
TLC	Thin layer chromatography
TNF α	Tumour Necrosis Factor Alpha

INTRODUCTION

Feretia apodanthera Del, known as kuru-kuru (Hausa/Fulani - Northern Nigeria) is from the family Rubiaceae. It is a bushy shrub that can grow up to 6 metres high and is found in a variety of locations in the savannah bushland and woodland. It is distributed along tropical Africa including Mauritania to Nigeria and across the Congo basin to Sudan and East Africa. The plant is myremcophyllous, being sometimes found on ant-hills [1]. In addition to feretoside and gardenoside, other iridoids that have been isolated from stem and root barks are geniposidic acid 1 and 10–desacetylasperulosidic acid 2, 11–methyl ixoside 3, apodanthoside 4 and 10–ethyl apodanthoside 5 [2]. Coulibaly [3] established the presence of phenols, flavonoids and β-carotene such as harman and tetrahydroharman in Feretia apodanthera [4]. Research has shown that the aqueous extract of *F. apodanthera* contained flavonoids, alkaloids, saponins, tannins, glycosides, anthraquinones, and phenols. The bark, root and leaves have previously been used by traditional folks to treat infective wounds, gastrointestinal problems such as nausea, stomach upset and some infections of the renal and urinary systems. It has also been used to correct cognitive malfunctions and to improve erection dysfunction in the penis [5].

Inflammation is described as a metabolic imbalance towards catabolism due to tissue injury resulting in its classification as a pathological process and not just a defensive reaction [7,6]. It serves to isolate and eliminate infectious agents, induce repair and give protective response. Inflammation is diverse, ranging from the healing process after physical injury and infection; to the acute inflammation associated with S. aureus infection of the skin; through to chronic inflammatory processes resulting in remodeling of the artery wall in atherosclerosis; the bronchial wall in asthma and chronic bronchitis; and the debilitating destruction of the joints associated with rheumatoid arthritis [8]. These processes involve the major cells of the immune system, including neutrophils, basophils, mast cells, T-cells, and B-cells [9,10]. Inflammatory responses can be triggered by a lot of factors including injury, foreign bodies among others. Feretia apodanthera has been shown to control inflammation due to its ability to reduce the activity of nuclear factor kappa β and nitric oxide which play significant roles in inflammation [11]. This study is to determine the active component responsible for the anti-inflammatory activity of *Feretia apodanthera*.

Fourier transform infrared (FTIR) and gas chromatography coupled with mass spectrometry (GCMS) are methods used in identifying functional groups and unknown compounds present in a sample. Fourier Transform InfraRed (FTIR) method applies the principle of infrared spectroscopy while Gas chromatography–mass spectrometry (GC-MS) pools together the features of gas-chromatography and mass spectrometry to detect distinct molecules in the test sample.

MATERIALS AND METHODS

Plant material collection and identification

Feretia apodanthera Del roots were harvested from its natural habitat at a local farm at Magami village, Gusau Local Government Area, Zamfara State, Nigeria (Latitude: 12.1702 Longitude: 6.6641). Identification and authentication was done at the herbarium located at Biological Sciences department, Faculty of Science, Ahmadu Bello University, Zaria, Nigeria and the voucher number 930 was deposited for the specimen.

Experimental animals

In this study, sixty-two (62) albino wistar rats of either sex were obtained from the Animal house of the National Institute for Trypanosomiasis Research, Kaduna and their weight was between 100g and 200g. The rats were housed at room temperature, humidity of 50% to 60% in polypropylene cages with paddy house bedding for 14 days to allow acclimatization prior to experimentation. All procedures were approved by the Scientific and biochemical ethics committee for the Faculty of Science, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. The animals were provided with water ad libitum and laboratory chow.

Determination of Median Lethal Dose (LD50) for the Crude Extract

The mean lethal dose of ethanol root bark extract of *Feretia apodanthera Del* was determined in albino rats (weighing 150g-200g) after two weeks of acclimatization using the methods described by Lorke, 1983. A total of thirteen rats were used in the two

phases of this study. At the initial step, 3 groups with 3 rats each, making a total of nine animals was used. Groups 1, 2 and 3 rats had a single dose of 10, 100 and 1000 mg/kg body weight (b.w.) of the extract administered to them respectively to determine the range of doses that may produce toxic.

In the second phase, another set of 4 rats were each given doses of 1200, 1600, 2900 and 5000 mg/kg body weight each of the extract respectively to ascertain the right LD50 value. Extracts were dissolved in saline water solution and administered orally [12,13].

Partial Purification of Ethanol Extract

Thin layer chromatography and column chromatography techniques were used to partially purify the crude ethanol extract of *feretia apodanthera*. A preliminary TLC was carried out on the crude ethanol extract to determine the best solvent system that will give the highest number of distinct band for components. Pre-coated aluminum chromatographic plate with silica gel was used as the stationary phase. The crude ethanol extract was dissolved in ethanol and applied several times on the plates using a micro haematocrit capillary tube until the quantity loaded was adjusted sufficiently for the experiment. As a follow up to the TLC plate result, the solvent system of ratio 3:2 of n-hexane and acetone respectively was used as the eluent for column chromatography because it gave the highest separation bands. The column was conditioned using n-hexane to pack the silica gel of 50-200µm mesh size. The ethanol crude extract (2.0g) of *F. apodanthera* was emptied into a porcelain mortar, 5g of silica gel was added and a pestle was used to macerate the mixture to homogeneity, to prevent pack on one side of the column. The mixture was carefully packed on top of the column chromatography to partially separate the compound(s) responsible for the highest anti-inflammatory activity. The column was then eluted with the solvent system (graded) solution of % (v/v) Acetone in n-Hexane, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100% (v/v) and the column was finally washed with ethanol. Approximately 50ml/18min flow rate was maintained. The collected elutes (fractions) were evaporated to near dryness. Each of the fractions was weighed and spotted on TLC plate. The developed fraction was then viewed under UV radiation (λ=254 nm and 365 nm). The plate was further sprayed with 20% of concentrated sulfuric acid in methanol solution and heated for 15 minutes at 110°C [14]. This TLC procedure enabled similar fractions to be pooled together based on their profile and Rf values of the spots on the TLC plate into main fractions respectively. The Rf values was calculated using the formula below:

Rf =Dc /Ds

Where Dc is distance travelled by component and Ds is distance travelled by solvent front

The combined yield of fractions with the same Rf value and combined yield as percentage (%) of the total crude fraction fractionated were calculated. These intermediate pooled fractions were kept in a refrigerator for further in-vivo anti-inflammatory analysis.

Induction of Oedema

Anti-inflammatory test on carrageenan induced hind paw inflammation was carried out on the Ethanol extract and fractions using a modified method of Kataki [15]. The experimental animals were fasted overnight before edema induction but water was available ad libitum.

Weights of rats were taken and rats were separated into 12 groups consisting of 4 each. The extract and fractions were administered orally to the animals in their designated groups at 50mg/kg body weight one hour before carrageenan injection. The standard drug was administered to the Standard group while the inflammation control group was not treated with any drug or extract. Sub-plantar injection of 0.1ml of 1% carrageenan in distilled water was introduced into the right hind paws of the animals to induce acute paw edema [16,17]. Paw edema (expressed as an increase in paw volume in mm) was measured using a digital caliper. Animals were deprived of water during this experimental period to reduce variability and ensure uniform hydration as edema is developed. The animals were grouped as follows:

Group A: Inflammation Control. Rats were induced with 0.01ml of 1% carrageenan in distilled water (IC1).

Group B: Standard Control. Rats were induced with 0.1ml of 1% carrageenan in distilled water and treated with 10mg/kg of standard drug Ketoprofen (IS).

Group C: Rats were treated with 50mg/kg of the Fraction 1 of ethanol extract of Feretia apodanthera and induced with 0.1ml of 1% carrageenan (IF1).

Group D: Rats were treated with 50mg/kg of the Fraction 2 of ethanol extract of Feretia apodanthera and induced with 0.1ml of 1% carrageenan (IF2).

Group E: Rats were treated with 50mg/kg of the Fraction 3 of ethanol extract of Feretia apodanthera and induced with 0.1ml of 1% carrageenan (IF3).

Group F: Rats were treated with 50mg/kg of the Fraction 4 of ethanol extract of Feretia apodanthera and induced with 0.1ml of 1% carrageenan (IF4).

Group G: Rats were treated with 50mg/kg of the Fraction 5 of ethanol extract of Feretia apodanthera and induced with 0.1ml of 1% carrageenan (IF5).

Group H: Rats were treated with 50mg/kg of the Fraction 6 of ethanol extract of Feretia apodanthera and induced with 0.1ml of 1% carrageenan (IF6).

Group I: Rats were treated with 50mg/kg of the Fraction 7 of ethanol extract of Feretia apodanthera and induced with 0.1ml of 1% carrageenan (IF7).

Group J: Rats were treated with 50mg/kg of the Fraction 8 of ethanol extract of Feretia apodanthera and induced with 0.1ml of 1% carrageenan (IF8).

Group K: Rats were treated with 50mg/kg of the Fraction 9 of ethanol extract of Feretia apodanthera and induced with 0.1ml of 1% carrageenan (IF9).

Group L: Rats were treated with 50mg/kg of the crude ethanol extract of Feretia apodanthera and induced with 0.1ml of 1% carrageenan (IC2)

The volume of right hind paw was measured at 1st, 2nd, 3rd, 4th and 5th hours after carrageenan injection. Increase in paw thickness and percent inhibition was calculated as below;

Increase in Paw Volume = vt - vo

Where;

Vt = Paw Volume at Time t

V0 = Paw Volume at Time 0

% Paw *Volume inhibition = vc - vT/vc X100*

Where;

Vc = Paw Volume Increase in Control Animals

VT = Paw Volume Increase in Treated Animals

Characterization of the Bioactive Component by FTIR and GCMS

Fourier transform infra-red spectroscopy analysis

The FTIR spectra of the fractions that gave the best inhibitory activity against inflammation were carried out using FTIR-8400S spectrophotometer (Shimadzu). Two potassium bromide discs held a thin film of the crude ethanol and ethanol fraction paste respectively and mounted in the FTIR spectrometer [Table 1]. Absorption is written in terms of wave length (units cm-1).

Gas chromatography mass spectroscopy analysis

Ethanol fractions with the highest anti-inflammatory potential were analysed using GC-MS to determine the compounds present. 1µl of each sample was injected into a GC-MS (model GC-MS-QP-2010SE, Shimadzu) equipped with RTX-5 column (60 m X 0.25 mm i.d., film thickness 0.25µm). Helium gas was used as carrier gas with an inlet pressure of 108 kpa and a constant column flow rate of 1.58 ml/min. The temperature was programmed to maintain a range of 100 °C to 200 °C with consistent rise of 5 °C/ min and thereafter maintained at a constant temperature of 200 °C for 6 minutes; later, the temperature was elevated up to 290 °C at the rate of 10 °C/min and at that point maintained a constant temperature of 290 °C for another 10min. The ion source and injector temperatures were 250 °C and 270 °C, respectively. 2 mg/ml of each fraction with the highest anti-inflammatory activities were dissolved in ethanol and n-hexane (HPLC grade, Merck, India) respectively and were injected for analysis. The Spectra was taken at 70 eV; at scan interval of 0.5 seconds and fragments from 40 to 800 Dalton and matched using the 2011 National Institute of science and Technology (NIST) library.

Statistical Analysis

The results were expressed as mean ± SD. Analysis of variance (ANOVA) was used to analyse data using SPSS (version 20.0 SPSS Inc. Chicago, IL, USA). Duncan Multiple Range Test was used to determine if the difference between the action of the different extracts across the animal groups were significant or not. P values less than 0.05 was considered significant ($p<0.05$).

RESULTS AND DISCUSSION

Acute toxicity (LD50) of Ethanol Extract of Feretia apodanthera

No death was recorded after the extract was administered, even at doses up to 5000mg/kg. The Organization for Economic Cooperation and Development (OECD), Paris, France, recommended chemical labelling and classification of acute systemic toxicity based on oral median lethal dose values as: very toxic if ≤ 5 mg/kg, toxic if > 5 mg/kg but ≤ 50 mg/kg, harmful if > 50 mg/kg but ≤ 500 mg/kg, and non-toxic or not harmful if > 500 mg/kg or ≤ 2,000 mg/kg [14]. Based on this classification, this result suggests that the ethanol extract of *Feretia apodanthera* was relatively safe for oral ingestion .ie. with a LD50 greater than 5,000 mg/kg. LD50 is a useful index in assessing the safety margin of a substance although it may not accurately reflect the full spectrum of toxicity or hazard associated with drug or chemical [18,19].

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Table 1: Fraction yield from crude ethanol extract of *F. apodanthera*

Fraction number	Combined Fraction(s)	Solvent System	Yield (%)	Rf Value
FA1	F3	nH 4:1 A	20.5	0.45
FA2	F4, F5	nH 4:1 A	7	0.8
FA3	F6	nH 7:3 A	3.5	0.62
FA4	F7, F8, F9, F10, F11	nH 7:3 A; nH 3:2 A	10	0.78
FA5	F12, F13, F14	nH 3:2 A	3.5	0.77
FA6	F15, F16	nH 1:1 A	3.5	0.69
FA7	F17, F18, F19, F20	nH 1:1 A	4	0.66
FA8	F21	100% ETH	3.5	0.12
FA9	F22, F23, F24	100% ETH	11	0.07
Discard	F0, F1, F2, F25, F26			

nH=n-Hexane, A=Acetone, ETH=Ethanol

Partial Purification of Ethanol Extract

The n-Hexane and acetone mixture of ratio 3:2 was found to be most suitable for partial purification as seen in Figure 1(a), it gave six distinct bands. A column was used to separate the (crude) ethanol extract to fractions. A total of 27 fractions were gotten. A thin layer chromatogram was then used to pool similar fractions together based on a similarity in colour, shape and Rf value as shown in Figure 1(b) and [Table 2]. A total of 9 fractions remained after pooling. Out of the nine (9) fractions, two (2) has low affinity for the polar stationary phase, four spots (4) had polarity falling between the polarity range of both the mobile and stationary phase while three fractions showed they have higher affinity for the mobile phase. This is because silica gel is a relatively polar stationary phase which has OH groups on their surfaces that interacts strongly with polar compounds. As such, more polar compound move along the plate slowly while less polar compounds move faster along the plate because they are weakly absorbed [20].

Effect of the Crude and Fractions of Ethanol Extract of Feretia Apodanthera on Carrageenan Induced Inflammation

Inflammation was induced and treated in albino rats for this study because these rats have been shown to adequately model the human system making it the leading animal used experimentally [21,22]. [Table 3] reveals the inhibitory effect of 50mg/kg of the nine ethanol fractions of *Feretia apodanthera* on carrageenan induced inflammation in comparison with the crude extract and the standard drug (ketoprofen) on carrageenan induced inflammation. In the first hour, the percentage inhibition of fraction 8 was significantly higher than that of the crude extract but no significant ($p>0.05$) difference when compared with the percentage inhibition of the ketoprofen (standard drug). In the second hour, the percentage inhibition of fractions 5, 8 and 9 were not significantly ($p>0.05$) different from that of the standard drug but they were significantly ($p<0.05$) higher than the percentage inhibition of crude extract. In the third hour, the percentage inhibition of all the extracts were significantly ($p<0.05$) higher than that of the crude but lower significantly ($p<0.05$) when compared with the standard drug. The percentage inhibition at the fourth hour showed that fraction 9 exhibited a significantly ($p<0.05$) higher percentage inhibition than the crude but it was not significantly ($p>0.05$) different from the standard and other fractions. In the fifth hour, fraction 5 [Figure 2] exhibited a significantly ($p<0.05$) higher percentage inhibition than fractions 1, 2 and the crude extracts but was not significantly ($p>0.05$) different from that of the standard drug.

The effect of the fractions on carrageenan induced inflammation in rats showed that fraction 8 [Figure 3] was most effective to reduce oedema at the first and second hours (66.21 and 72.47 percent respectively). This suggests that it may be effective in inhibiting the release of bradykinin, histamine and serotonin. Histamine contributes to the progression of inflammation by enhancing the secretion of proinflammatory cytokines like IL-1α and chemokines, it also regulates granulocyte accumulation to tissues [23]. Necas and Bartosikova (2013) in their review mentioned that some NSAIDs such as aspirin or indomethacin do not inhibit the first phase of edema formation, this suggests that fraction 8 is better as it was able to significantly offset initial phase of oedema formation. Fraction 9 [Figure 4] was most effective in the fourth hour (67.19 percent inhibition) while fraction 5 was most effective in the third and fifth hours (69.61 and 78.54 percent respectively). This shows that fractions 5 and 9 most likely act by inhibiting the induction of COX-2 thereby limiting the release of prostaglandins. Prostaglandins, which are synthesized from the oxidative metabolism of arachidonic acid in a reaction mediated by cyclooxygenase, generally evoke increased vascular permeability and vasodilation hereby contributing to erythema, oedema and pain as seen in inflammation [24,25]. This led to a selection of fractions 5, 8 and 9 for further analysis.

FTIR Spectra for the most Effective Fractions

The FTIR spectrum revealed the presence of functional groups characteristic of aromatics, ethers, esters, alcohol, alkanes, ketones, alkenes, alkynes, amines and carboxylic acid [26]. Many of the functional groups identified in the fractions have been linked with anti-oxidant and anti-inflammatory responses. Fink [27] in his report stated that the presence of keto carboxylate elicits antioxidant effect by scavenging hydrogen peroxide. Compounds with aromatic rings and alcohol groups as seen in phenols have been known to modulate inflammation at different levels by decreasing the production of reactive nitrogen and oxygen

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Figure 1. a) Six distinct bands of n-Hexane and acetone mixture of ratio 3:2, **(b)** chromatogram used to pool similar fractions together based on a similarity in colour, shape and Rf value

Table 2: The Inhibitory Effect of the Fractions of Ethanol Extract, the Crude Ethanol Extract of *F. apodanthera* and the Standard Drug (Ketoprofen) on Carrageenan Induced Inflammation.

Sample (50mg/kg)	1st Hour (%)	2nd Hour (%)	3rd Hour (%)	4th Hour (%)	5th Hour (%)
Fraction 1	50.00±17.62ab	61.87±6.08cd	50.00±6.53bc	39.58±22.56ab	29.55±12.97a
Fraction 2	36.13±22.24ab	55.22±11.44cd	68.14±9.30bc	46.88±12.70ab	41.08±8.41 a
Fraction 3	32.62±21.70ab	56.33±6.35 cd	48.53±6.16bc	31.51±14.69ab	46.21±8.43ab
Fraction 4	41.02±21.70ab	59.18±10.60cd	63.24±21.53bc	51.56±34.78ab	46.21±25.82ab
Fraction 5	41.67±14.50ab	70.41±11.91d	69.61±3.90bc	45.57±7.18 ab	78.54±12.68b
Fraction 6	35.16±18.30ab	28.64±26.15ab	44.61±28.03b	53.65±21.88ab	63.89±18.43ab
Fraction 7	49.02±25.60ab	42.88±9.15bc	43.14±21.06b	46.61±22.49ab	51.26±27.28ab
Fraction 8	66.21±26.80b	72.47±16.70 d	64.71±17.32bc	58.07±27.90ab	56.31±21.45ab
Fraction 9	54.49±5.39ab	71.84±16.83 d	65.69±10.30bc	67.19±17.06 b	51.52±19.60ab
Crude	24.01±16.50a	19.81±15.76a	18.72±15.31a	24.72±21.19a	39.27±27.88a
Standard	45.90±11.17ab	69.94±12.49d	72.55±18.21 c	40.10±17.02ab	48.23±23.78ab

Values are written as means ± SD n=3 replicate determinations values with different superscript down the column are significantly different ($p<0.05$). Values with similar superscripts are not significantly different.

Table 3: Functional groups identified by FTIR from the most potent fractions of column chromatography of *F. apodanthera* ethanol extract.

S/No	Absorption peak (cm-1)	Fraction	Bond	Functional Group
1	440.75-567.09	5,8 and 9	R-I	Halide
2	842.92-875.71	5 and 9	CH (para)	Aromatics
3	1100.43-1106.21	5,8 and 9	R-O-R', RC(O)OR	Ethers, esters
4	1263.42-1275.95	5 and 8	R-O stretch	Alkyl aryl ether
5	1375.29	5	-CH3, C-H bend	Alkanes.
6	1419.66-1450.52	5,8 and 9	0	Methyl bend
7	1641.48-1642.44	5,8 and 9	C=C	Alkenyl stretch
8	1717.67	5	C=O	Ketones
9	2114.05-2138.16	5,8 and 9	R-C≡C-H	Terminal alkyne
10	2927.08-2933.83	5,8 and 9	>CH2, C-H, ,	Alkane
11	3423.76- 3444.02	5,8 and 9	R-C(O)-OH	Carboxylic acid
12	3798.93	5	N-H Stretches	Amines

species, limiting the activity of iNOS and COX, suppressing inflammatory chemokines and cytokines synthesis as well as controlling pathways for NF-κβ signaling [28,29].

Gas chromatography mass spectrometry analysis of the most effective fractions

The results of the GC-MS analysis identified the various compounds present in the partially purified fraction as seen in [Figure 5] to. GCMS analysis of fractions 5, 8 and 9 [Figures 6, 7] revealed the presence of compounds which had a high similarity index with 9-Octadecenoic acid [Figures 8, 9]. 9-Octadecenoic acid is similar to ricinoleic acid (12-hydroxy-9-octadecenoic acid), an unsaturated omega-9 fatty acid, which has been proven to exert remarkable anti-inflammation effects in acute and sub chronic experimental models of inflammation [30]. In their report, Vieira [31] showed ricinoleic acid maybe seen as a new non pungent, capsaicin-like anti-inflammatory agent by inhibiting histamine and bradykinin. Other compounds identified by GCMS of fraction 5 were Eicosanoid acid, tetracosanol [Figure 10], hexadecanoic acid, phthalic acid, heptaethylene glycol [Figure 11] and 13-docosenamide. Tetracosanol is a long fatty chain alcohol (LCFA) which has been proven to inhibit the release of different pro-

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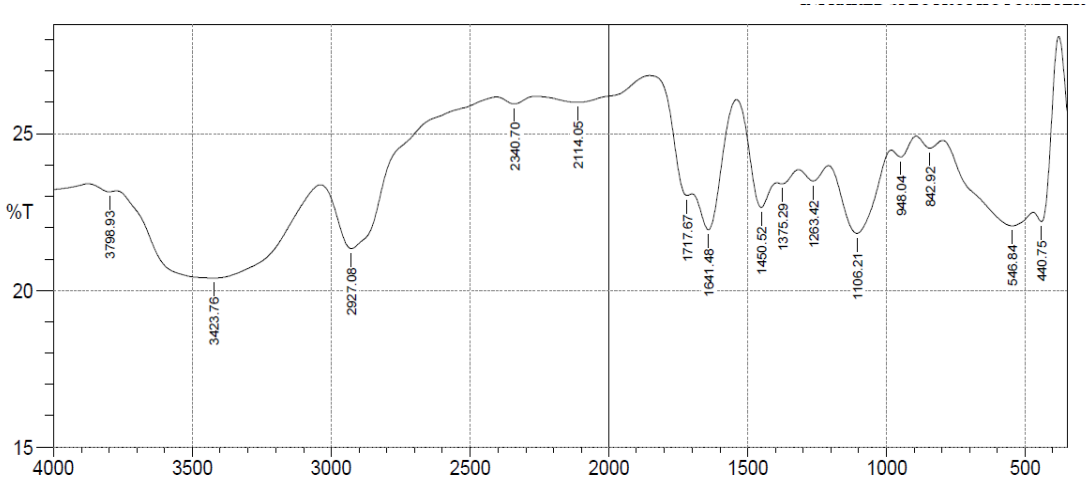

Figure 2. FTIR Spectra for fraction 5

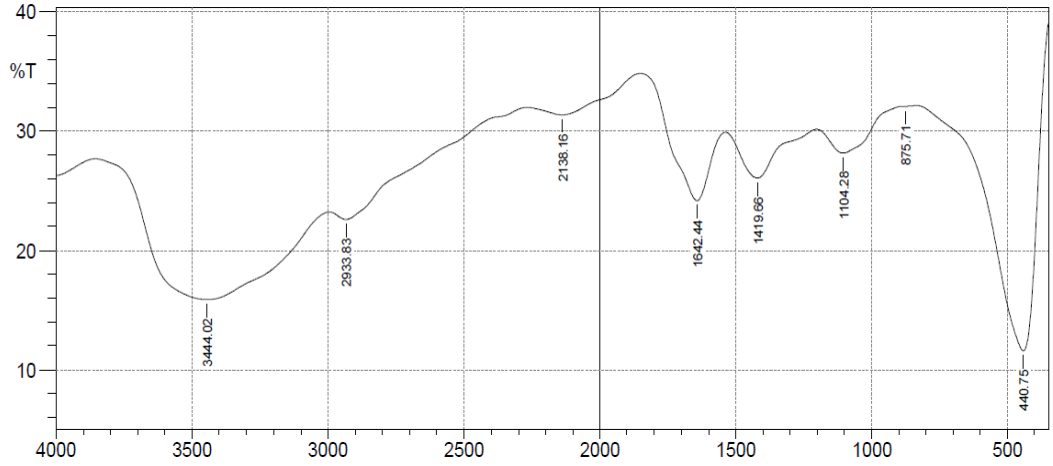

Figure 3. TIR Spectra for fraction 5

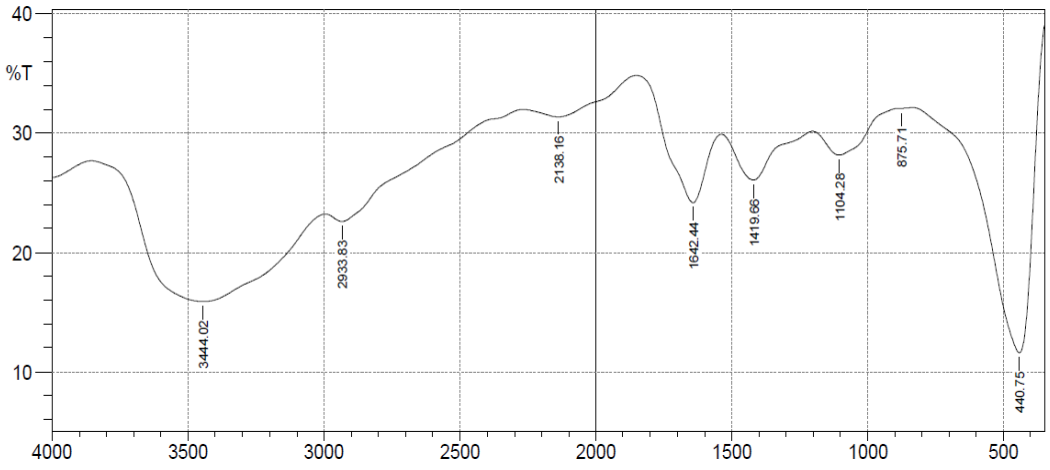

Figure 4. FTIR Spectra for fraction 9

inflammatory mediators including a reduced tumor necrosis factor alpha (TNF-α), thromboxane and prostaglandin production as well as a dose dependent decrease of nitric oxide production [32]. Previous studies have also shown heptaethylene glycol which is a polyethylene glycol (PEG) to be able to reduce inflammation [33,34].

In fraction 8, other compounds identified were cis-Vaccenic acid, Cyclopropa[5,6]stigmast-22-en-3-one [Figure 12], Cyclohexanecarboxylic acid, Hexadecanoic acid, Stigmasta-3,5-dien-7-one [Figure 13], 11-Eicosenoic acid and Octadecanoic acid. cis-Vaccenic acid have been shown to suppress vascular cellular adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1) making it a possible prophylaxis agent in cases of arteriosclerosis which is an inflammatory disease[25]. Cyclopropa[5,6]stigmast-22-en-3-one and Stigmasta-3,5-dien-7-one, are corticosteroid-like and corticosteroids [Figure 14] have been known to be effective in many inflammatory and immune disease [35].

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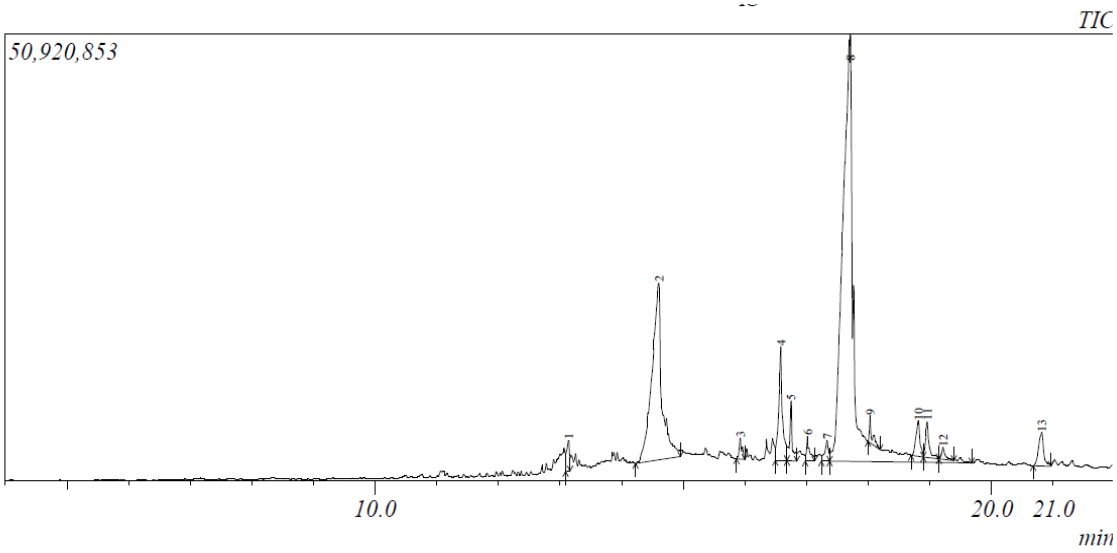

Figure 5. GCMS analysis spectra for fraction 5

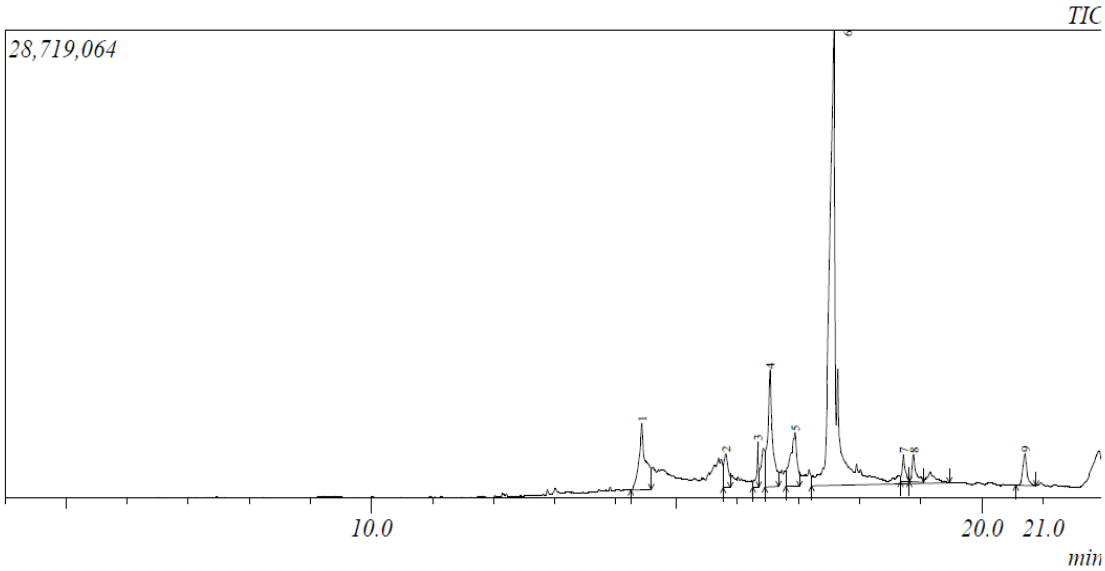

Figure 6. GCMS analysis spectra for fraction 8

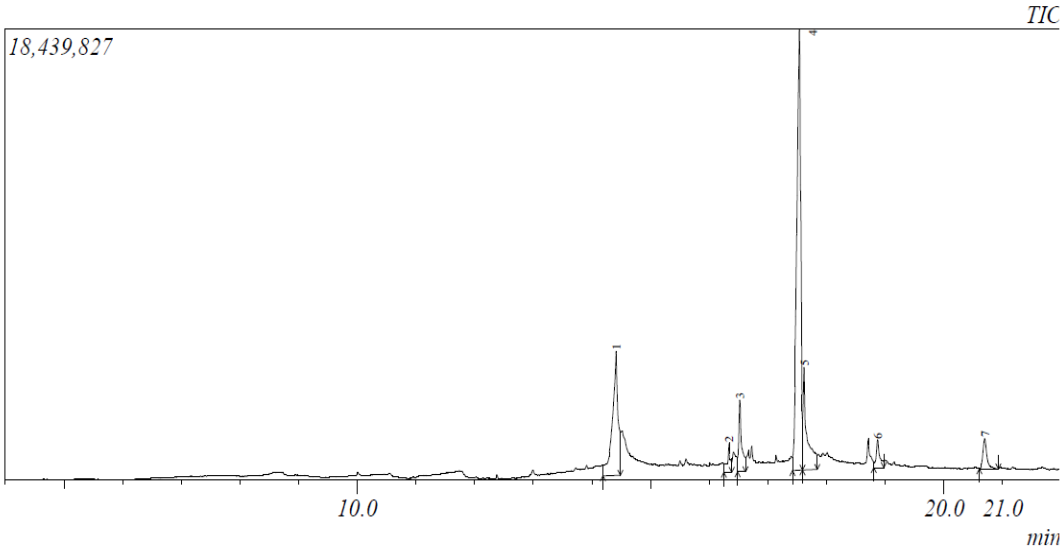

Figure 7. GCMS analysis spectra for fraction 9

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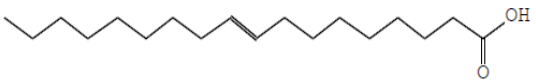

Figure 8. Structure of 9-Octadecenoic acid (E)

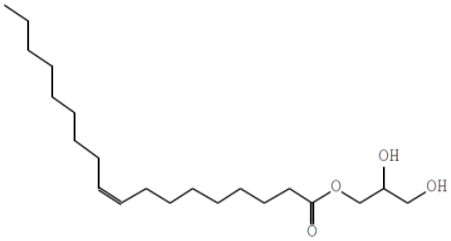

Figure 9. Structure of 9-Octadecenoic acid (Z))-, 2,3-dihydroxypropyl ester

Figure 10. Structure of Tetracosanol

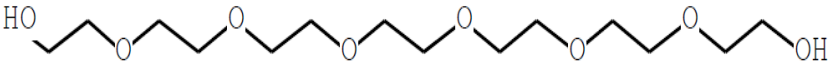

Figure 11. Structure of Heptaethylene glycol

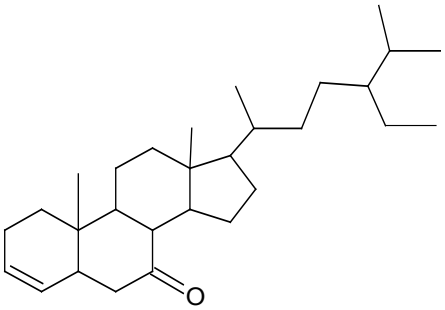

Figure 12. Structure of Cyclopropa[5,6]stigmast-22-en-3-one

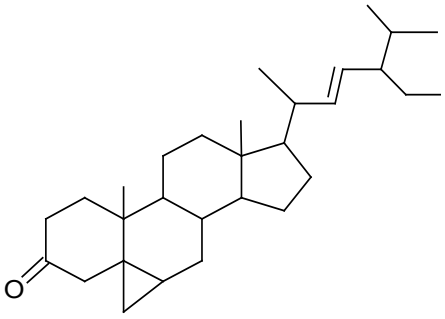

Figure 13. Structure of Stigmasta-3,5-dien-7-one

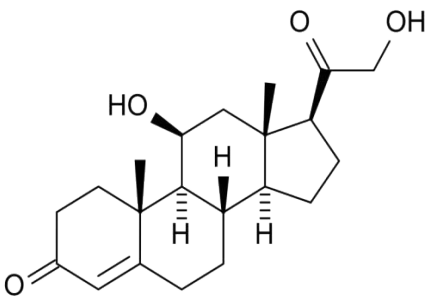

Figure 14. Structure of Corticosterone (corticosteroid)

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Table 4: Activity of Compounds Identified in the GCMS Study of Feretia Apodanthera Root Bark.

S/No	Name of Compound	Molecular formula	M/Z value	Compound nature	Activity
1	9-Octadecenoic acid	C18H34O2	356	Unsaturated fatty acid , ricinoleic acid	Anti-inflammatory, Antiandrogenic, Cancer preventive, Dermatitigenic. Hypocholesterolemic, flavor, 5-Alpha reductase inhibitor, Anemiagenic, Insectifuge.
2	Eicosanoic acid	C20H40O2	312	Arachidic acid	Photographic material, detergent and lubricants
3	Tetracosanol	C24H50O	354	Long chain fatty alcohol	Anti-inflammatory, cholesterol lowering, antihepatotoxic
4	Hexadecanoic acid	C18H36O2	330	Palmitic acid ester	Antioxidant, Nematicide, Pesticide, Hypercholesterolemic, Soap, Flavor, Lubricant, 5-Alpha reductase inhibitor, Antiandrogenic, Hemolytic, antialopecic, antifibrinolytic, propecic
5	Phthalic acid	C24H38O4	390	-	-
6	Heptaethylene glycol	C14H30O8	326	Monounsaturated omega-9 fatty acid	Antibiotics, immunosuppressants, cosmetics, antimycotics
7	13-docosenamide	C22H43NO	337	Docosenoic acid	Reduced mobility, lessened awareness in rats
8	Octadecanoic acid (Z)-, 2,3-dihydroxypropyl ester	C18H36O2	356	Stearic acid	5-Alphareductase inhibitor, Cosmetic, Hypocholesterolemic, Flavor
9	cis-Vaccenic acid	C18H34O2	282	omega-7 fatty acid	Hypocholesterolemic, Prophylaxis agent
10	Cyclopropa[5,6]	C30H48O	424	Corticosteroids	Analgesic, antiprostatitic
	stigmast-22-en-3-one				
11	Cyclohexanecarboxylic acid	C18H32O2	280	Abscisic acid	Flavor, anti-inflammatory and anti-diabetic effects
12	Stigmasta-3,5-dien-7-one	C29H46O	410	Corticosteroids	Analgesic, antiprostatitic
13	11-Eicosenoic acid	C20H38O2	324	Gondoic acid	Painting

The major action of plant steroids is to switch off multiple inflammatory genes (proteins, inflammatory receptors, enzymes, adhesion molecules, chemokines and encoding cytokines) that have been activated during chronic inflammation process [36]. In higher concentrations corticosteroids have additional effects on anti-inflammatory proteins synthesis and postgenomic effects by inhibiting the proteins that stabilise mRNA, leading to an increased catabolism of mRNA and hence a reduced inflammatory protein expression [37]. The presence of corticosteroid in fraction 8 might also be responsible for giving the best anti-inflammatory effects in the first and second hours and a good inflammatory inhibitory action in the third, fourth and fifth hours. Apart from 9-Octadecenoic acid, compounds identified in fraction 9 are cyclohexanecarboxylic acid, palmitic acid and octadecanoic acid. [Table 4] shows medicinal and other benefits of each compound identified in the fractions of the ethanol extract of *Feretia apodanthera.* These results are in agreement with the report by Coulibaly (2019) that showed the presence of Octadecenoic acid methyl esters in aqueous-acetone extracts and fractions of *feretia apodanthera*.

CONCLUSION

The ethanol fractions of *F. apodanthera* root bark possess anti-inflammatory activity against carrageenan induced inflammation. This activity may be related to its components such as corticosteroids, tetracosanol, 9-octadecenoic acid and its derivatives; which may be able to mediate different stages of inflammation. This may justify the use of the plant as herbal medicine to treat wounds. Further studies can be done to determine the mode of action of the extract.

Conflict of Interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no financial support for this work that could have influenced its outcome.

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