

(RESEARCH ARTICLE)

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# Studies on phytochemical constituents of *Acioa barteri* (Chrysobalanaceae) leaf extract and its anti-inflammatory properties

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World Journal of Biology Pharmacy and Health Sciences, 2023, 14(02), 200-211

Publication history: Received on 22 March 2023; revised on 06 May 2023; accepted on 09 May 2023

Article DOI: https://doi.org/10.30574/wjbphs.2023.14.2.0205

#### Abstract

Bioactive compounds isolated from leaves of *Acioa barteri* are responsible for its antiinflammatory activity. The result of qualitative and quantitative phytochemical analysis confirmed the presence flavonoids, alkaloids, Tannins, Resin, Saponins, Cardiac glycosides, Terpenoids, Steroids and Phenols. The result of anti-inflammatory activity testing showed that the plant extract possessed anti-inflammatory activity which was found to be dose dependent. At the doses of 250 mg/kg, 500 mg/kg, and 1000 mg/kg the percentage inhibition after 24 hours of administration was found to be 19.6%, 29.64%, and 49.75% respectively. The percentages 29.64% and 49.75% were found to be higher than 27.64% produced by the standard drug (Diclofenac) at 15 mg/kg. A good number of active compounds were isolated and identified by GC-MS, which include, Trilinolein, Pregn-4-ene 3, 20-dione, 17 epoxy, Octadecanoic acid 2-(hexadecyloxy) ethyl ester, Limonin, Colchicine, Paromomycin and Oleic acid eicosyl ester. Pregn-4-ene, 20-dione, 17 epoxy, Limonin, Colchicine and Octadecanoic acid 2-(hexadecyloxy) ethyl ester have been reported to possess antinflammatory activities. The result of the percentage inhibition on the Carrageenan induced paw edema albino rats obtained showed that the leaves extract of the plant possessed anti-inflammatory activity and this justified the use of this plant in ethnomedicine.

Keywords: Anti-inflammatory; Acioa barteri; Paw Oedema; Acute toxicity; Phytochemical; Flavonoids; GC-MS

### 1. Introduction

Natural products can be seen as substances which include metabolites, bioactive molecules or moieties obtained from natural sources like plants, fungi, micro-organisms, animals and marine organisms. Researchers (Folashade *et al.*, 2012) have shown us that 80% of the world population depends on natural products as source of medicine for healthy living. *Acioa barteri* (Chrysobalanaceae) is an evergreen shrub or small tree of 12m high, with slender branches more or less scandent, commonly found in Cameroon and South East Nigeria.

Kingdom. Plantae Phylum. Tracheophyta Class. Magnoliopsida Order. Malpighiales Family .Chrysobalanaceae Genus. Acioa

#### Botanical name: Acioa barteri

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### Part used: leaves



Figure 1 The leaf of Acioa barteri

Studies conducted by Anyanwu *et al.*, (2020), ascertain that the phytochemical composition of *Acioa barteri* includes the presence of the following secondary metabolites; flavonoids, terpenoids, saponins, alkaloids, Steroids, Cardiac glycosides, Resins. The presence of alkaloids (spartein) is envisaged to be responsible for antihypertensive and anticonvulsant activity to the plant, balancing nervous system and blood pressure lowering. According to authors (Kumar *et al.*, 2012; Fraga-Corral *et al.*, 2021; Aleksandra *et al.*, 2019) Tannin is known for its astringent and antiparasitic activity, therefore will facilitate wound healing. The presence of terpenoids suggests its use as anti-tumor and anti-viral agent (Cox-Georgian *et al.*, 2019). According to studies conducted by several authors (Uzor, 2020; Amoa Onguéné *et al.*, 2013; Bekono *et al.*, 2020), Alkaloids are known to possess anti-malaria property, so this plant may be a good source of antimalarial. Cardiac glycosides have been effective in the treatment of congestive heart failures, cancer and as anti-arrhythmia agent (Newman et al., 2008). Anyanwu *et al.*, 2020 proposed that since the leaf extract of this plant (*Acioa barteri*) contains saponins and flavonoids, they are believed to have antioxidants, anti-cancer, antiinflammatory and anti-viral properties. Inflammatory response is a coordinated activation of signaling pathways that regulate inflammatory mediator levels in resident tissue cells and inflammatory cells recruited from the blood (Chen *et al.*, 2017). Inflammation is a common pathogenesis of many chronic diseases, including cardiovascular and bowel diseases, diabetes, arthritis, and cancer (Furman *et al.*, 2019; Pahwa *et al.*, 2021).

# 2. Material and methods

### 2.1. Plant sample collection

The leaves of *Acioa barteri* were collected in a controlled field from Amudi Obizi village Ezinihitte Mbaise, Imo State and were identified by Dr. Garuba Omosun, a taxonomist at the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria.

### 2.2. Drying and pulverization of the leaves.

The collected leaves samples were dried properly at room temperature and pulverized to the desired size using a suitable pulverizer.

#### 2.3. Extraction using cold maceration method

The pulverized sample weighed 1.5 kg in total, was divided into three (3) portions of 500 mg each, they were transferred into different beakers; 1500 ml of methanol (extracting solvent) was added to each of the pulverized leaves stirred for few minutes, kept for 72 hours. Subsequent to this, the macerated sample (the three mixtures) were sieved with muslin cloth; the filtrate was further filtered with Whatman (No. 1) filter paper. The resultant filtrate was concentrated in-vacuo using a rotary evaporator maintained at temperature 40-50 °C.

### 2.4. Phytochemical analysis ( quantitative)

Using a sensitive weighing balance, 1 g of the extract was weighed and transferred in a test tube and 15 ml of methanol was added. The mixture was allowed to react in a water bath at 60 °C for 60 minutes. After the reaction time, the reaction product contained in the test tube was transferred to a separating funnel. The tube was washed successfully with 20 ml of ethanol, 10 ml of cold water, 10 ml of hot water and 3 ml of hexane, which was all transferred to the funnel. This extracts were combined and washed three times with 10 ml of 10 %v/v ethanol aqueous solution. The solution was

dried with anhydrous sodium sulfate and the solvent was evaporated. The sample was solubilized in 1000 ul of hexane of which 200 ul was transferred to a vial for analysis.

## 2.5. Quantitative phytochemical analysis by Gas chromatography

The quantitative phytochemical analysis was performed on a BUCK M910 Gas chromatography equipped with a flame ionization detector. A RESTEK 15 meter MXT-1 column (15m x 250 um x 0.15 um) was used. The injector temperature was 280 °C with splitless injection of 2 ul of sample and a linear velocity of 30 cms<sup>-1</sup>, Helium 5.0 pa.s was the carrier gas with a flow rate of 40mlmin<sup>-1</sup>. The oven operated initially at 200 °C, it was heated to 330 °C at a rate of 3° c min<sup>-1</sup> and was kept at this temperature for 5 minutes the detector operated at a temperature of 320 °C.

Phytochemical were determined by the ratio between the area and mass of internal standard and the area of the identified phytochemical.

## 2.6. Animal studies (Experimental animals)

Forty-three (43) albino rats were out-sourced and were housed in the animal house of Department of Pharmacology and Toxicology, Faculty of Pharmaceutical sciences Agulu, Nnamdi Azikiwe University Awka. The rats were kept in the animal house for one (1) week for them to acclimatized with the new environment during which they were fed with commercial pellet diet and water, the animals were handled in compliance with Animal Research Reporting of In Vivo Experiments (ARRIVE) guidelines while the experiments was conducted according to standard protocol of National Institute of Health (NIH) guidelines for use and care of laboratory animals.

## 2.7. Evaluation of anti-inflammatory activity of plant extract

Thirty animals weighing 120-150 g were grouped randomly into six (6) groups of five animals per group. Animals were weighed and labeled; a solution of the substance (carrageenan) was prepared, administered intra peritoneal to the animal groups (group 2 -6), after few minutes the paw edema was measured and recorded. Treatment groups are as follows:

- Group 1; normal control received 10 ml of distil water, Group 2; positive control carrageenan + 15 mg/kg Diclofenac, Group 3; negative control carrageenan only, Group 4; carrageenan + 250 mg/kg of the plant extract
- Group 5; carrageenan + 500 mg/kg of the plant extract, Group 6; carrageenan + 1000 mg/kg of the plant extract.

Both the plant extract and diclofenac (an anti-inflammatory drug) were given orally to the corresponding groups as shown above. The paw edema was measured firstly on day 0 at 1hour interval for five (5) consecutive hours, then for five (5) consecutive days. The readings were recorded.

### 2.8. Vacuum Liquid Chromatography (VLC) of the ethyl acetate fraction

The extract (2 g) was dissolved in 1 ml of methanol and absorbed on about 25 g silica gel (200 – 400 mesh size) by carefully triturating in a mortar to form a homogenous mixture. The glass column (diameter 2.5 cm x 30 cm height) was packed with silica gel (200 – 400 mesh size) to the bed size of 14 cm height. The adsorbed fungi extract admixture was introduce from the top of the column and a small amount of silica gel (in place of sea sand) was added on top of the adsorbed fungi extract admixture. Cotton wool was used to cover the silica gel to prevent distortion of the silica gel bed when the solvent system will be introduced and vacuum pump was connected to the column. The column was eluted gradually with increasing order of polarity of solvent from non-polar to highly polar solvent system. The column was developed with a gradient mixture of 500 ml of n-hexane and ethyl acetate solvent system in the ratio of 10:0, 9:1, 8:2, 6:4, 4:6, 3:7, 2:8, 0:10 using negative pressure created by the vacuum pump. Furthermore, the column was developed with a gradient mixture of 500 ml of dichloromethane and methanol solvent system in the ratio of 10:0, 9:1, 8:2, 6:4, 4:6, 2:8, 0:10 to give successive fractions. The column was allowed to run dry after each fraction was collected. The eluate was collected separately with a round bottom flask, evaporated using a rotary evaporator and labeled appropriately.

### 2.9. Gas Chromatography - Mass Spectrometry (GC/MS) method

The Gas chromatography GC (Model – Agilent 6890N) coupled with EI mass spectrometer MSD (Model – 7693N) as the detector was used in order to analyze the plant extract. The samples of the extracts were diluted with solvent and internal standard solution before making the analysis in the GC-MS. 0.1ml of extract was dissolved in 0.5 ml of solvent used in extracting the sample. The GC device is connected to computer program and the analysis results are obtained as graphics. A stream of an inert gas (helium) acted as carrier pass. The detector gives analysis as graphics using the

National Institute of Standards and Technology Library (NIST). Before the use of GC for Analysis of the extracts, the carrier gas (He and  $N_2$ ) tanks were opened before start at least for 5 minutes. The GC was powered on using on button (power button) and Waited until the machine has checked each process. The online access page on the GC computer was opened when GC was ready. The following GC conditions were fulfilled before analysis was commenced:

# 2.10. Statistical Analysis

The results in were analyzed with Statistical Package for Social Sciences (SPSS) version 16.0 and presented as mean  $\pm$  standard error of mean (SEM) inhibition zone diameters (IZD) using One-Way ANOVA and further subjection to Tukey's post hoc test. Differences between means were accepted at significance of p<0.05.

# 3. Results

# **3.1. Phytochemical Result**

## 3.1.1. Quantitative phytochemistry using GC-FID



Figure 2 Result of phytochemicals obtained from gas chromatography

Table 1	Hourly study	of the effect	of administrati	on of Acioa	<i>barteri</i> on	inflammation
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Group	Treatment	Chan	Change in paw diameter in (mm) after dose administration				
		0hr	1hr	2hr	3hr	4hr	5hr
Normal Control	2 ml Distill water	.00	2.90 ± 0.06	2.95 ± 0.07	3.06 ± 0.07	3.15 ± 0.11	3.27 ±0.06
Negative Control	Carrageenan	0.00	3.01±0.76 (-3.79%)	.09±2.00 (-4.75%)	3.60±1.07(- 4.35%)	3.98±0.09 (-17.65%)	4.09± 1.54 (-25.08%)

Positive Control	Carrageenan + Diclofenac (15 mg/kg bw)	0.00	2.65±0.05 (8.62%)	2.66±0.10 (8.81%)	2.76 ±0.03 (9.80%)	2.84±0.02 (9.84%)	2.59±0.02 (21.41%)
Crude extract low dose	Carrageenan +(250 mg/kg)	0.00	2.77±0.01 (4.48%)	2.78±0.02 (5.76%)	2.79 ±0.05 (8.82%)	2.86±0.06(9.21%)	2.90±0.02 (11.31%)
Crude extract medium Dose	Carrageenan+(500 mg/kg bw)	0.00	2.65 ± 0.04 (8.62%)	2.68 ± 0.04 (9.15%)	2.77 ±0.07 (9.48%)	2.82±0.09 (10.47%)	2.55±0.06 (22.02%)
Crude extract large dose	Carrageenan +(1000 mg/kg bw)	0.00	2.70± 0.04 (6.89%)	2.67 ± 0.04 (9.49%)	2.75 ± 0.03 (10.13%)	2.78 ± 0.06 (11.75%)	2.11 ± 0.06 (35.47%)

Values are expressed as Mean ± SEM for five animals per group (one-way ANOVA followed by Tukey"s test);p<0.05



Keys; Ab = Acioa barteri

Figure 3 Hourly study of the effect of administration of Acioa barteri on inflammation

Group	Treatment	change in paw diameter in (mm) after dose administration				
		Day 1	Day 2	Day 3	Day 4	Day 5
Normal Control	2 ml Distill water	1.99 ± 0.26	3.55 ± 0.43	3.58 ± 0.29	3.77 ± 0.22	4.04 ± 0.29
Negative Control	Carrageenan	2.07±0.43 (-4.02)	3.80±0.42 (-7.42)	3.90±0.34 (-8.94)	3.95±0.32 (-4.77)	4.56±0.64 (-8.91)

Positive Control	Carrageenan + Diclofenac (15 mg/kg bw)	1.44±0.21 (27.64%)	1.96 ± 0.11 (44.79%)	1.52 ± 0.16 (57.54%)	1.23±0.16     1.05±0.21       (67.37%)     (74.00%)
Crude extract low dose	Carrageenan +(250 mg/kg)	1.60 ± 0.55 (19.60%)	2.50 ± 0.54 (29.57%)	2.00±0.24 (41.13%)	1.35±0.141.20±0.75(64.19%)(70.29%)
Crude extract medium dose	Carrageenan+(500 mg/kg bw)	1.40±0.65 (29.64%)	1.91 ± 0.87 (46.20%)	1.69 ± 0.54 (52.79%)	1.22 ± 0.81     0.80 ± 0.19       (67.63%)     (80.20%)
Crude extract large dose	Carrageenan +(1000 mg/kg bw)	1.00 ± 0.23 (49.75%)	1.45± 0.15 (59.15%)	1.35 ± 0.19 (62.29%)	0.90 ± 0.33       0.45 ± 0.42         (76.13%)       (88.86%)

Values are expressed as Mean ± SEM for five animals per group (one-wsay ANOVA followed by Tukey's test (p < 0.05)



Key; Ab = Acioa barter



## 3.2. Result of GC-MS analysis

**Table 3** Identified compounds from extract A0

NO.	RT. (min)	Chemical Name	Molecular Formular	Molecular Weight (g)
1	12.742	Phorbol	С20Н28О6	364
2.	13.586	Trilinolein	С57Н 9806	878
3.	13.936	Pregn-4-ene-3,20-dione, 16,17-epoxy-, (16α)-	C21H28O3	328
4.	17.823	Docosahexaenoic acid, 1,2,3-propanetriyl ester	С69Н98О6	1022



Figure 5 Total Ion Chromatogram (TIC) of A0 (Crude Extract)



Figure 6 TIC of AB3 (N-hexane /Ethyl acetate VLC fraction (150:350)

NO.	RT. (min)	Chemical Name	Molecular Formular	Molecular Weight (g)
1.	12.817	1-(4-Hydroxyphenylmethyl)-3,6- diazahomoadamantane	C <sub>16</sub> H <sub>22</sub> N <sub>2</sub> O	258
2.	14.002	Octadecanoic acid, 2-(hexadecyloxy) ethyl ester	C36H72O3	552
3.	14.104	5Alpha-androstane-3,17-dione 17-monooxime	C19H29NO2	303
4.	16.304	Colchicine, 9(2H)-deoxy-10-demethoxy-9,10- dichloro-7,9-didehydro-	C <sub>21</sub> H <sub>21</sub> Cl <sub>2</sub> NO <sub>4</sub>	421

Table 4 Identified compounds from AB3



Figure 7 TIC of sample AB5 {N-hexane /Ethyl acetate fraction (50:450)}

Table 5 Identified compounds from AB5
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NO.	RT. (min)	Chemical Name	Molecular Formular	Molecular Weight (g)
1.	11.560	7,10,13-Eicosatrienoic acid, methyl ester	C <sub>21</sub> H <sub>36</sub> O <sub>2</sub>	320
2.	11.648	Batrachotoxinin A, 7,8-dihydro-O3-methyl-, (8β,20 )	C25H39NO5	433
3.	12.224	Tetradecanoic acid, $3,3a,4,6a,7,8,9,10,10a,10b$ -decahydro- $3a,10a$ -dihydroxy- $5$ -(hydroxymethyl)- $2,10$ -dimethyl- $3$ -oxobenz[e]azulen- $8$ -yl ester, [ $3aR$ -( $3a\alpha,6a\alpha,8\alpha,10\beta,10a\beta,10b\beta$ )]-	$C_{31}H_{50}O_6$	518
4.	12.707	$\begin{array}{llllllllllllllllllllllllllllllllllll$	C24H34O6	418
5.	13.592	Oxiraneoctanoic acid, 3-octyl-, cis-	C <sub>18</sub> H <sub>34</sub> O <sub>3</sub>	298
6.	13.906	2,4,6-Decatrienoic acid, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a- dihydroxy-4-(hydroxymethyl)-1,1,7,9 -tetramethyl-11-oxo-1H-2,8a- methanocyclopenta[a]cyclopropa[e]cyclodecen-6-yl ester, [1aR- $(1a\alpha,2\alpha,5\beta,5a\beta,6\beta,8a\alpha,9\alpha,10a\alpha)]$ -	C <sub>30</sub> H <sub>40</sub> O <sub>6</sub>	496
7.	17.835	Limonin	C <sub>26</sub> H <sub>30</sub> O <sub>8</sub>	470



Figure 8 TIC of Sample AB8 {DCM/Methanol (350:150)}

Table 6 Identified compoun	nds from AB8
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NO.	RT. (min)	Chemical Name	Molecular Formular	Molecular Weight (g)
1	11.560	Paromomycin	$C_{23}H_{45}N_5O_{14}$	615
2.	12.707	5H-Cyclopropa(3,4)benz(1,2-e)azulen-5-one, 1,1a- $\alpha$ ,1b- $\beta$ ,4,4a,7a- $\alpha$ ,7b,8,9,9a-decahydro-4a- $\beta$ ,7b- $\alpha$ ,9a- $\alpha$ -trihydroxy-3-(hydroxymethyl)-1,1,6,8- $\alpha$ -tetramethyl-, 9a-isobutyrate	C24H34O6	418
3.	13.603	Corynan-17-ol, 18,19-didehydro-10-methoxy-, acetate	C22H28N2O3	368
4.	13.941	Oleic acid, eicosyl ester	C <sub>38</sub> H <sub>74</sub> O <sub>2</sub>	562
5.	15.204	Milbemycin b, 13-chloro-5-demethoxy-28-deoxy-6,28-epoxy-5- (hydroxyimino)-25-(1-methylethyl)-, (6R,13R,25R)-	C <sub>33</sub> H <sub>46</sub> ClNO <sub>7</sub>	603
6.	16.251	Adenosine, N-(2,3-dihydroxy-3 methylbutyl)	C15H23N5O6	369
7.	17.829	Pregnan-20-one, 5,6-epoxy-3,17-dihydroxy-16-methyl-, (3β,5α,6α,16α)-	C22H34O4	362

 Table 7 Active compounds from the plant extract

NO.	Compound	Biological activity	Reference
1	Trilinolein	Anti-ischemic, Antiarrhythmic, and Antioxidant	(Srivastava <i>et al.,</i> 2015)
2.	Pregn-4-ene 3, 20-dione, 17 epoxy	Anti-inflammation anti-asthma, antihistamine	(Ramamoorthy & Cidlowski, 2016)
3.	Octadecanoic acid, 2- (hexadecyloxy) ethyl ester	Antifungal, Antimicrobial and Antimalarial	(S Metwally <i>et al,</i> 2020)
4.	Limonin	anti-cancer, anti-inflammatory, analgesic, anti- bacterial, anti-viral, anti-oxidation, liver protection properties	(Fan <i>et al.</i> , 2019)

5.	Colchicine	Anti-inflammation, analgesic.	(Weng <i>et al.</i> , 2021)
6.	Paromomycin	Antimicrobial	(Wiwanitkit, 2012)
7.	Oleic acid, eicosyl ester	Anti-inflammation, Cancer preventive.	(Gurunathan <i>et al.,</i> 2016)

# 4. Discussion

The qualitative and quantitative phytochemicals analysis identified the phytochemicals present in this plant, which include, flavonoids, alkaloids, Tannins, Resin, Saponins, Cardiac glycosides, Terpenoids, Steroids, Phenols.

The result obtained from quantitative phytochemicals analysis of the plant extract which was carried out using gas chromatography, showed how rich the leaves of *Acioa barteri* is in various secondary metabolites or rather quantifies the various secondary metabolites in the leaf extract. It was found that different sub classes of flavonoids were found to be numerous; they include rutin, catechin, flavones, anthocyaninm, naringin, naringenin, epicatechin, kaempferol etcetera. The anti-inflammatory activity of flavonoids has been reported by Osadebe and Okoye (2003). Also phytochemicals such as saponins, steroids, flavonoids, terpenoids, tannins and glycosides had been documented to have anti-inflammatory activities (Ahmadiani *et al.*, 1998)

The effect of methanol extract of *Acioa barteri* carrageenan-induced edema is as shown in Table 1 for hourly study. The extract (250 - 1000 mg/kg) exerted a significant (p < 0.05 - 0.001) anti-inflammatory effect in a dose-dependent manner which was comparable to the standard drug, Diclofenac, 15 mg/kg. For the hourly study the highest percentage reduction was seen in 1000 mg/kg dose of the extract. The mean and percentage reduction was (2.11 ± 0.06 (35.47%)), this was higher than the standard drug diclofenac (2.59±0.02 (21.41%)).

This was also the same in the case of the daily study; the extract also displayed a significant reduction of paw edema. The percentage reduction was also dose dependent. The highest activity was shown at 1000 mg/kg dose of the extract. The mean and percentage reduction was  $(0.45 \pm 0.42 \ (88.86))$ , it appears to be the highest reduction when compared to the standard intervention with mean and percentage value of  $(1.05\pm0.21 \ (74.00\%))$ 

The activity was found to be dose dependent, in that the paw edema was substantially decreased as the dose was increased from 250mg to 1000mg. In addition, it was also observed that the dose 1000 mg/kg elicit a greater reduction in the paw edema than the standard drug (positive control). It can be seen that the percentage (%) inhibition of the induced inflammation increased with time, providing knowledge about the duration of action of the plant extract.

From the anti-inflammatory activity results, it can be inferred that the methanol extract of the tested plant possessed anti-inflammatory activity, as there was a significant reduction or decrease in the paw edema induced in the animals. According to Chen *et al.*, 2018, inflammatory response is the coordinate activation of signaling pathways that regulate inflammatory mediator levels in resident tissue cells and inflammatory cells recruited from the blood. However, the result showed that *Acioa barteri* extract could reduce the coordinate activation of signaling pathways that regulate inflammation in tissue cells.

The results of GC-MS analysis also supported the anti-inflammatory analysis results since the major compounds like Pregn-4-ene, 20-dione, 17 epoxy, Limonin, Colchicine and Octadecanoic acid 2-(hexadecyloxy) ethyl ester contributed to reduction of inflammatory disorder in the experimental animal.

# 5. Conclusion

The qualitative and quantitative phytochemical analysis of leaves extract of *Acioa barteri* was successfully carried out. The result of the percentage inhibition on the Carragenan induced paw edema albino rats obtained showed that the leaves extract of the plant possessed anti-inflammatory ability which was found to be higher than the standard drug (Diclofenac 15 mg/kg). The GC-MS analysis revealed that most of the compounds detected are flavonoid and phenolics and it is well known that these compounds are rich sources of valuable potential therapeutic agents. Bioactive compounds isolated from the plant have proven its efficacy to be considered a good anti-inflammatory agent. *Acioa barteri* leaf has good antiinflammatory property and thus validates the ethnomedicinal use of its leaves in detoxifying the body system of post-natal women in Nigeria.

#### **Compliance with ethical standards**

#### Acknowledgments

The authors express there thanks to Springboard Laboratory Awka, Anambra State where the GC-FID was done and BGI laboratory Port Harcourt where GC-MS analysis was conducted.

#### Disclosure of conflict of interest

The authors declare no conflict of interest.

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