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Original Article

Pharmacological studies of anti-inflammatory, anti-nociceptive and anti-pyretic compounds found in chromatographic fractions of *Anogeissus leiocarpa* (DC). Guill. & Perr. leaves

[Estudios farmacológicos de compuestos antiinflamatorios, antinociceptivos y antipiréticos encontrados en fracciones cromatográficas de hojas de *Anogeissus leiocarpa* (DC). Guill. & Perr.]

Precious A. Idakwoji[1*], Francis O. Atanu[1], Paul C. Nweje-Anyalowu[2,9], Theophilus B. Momoh[3], Wisdom O. Oniwon[1], Sara T. Elazab[4], Souty M.Z. Sharkawi[5,10], Rania M. Waheed[6], Amal Youssef[7], Gaber El-Saber Batiha[8]

[1]Department of Biochemistry, Faculty of Natural Science, Kogi State University, P.M.B. 1008, Anyigba, Kogi State, Nigeria.
[2]Biochemistry Programme, Department of Chemical Sciences, Faculty of Science, Clifford University, Owerrinta, Abia State, Nigeria.
[3]Department of Plant Science and Biotechnology, Faculty of Natural Science, Kogi State University, P.M.B. 1008, Anyigba, Kogi State, Nigeria.
[4]Department of Pharmacology, Faculty of Veterinary Medicine, Mansoura University, Mansoura 35516, Egypt.
[5]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Nahda University, Egypt.
[6]Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Benha University, Toukh 13736, Egypt.
[7]Medical Pharmacology Department, Faculty of Medicine, Cairo University, Egypt.
[8]Department of Pharmacology and Therapeutics, Faculty of Veterinary Medicine, Damanhour University, Damanhour 22511, Albeheira, Egypt.
[9]Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria.
[10]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Beni-Suef University, Egypt.
*E-mail: precious.ia@ksu.edu.ng

Abstract

Context: The anti-nociceptive and anti-pyretic properties of the crude aqueous extract of *Anogeissus leiocarpa* (DC). Guill. & Perr. leaves have been reported.

Aims: To investigate the anti-inflammatory, anti-nociceptive, and anti-pyretic properties of aqueous extract of *A. leiocarpa* leaves (AEAL) and its fractions to identify the bioactive compound(s).

Methods: AEAL was fractionated successively and eluted in gradients of solvent mixture (n-hexane, ethylacetate, and methanol). Eluates, which showed similar TLC profiles were pooled to afford 5 fractions (F1-F5). AEAL and the fractions were subjected to phytochemical analysis and investigated for anti-inflammatory, anti-nociceptive, and anti-pyretic effects using carrageenan-induced paw edema and Brewer's yeast-induced pyrexia in rats, and acetic acid-induced writhing in mouse models, respectively.

Results: Phytochemical analysis indicated presence of terpenoids, saponins, steroids, glycosides, flavonoids, tannins and alkaloids. At 200 mg/kg b.w, AEAL and F1-F5 recorded significant ($p<0.05$) improvements in paw edema, abdominal constrictions and pyrexia, which is comparable to the standard (ketoprofen or Aspirin), relative to the control groups, with F4 observed to be the most potent fraction. GC-MS analysis of F4 reveal the presence of hexadecenoic acid, 15-methyl-, methylester, 9-oxabicyclo (6.1.0) nonane, cis-, cholest-4-en-3-one, cholesterol and 3-diazo-1-methyl-1, 3-dihydro-indol-2-one.

Conclusions: The results indicate that identified compounds, particularly 3-dihydro-indol-2-one, could be responsible for the anti-inflammatory, anti-nociceptive, and anti-pyretic properties of the extract.

Keywords: *Anogeissus leiocarpa*; anti-inflammatory; antinociception; antipyretic; inflammation; NSAID; pyrexia.

Resumen

Contexto: Se han reportado las propiedades anti-nociceptivas y antipiréticas del extracto acuoso crudo de hojas de *Anogeissus leiocarpa* (DC). Guill. & Perr.

Objetivos: Investigar las propiedades antiinflamatorias, anti-nociceptivas y antipiréticas del extracto acuoso de hojas de *A. leiocarpa* (AEAL) y sus fracciones para identificar los compuestos bioactivos.

Métodos: AEAL se fraccionó sucesivamente y se eluyó en gradientes de mezcla de solventes (n-hexano, acetato de etilo y metanol). Los eluatos que mostraron perfiles de TLC similares se agruparon para proporcionar 5 fracciones (F1-F5). AEAL y las fracciones se sometieron a análisis fitoquímicos y se investigaron los efectos antiinflamatorios, anti-nociceptivos y antipiréticos utilizando edema de la pata inducido por carragenina y pirexia inducida por levadura de Brewer en ratas, y retorcimiento inducido por ácido acético en modelos de ratón, respectivamente.

Resultados: El análisis fitoquímico indicó presencia de terpenoides, saponinas, esteroides, glucósidos, flavonoides, taninos y alcaloides. A 200 mg/kg p.c., AEAL y F1-F5 registraron mejoras significativas ($p<0,05$) en el edema de la pata, constricciones abdominales y pirexia que es comparable al estándar (ketoprofeno o Aspirina), en relación con los grupos de control, y se observó que F4 era la fracción más potente. El análisis GC-MS de F4 revela la presencia de ácido hexadecenoico, 15-metil-, éster metílico, 9-oxabiciclo (6.1.0) nonano, cis-, colest-4-en-3-ona, colesterol y 3-diazo-1 -metil-1,3-dihidro-indol-2-ona.

Conclusiones: Los resultados indican que los compuestos identificados, particularmente la 3-dihidro-indol-2-ona, podrían ser responsables de las propiedades anti-inflamatorias, anti-nociceptivas y anti-piréticas del extracto.

Palabras Clave: AINE; *Anogeissus leiocarpa*; anti-inflamatorio; anti-nocicepción; antipirético; inflamación; pirexia.

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INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are used throughout the world to treat and manage inflammation, pain and fever. These drugs mainly target the cyclooxygenases (COX-1 and COX-2) via the eicosanoid biosynthetic pathway. Despite their role in alleviating inflammation, the use of NSAIDs could also result in ulcers as a side effect (Deghrique et al., 2015). They are also associated with side effects such as nausea, respiratory depression, gastrointestinal bleeding, and toxicity to the brain cortex, hepatocytes, cardiac muscles, and glomeruli. Therefore, there is a need for new anti-inflammatory, analgesics, and anti-pyretic drugs with improved efficacy and safety (Almgeer et al., 2015).

Medicinal plants possess numerous phytocompounds that might lead to the discovery of new drugs that may be used to prevent and manage diseases (Lahlou, 2013; Idakwoji et al., 2021a; 2021b). *Anogeissus leiocarpa* (DC.) Guill. & Perr. (family *Combretaceae*) (Fig. 1) is a medicinal plant commonly found in west and central Africa, where it has a wide range of uses (Burkill, 1985). It is commonly called the African Birch. In Nigeria, it is known as Otra in Idoma, Marke (or kwankila) in Hausa, Atara in Ibo and Orin-odan in Yoruba. It has numerous medicinal applications all over Africa (Adigun et al., 2000). The plant parts (leaf, stem, bark and root) are used in traditional medicine for the treatment of some ailments (fever, diarrhea and stomach infections) as they have been reported to possess antimicrobial, hepatoprotective, antioxidant and antidiabetic properties (Batawila, 2005; Etuk and Mohammed, 2009; Atawodi et al., 2011). Our previous study also reported the antinociceptive and anti-pyretic properties of the crude aqueous extract of *A. leiocarpa* leaves (Idakwoji et al., 2019). This present study further evaluated the fractions of the aqueous leaf extract of the plant against experimental models of inflammation, pain, and pyrexia to identify the active principles responsible for these pharmacological activities.

MATERIAL AND METHODS

Collection, identification and authentication of plant material

Fresh leaves of *A. leiocarpa* were harvested from the natural habitat in Agbeji, Dekina Local Government Area, Kogi State, Nigeria (41°24′12.2″N 2°10′26.5″E). The plant sample was identified and authenticated at the Herbarium of the Department of Biological Sciences, Federal University, Lokoja, Kogi State, Nigeria, by an ethnobotanist Mr. Gbenga Akanni. A voucher specimen (FU/1681) was deposited for future reference.

Figure 1. *Anogeissus leiocarpa* (DC.) Guill. & Perr.

Chemicals and drugs and equipment

Hexane, ethylacetate, methanol, acetic acid, carrageenan, Brewers' yeast and methylcellulose were purchased from Sigma Chemical Co. Ltd (U.S.A), ketoprofen and Aspirin were purchased from Health seal® Pharmacy Ltd., Lokoja, Nigeria. Silica gel (70-230 mesh) and pre-coated silica gel $60F_{254}$ on aluminum sheets were purchased from Sigma Chemical Co. Ltd. (USA). Clinical thermometer (Boots, Birmingham, England) and Vernier caliper (Mitutuyo corporation, Japan).

Study animals

Healthy adult Wistar rats (130-170 g) and mice (24-30 g) were used for this study. They were purchased from the Animal House Facility of Salem University, Lokoja, Kogi State, Nigeria, and kept in stainless steel cages under standard laboratory conditions with a 12 h dark/light cycle guaranteed at the facility. They were maintained on standard rodent feed and potable drinking water *ad libitum* and were allowed to acclimatize to the laboratory environment for a period of 7 days prior to the experiment. The study animals were accorded humane care in accordance with the recommendations of the institutional ethical committee and the International Guidelines for Handling of Laboratory Animals (National Research Council, 1996). The Institutional Ethics and Biosafety Committee of the Faculty of Biological Sciences, University of Nigeria, Nsukka, Nigeria, with Ethics Committee Approval No. UNN/FBS/EC/1020 on April 5th, 2019.

Preparation of crude extract

The leaves of *A. leiocarpa* were rinsed with distilled water in order to remove all debris. The leaves were shade-dried for seven days and subsequently pulverized using an electric blender. Two thousand (2000) grams of the pulverized leaves were soaked in distilled water for 72 h. The resulting mixture was filtered using Whatman No. 1 filter paper, and the extract was concentrated using a freeze-dryer and thereafter referred to as AEAL.

Fractionation

Wet silica gel (70-230 mesh) was loaded into a column, and AEAL was added on the upper layer (Adzu et al., 2007). The crude extract was eluted successively in gradients of solvent mixture (hexane, ethyl acetate, and methanol) in multiples of 100 mL and each fraction was collected separately. Thin layer chromatographic (TLC) was carried out on aluminum TLC sheets precoated with silica gel $60PF_{254}$. The plates were dried using an air blower and developed at room temperature using a Shandon chromatographic tank. Spots on TLC plates were visualized under UV light (254 and 366 nm) and spraying with 10% sulphuric acid, followed by heating at 110°C for 5 min. Eluates that showed similar TLC profiles were combined to produce 5 main fractions, which were tested separately for anti-inflammatory, anti-nociceptive, and anti-pyretic activities.

Phytochemical analysis of fractions

The fractions obtained were subjected to phytochemical screening following the procedures described by Sofowora (1993) to determine the predominant secondary metabolites in each fraction.

Carrageenan-induced paw edema in rats

The study was carried out according to the method described by Winter et al. (1962). A total of forty (40) Wistar rats were divided into eight groups of five rats each. Group 2 was pre-treated with the 200 mg/kg AEAL, groups 3-7 were pre-treated with 200 mg/kg to F1-5, respectively, while groups 8 and 1 received ketoprofen (10 mg/kg body weight) and normal saline (1 mL/kg body weight) as positive and normal controls respectively. All doses were given via intraperitoneal route. After 30 min, 0.1 mL of freshly prepared carrageenan suspension (1% w/v in normal saline) was injected into the plantar region of the left hindpaw of each rat. The paw diameter was measured with the aid of Vernier caliper at 0, 1, 2, 3, and 4 h after injection of the carrageenan. Percentage inhibition of edema was calculated using the following formula [1].

$$\text{Edema inhibition (\%)} = \frac{\text{Mean edema increase(Ct)} - \text{Mean edema increase (Tt)}}{\text{Mean edema increase (Ct)}} \times 100 \quad [1]$$

Where Ct: Control group at time 1, 2, 3 and 4 h. Tt: Treated groups at times 1, 2, 3, and 4 h.

Acetic acid-induced writhing test

Forty randomly selected adult Swiss mice of both sexes were used for this analgesic study. The mice were subjected to 24 h fast (but had free access to water and later) and subsequently divided into 8 groups of 5 mice per cage. According to the procedure described by Akuodor et al. (2011), control groups 1 and 8 received orally 20 mL/kg of distilled water and 150 mg/kg of Aspirin, respectively, group 2 received orally 200 mg/kg of AEAL while groups 3-7 received 200 mg/kg of F1-5, respectively. Thirty minutes after treatment, each mouse in all groups was treated with an intraperitoneal injection of 20 mL/kg b.w. of 0.7% acetic acid to induce pain sensation. Each mouse was then placed in a transparent observation chamber. Five minutes post acetic acid administration, the number of abdominal constrictions or writhing behavior for each mouse was noted, counted, and recorded for a period of 30 min. The mean number of writhes was calculated for each group, and the percentage inhibition of writhes was determined using the formula [2].

$$\text{Inhibition (\%)} = \frac{\text{Mean of control} - \text{Mean of test}}{\text{Mean of control}} \times 100 \quad [2]$$

Yeast-induced pyrexia

The modified methods by Mukherjee et al. (2002), Akuodor et al. (2011) and Essien et al. (2015) were used for this study. Forty Wistar rats were randomly selected and divided into 8 groups of 5 rats per cage. A clinical thermometer (Boots, Birmingham, England) was used in measuring their initial basal rectal temperature. Thereafter, pyrexia was induced in rats by injecting subcutaneously 20 mL/kg of 15% brewer's yeast suspended in 0.5% methylcellulose solution. After 24 h, rectal temperature was again measured, and any rat(s) without elevated temperature above by 0.5°C was disregarded for the study. Thereafter, 200 mg/kg AEAL were administered orally to group 2 and 200 mg/kg F1-5 was administered to groups 3-7, respectively. The control groups 1 and 8 received distilled water (5 mL/kg) and Aspirin (150 mg/kg), respectively. Their rectal temperature was again recorded at 1 h interval and for 6 h after drug administration.

GC-MS analysis of active components of F4 isolated from AEAL

The phytochemical composition of F4 of AEAL was using a gas chromatograph in tandem with a

mass spectrometer, GC/MS-QP-2010 plus Ultra (Shimadzu, Kyoto Japan using a DB-5 MS fused silica capillary column (30 × 0.25 m internal diameter, film thickness 0.25 µm). For GC-MS detection, an electron ionization system with an ionization energy of 70 eV was used. The carrier gas was helium gas, and the flow rate was 1.2 mL/min. Injector and MS transfer line temperature were set at 260°C and 270°C, respectively. The oven temperature was initially maintained for 2 min and then increased to 210°C at a rate of 8°C/min to 280°C; the hold time was 10 min. Samples were completely dissolved in absolute ethanol, and 0.3 µL was injected through an auto-sampler in the split mode. The split ratio was 1:100. The relative percentage of each constituent was expressed as percentages by peak area normalization. Each component was identified based on its column retention time relative to the computer-based matching of mass spectra with those of standards (NIST and Wiley libraries for GC-MS system).

Statistical analysis

The data obtained from this study were analyzed using IBM Statistical Product and Service Solution (SPSS), version 20 (Chicago, IL). Significant differences in the means were established by the one-way analysis of variance (ANOVA), followed by the Duncan's *post hoc* multiple comparison. The results were expressed as mean ± standard error of replicate determinations. Differences between the means of treated groups and the control were considered significant at $p<0.05$.

RESULTS

Fractionation of AEAL

After fractionation, eluates that showed similar TLC profiles were combined to afford 5 main fractions (F1-F5). The TLC profiles of the pooled fractions are presented in Table 1.

Table 1. TLC profiles of fractions of AEAL.

Fraction	No. of major TLC spots	R_f values
1	2	0.31, 0.52
2	2	0.73, 0.81
3	4	0.34, 0.43, 0.61. 0.76
4	2	0.51, 0.81
5	3	0.43, 0.69, 0.78

Quantitative phytochemical composition of AEAL and its fractions

Table 2 presents the quantitative phytochemical composition of AEAL and its fractions. From the result obtained, AEAL and its fractions indicated the presence of phenols, terpenoids, saponins, steroids, glycosides, flavonoids, tannins and alkaloids in varying proportions. Results also showed the presence of these phytochemicals in the fractions but to a lesser extent when compared to AEAL. However, among the fractions, F4 had the highest quantity of these phytochemicals.

Anti-inflammatory property of AEAL and its fractions

Table 3 presents the anti-inflammatory property of AEAL and its fractions. AEAL and its fractions indicated a significant difference ($p<0.05$) reduction in the carrageenan-induced rat paw edema at each time interval monitored (Table 3). The percentage inhibition of carrageenan-induced rat paw edema by AEAL and its fractions are indicated in Table 4. The results showed that at 200 mg/kg b.w., AEAL produced the highest percentage (61.83%) inhibition of edema followed by F3 and F4 (51.08 and 58.06%, respectively). These effects were comparable to that of ketoprofen (10 mg/kg) (53.23%) after 4 h as shown in Table 4.

Acetic acid-induced writhing/abdominal constriction of AEAL and its fractions

Table 5 shows the effect of AEAL and its fractions (F1-F5) on acetic acid-induced writhing in mice. At a dose of 200 mg/kg b.w., AEAL and its fractions led to significant ($p<0.05$) decreases in abdominal constrictions (writhes) in mice. The crude extract and F4 produced percentage inhibition of abdominal constrictions of 86.54% and 81.52%, respectively, comparable to Aspirin (85.52%).

Brewer's yeast-induced pyrexia effects of AEAL and its fractions

Table 6 shows the effect of AEAL and its fractions on brewer's yeast-induced pyrexia in rats. Administration of the AEAL and the fractions led to a significant ($p<0.05$) reduction in pyrexia induced by brewer's yeast. At the dose administered (200 mg/kg b.w., the AEAL and its fractions produced a significant difference ($p<0.05$) reduction in body temperature after 1 h up to 6 h. The anti-pyretic activity of the extract and the fractions at 200 mg/kg b.w., was comparable to that of Aspirin.

Identification of active components of F4

The GC-MS fingerprint of AEAL F4 (Fig. 2) and chemical composition (Table 7) indicated presence of 5 peaks identified as hexadecanoic acid, 15-methyl-, methyl ester (1.6%) at retention time 33.6 mins, 9-oxabicyclo (6.1.0) cis-nonane, (1.5%) at retention time 37.1 min, cholesterol (64.5%), which retained at 45.5

min, 3-diazo-1-methyl-1,3-dihydro-indol-2-one (1.4%) with retention time 46.5 min, and cholest-4-en-3-one (31.0%) with retention time of 49.9 min.

Table 2. Quantitative phytochemical composition (mg/100g) of AEAL and its fractions.

Phytochemical	AEAL	F1	F2	F3	F4	F5
Phenols	9.61 ± 0.0011	1.56 ± 0.0043	1.45 ± 0.0043	1.43 ± 0.0018	4.46 ± 0.0055	1.45 ± 0.0011
Terpenoids	0.91 ± 0.0026	0.14 ± 0.0033	0.20 ± 0.0023	0.86 ± 0.0013	0.15 ± 0.0018	0.18 ± 0.0021
Saponins	2.02 ± 0.0019	0.03 ± 0.0028	0.67 ± 0.0011	0.44 ± 0.0057	1.23 ± 0.0023	0.45 ± 0.0019
Steroids	1.55 ± 0.0023	0.77 ± 0.0014	0.34 ± 0.0022	0.36 ± 0.0014	0.21 ± 0.0012	0.10 ± 0.0010
Glycosides	1.65 ± 0.0015	0.21 ± 0.0015	0.15 ± 0.0010	0.29 ± 0.0023	1.44 ± 0.0011	0.11 ± 0.0018
Flavonoids	6.22 ± 0.0012	1.11 ± 0.0020	1.34 ± 0.0012	0.54 ± 0.0027	3.32 ± 0.0018	1.32 ± 0.0043
Tannins	6.88 ± 0.0081	1.45 ± 0.0014	1.67 ± 0.0056	1.57 ± 0.0018	1.83 ± 0.0054	4.43 ± 0.0019
Alkaloids	4.33 ± 0.0012	1.21 ± 0.0047	1.27 ± 0.0012	0.47 ± 0.0029	0.05 ± 0.0034	2.55 ± 0.0056

Data represent the mean ± SD, n = 3.

Table 3. Anti-inflammatory activity of AEAL and its fractions.

Treatment	Mean changes (mm) in paw edema ± standard error of mean			
	1 h	2 h	3 h	4 h
Control (5mL/kg NS)	0.182 ± 0.011	0.178 ± 0.015	0.178 ± 0.031	0.186 ± 0.021
AEAL	0.131 ± 0.032***	0.110 ± 0.028***	0.093 ± 0.012***	0.071 ± 0.032***
F1	0.161 ± 0.013**	0.158 ± 0.015**	0.150 ± 0.014**	0.099 ± 0.021***
F2	0.170 ± 0.015*	0.168 ± 0.022*	0.153 ± 0.018**	0.100 ± 0.031***
F3	0.146 ± 0.034**	0.138 ± 0.021**	0.100 ± 0.026***	0.091 ± 0.013***
F4	0.137 ± 0.012***	0.120 ± 0.018***	0.096 ± 0.020***	0.078 ± 0.018***
F5	0.156 ± 0.014**	0.150 ± 0.023**	0.132 ± 0.024**	0.101 ± 0.024***
Ketoprofen (20 mg/kg)	0.140 ± 0.019**	0.130 ± 0.024**	0.099 ± 0.018***	0.087 ± 0.021***

Data are expressed as mean ± standard error of mean (n = 5). *p<0.05, **p<0.01, ***p<0.001 statistically significant differences with respect to the control. Dose of AEAL and fractions 200 mg/kg b.w.

Table 4. Percentage inhibition of carrageenan-induced rat paw edema by AEAL and its fractions.

Treatment	Inhibition of edema (%)			
	1 h	2 h	3 h	4 h
AEAL	28.02	38.20	47.75	61.83
F1	11.54	11.24	15.73	46.77
F2	06.59	05.62	14.04	46.24
F3	19.78	22.47	43.82	51.08
F4	24.73	32.58	46.06	58.06
F5	14.29	15.73	25.84	45.70
Ketoprofen (20 mg/kg)	23.08	26.97	44.38	53.23

Dose of AEAL and fractions 200 mg/kg b.w. (n = 5).

Table 5. Effect of the administration of AEAL and its fractions on acetic acid-induced writhing in mice.

Treatment	Writhes	% Inhibition
Control (5ml/kg NS)	33.28 ± 0.54	0.00
AEAL	4.48 ± 0.13*	86.54*
F1	15.22 ± 0.21*	54.27*
F2	14.15 ± 0.26*	57.48*
F3	16.40 ± 0.18*	50.72*
F4	6.15 ± 0.23*	81.52*
F5	11.89 ± 0.36*	64.27*
Aspirin (20 mg/kg b.w)	4.82 ± 0.31*	85.52*

Data are expressed as mean ± standard error of mean (n = 5). *p<0.05 statistically significant differences with respect to the control. Dose of AEAL and fractions 200 mg/kg b.w.

Table 6. Effect of the administration of AEAL and its fractions on Brewer's yeast-induced pyrexia in rats.

Treatment	0 h	1 h	2 h	3 h	4 h	5 h	6 h
Control (NS) (5 mL/kg)	37.41 ± 0.05	37.63 ± 0.21	37.58 ± 0.16	37.23 ± 0.28	37.45 ± 0.23	37.77 ± 0.41	37.28 ± 0.45
AEAL	37.23 ± 0.34	36.08 ± 0.11*	37.45 ± 0.12*	36.38 ± 0.07*	36.48 ± 0.15*	36.36 ± 0.06*	35.88 ± 0.12*
F1	37.44 ± 0.26	37.01 ± 0.22*	36.23 ± 0.09*	36.42 ± 0.05*	36.34 ± 0.09*	35.31 ± 0.06*	35.41 ± 0.15*
F2	37.80 ± 0.13	37.08 ± 0.11*	37.19 ± 0.09*	36.28 ± 0.15*	36.11 ± 0.13*	36.08 ± 0.08*	35.99 ± 0.08*
F3	37.55 ± 0.06	36.71 ± 0.14*	37.23 ± 0.08*	36.88 ± 0.14*	36.23 ± 0.06*	35.23 ± 0.07*	35.82 ± 0.09*
F4	37.48 ± 0.07	37.20 ± 0.05*	36.23 ± 0.08*	36.48 ± 0.11*	36.33 ± 0.15*	36.75 ± 0.03*	35.48 ± 0.02*
F5	37.78 ± 0.06	37.39 ± 0.05*	36.44 ± 0.05*	36.61 ± 0.09*	36.36 ± 0.05*	35.33 ± 0.04*	35.38 ± 0.11*
Aspirin (20 mg/kg)	37.34 ± 0.07	37.50 ± 0.09*	36.88 ± 0.11*	36.76 ± 0.05*	36.34 ± 0.56*	35.88 ± 0.08*	35.22 ± 0.17*

Data are expressed in °C as mean ± standard error of mean (n = 5). *p<0.05 statistically significant differences with respect to the control. NS: normal saline; dose of AEAL and fractions 200 mg/kg b.w.

Table 7. GC-MS profile of bioactive compounds in F4.

Peak No.	Retention time (min)	Area (%)	Name of compound	Ref	CAS	Quality
1	33.6281	1.6272	Hexadecanoic acid, 15-methyl-, methyl ester	144336	006929-04-0	74
2	37.1472	1.5158	9-Oxabicyclo[6.1.0]nonane, cis-	11674	004925-71-7	37
3	45.5413	64.4608	Cholesterol	231246	000057-88-5	98
4	46.5426	1.3785	3-Diazo-1-methyl-1,3-dihydro-indol-2-one	42696	003265-14-3	35
5	49.8614	31.0177	Cholest-4-en-3-one	229991	000601-57-0	99

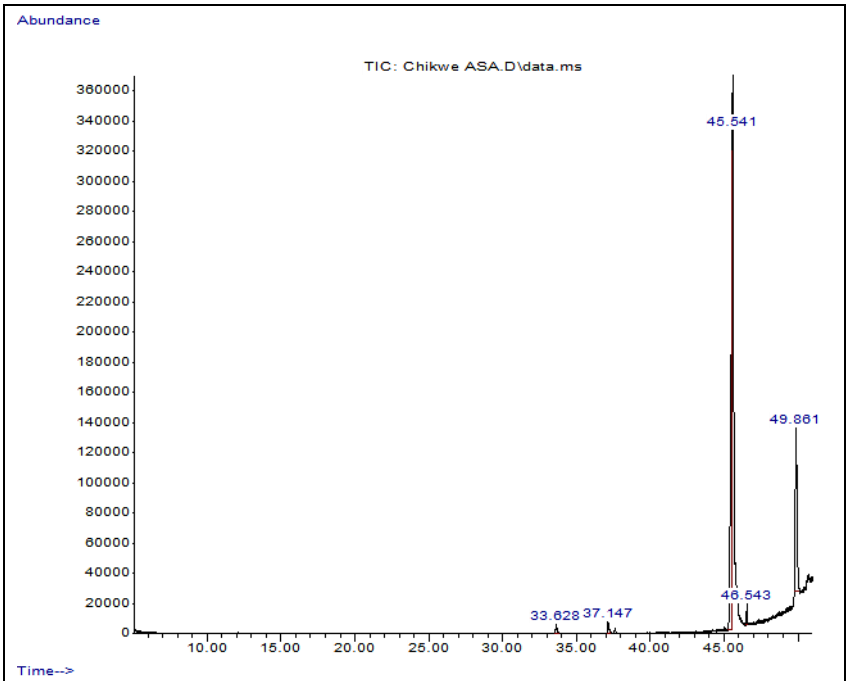

Figure 1. GC-MS fingerprint of F4 of AEAL.

DISCUSSION

Our previous study on the leaf extract of *A. leiocarpa* reported relevant anti-nociceptive and anti-pyretic activities (Idakwoji et al., 2019). This observation as well as the traditional use, encouraged us to extend our study by partially purifying and evaluating its fractions using *in vivo* models of inflammation, nociception and pyrexia.

The crude aqueous extract of *A. leiocarpa* was fractionated successively using gradients of solvent mixture (hexane, ethyl acetate, and methanol) and eluates that showed similar TLC profiles were combined to afford 5 major fractions (F1-F5). Preliminary phytochemical analysis revealed the presence of phenols, terpenoids, saponins, steroids, glycosides, flavonoids, tannins and alkaloids. SF4, which gave the best activity in the anti-inflammatory and anti-nociceptive studies, appeared to have the highest concentration of the phytochemicals among the fractions.

The anti-inflammatory activity of the fractions of *A. leiocarpa* was evaluated using the carrageenan-induced paw edema test. The edema induced by carrageenan corresponds to the acute phase of inflammation in which various mediators operate to produce the inflammatory response. Carrageenan-induced paw edema occurs in two phases (early and late phase). The early phase begins after the administration of the irritant and lasts for an hour. It is characterized by the release of serotonin, histamine, and bradykinins (Samriti et al., 2016). Non-steroidal anti-inflammatory drugs such as indomethacin or Aspirin do not inhibit this phase. The late phase occurs from the second to the fourth hour after the administration of the irritant and is ascribed to the release of prostaglandins, oxygen-derived free radicals, lysosome enzymes, and proteases. Most drugs show their anti-inflammatory response at this phase (Samriti et al., 2016). Moreover, carrageenan-induced edema is a standard experimental model for assessing the anti-inflammatory activities of natural products as well as synthetic chemical compounds (Samriti et al., 2016). The crude extract and fractions of *A. leiocarpa*s lightly inhibited inflammation in the early phase and significantly inhibited it in the late phase, with the highest anti-inflammatory effect of 24.73% and 58.06% respectively produced by F4. The herbal extract's ability to reduce paw edema in both phases of the carrageenan-induced edema in mice suggests the active principles of the herbal extract in inhibiting the release or action of the early and late phase mediators of inflammation suppressing edema. The active principles with good anti-inflammatory potential include flavonoids, terpenoids, saponins, and tannins. Flavonoids and saponins may have acted synergistically to reduce inflammation by inhibiting key enzymes such as cyclooxygenase, lipoxygenase, and nitric oxide synthase, which are involved in the production of inflammatory mediators and metabolism of arachidonic acid.

The fractions of *A. leocarpus* also possess anti-nociceptive activity, as evident in this study. The acetic acid-induced writhing test is a well-established experiment to investigate the peripheral analgesic activity of drugs. Evidence suggests that the pain caused by acetic acid is due to the secretion of endog-

enous substances and pain mediators, such as bradykinin, serotonin, substance P, histamine, prostaglandins (PGE2 and PGF2α), and pro-inflammatory cytokines including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and IL-8, which activate and sensitize peripheral nociceptive neurons (Hijazi et al., 2017; Gaber et al., 2020). Our study shows that all the fractions reduced the abdominal contractions of the mice with the greatest inhibition (81.52%) produced by F4. This was comparable to the inhibition (85.52%) produced by the standard drug Aspirin. The significant reduction in acetic acid-induced writhes by F4 suggests that the analgesic effect may be peripherally mediated via the inhibition of synthesis and release of PGs and other endogenous substances.

Pyrexia begins whenever exogenous or/and endogenous stimuli, which may include pyrogens are exposed to host cells-monocytes and macrophages (Arai et al., 1990). Formation of cascade of other pyrogenic cytokines like interleukin-1, TNF-α, and interleukin-6, among others follow. As a result of an interaction of cytokines and their receptors in the preoptic region of the anterior hypothalamus, phospholipase A is activated to catalyze arachidonate (substrate for COX), leading to the synthesis of prostaglandins, that could further trigger the temperature to be elevated. The fractions of *A. leiocarpa* exhibited significant antipyretic activity against Brewer's yeast-induced pyrexia. The phytochemicals steroids, tannins, alkaloids, flavonoids, saponins, and terpenoids detected in the fractions are associated with good anti-pyretic activity. Steroids, tannins, alkaloids and terpenoids are predominant inhibitors of PG synthetase, while flavonoids inhibit the production of tumor necrosis factor-α, which stimulates the synthesis of PGE2 necessary for fever induction. In addition, saponins inhibit the enzymes cyclooxygenase and phospholipase A_2, which are involved in developing pyrexia (Kumar et al., 2015; Kamau et al., 2016). Hence, the fractions of *A. leiocarpa* showing high efficacy similar to Aspirin in inhibiting the elevated temperature in the yeast-induced fever model suggests a similar possible mechanism of action.

Observations from the anti-inflammatory, anti-nociceptive and anti-pyretic studies revealed that, among the fractions, F4 was the most potent. On this note, F4 was selected and subjected to GC-MS analysis to identify the active principles. The GC-MS analysis revealed a number of bioactive compounds (Table 7), which might have directly or indirectly contributed to the anti-inflammatory, anti-nociceptive and anti-pyretic activity of the aqueous leaf extract of *A. leiocarpa*. The compound 3-diazo-1-methyl-1,3-dihydro-indol-2-one is a derivative of indolone. Indole-indolone is an important scaffold in the field of medicinal chemistry. Indole derivatives have been reported to possess significant pharmacological activities. Derivatives of indole have been used as anti-inflammatory, analgesic and anti-pyretic agents (Rani et al., 2004; Lamie et al., 2016). Schiff bases have been reported to possess various pharmacological activities, e.g., anti-inflammatory activity (Bhat et al., 2015) anti-tubercular activity (Bhat et al., 2011; Sharar et al., 2017) and anticonvulsant activity (Bhat and Al-Omar, 2011). Indomethacin is an NSAID and one of the indole acetic acid derivatives, which are known to cause ulcers for its users. However, its safety profile has been improved by chemical modifications (Amir and Kumar, 2005). This has shown that modification by synthesis has a high possibility to provide derivatives with significant anti-inflammatory activity and fewer side effects. Also, hexadecanoic acid methyl ester was implicated in the anti-inflammatory effect of *Jatropha curcas* L. roots (Othman et al., 2015) as several reports implicated the role of saturated fatty acids in regulating the inflammatory process (Rajeswari et al., 2012; Vasudevan et al., 2012).

CONCLUSION

From the data obtained in our study, F4 of *A. leiocarpa* possesses significant anti-inflammatory, anti-nociceptive and anti-pyretic activities in the animal model studied. This we accrue to the presence of the identified compounds such as 3-diazo-1-methyl-1, 3-dihydro-indol-2-one. Through synergistic and/or additive pharmacological effects, the bioactive compounds present in F4 might have contributed to the anti-inflammatory, anti-nociceptive and anti-pyretic properties of the aqueous extract of *A. leiocarpa* leaves.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTION:

Contribution	Idakwoji PA	Atanu FO	Nweje-Anyalowu PC	Momoh TB	Oniwon WO	Elazab ST	Sharkawi SMZ	Waheed RM	Youssef A	Batiha GE-S
Concepts or ideas	x	x								
Design	x	x								
Definition of intellectual content	x	x	x	x	x	x	x	x	x	x
Literature search	x			x	x					
Experimental studies	x	x		x			x	x	x	
Data acquisition	x		x	x	x					
Data analysis	x		x		x	x		x	x	x
Statistical analysis	x		x		x	x		x	x	
Manuscript preparation	x						x			x
Manuscript editing	x	x								
Manuscript review	x	x	x	x	x	x	x	x	x	x

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