



Acute toxicity and anti-inflammatory activity of hydro-methanol leaves extract of *Allophylus africanus* Beauv in rats

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ABSTRACT

Introduction: The leaves of *Allophylus africanus* are traditionally used for the treatment of various ailments such as arthritis, rheumatism, gout, hemorrhoids, dysentery, venereal diseases and malnutrition. This study was carried out to evaluate the acute toxicity and anti-inflammatory activity of the hydro-methanol leaves extract of *A. africanus* on laboratory rats.

Methods: *Allophylus africanus* leaves were extracted with 80% methanol using cold maceration for 5 days. The extract was subjected to phytochemical analysis, acute toxicity study and anti-inflammatory evaluation using carrageenan induced paw edema in laboratory rats.

Results: The phytochemical screening of the aqueous methanol leaves extract revealed the presence of carbohydrates, tannins, steroids/triterpenes, flavonoids, alkaloids and cardiac glycosides. The extract was found to have median lethal dose (LD50) of 3807.89 mg/kg body weight orally and the aqueous methanol leaves extract at doses 250 and 1000 mg/kg produced significant anti-inflammatory effect at the 3rd, 4th and 5th hours with the effect being dose dependent at the 4th and 5th hours. There were remarkable reductions of paw edema in the rats.

Conclusion: *Allophylus africanus* leaves has anti-inflammatory activity which explains the basis of its use in traditional medicine in the management of inflammation and related inflammatory disorders.

Implication for health policy/practice/research/medical education:

Allophylus africanus leaves have anti-inflammatory activity which explains the basis of its use in traditional medicine in the management of inflammation but caution is required due to its slight toxic effects. This knowledge can be tapped to formulate new agents to treat inflammatory and related inflammatory disorders.

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Introduction

Traditional medicine includes diverse health practices, approaches, knowledge and beliefs (1). The use of traditional medicines and medicinal plants in most developing countries as therapeutic agents for the maintenance of good health has been widely observed (2), with herbal medicine being an integral element, meet about 75% of the population's health care needs in Nigeria and up to 80% in Africa (3). The only true medicines ever used initially are plants (4) and some chemically useful plant drugs have been identified from the lead provided by their ethno-medical uses (5). Inflammation is a protective response that involves immune cells, blood vessels

and molecular mediators and its disorders bring about enormous array of human diseases (6). The major classes of drugs to suppress inflammation are non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids but their toxic adverse effects such as epigastric distress, peptic ulceration, osteoporosis, and iatrogenic Cushing's syndrome have limited their uses (7,8). There is hence, a need to intensify researches for plant based anti-inflammatory agents that are efficacious, safe, affordable, and accessible for patients.

Allophylus africanus is a specie of the genus *Allophylus* of the family Sapindaceae. It is a shrub that grows in the reverine thickest, open wood land and forest edges at

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altitudes of 960-1540 m. It is widely distributed throughout tropical Africa (9). In Nigeria, it is locally known as Akanro, Akaraesu (in Yoruba), Akaito (in Igbo), Karki (in Hausa) and Ebe/ukpe (in Esan tribe in Edo state). In ethno-medicine, the boiled leaves are used in aches, fever and rheumatic pains (10), in gout, hemorrhoids (9) while root and twigs are used for diarrhea, dental and oral health care (10).

Previous pharmacological studies on the leaves extract of this plant showed that it possessed antimalarial, antimicrobial and anti-oxidant activities (11). The present study was carried out to evaluate the acute toxicity and anti-inflammatory effects of the extract in animal model, so as to validate the ethno-medicinal claim of the use of the plant in treatment of inflammation.

Materials and Methods

Collection, identification and preparation of the plant material

Fresh leaves of *A. africanus* were collected from "Samaru" village, Zaria, Kaduna State, Nigeria in March, 2014. The plant leaves were identified by Mallam Musa of the herbarium section of the Department of Biological Sciences, Ahmadu Bello University Zaria and a voucher specimen was deposited there with number 1540.

The plant leaves were cleaned, air dried under shade and powdered using mortar and pestle to a suitable size and stored in appropriate container for further use.

Extraction procedure

Analytical grade solvents and freshly prepared solutions were used throughout the study. One thousand gram (1000 g) of the air dried powdered plant material was extracted by cold maceration in hydro-methanol mixture (methanol-water 80:20 v/v) for 120 hours using 5 L. The filtrate obtained was concentrated to dryness in a rotor evaporator under vacuum and later in oven (45°C). The dried powder was used for toxicological tests and anti-inflammatory activity (12).

Qualitative phytochemical screening

Preliminary phytochemical screening of the aqueous methanol leaf extract was carried out for detection of carbohydrates, tannins, steroids/triterpenes, glycosides, flavonoids, alkaloids using standard test methods (13-15).

Experimental animals

Adult rats of both sexes (95-105 g) were obtained from National Institute for Trypanosomiasis Research (NITR), Kaduna State, Nigeria and kept in the animal house of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The animals were maintained under standard conditions (12 hours light/12 hours dark cycle, temperature of $37 \pm 2^\circ\text{C}$, 35-60% humidity). The rats were fed with standard (grower) mash (Vital feed,

Jos, Nigeria) and water - *ad libitum*. Ethical rules guiding the use of animals for the experimentation were strictly adhered to (16) and with approval of Animal Ethics and Care Committee, Ahmadu Bello University, Nigeria (ABUCAUC/2016/018).

Acute toxicity study

The acute toxicity study of the aqueous methanol leaves extract of *A. africanus* was determined using previously described method (17). The study was carried out in two phases. The first phase consisted of 9 rats divided into 3 groups of three rats each and were treated with the aqueous methanol extract at doses of 10, 100 and 1000 mg/kg body weight per oral. They were observed for 24 hours for signs and symptom of toxicity and death. In the second phase, three rats divided into 3 groups of one rat each were treated with the aqueous methanol extract at doses of 1600, 2900 and 5000 mg/kg body weight per oral and observed for 24 hours for signs and symptom of toxicity and death. The oral median lethal dose (LD_{50}) was calculated as the geometric mean of the minimum toxic dose and maximum tolerated dose.

Anti-inflammatory study

Thirty adult rats of both sexes of weight ranges (85-105 g) were divided into five groups of six animals each. Stock solution of the extract, aspirin and carrageenan were prepared. To group I of the animals, 1 mL/kg of normal saline (negative control) was administered to each animal. To group II, aspirin (positive control) at dose of 300mg/kg was administered. To groups III, IV and V, the extracts at doses 250, 500 and 1000 mg/kg were administered, respectively. After an hour, 0.1ml of sterile saline suspension of 1% carrageenan was injected into the sub planter surface of the left hind paw of all animals in each group. Paw size was measured using venier caliper at time 0, 1, 2, 3, 4 and 5 hours after carrageenan administration. The percentage inhibition (PI) at each time interval was calculated (18).

$$PI = \frac{\text{Control group} - \text{treated group}}{\text{Control group}} \times 100$$

Statistical analysis

The results were expressed as mean \pm standard error of the mean (SEM) for all values. The data were statistically analyzed using one-way ANOVA (SPSS version 20.0) followed by Tukey's post hoc multiple comparison tests. The results were considered to be significant at $P < 0.05$.

Results

Extraction yield

The total solid of *A. africanus* crude extract recovered from maceration was 71 g. The extract was dark green in color with pleasant smell and hard.

Qualitative phytochemical screening

The results of the phytochemical screening of aqueous methanol leaves extract of *A. africanus* revealed the presence of secondary metabolites namely carbohydrates, tannins, steroids/triterpenes, flavonoids, alkaloids, cardiac glycosides and absent of saponins as shown in Table 1.

Acute toxicity

The acute toxicity of the extract was done per oral in rats. The LD₅₀ was found to be 3807.89 mg/kg (the following formula) and there was death of an animal at dose 5000 mg/kg in phase II (Table 2).

Anti-inflammatory activity of aqueous methanol leaves extract of *Allophylus africanus*

In the normal saline treated rats, sub-planter injection of 1% carrageenan suspension produced a local edema reaching its maximum effect at 3 hours. The aqueous methanol extract at doses 250 mg and 1000 mg/kg was able to significantly produce anti-inflammatory effect (at the 3rd, 4th and 5th hours). At the 4th and 5th hours, the effect was dose dependent. The extract at dose 1000 mg/kg showed higher inhibition of induced edema compared to standard drug (aspirin) at the 5th hour as shown in Table 3.

Discussion

Preliminary phytochemical screening provides a brief idea about the qualitative nature of active phytochemical constituents present in plant extract. The result of the preliminary phytochemical screening of the aqueous methanol extract indicated the presence of carbohydrates, steroids, triterpenes, glycosides, tannins, flavonoids and alkaloids (Table 1). These results other than conforming the previous findings (11) which reported the presence of tannins, saponins, flavonoids and carbohydrates in *A. africanus* plant, were necessary for standardization of the plant. These secondary plant metabolites are known to have various pharmacological effects and may be responsible for various activities of *A. africanus*.

The acute toxicity of the extract was done per oral in rats. The median lethal dose LD₅₀ of aqueous methanol leaves extract of *A. africanus* was found to be 3.807.89 mg/kg. This suggests that the extract is slightly toxic, based on the recommended classification (19) which states that substances with LD₅₀ values of 2000 to 5000 mg/kg body weight is slightly toxic.

The hydro-methanolic leaf extract of *A. africanus* was able to significantly ($P < 0.05$) produce anti-inflammatory effect at the peak of carrageenan induced edema but with less activity than that of the standard anti-inflammatory agent (aspirin). The extract at doses 250 mg/kg and 1000 mg/kg was able to significantly produce anti-inflammatory effect at the 3rd, 4th and 5th hours with the effect being dose dependent at the 4th and 5th hour. The extract however

Table 1. Qualitative phytochemical constituent of aqueous methanol extract of *Allophylus africanus* leaves

Constituents	Inference
Carbohydrates	+
Tannins	+
Saponins	-
Steroids/Triterpenes	+
Flavonoids	+
Cardiac glycosides	+
Alkaloids	+

Table 2. Median lethal dose (LD₅₀) of aqueous methanol leaves extract of *Allophylus africanus* administered orally

Experiment	Dose (mg/kg)	Number of dead mice after 24 h
Phase I	10	0/3
	100	0/3
	1000	0/3
Phase II	1600	0/1
	2900	0/1
	5000	1/1

$$LD_{50} = \sqrt{\text{Min. toxic dose} \times \text{Max. tolerated dose}}$$

$$LD_{50} = \sqrt{(2900 \times 5000)}$$

$$LD_{50} = 3807.89 \text{ mgkg}^{-1}$$

showed higher inhibition significantly ($P < 0.01$) of induced edema compared to the standard drug (Aspirin) at the 4th and 5th hour with percentage inhibition of 62.57% and 82.88% respectively (Table 3). Inflammation induced by carrageenan is thought to be biphasic (20). The early phase is mediated by histamine, serotonin, and prostaglandin increased synthesis in the damaged surrounding tissues. The late phase is sustained by prostaglandins produced by tissue macrophages and prostaglandins released and mediated by polymorphonuclear cells, leukotrienes and bradykinins. Mona et al (6) reported that secondary metabolites like flavonoids, tannins, saponins, alkaloids found in plants have anti-inflammatory effects. The mechanism underlying the anti-inflammatory effects of tannins includes the scavenging of radicals and inhibition of the expression of inflammatory mediators such as some cytokines, inducible nitric-oxide synthase and COX-2 (21). Some flavonoids have anti-inflammatory property and produce activity by inhibiting molecular targets of pro-inflammatory mediators in inflammatory responses (21). Wang et al (22) has reported that the anti-inflammatory effects of steroids could be attributed to a counter-irritation effect.

Conclusion

The aqueous methanol extract of *A. africanus* leaves had

Table 3. Effect of the aqueous methanol extract of *Allophylus africanus* on carrageenan induced paw edema in rats

Treatment	Dose	Mean paw edema thickness in diameters \pm SEM (mm)				
		1 h	2 h	3 h	4 h	5 h
Normal saline	1 mL/kg	1.76 \pm 0.26	2.29 \pm 0.15	2.40 \pm 0.37	1.71 \pm 0.35	1.11 \pm 0.17
Aspirin	300 mg/kg	0.89 \pm 0.18* (49.43)	1.36 \pm 0.23* (40.61)	0.94 \pm 0.06** (60.83)	0.75 \pm 0.11* (56.14)	0.58 \pm 0.12* (47.75)
Extract	250 mg/kg	1.46 \pm 0.19 (17.05)	1.65 \pm 0.17 (27.95)	1.39 \pm 0.19* (42.08)	1.26 \pm 0.16 (26.32)	0.58 \pm 0.12 (47.75)
Extract	500 mg/kg	1.46 \pm 0.16 (17.05)	1.99 \pm 0.24 (13.10)	1.68 \pm 0.22 (30.0)	0.95 \pm 0.23 (44.44)	0.60 \pm 0.18 (45.95)
Extract	1000 mg/kg	1.22 \pm 0.15 (30.68)	1.81 \pm 0.24 (20.96)	1.40 \pm 0.25* (41.67)	0.64 \pm 0.24* (62.57)	0.19 \pm 0.13** (82.88)

* $P < 0.05$, compared with normal saline group, ** $P < 0.01$ compared with normal saline group (One-way ANOVA followed by Tukey's post hoc multiple comparison test).

Data expressed as Mean \pm SEM. values given in parentheses represent PI – Percentage inhibition.

anti-inflammatory activity which may explain the basis of its use in traditional medicine in the management of inflammation and related inflammatory disorders. The significant anti-inflammatory activity of the extract observed might be associated to the secondary metabolites present in the plant and this may proffer scientific basis for its use. But, systemic important dose should be avoided due to the toxicity risk. Further bioassay-led fractionation and isolation of active principles are to be undertaken.

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Authors' contributions

ISF, ZM and NI conceived the idea and designed the study. AN and SS assisted during the literature search, data analysis and preparation of the manuscript. All read and confirmed the final version of the manuscript for publication.

Conflict of interests

The authors declare no conflict of interest.

Ethical considerations

Ethical issues have been observed by the authors. The protocol was confirmed by Animal Ethics and Care Committee, Ahmadu Bello University, Nigeria (ABUCAUC/2016/018).

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