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Short Communication

In Vivo Investigation of the Aqueous Methanol Extracts of Leaves of *Millettia aboensis* (Hook. F.) Baker (Fabaceae) for Anti-Inflammatory Potentials

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ABSTRACT

Inflammation is a serious disorder that is often accompanied by pain and discomfort. *Millettia aboensis* leaves are widely used traditionally in folkloric medicine in many parts of Africa for the management of different human ailments like inflammatory disorders, constipation, liver diseases. The phytochemical constituents and anti-inflammatory properties of aqueous methanol extract of *Millettia aboensis* leaf were investigated. The powdered leaf was exhaustively extracted with 95% aqueous methanol by cold maceration. The crude extract was concentrated *in vacuo*, weighed and percentage yield determined. Acute toxicity test was performed in accordance with the Lorke's method. The phytochemical composition was screened using standard procedures, while the anti-inflammatory property was screened using the carrageenan-induced paw oedema model in rats. The acute toxicity test revealed that the plant has a high margin of safety. The phytochemical studies of the crude extract showed the presence of alkaloids, steroids, terpenoids, glycosides, tannins, flavonoids and saponins. The *in vivo* anti-inflammatory study revealed that at 200 mg/kg dose there was significant ($p<0.05$) inhibition of 79.06% on the inflammation of carrageenan-induced paw oedema after 4 hours as compared to the standard drug (piroxicam) that had 62.79% inhibition. These findings suggest that *Millettia aboensis* possesses anti-inflammatory potentials.

Keywords: Anti-inflammatory activity, *Millettia aboensis*, Inflammation, Phytoconstituents, Prostaglandins.

Introduction

Inflammation is associated with increase in blood flow which leads to the reddening of the area due to erythrocyte accumulation and oedema.[1] This could arise as a result of various ailments such as diabetes, ankylosing spondylitis, multiple sclerosis, cancer etc.[1-6] Phospholipase A_2 (PLA_2) is very vital in the formation of inflammatory mediators which forms in the arachidonic pathway, these mediators controls numerous physiologic events in some disease conditions e.g prostaglandins, bradykinins, kinins etc.[7] The production of inflammatory mediators can be prevented by inhibiting the metabolism of arachidonic acid with the use of medicinal plants and drugs with low side effects that can reduce inflammation and pain.[8]

Nigeria is richly blessed with diverse anti-inflammatory plants used in traditional medicine. [9] *Millettia aboensis* have several traditional names in different parts of Nigeria which include; Igbo "*Otikpo",* Edo "*Ukperurumwesi",* Efik "*Odudu",* Ijo-izon "*Ofoni"* .[9,10] The leaf and root decoction has been widely used by traditional herbalist in treatment of ulcer, gastrointestinal disturbances, laxatives, liver disease, inflammatory disease and as well can be chewed and rubbed on the painful spots. [12] Among the people of Nsukka in Enugu State Nigeria, the leaves are masticated, mixed with saliva and spit on swollen body parts, hence the name *otikpo* (breaker).

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Pharmacological studies on *M. aboensis* includes antioxidant and immune enhancing properties,[13] hepatoprotective activity,[14] hepatotoxicity,[15] Contraceptive activity,[16] hematopoietic property,[17] Antimicrobial property,[18] laxative property.[19] The systemic evaluation of anti- inflammatory activity of *M. aboensis* in rats has not been described in the literature. This study investigated the acute toxicity, phytochemical constituents and anti-inflammatory properties of the leaves of *M. aboensis*.

Materials and Methods

Chemicals, reagents and drugs

The following reagents were used: sodium hydroxide, ammonia solution, ferric chloride solution, normal saline, carrageenan, hydrochloric acid, ethanol, potassium hydroxide, ethyl acetate, Molisch`s reagent, Million`s reagent, Wagner's reagent, Liebermann-Burchard reagent, 95% aqueous methanol (JHD UN1230, China), distilled water, Tween 80 and piroxicam capsule (Pfizer, U.S.A).

Experimental animals

Adult albino rats (25) and adult Swiss albino mice (15) were purchased from University of Nigeria, Nsukka, the animal facility of the Department of Pharmacology and Toxicology. The experimental protocol was in accordance with the guidelines of the ethics committee of the University of Nigeria (ref: NHREC/05/01/2008B) as registered by the National Health Research Ethics Committee of Nigeria.

Preparation of plant

M. aboensis leaves were collected from Ihe/Owerre, Nsukka Local Government Area, Enugu State in April 2017, and authenticated by Mr. Alfred E. Ozioko. An herbarium specimen with the voucher specimen number: InterCEDD/302B was deposited in the herbarium at International Center for Ethnomedicine and Drug Development (InterCEDD).

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Extraction

M. aboensis leaves were washed and air-dried at 29 ± 1°C for fourteen days. The dried leaves were grounded with electric blender. Cold maceration was used in the extraction process. A 500 g of the plant materials was soaked in 2.5 L of 95% methanol for 72 h with intermittent shaking. The extract was filtered using a Whatmann filter paper No.42, 125 mm (Whatman, England) after which it was concentrated *in vacuo* at 40°C.

Phytochemical analysis

Phytochemical screening was done using standard procedure as described by Trease and Evans.[20] The methanol extract of *M. aboensis* were screened for alkaloids, glycosides, saponin, tannins, terpenoids, steroids, phenols, flavonoids and carbohydrates. [20]

Acute Toxicity Test and determination of the median lethal dose (LD_{50})

The acute toxicity of the extract was determined using standard procedure as described in Lorke's method. [21] A total of 15 Swiss albino mice (16-29 g) were used in the experiment. The experiment was divided into two phases. In the first phase, nine mice were divided into three groups of three mice each which were given 10, 100 and 1000 mg/kg body weight (b.w.) of the extract respectively. In the second phase, 1600, 2900 and 5000 mg/kg b.w. of the extract were given to the remaining three mice (one mouse per dose). A control group of three mice were not given the extract.

The LD_{50} for the crude extract was calculated using the formular:

$$LD_{50} = \sqrt{Minimum\ toxic\ dose \times Maximum\ tolerated\ dose}$$

Anti-inflammatory activity test

The carrageenan-induced rat paw oedema model was used to assess for the anti-inflammatory potentials of the methanol extract. Albino rats (25) weighing 100-200 g were grouped into five groups of five rats each. Groups 1, 2, and 3 received 50, 100 and 200 mg/kg, respectively of methanol extract of *M. aboensis*. The 4th group received 10 mg/kg of piroxicam while the 5th group received 2.5 mL of the vehicle and served as the positive and negative control groups respectively. In each case, administration was via the oral route. A 0.1 mL of 1% w/v carrageenan (in normal saline) was injected in the sub-plantar region of the left hind paw of each rat to induce acute inflammation.[22] Changes in paw size were determined by wounding a thin thread round the paw and then determining the length of paw circumference, before carrageenan injection, and at 1, 2, 3, and 4th hour after carrageenan injection. The difference between the zero-time paw circumference length of the treated paw and the paw circumference length at different time intervals determines the oedema formation.[23] Inhibition of oedema was determined using the following formula:

$$\text{Inhibition of oedema (\%)} = \frac{Co - Ct}{Co} \times 100$$

Where C_O= paw circumference length of the control (negative) group at time t;

Ct= paw circumference length at corresponding time of the treated rats.

Statistical Analysis

The data were presented as Mean ± SEM and were analysed with one way analysis of variance. The difference between the mean of the treated and the control groups were considered significant at $p < 0.05$.

Results and Discussion

Phytochemical studies of *M. aboensis* leaves using the standard procedure as described by Trease and Evans showed the presence of alkaloid, tannins, saponins, terpenoids, glycosides, steroids, phenols and flavonoids (Table 1). Phytochemical analysis showed that the extract contains phytochemicals that have been documented to have anti-inflammatory activities and it includes flavonoids, saponins, steroids, terpenoids, phenols, tannins and glycosides have all been shown to have good anti-inflammatory properties.[24-26] The exact mechanism of suppression of inflammation by *M. aboensis* is not known however, the possible inhibition of prostaglandin synthesis may be traced to the high content of flavonoids and phenols in the extract that may have contributed to its anti-inflammatory actions. Flavonoids are known to inhibit the enzyme cyclooxygenase and lipoxygenase. [27-28]

The acute toxicity of *M. aboensis* on mice shows that the extract is relatively safe since there was no record of death as suggested in Lorke method. [21] The carrageenan-induced rat paw oedema model were used to assess for anti-inflammatory potentials of the methanol extract. This model used in the study is bi phasic; the first phase which spans over 60-150 min is attributed to the release of serotonin, kinins and histamine, while the second phase which starts from the 3rd hour is attributed to the release of prostaglandins and bradykinins.[29-30] The methanol extract of *M. aboensis* at 100 mg/kg reduced the paw circumference by 53.33% whereas at 200 mg/kg b.w. dose shows 62.22% inhibition after 3 h indicating that the effect of aqueous methanol extract of *M. aboensis* (Figure 1) is reflected in dose dependent manner. The inhibition of acute inflammation by the extract became pronounced at the 3rd and 4th hour as piroxicam (Figure 1), suggesting that the extract may have inhibited the second stage of acute inflammation induced by carrageenan which is essentially mediated by prostaglandins.[30] These potentials may be credited to the presence of flavonoids, phenols and saponins which are known anti-inflammatory phytochemicals.

Table 1: shows the qualitative phytoconstituents of the extract of *Millettia aboensis*.

S/N	Phytoconstituents	Relative Abundance
1.	Saponins	+
2.	Phenols	+
3.	Flavonoids	+
4.	Alkaloid	+
5.	Glycosides	+
6.	Terpenoids	+
7.	Steroids	+
8.	Tannins	+

Key: presence = + and absent = -.

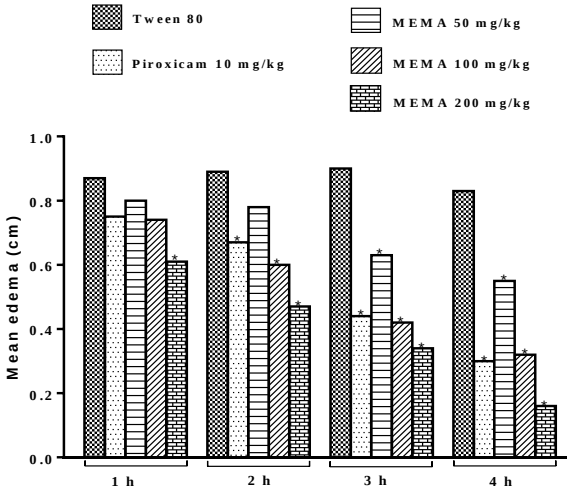

Figure 1: Effect of extract on the carrageenan induced paw oedema.

n=5. *Values are significantly different from the negative control group at ($p < 0.05$)

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Conclusion

The methanol leaf extract of *M. aboensis* possesses anti-inflammatory potentials. The methanol extract can be used for the management of inflammatory diseases in herbal medicine and can lead to the development of lead compound.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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