



## ANTI-INFLAMMATORY AND ANALGESIC POTENTIALS OF THE ROOT BARK EXTRACT OF *SABA COMORENSIS* (BOJER) PICHON (APOCYNACEAE) USED IN NIGERIAN TRADITIONAL MEDICINE

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### ABSTRACT

Painful and inflammatory diseases such as arthritis, backache, headaches, asthma and peptic ulcer disease are widespread and highly prevalent. The existing drugs for these conditions are liable to sub-optimal efficacy, intolerable adverse effects, prohibitive costs or limited availability. This scenario calls for renewed search for additional anti-inflammatory and analgesic agents. Hence, in line with the Traditional uses of plants, this study investigated the ethanol extract of *Saba comorensis* (Bojer ex. A.DC.) Pichon, for its safety, anti-inflammatory and analgesic effects in rodents using standard experimental protocols. The LD<sub>50</sub> (*i.p.*) for the ethanol extract obtained from the geometric mean of doses was 770 mg/kg. Compared with the negative controls, the ethanol extract exhibited significant ( $p < 0.05$ ) and dose-dependent anti-inflammatory, ulcerogenic and dose-independent analgesic effects, while the petroleum extract did not induce any significant ulcerogenic effects. These findings suggest *Saba comorensis* ethanol root extract may be relatively safe and possesses anti-inflammatory and analgesic properties and may justify its use as traditional remedy for painful and inflammatory conditions. There is the need to investigate the study plant further for anti-inflammatory and analgesic drug discovery.

**Key words:** Ethnomedicine, inflammation, gastric ulcers, nociception, Rodents.

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### 1.0 INTRODUCTION

Inflammation is a natural response to trauma, infection or certain stimuli by an organism, which is often associated with redness and heat, swelling, and pain (Calixto *et al.*, 2003; Maione *et al.*, 2016). These painful and, often time, progressively

deteriorating conditions such as rheumatoid arthritis suffered by over 17.5 million people globally affect the quality of life of sufferers resulting in both societal and economic burdens (CDC, 2017). These symptoms are often managed by numerous synthetic anti-inflammatory drugs-steroids (including

cortisone, prednisone and methylprednisolone) and non-steroidal anti-inflammatory drugs (NSAIDs such as aspirin, diclofenac, etodolac, ibuprofen and indomethacin)-on the market, but these drugs are usually associated with numerous side effects and high cost (Blanca-Lopez *et al.*, 2019; Calixto *et al.*, 2004). Interestingly, anti-inflammatory phytochemicals, present in extracts from medicinal plants, such as flavonoids, saponins, alkaloids, tannins, usually follow the same mechanism of action as NSAIDs, by inhibiting the inflammatory processes in the body (Iwalewa *et al.*, 2007; Oguntibeju, 2018), but, would serve as a safe (Oguntibeju, 2018) and ideal alternative to synthetic anti-inflammatory drugs. It therefore calls for a search for more ethnomedicinal plants with anti-inflammatory effect, to help curb the negative effects of prolonged use of NSAIDs and their likes, which is the focus of the current study.

*Saba comorensis* (Bojer ex A.DC.) Pichon also known as *Saba florida* (Benth.) Bullock, *Landolphia florida* Benth. var. *leiantha* Oliv. and *Landolphia comorensis* (Bojer ex A. DC.) K. Schum (Family: Apocynaceae), is an important food/nutraceutical plant widely distributed in Tropical Africa, Madagascar and the Comoro Islands (Leeuwenberg and van Dilst, 1989; Wakibara, 2005; Burrows *et al.*, 2018). The species under the genus *Saba*, are African indigenous plants (Leeuwenberg and van Dilst, 1989), whose fruits are consumed by humans as sources of nutrients (Omujal *et al.*, 2014; Sarr *et al.*, 2018), as well as medicine (Anthony *et al.*, 2017), while other parts of the plants are mainly used in ethnomedicine for management of different ailments including rheumatism and pain. These plant species are macromorphologically similar (Leeuwenberg and van Dilst, 1989), which often leads to herbalists prescribing one

species for another, however, extracts of different parts of *S. senegalensis* and *S. thompsonii* have been reported to show some differences in their TLC profile (Mireku-Gyimah *et al.*, 2018). It is also on record that different organs (leaves, stem and roots) of these species contain some useful phytochemicals like alkaloids, triterpenoids, tannins, saponin and anthracene glycosides (Mireku-Gyimah *et al.*, 2018), which support their usefulness in traditional medicine in different parts of Africa. The unripe fruit decoction of *S. senegalensis* is used for washing small boils (Inngjerdingen *et al.*, 2004), while the ripe fruit is used as an appetite suppressant in the management of obesity in Burkina Faso (Pare *et al.*, 2016). The fruit of *Saba comorensis* is used as a diagnostic tool for enlarged prostate in a community in Kenya (Kipkore *et al.*, 2014), as well as in the treatment of syphilis, stomach ache and diarrhea with blood, in some parts of Ethiopia (Teklehaymanot and Giday, 2010). However, the ethnomedicinal importance of these species is not limited to their fruits, other organs of the plants also play important roles in health promotion of both humans and animals in the traditional African medicine. For instance, the powdered root decoction of *S. comorensis* is used as labor inducer in northern part of Nigeria (Attah *et al.*, 2012), and its root and root bark aqueous decoctions are used in the treatment of rheumatism in Kenya (Omino and Kokwaro, 1993); the leaf decoction of *S. senegalensis* is used in ethnoveterinary medicine for the treatment of diarrhea in livestock (Koné and Atindehou, 2008).

A number of studies have been conducted to validate the wide ethnomedicinal applications of this plant and related species. The leaf decoction of *S. senegalensis* and the aqueous and chloroform extracts of the leaf and stem of *S. comorensis* have been reported to possess in-vitro anthelmintic

activity (Belemlilga *et al.*, 2016; Omale and Ubimago, 2014) while leaf and stem extracts of a closely related *S. senegalensis* have been shown to have in-vitro anti-inflammatory and antioxidant effects, and in-vivo analgesic effects in mice, in another study (Yougbaré-Ziébrou *et al.*, 2015). Additionally, *S. comorensis* fruit pericarp, stem bark and leaf extracts have been previously reported for antifungal, antibacterial and antioxidant effects (Bagre *et al.*, 2014); Diabagate *et al.*, 2019; Omale *et al.*, 2010; Omale and Omajali, 2010). However, despite the numerous reported pharmacological effects of different parts of the *Saba* species, the anti-inflammatory and analgesic property of the roots of *Saba comorensis*, within the available literature, has not been investigated and this study sets out to do justice to that in the face of high prevalence of arthritis and other inflammatory diseases on one hand, and the above-stated shortcomings of the existing anti-inflammatory and analgesic agents.

## **2.0 MATERIALS AND METHODS**

### **2.1 MATERIALS**

#### **2.1.1 Plant material**

*Saba comorensis* roots were collected from their natural habitat in Samaru – Zaria, Kaduna state, Nigeria between October and November, 2006 and authenticated by Mr Gallah of the Herbarium unit, Biological Science Department, Ahmadu Bello University, Zaria with a sample voucher deposited at the herbarium unit of the University.

#### **2.1.2 Animals**

Swiss albino mice of both sex weighing between (18-22 g) and albino Wistar rats of the either sex (180-200 g) which were kept under good laboratory practices were used for the present study. Ethical approval was obtained from the Department of

Pharmacognosy was granted which is in line with the guidelines of Ahmadu Bello University animal use and care policy.

#### **2.1.3 Drug and chemicals**

Acetyl salicylic acid 300 mg (Sigma U.S.A), piroxicam 20 mg (Pfizer New York), formalin (2.5%) and glacial acetic acid were used for the animal investigations.

## **2.2 METHODS**

### **2.2.1 Plant material processing**

Collected roots of *S. comorensis* were cleaned and washed with water, air-dried during harmattan season under shade at room temperature and further pulverized using a milling machine. The powdered sample was preserved in a clean airtight container, kept away from light, heat and moisture until further use. Dried powdered material of the root was extracted independently with ethanol and petroleum ether, using cold maceration for 72 h. While the exhausted marcs were discarded, filtrates obtained were dried over water bath at 50 °C and the moisture-free yield obtained was refrigerated at 4 °C until further use (Sireeratawong *et al.*, 2012). Only the ethanol extract was used for the assays while the petroleum ether extract was exclusively used to validate the inactivity of the lipid portion of the extract in inducing gastric ulceration.

### **2.2.2 Acute Toxicity Studies**

The method described by Lorke (1983) was used to determine the LD<sub>50</sub> of the extract. This was accomplished in two phases, 1 and 2; where phase 1 animals (3 per dose group) received graded doses (10,100, 1000 mg/kg body weight) of extract, while phase 2 test depended on the outcome from the phase 1 test. All animals received extracts or normal saline intraperitoneally (i.p). The LD<sub>50</sub> value was calculated from the geometric mean of

the doses that produced a transition from survival to death. This is expected to give information on doses to be chosen for the pharmacological studies.

### 2.2.3 Acetic acid induced writhing

The response to an intraperitoneal (*i.p.*) injection of acetic acid solution, manifesting as a contraction of the abdominal muscles and stretching of the hind limbs, was carried out according to procedures described by Hendershot and Forsaith (1959) and Koster *et al* (1959). Groups (n=5) of Swiss albino mice (5 per group) were randomized into 5 experimental groups (n=5) with a group receiving *i.p.* normal saline (10 ml/kg), extract (50, 100, 150 mg/kg) or acetyl salicylic acid (150 mg/kg). After thirty minutes, 10 mL/kg of 0.6% v/v acetic acid solution was administered intraperitoneally to all groups. The number of writhing movements produced in each group for the succeeding 5 – 15 minutes after the administration of acetic acid was counted (Vacher *et al*, 1964; Gwade, 2012; Shama *et al.*, 2019) and the results were expressed as percentages relative to the control group according to the formula below. A significant reduction in the number of writing movements in the groups tested with Ethanol extract compared with control was considered to be a positive analgesic response.

$$\% \text{ inhibition of Writhing} = \frac{\text{mean number (Control)} \times \text{mean number (test)}}{\text{Mean number of control}} \times 100$$

### 2.2.4 Acute and chronic anti-inflammatory analysis of the root bark extract of *Saba comorensis*

Acute and chronic anti – inflammatory studies were carried out in formalin – induced paw oedema in Wister rats as described by Ashok *et al* (2006). Overnight – fasted, Wistar rats that have been fasted overnight were randomly assigned to five groups of five animals each. Groups I and II

received normal saline and piroxicam respectively while groups III – V received graded doses of the ethanol extract of the root bark. inflammation was produced in these animals by injection of 0.1 mL of 1% w/v formalin into the sub planter region of left hind paw. The volume was measured using Vernier Caliper, at 0, 1, 2, 3 and 4 hours of formalin injection; this represents the acute phase. The difference between the 0- and 3-hours reading was taken as the volume of oedema and the percentage of oedema inhibition was calculated for each group. In the chronic phase, the drug treatment started 30 minutes before the injection of formaldehyde and continued every 24 hours (just after measurement of paw volume) until day 5. The paw volume was measured on day zero and continued every day until the fifth day. The volume of oedema and percentage of oedema inhibition were calculated for each group. The difference between day 0 and 5 was taken as the volume of oedema.

### 2.2.5 Ulcerogenic assay

The method described by Cashin *et al* (1977) was employed for this assay. To exclude the effect of the lipid portion of the study plant on gastric ulceration, petroleum ether extract was compared with the ethanol extract. Randomised groups (n=4) of Wistar rats were fasted for 18 h and thereafter one group was administered normal saline (10 ml/kg) (negative control), ethanol extract or petroleum ether fraction (150 or 500 mg/kg), or acetyl salicylic acid (150 mg/kg) (positive control). Three hours later, the animals were sacrificed and the stomachs removed and cut open at regions of less curvature. Normal saline was used to wash the opened stomach and viewed with a magnifying lens (x 10). The arbitrary scales described by Bani *et al.* (2000) were used to score the gastric lesions.

### 3.0 Statistical Analysis

The values of the mouse paw oedema (paw volume) for the anti-inflammation assay and the percentage writhing reflex for the analgesic assay were expressed as mean  $\pm$  standard error of mean (S.E.M). Data between experimental groups were analysed using analysis of variance (ANOVA) with intergroup comparisons made using student t-test. Values with  $p$ -values less than 0.05 were considered significant

## 4.0 RESULTS

**Table I: Effect of the Ethanol Extract on Acetic Acid-induced Writhing Test in Mice**

| Treatment (mg/kg) | No. of writhes | Mean no. of writhes | % inhibition |
|-------------------|----------------|---------------------|--------------|
| Normal saline     | 170            | 34.0 $\pm$ 3.4      | -            |
| Extract 50        | 63             | 12.6 $\pm$ 6.7*     | 62.9         |
| Extract 100       | 16             | 3.2 $\pm$ 3.6*      | 90.6         |
| Extract 150       | 91             | 18.2 $\pm$ 3.5*     | 46.5         |
| ASA 150           | 15             | 3.0 $\pm$ 1.0       | 91.2         |

The results given are mean  $\pm$  S.E.M \*t-test against control at  $P < 0.001$

### 4.3 Anti-inflammatory studies

The extract elicited significant ( $P < 0.05$ ) and dose-dependent inhibitory effect on both acute (Table II) and chronic (Table III) phases of inflammation. However, the extract's acute were greater than its chronic effects. The highest inhibition of acute oedema formation by the extract at 150 mg/kg was comparable to that of the standard drug, piroxicam.

### 4.4 Ulcerogenic assay

Compared with the negative control (normal saline), both extracts produced significant dose-dependent gastric ulcerations (Table IV) that were comparable to those of acetyl salicylic acid (positive control). The least

### 4.1 Acute Toxicity

The value obtained for the LD<sub>50</sub> (ip) following the geometric mean of doses was 770 mg/kg body weight.

### 4.2 Analgesic activity

The ethanol extract of *Saba comorensis* exhibited significant ( $p < 0.001$ ) dose-independent inverted-U analgesic effects (Table I). The highest analgesic effect of the extract at 100 mg/kg was comparable to that of 150 mg/kg dose of acetyl salicylic acid.

gastric ulceration was produced by the ethanol extract at 150 mg/kg.

## 5.0 Discussion

In this study, the acute toxicity, anti-inflammatory and analgesic activity of root bark of *Saba comorensis* (Bojer ex A.DC.) Pichon were investigated in rodents using the outlined standard experimental protocols in an attempt to validate their uses in traditional medicine. The LD<sub>50</sub> value (770 mg/kg i.p) obtained for *Saba comorensis* ethanol root extract in this study suggests that the plant extract is relatively safe in rodents and may lend credence to its use in ethnomedicine. Similar studies on other members of the genus *Landolphia*

(including *Landolphia owariensis* and *Landolphia dulcis*) reported an LD<sub>50</sub> value of > 5000 mg/kg in mice using ethanol extracts (Ilodigwe *et al.*, 2013; Coolborn and

Bolatito 2015; Ezike *et al.*, 2016) supporting the relative safety of the plants within the genus.

**Table II: Acute anti-inflammatory activity of ethanol extracts of *Saba comorensis* in formalin – induced inflammation.**

| <b>GROUPS</b>               | <b>Initial paw thickness (cm)</b> | <b>Paw thickness after 3hrs</b> | <b>Increase in paw thickness (cm)</b> | <b>% Inhibition</b> |
|-----------------------------|-----------------------------------|---------------------------------|---------------------------------------|---------------------|
| <b>Control</b>              | 0.42 ± 0.02                       | 0.78 ± 0.04                     | 0.36 ± 0.030                          | -                   |
| <b>Piroxicam 150 mg/kg.</b> | 0.43 ± 0.03                       | 0.48 ± 0.15                     | 0.05 ± 0.090                          | 86.1                |
| <b>Dose (I) 150 mg/kg</b>   | 0.44 ± 0.28                       | 0.52 ± 0.02*                    | 0.08 ± 0.150*                         | 77.8                |
| <b>Dose (II) 100 mg/kg</b>  | 0.42 ± 0.02                       | 0.54 ± 0.03*                    | 0.12 ± 0.025*                         | 66.7                |
| <b>Dose (III) 50 mg/kg</b>  | 0.42 ± 0.02                       | 0.60 ± 0.09*                    | 0.18 ± 0.050*                         | 50.0                |

The results given are mean ± S.E.M; \* t-test of extract versus control at  $P < 0.05$

**Table III: Chronic anti-inflammatory activity of ethanol extract of *Saba comorensis* in formalin – induced inflammation.**

| <b>Groups mg/kg</b>  | <b>Initial paw thickness (cm)</b> | <b>Paw thickness after 5 days</b> | <b>Increase in paw thickness (cm)</b> | <b>Inhibition (%)</b> |
|----------------------|-----------------------------------|-----------------------------------|---------------------------------------|-----------------------|
| <b>Control</b>       | 0.42 ± 0.02                       | 0.78 ± 0.04                       | 0.36 ± 0.03                           | -                     |
| <b>Piroxicam 150</b> | 0.43 ± 0.03                       | 0.56 ± 0.04                       | 0.13 ± 0.03                           | 63.9                  |
| <b>Dose (I) 150</b>  | 0.44 ± 0.28                       | 0.60 ± 0.05*                      | 0.16 ± 0.16*                          | 55.6                  |
| <b>Dose (II) 100</b> | 0.42 ± 0.02                       | 0.64 ± 0.06*                      | 0.22 ± 0.04*                          | 38.9                  |
| <b>Dose (III) 50</b> | 0.42 ± 0.02                       | 0.64 ± 0.06*                      | 0.22 ± 0.04*                          | 38.9                  |

The results given are mean ± S.E.M; \* t-test of extracts versus control at  $P < 0.05$ .

**Table IV: Effect of ethanol extract and petroleum ether extracts on gastric irritation in rats**

| Extract | Dose (mg/kg) | Ulcer incidence | Ulcer score |
|---------|--------------|-----------------|-------------|
| EE      | 500          | 3/4             | 2.75 ± 0.70 |
|         | 150          | 2/4             | 0.5 ± 0.70  |
| PE      | 500          | 4/4             | 1 ± 0.70    |
|         | 150          | 2/4             | 1 ± 0.70    |
| ASA     | 150          | 4/4             | 3.00 ± 0.90 |
| Control | -            | 0/4             | 0.00 ± 0.00 |

n = 4. Lesions on the mucosal surface were scored according to arbitrary scale: 0 = no lesion; 0.5 = hyperemia; 1 = one or two lesions; 2 = severe lesion; 3 = very severe lesions; 4 = mucosal full of lesions. EE, Ethanol extract; PE, Petroleum ether extract; ASA, Acetylsalicylic acid.

The acetic acid-induced writhing test is also called the abnormal constriction test (Aliyu *et al.*, 2005). It is a sensitive procedure to establish peripherally acting analgesics which involve local peritoneal receptors (Bentley *et al.*, 1983; Vogel and Vogel 1997; Ashok *et al.*, 2006). This method has been applied to understand the mechanism(s) by which plant metabolites act against pain of peripheral origin (Okpoko *et al.*, 2020; Nguyen *et al.*, 2020). Its activity in-vivo causes the release of endogenous mediators like prostaglandins, which stimulate peripheral nociceptive receptors, and this writhing behavior produced is blocked by NSAIDs. It is a well-established fact that NSAIDs exert their analgesic and anti-inflammatory activity by the inhibition of cyclooxygenase activity (Vane, 1971). Documented evidence strongly supports the use of herbal remedies and medicinal plants such as the extracts of *Salvia divinorum*, *Periplaneta americana*, *Terminalia avicennioides* in the management of pain (Tlacomulco-Flores *et al.*, 2019; Sharma *et al.*, 2019; Okpoko *et al.*, 2020; Nguyen *et al.*, 2020). Interestingly, *Landolphia owariensis*, a

member of the same genus *-Landolphia*, to which the study plant belongs, has been reported to have analgesic property in rat models (Owoleye *et al.*, 2001) and this lends support to the findings of this study. The inverted U-shaped dose-independent analgesic activity of extract of *S. comorensis* is consistent with the vast body of literature concerning hormetic dose responses (Calabrese and Blain, 2005) and has been observed with plant extracts, plant derived compounds and polyherbal formulations (Calabrese, 2008; Heidari *et al.*, 2009; Nowacki *et al.*, 2015; Linares *et al.*, 2019; Wheeler *et al.*, 2020). Thus further mechanistic investigation is encouraged as the extract may be acting via different types of receptor mechanism (Calabrese, 2008).

The result obtained in this study showed that the ethanol extract of the root of *Saba comorensis* possesses anti-inflammatory effects observed in formalin-induced oedema test in rodents. Formalin induces peripheral tissue inflammation, paw oedema and causes local injury of the paw making it a suitable model for localized inflammatory pain (Tjoslen *et al.*, 1992; Ahsan *et al.*, 2019; Tlacomulco-Flores *et al.*, 2019).

Formalin-induced paw oedema is most suitable as it closely resembles human arthritis (Greenwald, 1991; Okpoko *et al.*, 2020). The anti-inflammatory and antinociceptive effects of *Saba comorensis* may be due to their phytochemical content including flavonoids, tannins, alkaloids, and saponins, which have been reported in our previous study (Attah *et al.*, 2007). For instance, polyphenols such as phenolic acids, proanthocyanidins, and flavonoids have been documented to have a modulatory effect on pain and inflammation via interference with the cytokine cascade pathways (Calixto *et al.*, 2004). The ability of the extracts to reduce inflammation by inhibiting oedema formation during the inflammatory phase may be due to the inhibition of the release of pro-inflammatory mediators of acute inflammation such as histamine and prostaglandin (Okpoko *et al.*, 2020). Moreover, the extracts elicited a moderate gastrointestinal irritation in rats typical of anti-inflammatory prostaglandin inhibitors such as the non-steroidal anti-inflammatory drugs, NSAIDs (Rang and Dale, 1988). Thus, active metabolite(s) in the extract may exert anti-inflammatory effects by inhibiting the synthesis of prostaglandin.

The association of analgesic and anti-inflammatory effects observed with the extract of this study plant has been documented for other plant species and non-steroidal anti-inflammatory drugs (NSAIDs) (Seddighfar *et al.*, 2020; Sundar and Arunachalam, 2020). The analgesic and anti-inflammatory metabolites responsible for the plant's activity could represent potential molecules for the development of new, safe and effective anti-inflammatory agents to combat various inflammatory conditions currently plaguing mankind (Calixto *et al.*, 2004; Zahra *et al.*, 2020). However, the phytochemical group, specific biologically active chemical group and

mechanism(s) responsible for the pharmacological activities of *Saba comorensis* remain to be well investigated.

## 6.0 Conclusion

This study has shown *Saba comorensis* root extracts to be relatively safe, and to possess significant anti-inflammatory and analgesic activities, which may justify its traditional use as a pain reliever. However, there is a need to investigate it further as a potential source of novel anti-inflammatory and analgesic agents.

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