Anti-inflammatory activity of *Sabicea brevipes* Wernharm (Rubiaceae)

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Abstract

Over the years, medicinal plants have been employed in the treatment of inflammation and related ailments. This study evaluated the anti-inflammatory potential of the aerial parts of *S. brevipes*. The extracts and fractions were further evaluated for anti-inflammatory activity in carrageenan-induced rat model at varying doses (200 and 400 mg/kg doses, orally) for 5 h of treatment. The result of the phytochemical screening showed the presence of alkaloids, terpenoids, glycosides, flavonoids and tannins in the aerial parts of the plant. The *in vivo* anti-inflammatory study exhibited inhibition of 42% and 44%, 47% and 36%, 33% and 31%, and 43% and 42% for methanol extract *n*-hexane fraction, ethyl acetate fraction, and methanol fraction, at 200 and 400 mg/kg doses, respectively. The positive control (diclofenac sodium) showed an inhibition value of 51% at 5 mg/kg dose. Finally, it is concluded that *S. brevipes* possesses anti-inflammatory potential which validates the ethnomedicinal claim of the plant.

Keywords

*Sabicea brevipes*, phytochemicals, anti-inflammatory, paw edema, carrageenan

Introduction

Over the years, medicinal plants have been employed in the traditional treatment of human disease, which include but is not limited to wounds, malaria, fever, diarrhea, dyspepsia, gonorrhea and leprosy (Hussain et al. 2021).

Various phytochemicals contained in medicinal plants are responsible for these therapeutic activities. There are fewer records of side effects arising from the treatment of diseases using herbs and herbal medicines (Oguntibeju, 2018). Phytoconstituents such as alkaloids, tannins, terpenoids, flavonoid glycosides and saponins have various
attributes of physiological responses in the human body (Junejo et al. 202a; Junejo et al. 2020b). The development of anti-inflammatory drugs derived from natural sources has been described as a rational and productive strategy towards the treatment of inflammatory diseases, and plant-derived natural products are known to inhibit specific inflammatory mediators (Lahare et al. 2021).

Inflammation which is a local response of living mammalian tissues due to foreign agents often occurs to eliminate or limit the spread of injurious substances (Zhao et al. 2021). It eradicates microbes or irritants and potentiates tissue repair (Fung et al. 2020). Rubor (redness), tumor (swelling), calor (heat), dolor (pain) as well as function laesa (functional impairment) are the signs of inflammation. Inflammation protects by diluting, destroying harmful agents like toxic microbes, before healing and repairing take place (Chu et al. 2020). Many disease conditions such as arthritis, prostatitis, anaphylaxis, diabetes, chronic kidney disease, digestive disorders, Alzheimer’s disease, bacterial infections and novel coronavirus infection are all associated with inflammation especially at the cellular level which usually results in serious health problems (Junejo et al. 2021; Bandyopadhyay et al. 2021). Over and above this, it has been reported that more than 3 million people are affected by inflammatory bowel disease (IBD), a chronic inflammatory illness, in United States and that about 1.6 million patients have IBD in the region (Kumar 2021).

The aerial parts of *S. brevipes* (Family: Rubiaceae) (Fig. 1) has been reported to contain alkaloids, glycosides, saponins, tannins, triterpenoids, antracene, flavonoids and volatile oils (Ogbuanu et al. 2014; Chimaobi et al. 2019). The extract of this plant is used in preventing oxidative stress and in the treatment of common infectious illness. The root (aerial parts) enhances male potency probably due to their stimulating and tonifying effects on skeletal muscles (Ogbuanu et al. 2021). The leaves are employed in ethnomedicine to heal wounds, and also to treat bacterial and fungal infections of which most of these claims have not been experimentally validated. In an attempt to validate a part of the claims mentioned above, this study was aimed to investigate the anti-inflammatory potential of the aerial parts of *S. brevipes*.

**Materials and methods**

**Chemicals and reagents**

All chemicals and reagents used in the study were of analytical grade, supplied by (Sigma-Aldrich Inc, St. Louis, USA) and Nigerian-German Chemicals, plc. They include 95% aqueous methanol, ethanol, ferric chloride, glacial acetic acid, Mayer’s reagent (potassium mercuric iodide solution) and Wagner’s reagents (iodine in potassium iodide solution), ethyl acetate, sodium hydroxide, Fehling’s solution A and B, concentrated tetraoxosulphate (vi) acid, acetic anhydride, hydrochloric acid, carrageenan, dichloromethane, and *n*-hexane.

![Figure 1. *S. brevipes* plant.](image)
**Plant material**

The aerial parts of *S. brevipes* was collected in July 2019 from Leija in Enugu State Nigeria, and was identified and authenticated by Mr. Felix Nwafor of the Department of Pharmacognosy and Environmental Medicines, University of Nigeria, Nsukka with a Voucher number of PPC/UNN/O331. A voucher specimen was deposited at the herbarium of the Department. The collected plant material was air-dried at room temperature under shade and reduced to the appropriate size using a milling machine (Junejo et al. 2017).

**Extraction**

A 400 g dried aerial parts of the plant was extracted by cold maceration with 95% ethanol (1:6, w/v) (× 2) at room temperature for 48 h. The extraction process was facilitated by periodic shaking. The crude extract was first filtered using gauze followed by with Whatman filter paper No. 1 (Whatman, England) (Junejo et al. 2018). The residue was re-macerated for another 48 h twice and filtered. The combined filtrates were then dried by a rotary evaporator (Buchi Rotavapor, Switzerland) to a constant weight of 25 g of dry extract (6.25% w/w yield) and the dried *S. brevipes* extract (SBE) was kept at –20 °C before further use (Mohammadi et al. 2020).

**Fractionation of the crude extract**

The crude extract was subjected to successive extraction using solvents of graded polarities (*n*-hexane, ethyl acetate and absolute methanol). The fractions were dried to obtain *n*-hexane (HF), ethyl acetate (EF) and methanol (MF) fractions, transferred into separate vials and stored in a refrigerator for further use (Lekouaghet et al. 2020).

**Qualitative phytochemical analysis**

The preliminary phytochemical screening of the crude extract and fractions were carried out using the standard methods reported in literature. Tests for the detection of alkaloids (Chen et al. 2006; Hossain et al. 2013), flavonoids (Junejo et al. 2014a), saponins (Sheel et al. 2014), tannins, terpenoids (Junejo et al. 2014b) and glycosides (Hussain et al. 2020c) were performed.

**Experimental animals**

The animals were eight weeks old Wister albino rats (66.5 ± 18.5 g) and of both sexes. They were kept in clean cages under normal laboratory conditions of 25 ± 2 °C temperature and 12 h day light and night cycle in a well-ventilated room. Animals were allowed to acclimatize for seven days at the animal house with free access to clean drinking water and commercial pelleted food.

The experimental protocol was in accordance with the guidelines of the ethics committee of the University of Nigeria as registered by the National Health Research Ethics Committee of Nigeria (Ref. No.: NHREC/05/01/2008B). The research was conducted in accordance with the internationally accepted principle for laboratory animal use and care as found in European Community Guidelines (EEC Directive of 1986; 86/609/EEC). The ethics for the use of experimental animals were followed carefully.

**Acute toxicity test**

The oral acute toxicity (LD$_{50}$) of the methanol extract (SBE) was evaluated in Wister albino rats weighing between 47 ± 7 g following Lorke's method. (Usman et al. 2020; Hussain et al. 2020a, b). The methanol extract was dissolved in 10% v/v Tween 80 and distilled water. Dose levels used ranged from 10 – 5000 mg/kg of the methanol extract. The test comprises two phases. In the first phase, twelve rats were randomly divided into three groups (n=4). Each group received 10, 100 and 1000 mg/kg of the extract. Signs of toxicity such as death, change in physical appearance and behavioral changes were observed for 24 h. In the second phase, each rat received different doses of 1600, 2900 and 5000 mg/kg of the extract per oral. The rats were monitored for 24 h for lethality. The LD$_{50}$ was calculated as the geometric mean of the maximum dose of the extract that caused zero percent lethality (0% death) and the maximum dose that resulted in 100% lethality. A total of fifty Wister albino rats were randomly divided into ten groups. Group A received normal saline (untreated). Group B received diclofenac sodium (5 mg/kg). Groups C and D were treated with 200 and 400 mg/kg doses of SBE respectively. Groups E and F were treated with 200 and 400 mg/kg doses of HF respectively. Groups G and H received 200 and 400 mg/kg doses of EF, respectively, while groups I and J received 200 and 400 mg/kg doses of MF, respectively. The rats were treated according to Winter's method. Inflammation was induced by injecting 0.1 ml of 1% carrageenan in sterile normal saline into the sub-plantar region of the right hind paw of the rat. Rats were pre-treated orally with crude extract, fractions, and positive control 1 h before the carrageenan injection. The paw volume was measured from 0–5 h, at 1 h interval using a water plethysmometer. The mean changes in injected paw volume with respect to initial paw volume were calculated.

The percentage inhibition of paw edema was calculated by the formula (Munir et al. 2020; Junejo et al. 2022).

\[
\text{Percentage inhibition} = \frac{C_0 - C}{C_0} \times 100
\]

Where, $C_0$ = average paw volume of the treated group, $C$ = average paw volume of the control group.
Results were expressed as mean ± SD (n=5). The variation in paw volume across the experimental duration (between successive paw volume) was analyzed using one-way analysis of variance (ANOVA) at p < 0.05 confidence interval, while the variation of each paw volume means from the negative control was analyzed at p < 0.01 and p < 0.02 confidence interval followed by a two-sample t-test independent measures which compared the chosen dosages (200 and 400 mg/kg) for each fraction at p < 0.05.

Results and discussion

Phytochemical constituents of *S. brevipes*

The qualitative phytochemical screening of the crude extract and fractions showed the presence of alkaloids, flavonoids, saponins, tannins, terpenoids and glycosides (Table 1).

Table 1. Phytochemical constituents of *S. brevipes*.

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>SBE</th>
<th>HF</th>
<th>EF</th>
<th>MF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Steroid</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Saponin</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Tannin</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = Present, – = Absent
SBE = 95% methanol crude extract; HF = n-hexane fraction; EF = ethyl acetate fraction; MF = methanol fraction

Oral acute toxicity

Results (Table 2) of acute toxicity showed no obvious signs of toxicity in all treatment groups in both phases of the study following the administration of the SBE. The LD₅₀ of the extracts was greater than 5000 mg/kg body weight (1).

Effect of *S. brevipes* on carrageenan-induced paw edema in rat

Table 3 shows the effect of *S. brevipes* extract and fractions on carrageenan-induced paw edema in rats with time. A measure of significant difference between dosages determines the dose effect for each fraction. Fig. 2 shows the rate at which the developed carrageenan-induced inflammation is being inhibited by the extract and fractions. The measure of the steepness of each plot determines the rate and extent to which the associated fraction inhibits inflammation.

*S. brevipes* is reportedly used in preventing oxidative stress and in the treatment of common infections such as enhancement of male potency (Chimaobi et al. 2019). This in addition to the ethnomedical uses in the healing of wounds and treatment of bacterial infection necessitated this study. From results, it is apparent that he methanol extract of *S. brevipes* could possess anti-inflammatory activity. The distribution of the phytochemicals in the fractions is a consequence of the polarity difference of the fractionating solvents; as polar phytochemicals are soluble in polar solvents and non-polar phytochemicals are not. This could be the reason behind varying anti-inflammatory property of the fractions.

The safety of plant-based drugs is a major concern as far as their clinical utility is concerned. The result of acute toxicity showed no mortality record up to 1000 mg/kg for 24 h. The safety of the plant extract could explain its extensive usefulness in ethnomedicine as anti-inflammatory agent. There is no report of acute toxicity on the plant's aerial parts (*Sabicea brevipes*) in previous studies.

Carrageenan induced paw edema is widely used to assess the anti-inflammatory activity of natural and synthetic compounds (Cordaro et al. 2020). As a result of its high reproducibility, it stands as a distinctive anti-inflammatory model (Vyshtakalyuk et al. 2020). The first phase which ranges from 0–1 h are mediated by histamine, serotonin and bradykinin, whereas prostaglandin and several cytokins (IL- β, IL-6, IL-10 and TNF-α) account for the second phase which occurs around 3 h after carrageenan injection. The effect of these phases was observed among the paw volumes in the reported time intervals.

Except for the 400 mg/kg dosage of EF, all other fractions showed significant differences in the decrease of paw volume across the experimental duration (between successive paw volumes). However, for comparison with control, paw volume recorded at the 2 h for 200 mg/kg and 400 mg/kg SBE, 400 mg/kg HF, 200 mg/kg and 400 mg/kg EF, and all paw volumes recorded at the 1 h duration showed no significant difference at either 99% or at 98% confidence interval. Except for 200 mg/kg MF which has a significant difference at 98% confidence interval, all other paw volumes recorded showed significant difference at 99% confidence interval.

For dose-to-dose comparison (200 and 400 mg/kg), only HF has a significant difference in dosage, which implies that only this fraction is dose-dependent. Moreover, HF extract contains terpenoids. The presence of terpenoids might be responsible for the anti-inflammatory effect of the extract. Las Heras et al. (2003) also reported the anti-inflammatory potential...
of terpenoids. It is therefore assumed that the extract possesses anti-inflammatory effect due to the presence of terpenoids. The concentration of terpenoids in the extract is believed to be important for the therapeutic activity. A 200 mg/kg of HF significantly elicited higher inhibition of inflammation in paw edema induced rats than the corresponding 400 mg/kg dosage. Since other fractions showed no significant dose difference, it implies that inhibition of inflammation attained with 200 mg/kg of the fractions was not significantly different from the inflammatory inhibition attained with the corresponding 400 mg/kg dose across the experimental period.

Conclusion

In this study, the anti-inflammatory effect of the methanol and different solvent fractions (n-hexane, ethyl acetate, methanol) of S. brevipes Wernham aerial parts was investigated using carrageenan-induced paw edema. The extract and all the fractions inhibited carrageenan induced inflammation of paw edema in rats to an appreciable extent. The n-hexane fraction exhibited the highest average% inhibition at a dosage of 200 mg/kg, while the methanol extract and the methanol fraction showed significant inhibition at 200 and 400 mg/kg. Hence, the findings of our study validate the ethnomedicinal claim of S. brevipes.

Declaration of competing conflict of interest

The authors declare no conflict of interest.

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