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# Research Article
# Effect of Saponin-rich Fractions of *Neocarya macrophylla* on Murine Models of Pain and Inflammation

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## Abstract

**Background and Objective:** Saponins have a wide range of pharmacological properties such as analgesic and anti-inflammatory effects. *Neocarya macrophylla* tree has been used in ethnomedicine to treat pain and inflammation. The aim of this study was to investigate and compare the analgesic and anti-inflammatory effects of the saponin-rich fractions from the stem bark and root of *N. macrophylla*. **Materials and Methods:** Extraction of saponins and preliminary phytochemical screening was conducted using standard procedures; acute toxicity studies on the saponin-rich fractions was carried out according to Lorke's method. The analgesic and anti-inflammatory effects of the saponin-rich fractions of the stem bark and root of *N. macrophylla* were evaluated using acetic acid-induced writhing test in mice and carrageenan-induced paw oedema in rats, respectively. **Results:** The results of the study indicated that the saponin-rich fraction of the stem bark significantly ($p<0.05$) reduced the number of writhes by 87.7, 64.6 and 59.9% at 150, 300 and 600 mg $kg^{-1}$, respectively while the saponin-rich fraction of the root had 60.0, 66.2 and 81.5% inhibition of writhes at 75, 150 and 300 mg $kg^{-1}$, respectively; the standard drug, piroxicam (10 mg $kg^{-1}$) reduced the number of writhes by 73.8%. Both fractions significantly ($p<0.05$) and dose-dependently inhibited carrageenan-induced paw oedema at the 2nd, 3rd and 4th h. The intra peritoneal $LD_{50}$ of the saponin-rich fractions of the stem bark and root of *N. macrophylla* in mice were found to be 2154 and 1265 mg $kg^{-1}$, respectively suggesting that the fractions are fairly toxic. Similar phytochemicals were observed in the *n*-butanol fractions of the stem bark and the root. **Conclusion:** The saponin-rich fractions of the stem bark and root of *N. macrophylla* has significant analgesic and anti-inflammatory activities but the saponin-rich fraction of the stem bark has better effect compared to the root.

Key words: *Neocarya macrophylla*, saponins, analgesic, anti-inflammatory

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Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Saponins are a class of chemical compounds found in copious quantity in different plant species. To be more specific, they are amphipathic glycosides that are phenomenologically grouped by soap-like foaming they produce when shaken in an aqueous solution and structurally saponins have one or more hydrophilic glycoside moieties combined with lipophilic triterpene derivative[1]. Saponins in plants serve as anti-feedants and protect the plants against microbes[2]. In addition, saponins have historically been isolated from plants and marine organisms. Due to their extensive structural diversity, saponins have a wide range of pharmacological properties such as hemolytic, antimicrobial, molluscicides, anti-diabetic, anti-fungal, anti-tumor, anti-viral, allergic, analgesic, anti-inflammatory and anti-cancer[3-5]. Based on the information of their pharmacological activities, saponins from different sources provide lead for the development of new therapeutic agents[6].

Pain and inflammation form a significant component of many diseases and their drug management presents a serious challenge. The drug of choice NSAIDs and opioids are associated with GIT disturbance, aggravation of ulcer, tolerance, respiratory depression, constipation as well as psychological dependence[7]. Traditional medicine is still the mainstay of about 80% of the population especially in the developing countries, this is solely due to its acceptability, affordability and lesser side effects[8]. Medicinal plants have been a major source of most therapeutic agents[9]. Scientific evidence have shown that saponins constitute an effective analgesic and anti-inflammatory activity[10]. *Neocarya macrophylla* commonly known as ginger bread plum or neou oil tree (Gawasa in Hausa language) belongs to the chrysobalanaceae family. It has been used in traditional medicine (in northern Nigeria) to treat a number of ailments including pain and inflammation among others[11]. Due to its widespread usage in traditional medicine, the effect of the methanol stem bark extract of the plant was evaluated against *Naja nigricollis* venom[12]. The extract also demonstrated good anti-microbial activity on some selected micro-organism[13]. Chemical investigations on the stem bark of the plant is confined to the isolation of stigmasterol and a flavanol glycoside[14]. Recently, the anti-microbial activity of stigmasterol was reported by Yusuf *et al*.[15]. Furthermore, phytochemical studies on the methanol stem bark extract of the plant was also conducted by Yusuf *et al*.[12,13], saponins were found to be adequately present and the analgesic property of the plant have also been reported, providing scientific evidence for the use of the plant in the treatment of pain[11]. Extensive literature search revealed that, there is no report yet on the analgesic and anti-inflammatory properties of the saponin-rich fractions of the different parts of the plant. In view of the above, in this report herein, a comparative analgesic and anti-inflammatory studies of the saponin-rich fractions from the stem bark and root of *N. macrophylla*.

## MATERIALS AND METHODS

**Plant sample:** The plant sample of *N. macrophylla* was collected in October, 2015 at Jega Local Government Area of Kebbi state. It was identified by Namadi Sanusi at the Herbarium unit, Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria by comparing with herbarium reference voucher specimen (No. 3197). The stem bark (3000 g) and root (1280 g) were shade dried, pulverized to powder, labelled and stored at room temperature for use.

**Extraction of saponins:** The powdered materials were extracted exhaustively with 90% methanol. The extracts obtained were evaporated separately *in-vacuo* using rotary evaporator at 40°C and the crude extracts obtained were suspended in distilled water separately, filtered and successively partitioned with solvents of increasing polarity starting with *n*-hexane, dichloromethane/chloroform, ethyl acetate and finally *n*-butanol. The *n*-butanol fractions were further partitioned with 1% potassium hydroxide to remove polyphenols[16]. The fractions were evaporated to dryness under reduced pressure and the dried extract was dissolved in absolute methanol and diethyl ether[17] was used to evaporate the saponin components and were coded as $SRF_S$ for the stem bark and $SRF_R$ for the root of *N. macrophylla*.

**Experimental animals:** Swiss albino mice and adult Wister rats of either sex weighing 18-30 and 150-200 g, respectively were obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria. They were fed with laboratory diet and water *ad libitum* and maintained under standard conditions (12 h light and 12 h dark cycle) in propylene cages at room temperature. All experimental procedures were approved by the Animal Right and Ethics Committee of the University.

**Drugs and chemicals:** Acetic acid manufactured by BDH, England; Carrageenan 1%. Piroxicam manufactured by Pfizer Specialties LTD, Ikeja, Nigeria.

**Phytochemical tests:** A portion of the *n*-butanol (nBF) and saponin-rich (SRF) fractions of the different parts (stem bark and root) of *N. macrophylla* were subjected to phytochemical test to ascertain their chemical constituents based on standard procedures[18].

### Pharmacological studies

**Acute toxicity test:** The intraperitoneal $LD_{50}$ of the saponin-rich fractions was determined according to the method described by Lorke[19]. The route of administration was intra-peritoneal. The study was divided into two phases. In the first phase, 9 mice of either sex were divided into three groups containing three mice each. The first, second and third groups received 10, 100 and 1000 mg $kg^{-1}$ of the saponin-rich fractions ($SRF_S$ and $SRF_R$), respectively. Based on the result of the first phase, four animals were used in the second phase for $SRF_S$ in which each of the mice received 600, 1000, 1600 and 2900 mg $kg^{-1}$ of the fraction; three animals received 1600, 2900 and 5000 mg $kg^{-1}$ of $SRF_R$. Animals in both phases were observed for signs and symptoms of toxicity and mortality for 24 h. The median lethal dose was calculated using the following equation:

$$LD_{50} = \sqrt{\text{Minimal lethal dose} \times \text{Maximal survival dose}}$$

### Analgesic studies

**Acetic acid-induced writhing test in mice:** The method described by Koster *et al.*[20] was adopted; 25 albino mice were divided into 5 groups of 5 mice each. Group 1 was injected with 10 mL $kg^{-1}$ i.p., of normal saline (negative control), groups 2, 3 and 4 were injected i.p., with 150, 300 and 600 mg $kg^{-1}$ of $SRF_S$, respectively. Group 5 was injected with piroxicam 10 mg $kg^{-1}$ (positive control). Thirty minutes later, each mouse was injected with 10 mL $kg^{-1}$ of aqueous solution of acetic acid (0.6%). The number of writhing responses for each mouse was counted 5 min after injection of acetic acid for a period of 10 min. The procedure was repeated for $SRF_R$ using the doses of 75, 150 and 300 mg $kg^{-1}$.

The percentage inhibition of abdominal constrictions was calculated using the following equation:

$$\text{Inhibition (\%)} = \frac{\text{Mean No. of writhes (negative control)} - \text{Mean No. of writhes (Test)}}{\text{Mean No. of writhes (negative control)}} \times 100$$

### Anti-inflammatory studies

**Carrageenan-induced paw oedema:** The test was conducted according to the method described by Winter *et al.*[21]. Twenty five rats were divided into 5 groups of 5 animals each. Group one received 10 mL $kg^{-1}$ normal saline (negative control). Groups 2, 3 and 4 received 150, 300 and 600 mg $kg^{-1}$ of $SRF_S$, group 5 received piroxicam 10 mg $kg^{-1}$ (positive control). Thirty minutes later, 0.1 mL of sterile saline solution of 1% carrageenan was injected into the sub-planter surface of the left hind paw. Paw size was measured using vernier caliper at time 0, 1, 2, 3 and 4 h after the administration of the carrageenan. The procedure was repeated for $SRF_R$ using the doses of 75, 150 and 300 mg $kg^{-1}$.

**Statistical analysis:** The data was analyzed using one-way ANOVA followed by *post hoc* test and paired t-test using the SPSS software (version 22). The results were expressed as Mean±SEM. Values of $p<0.05$ were considered significant.

## RESULTS

**Phytochemical screening:** Preliminary phytochemical screening conducted on the *n*-butanol and saponin-rich fractions of the stem bark and root of *N. macrophylla* revealed the presence of similar phytochemicals including tannins, saponins, cardiac glycosides carbohydrates and flavonoids (Table 1).

**Acute toxicity studies:** The intraperitoneal $LD_{50}$ of the saponin-rich fractions of the stem bark and root of *N. macrophylla* in mice were found to be 2154 and 1265 mg $kg^{-1}$, respectively indicating the fractions to be fairly toxic (Table 2).

Table 1: Phytochemical constituents of the *n*-butanol and saponin-rich fractions of *N. macrophylla*

| Constituents | Inferences | | | |
|---|---|---|---|---|
| stem bark root | BFS | $SRF_S$ | BFR | $SRF_R$ |
| Tannins | + | - | + | - |
| Saponins | + | + | + | + |
| Alkaloids | - | - | - | - |
| Cardiac glycosides | + | - | + | - |
| Carbohydrates | + | - | + | - |
| Anthraquinones | - | - | - | - |
| Flavonoids | + | - | + | - |
| Steroids/triterpenes | - | - | - | - |

+: Present, -: Absent, BFS: N-butanol fraction of stem bark, BFS: N-butanol fraction of root, $SRF_S$: Saponin-rich fraction of stem bark, $SRF_R$: Saponin-rich fraction of root

Table 2: Median lethal dose of the saponin-rich fractions of *N. macrophylla*

| Fraction | $LD_{50}$ (mg $kg^{-1}$) |
|---|---|
| $SRF_S$ | 2154 |
| $SRF_R$ | 1265 |

$SRF_S$: Saponin-rich fraction of stem bark, $SRF_R$: Saponin-rich fraction of root

**Analgesic studies-acetic acid-induced writhing test:** The saponin-rich fractions of the stem bark and root of *N. macrophylla* significantly ($p<0.05$) and dose-dependently reduced the number of writhes induced by acetic acid at the graded doses employed. The $SRF_S$ reduced the number of writhes by 87.7, 64.6 and 59.9% at 150, 300 and 600 mg $kg^{-1}$, respectively (Table 3) while $SRF_R$ had 60.0, 66.2 and 81.5% inhibition of writhes at 75, 150 and 300 mg $kg^{-1}$, respectively; the standard drug, piroxicam (10 mg $kg^{-1}$) reduced the number of writhes by 73.8 mg $kg^{-1}$ (Table 4).

Table 3: Effect of $SRF_S$ on acetic acid-induced writhing in mice

| Treatments (mg $kg^{-1}$) | Mean No. of writhes | Inhibition (%) |
|---|---|---|
| Normal saline | 21.67±4.7 | - |
| $SRF_S$ (150) | 2.67±1.3* | 87.7 |
| $SRF_S$ (300) | 7.67±2.4* | 64.6 |
| $SRF_S$ (600) | 8.67±2.8* | 59.9 |
| Piroxicam (10) | 5.67±3.7* | 73.8 |

Each value represents Mean±SEM. *p = 0.05 compared with control (one-way ANOVA), n = 5. $SRF_S$: Saponin-rich fraction of stem bark

Table 4: Effect of $SRF_R$ on acetic acid-induced writhing in mice

| Treatments (mg $kg^{-1}$) | Mean No. of writhes | Inhibition (%) |
|---|---|---|
| Normal saline | 21.67±4.7 | - |
| $SRF_R$ (75) | 8.67±1.3* | 60.0 |
| $SRF_R$ (150) | 7.33±2.4* | 66.2 |
| $SRF_R$ (300) | 4.00±2.8* | 81.5 |
| Piroxicam (10) | 5.67±3.7* | 73.8 |

Each value represents Mean±SEM. *p = 0.05 compared with control (one-way ANOVA), n = 5. $SRF_R$: Saponin-rich fraction of root

**Anti-inflammatory studies-carrageenan-induced paw oedema:** The saponin-rich fractions ($SRF_S$ and $SRF_R$) and piroxicam significantly ($p<0.05$) decreased the mean paw oedema induced by carrageenan at the tested doses in a dose-dependent manner. The $SRF_S$ exhibited the highest inhibition of oedema (49.1%) at the 4th h (Table 5) which was higher than that observed by piroxicam (30.9%) while $SRF_R$ had 29.1% at the 4th h (Table 6).

**Comparative analysis using paired sample t-test:** The anti-inflammatory effect of the saponin-rich fraction of stem bark was only significant ($p<0.05$) when the mean paw diameter observed at the 1st h was compared to that observed at the 4th h i.e., pair 3 (Table 7), however, there was significant difference ($p<0.05$) when the mean paw diameter at the 4th h was compared to that observed at the 1st, 2nd and 3rd h i.e., pair 4-6 (Table 7).

Similar anti-inflammatory effect was observed for the saponin-rich fraction of root. There was significant difference ($p<0.05$) when the mean paw diameter observed at the 1st h was compared to that observed at the 4th h only i.e., pair 3 (Table 8); however, there was remarkable significant difference ($p<0.05$) when the mean paw diameter at the 4th h was compared to that observed at the 1st, 2nd and 3rd h i.e., pair 4-6 (Table 8).

Table 5: Effect of $SRF_S$ on carrageenan-induced paw oedema in rats

| | Mean paw diameter (cm) (inhibition %) | | | |
|---|---|---|---|---|
| | Time (h) | | | |
| Treatments (mg $kg^{-1}$) | 1 | 2 | 3 | 4 |
| Normal saline | 0.52±0.01 | 0.55±0.03 | 0.56±0.03 | 0.55±0.02 |
| $SRF_S$ (150) | 0.37±0.02* (28.9) | 0.36±0.02* (34.6) | 0.33±0.02* (41.1) | 0.28±0.03 (49.1) |
| $SRF_S$ (300) | 0.45±0.01* (13.5) | 0.43±0.02* (21.8) | 0.38±0.02* (32.1) | 0.30±0.03* (43.6) |
| $SRF_S$ (600) | 0.49±0.02 (5.8) | 0.48±0.02 (12.7) | 0.45±0.02* (19.6) | 0.40±0.03* (27.3) |
| Piroxicam (10) | 0.43±0.01* (17.3) | 0.44±0.01* (20.0) | 0.41±0.01* (26.8) | 0.38±0.00* (30.9) |

Each value represent Mean±SEM. *p<0.05 compared with control (One-way ANOVA), n = 5. Figures in parentheses represents percentage inhibition of inflammation. $SRF_S$: Saponin-rich fraction of stem bark

Table 6: Effect of $SRF_R$ on carrageenan-induced paw oedema in rats

| | Mean paw diameter (cm) (inhibition %) | | | |
|---|---|---|---|---|
| | Time (h) | | | |
| Treatments (mg $kg^{-1}$) | 1 | 2 | 3 | 4 |
| Normal saline | 0.52±0.01 | 0.55±0.03 | 0.56±0.03 | 0.55±0.02 |
| $SRF_R$ (75) | 0.47±0.02 (9.6) | 0.46±0.02* (16.4) | 0.46±0.01* (17.9) | 0.41±0.01* (25.5) |
| $SRF_R$ (150) | 0.45±0.01* (13.5) | 0.45±0.00* (18.2) | 0.46±0.00* (17.9) | 0.39±0.02* (29.1) |
| $SRF_R$ (300) | 0.45±0.01* (13.5) | 0.49±0.00 (10.9) | 0.44±0.01* (21.4) | 0.39±0.01* (29.1) |
| Piroxicam (10) | 0.43±0.01* (17.3) | 0.44±0.01* (20.0) | 0.41±0.01* (26.8) | 0.38±0.00* (30.9) |

Each value represent Mean±SEM. *p<0.05 compared with control (one-way ANOVA), n = 5. Figures in parentheses represents percentage inhibition of inflammation. $SRF_R$: Saponin-rich fraction of root

Table 7: Paired sample t-test of saponin-rich fraction of stem bark of *N. macrophylla*

| Anti-inflammatory effect of saponin-rich fraction of stem bark | Mean paw diameter (cm) | p-value |
|---|---|---|
| **Paired samples correlations at 1 h** | | |
| Pair 1-1 h | 0.45±0.01 | 0.67 |
| 2 h | 0.46±0.01 | |
| Pair 2-1 h | 0.45±0.01 | 0.06 |
| 3 h | 0.43±0.02 | |
| Pair 3-1 h | 0.45±0.01 | 0.00 |
| 4 h | 0.39±0.02 | |
| **Paired samples correlations at 4 h** | | |
| Pair 4-4 h | 0.39±0.02 | 0.00 |
| 1 h | 0.45±0.01 | |
| Pair 5-4 h | 0.39±0.02 | 0.00 |
| 2 h | 0.46±0.01 | |
| Pair 6-4 h | 0.39±0.02 | 0.00 |
| 3 h | 0.43±0.02 | |

Table 8: Paired sample t-test of saponin-rich fraction of root of *N. macrophylla*

| Anti-inflammatory effect of saponin-rich fraction of root | Mean paw diameter (cm) | p value |
|---|---|---|
| **Paired samples correlations at 1 h** | | |
| Pair 1-1 h | 0.46±0.00 | 0.07 |
| 2 h | 0.48±0.01 | |
| Pair 2-1 h | 0.46±0.00 | 0.69 |
| 3 h | 0.46±0.01 | |
| Pair 3-1 h | 0.46±0.00 | 0.00 |
| 4 h | 0.42±0.01 | |
| **Paired samples correlations at 4 h** | | |
| Pair 4-4 h | 0.42±0.01 | 0.02 |
| 1 h | 0.46±0.00 | |
| Pair 5-4 h | 0.42±0.01 | 0.00 |
| 2 h | 0.48±0.01 | |
| Pair 6-4 h | 0.42±0.01 | 0.00 |
| 3 h | 0.46±0.01 | |

## DISCUSSION

Phytochemicals are important constituents found in large quantity in medicinal plants; these constituents are responsible for most pharmacological actions of these medicinal plants in prevention of diseases[22]. The *n*-butanol fractions of the stem bark and root of *N. macrophylla* revealed the presence of similar constituents (Table 1), similar studies conducted on *Aegle marmelos* revealed the presence of similar phytochemicals in the aqueous extracts of both the stem bark and root of the plant[23]. The saponin-rich fractions of the stem bark and root were positive to frothing test indicating the presence of saponins[18]. Similar phytochemicals were also reported for the methanol stem bark extract of *N. macrophylla*[12-13].

Acute toxicity is defined as those adverse effects that occurs immediately or at a short time interval after single or multiple administration of a substance within 24 h, this study tends to give information about $LD_{50}$, therapeutic index and the degree of safety of a pharmacological agent[24]. Median lethal dose ($LD_{50}$) is the dose of any test substance required to kill half the number (50%) of animals. The saponin-rich fractions of the root of *N. macrophylla* was more toxic compared to that of the stem bark (Table 2). A relatively lower $LD_{50}$ value was reported for the methanol stem bark extract[12]. Thus, the extract was more toxic compared to the fractions. According to Lorke[19], the smaller the $LD_{50}$ value, the higher the toxicity and vice versa.

*Neocarya macrophylla* have been used in ethnomedicine to treat different ailments including pain and inflammation. Earlier studies on the methanol stem bark extract of the plant validated the analgesic effect of the plant[11]. In addition, saponins have proven to be effective against pain and inflammation[10]. Sequel to the above, this study compared the analgesic and anti-inflammatory activities of the saponin-rich fractions of the stem bark and root of *N. macrophylla*. Acetic acid-induced writhing test is a chemical method used to induce peripheral pain by injection of acetic acid in mice[25]. The nociceptive effect of the saponin-rich fractions of the stem bark and root of *N. macrophylla* was inferred from decrease in the frequency of writhing responses by mice i.e. arching of back, hind limb extension etc.[25]. The fractions were able to significantly ($p<0.05$) reduced the number of writhes induced by acetic acid at the graded doses in a dose dependent manner (Table 3, 4), however, the saponin-rich fraction of the stem bark ($SRF_S$) exhibited the highest percentage inhibition of writhes (87.7%) at the lowest dose (150 mg $kg^{-1}$), thus as the dose increases, activity decreases (Table 3). This was in contrast to what was observed for the saponin-rich fraction of the root ($SRF_R$), the number of writhes were reduced by 81.5% at the highest dose (300 mg $kg^{-1}$), however, activity was reduced as the doses were decreased (Table 4). The methanol stem bark extract recorded a lower analgesic effect[8] compared to the saponi-rich fractions. Thus, the fractions might elicit their analgesic effects by inhibiting the synthesis of prostaglandins, leukotrienes and other pain mediators[26-27], suggesting that the fractions possess peripherally-mediated analgesic activity.

The mechanism of action by which carrageenan induced inflammation is biphasic in which the first phase is attributed to the release of histamine, serotonin and kinins and it is mainly seen at the first hour while the second phase is related to the activation of prostaglandins and lysome enzymes and it is basically seen at the 2nd and 3rd h, thus most effective anti-inflammatory agents are sensitive to the second phase[28]. The saponin-rich fractions of the stem bark and root of *N. macrophylla* significantly and dose-dependently inhibited carrageenan-induced inflammation in the 2nd, 3rd and 4th.

However, $SRF_S$ exhibited highest inhibition of inflammation at the lowest dose (150 mg $kg^{-1}$) while $SRF_R$ showed higher inhibition of inflammation at the highest dose (300 mg $kg^{-1}$). The inhibition of inflammation by both fractions ($SRF_S$ and $SRF_R$) was more pronounced at the 3rd and 4th h suggesting the fractions to have longer onset and longer duration of action in a similar manner to most effective anti-inflammatory agents[29]. The saponin-rich fraction of the stem bark of *N. macrophylla* was more effective in the treatment of pain and inflammation compared to that of the root.

## CONCLUSION

The saponin-rich fractions of stem bark and root of *N. macrophylla* contained similar phytochemical constituents and both fractions were found to be toxic. Also, the fractions possess significant analgesic and anti-inflammatory activities but the saponin-rich fraction of the stem bark has better effect compared to the root.

## SIGNIFICANCE STATEMENT

This study discovered the effect of saponin-rich fractions of the stem bark and root of *Neocarya macrophylla* on pain and inflammation that can be beneficial for the use of the plant in the treatment of pain and inflammatory conditions. This study will help researchers to uncover the critical areas of natural product research in determining the toxicity, analgesic and anti-inflammatory effects of saponins that many researchers were not able to explore. Thus, a new theory on the search for better therapeutic agents with analgesic and anti-inflammatory effects may be arrived at.

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