



## Phytochemical, Quantitative Proximate and *In vitro* Anti-inflammatory Study of the Crude Methanol Extract of *Stachytarpheta indica* Leaves (Verbenaceae)

Cyril Ogbiko<sup>1,\*</sup>, Dickson Achimugu Musa<sup>2</sup>, Musa Usman Dabai<sup>1</sup>,  
Ibeabuchi Jude Ali<sup>3</sup>, Abubakar Sani Yelwa<sup>1</sup> and Hafsat Buhari Bature<sup>1</sup>

<sup>1</sup>Department of Pure and Applied Chemistry, Faculty of Science, Usmanu Danfodiyo University Sokoto, Sokoto State, Nigeria

<sup>2</sup>Department of Biochemistry, Ibrahim Badamasi Babangida University, Lapai, Nigeria

<sup>3</sup>Department of Pharmaceutical Chemistry, Enugu State University of Science and Technology, Enugu, Nigeria

\*Corresponding author e-mail: [cyril.ogbiko@udusok.edu.ng](mailto:cyril.ogbiko@udusok.edu.ng); [cyrilogbiko@gmail.com](mailto:cyrilogbiko@gmail.com)

### Abstract

Herbal medicine despite its increase usage globally is being plagued by inadequate research on their effectiveness. *Stachytarpheta indica* commonly known as snakeweed is used locally in the management of asthma, headache, alopecia, bronchitis, bruise, chest cold, constipation, itch, diarrhea, skin sore, vermifuge, dysentery, dysmenorrhea among others. This study investigated the phytochemical composition, quantitative proximate parameters and *in vitro* anti-inflammatory activity of the leaves of *S. indica* using established methods. Results confirmed the presence of alkaloids, saponins, carbohydrate, cardiac glycosides, terpenoid, tannin, anthraquinones, phenolics and flavonoid. Moisture content ( $9.16 \pm 0.04\%$ ), total ash ( $19.42 \pm 0.15\%$ ), water insoluble ash ( $7.17 \pm 0.22\%$ ), acid insoluble ash ( $3.63 \pm 0.26\%$ ), alcohol soluble extractive ( $0.99 \pm 0.06\%$ ) and water-soluble extractive ( $0.36 \pm 0.02\%$ ) values were obtained from the quantitative proximate analysis. An infusion of the methanol leaf extract of *Stachytarpheta indica* demonstrated significant anti-inflammatory activity in a dose dependent manner comparable to that of indomethacin standard.

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## Introduction

Medicinal plants are useful for healing as well for curing human diseases because of the presence of phytochemical constituents which naturally occur within them to offer defense and protection from various diseases [1-3]. Natural products, such as plants extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity [4]. Plants used for traditional medicine contain a wide range of substances that have been explored by researchers and industrialist as remedy for diverse diseases since the medicinal value of these plants lies in their phytochemical component, especially the secondary metabolites such as alkaloids, tannins, anthocyanins, anthraquinone derivatives, flavonoids and other phenolic compounds [5]. Natural compounds obtained from plants could be lead compounds allowing for the design and rational planning of new drugs, biometric synthetic development and the discovery of new therapeutic properties not yet attributed to known compounds [6]. Inflammation is the response of living tissues to injury. It involves a complex array of enzyme activation, mediator release, extravasations of fluid, cell migration, tissue breakdown and repair [7, 8]. Without inflammation wounds will never heal although chronic inflammation has been attributed to a host of diseases [9]. Non-steroidal anti-inflammatory drugs (NSAIDs) and opiates commonly used to treat pains have been established to cause gastric lesions, tolerance and dependence problem [10, 11] hence attention is now being focused on the investigation of the efficacy of plant-based drugs used in traditional medicine because they are not only cheap, but have little side effects as well as hold a large untapped source of structurally novel compounds that might serve as lead for the development of novel drugs [12, 13]. This is justifiably so since according to WHO, about 80% of the world population still rely mainly on herbal remedies [14, 15].

*Stachytarpheta indica* commonly known as snakeweed is belonging to the family Verbenaceae. It is a well branched herb 2-3 ft high with very long narrow spikes. The flowers are deep blue with white centre. They are drought tolerant suitable for xeriscaping. The plant has been used locally as an abortifacient and in the management of asthma, headache, alopecia, bronchitis, bruise, chest cold, constipation, itch, diarrhea, skin sore, vermifuge, dysentery, dysmenorrhea, erysipelas, fever, inflammation, liver disease, poisoning, tumor, venereal disease, cataract, sedative, anti-fertility, rheumatism among others [16, 17]. In northern Nigeria, a decoction of the leaves with natronis given

for dysentery in humans and for similar conditions in horses [16]. This study is an attempt to establish the phytochemical constituents, the quantitative proximate analysis as well as the *in vitro* anti-inflammatory potentials of the leaves of *Stachytarpheta indica*

## **Materials and Methods**

### ***Collection and preparation of plant materials***

Fresh *S. indica* leaves were collected in June 2018 from a forest in Othame village in Etsako East local government area of Edo State Nigeria. They were washed running tap water to remove earthy material before being transported in a closed sterile polythene bag to the laboratory for further analysis. They were identified and authenticated at the Forest Research Institute of Nigeria (FRIN) Ibadan where a herbarium specimen was deposited. The fresh leaves were air-dried for a period of two weeks before being reduced to fine powder with the aid of a mortar and pestle. The powdered sample was stored in an air tight container until used.

### ***Phytochemical analysis***

Simple chemical tests to detect the presence of secondary metabolites were done in accordance with standard methods [18-20].

### ***Proximate analysis***

The following quantitative parameters such as moisture content, total ash, water insoluble ash value, acid insoluble ash value, alcohol soluble extractive value and water-soluble extractive value of the powdered sample were carried out using standard methods [21, 22].

### ***Preparation of extract***

500 g of the pulverized material was extracted with 2.0 L of methanol by maceration at room temperature for 72 hours. The methanol extract of *S. indica* (MESI) was concentrated to dryness using a rotary evaporator at 40°C under reduced pressure. The concentrated extract was air dried, weighed, stored in an air-tight glass container before being preserved in a refrigerator at -4°C until ready for use.

### ***Evaluation of anti-inflammatory activity***

#### ***Carrageenan-induced rat paw oedema***

25 healthy rats of both sex and weight  $180 \pm 16$  g were procured and divided into

five groups containing five rats in each group. 0.1 ml of 1.0% carrageenan in normal saline (0.9% w/v NaCl) was injected to the sub plantar region of right hind paw. The MESI was administered to the rats 1 hour before carrageenan injection. Different groups were treated as follows:

Group I: 2 ml/kg of 0.9% normal saline in 3% Tween-80 (negative control)

Group II: 5 mg/kg *b.wt* Indomethacin (positive control).

Group III, IV and V: 100, 200 and 400 mg/kg *b.wt* of MESI (Treatment groups)

The paw volume was measured initially (basal time) and at 1, 2, 3 and 4 h after carrageenan injection, using a micrometer screw gauge and the increase in the linear diameter of the right hind paws were taken as an indication of paw oedema. The percentage inhibition of the inflammation was calculated using the expression:

$$\% \text{ inhibition} = \frac{A_0 - A_1}{A_0} \times 100,$$

where  $A_0$  is the average inflammation of the negative control group of rats at a given time while  $A_1$  is the average inflammation of the positive control group/treatment groups [23-25].

### ***Statistical analysis***

Data procured were analyzed using statistical package for social sciences (SPSS 20, Chicago, IL) at 0.05 level of significance. Results were presented as mean and standard deviation (Mean  $\pm$  SD). The statistical significance between the control and each of the treated groups was determined by Dennett's post hoc test after one-way ANOVA. The level of significance was set at  $p < 0.05$ .

## **Results and Discussion**

### ***Percentage yield and phytochemical screening***

The percentage yield of the crude methanol extract was found to be 4.13%. The phytochemical screening revealed the presence of important phytoconstituents presented in Table 1.

**Table 1.** Phytochemical screening of the methanol extract of *S. indica*.

Phytochemicals	Observation	Inference
Alkaloids	Yellow precipitate formed	+
Saponins	Yellow emulsion formed	+
Carbohydrate	Violet ring formed	+
Cardiac glycosides	Violet ring formed	+
Terpenoid	Brown ring formed	+
Tannin	Green black colour not seen	+
Anthraquinones	Green colour observed	+
Phenolics	Intense colouration	+
Flavonoids	Yellow colour persist	+

This result validates the findings of Kumar et al. [16] who reported the same phytochemicals in the methanol extract of the leaves of the plant. Secondary metabolites are known to impart characteristic odour, taste, colour, medicinal or poisonous properties on the plant as well as aid in the biological actions for which they are identified in folk medicine [4].

### *Quantitative proximate analysis*

The quantitative proximate analysis of the pulverized sample of the plant is presented in Table 2.

**Table 2.** Quantitative proximate analysis of *S. indica* leaves on dry matter basis.

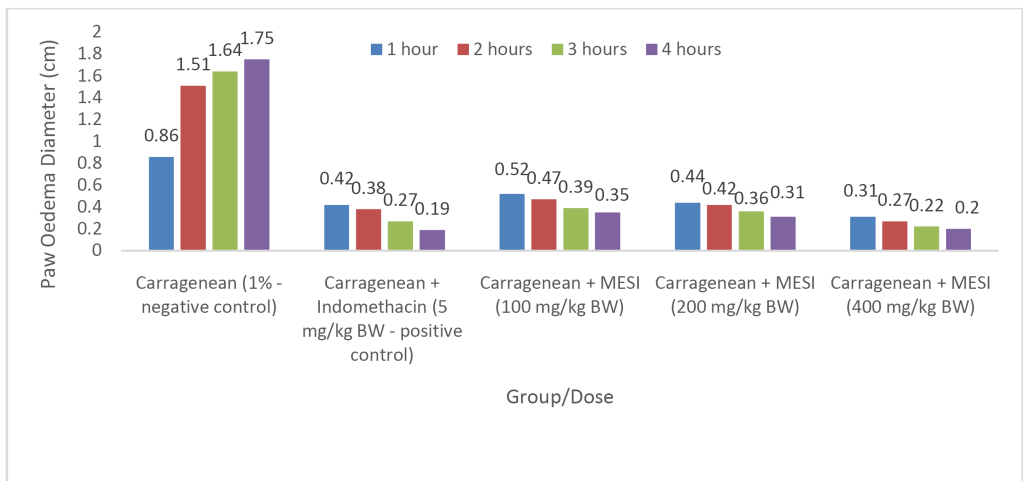
Parameter	Percentage Value
Moisture content	9.16 ± 0.04
Total ash	19.42 ± 0.15
Acid insoluble ash	3.63 ± 0.26
Water insoluble ash	7.17 ± 0.22
Alcohol extractive index	0.36 ± 0.02
Water extractive index	0.99 ± 0.06

Values expressed as % mean ± SD (Standard deviation),  $n = 3$

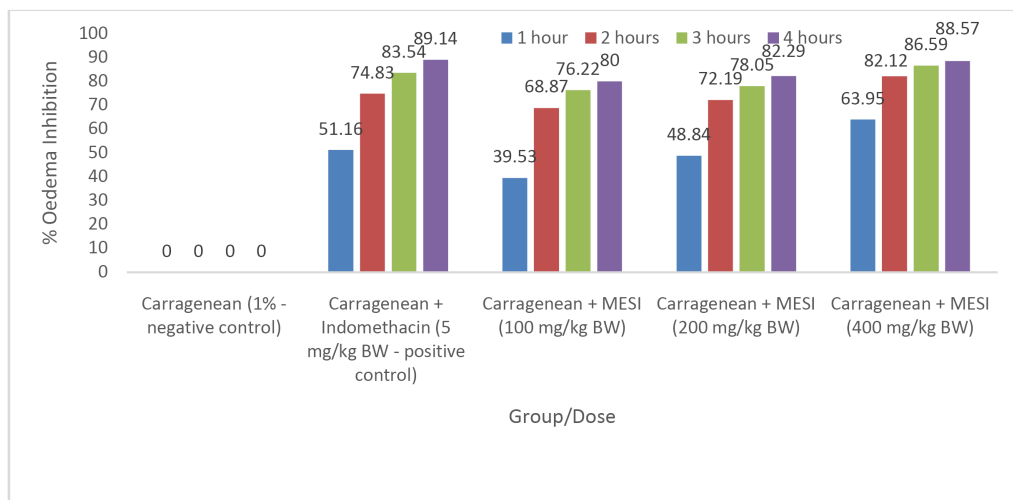
Although the moisture content in plants helps in maintaining the protoplasmic contents of cells, it also makes herbs perishable and susceptible to microbial degradation during storage [26, 4]. The moisture content is within the value set by Africa Pharmacopoeia, hence suggests that the powdered drugs from *S. indica* will not be prone to microbial attack and hence could have a long shelf life. The total ash value indicates that the powdered drug is rich mineral elements content since it is recommended that plants with ash content above 8.8% are useful health wise [27]. While the level of contamination or adulteration by sand (silicate) can be detected by the level of acid insoluble ash, the water and alcohol soluble extractive values aid in establishing the best solvent for the extraction of the inherent mineral in the plant. The water extractive value was observed to be higher than the alcohol extractive value indicating that alcohol is the best solvent for its extraction.

### Anti-inflammatory study

The result of the anti-inflammatory study of the methanol leaf extract of *S. indica* showing the paw oedema diameter and the percentage oedema inhibition is presented in Figures 1 and 2 respectively.



**Figure 1.** Paw oedema diameter of the MESI and indomethacin on carrageenan-induced oedema in the right hind-paw of rats. Data in mean  $\pm$ SD.  $n = 5$ .



**Figure 2.** Percentage oedema inhibition of the MESI and indomethacin on carrageenan-induced oedema in the right hind-paw of rats. Data in mean  $\pm$  SD.  $n = 5$ .

The carrageenan-induced hind paw oedema model is commonly used to evaluate acute inflammatory in animals since it has been shown to be sensitive to cyclooxygenase (COX) inhibitors [13]. Oedema development in the model is in two phases. While the first phase which involves the release of serotonin and histamine occurs within the first hour, the second phase is mediated by prostaglandins, the cyclooxygenase products and this occurs after the first hour.

When compared with the negative control, treatment with the extract (100, 200 and 400 mg/kg BW) as well as the reference drug reduced ( $p < 0.05$ ) the paw oedemas from the first hour after carrageenan injection. The anti-inflammatory effect of the extract and the reference drug were most pronounced after 4 hours. The anti-inflammatory activity of the extract was in a dose dependent manner and were all statistically significant from the negative control group but not statistically significant from the positive control group

## Conclusion

The leaves of *S. indica* contain several phytochemicals which may be responsible for its acclaimed ethnomedicinal use. The quantitative proximate analysis reveals the rich endowment of minerals in the plant leaves. The findings of the anti-inflammatory study have demonstrated that the methanol extract at the tested dose has a potent anti-inflammatory activity and this justifies its use in traditional medicine to treat

inflammatory. This could among other reasons be attributed to the presence of tannins which are known to be potent cyclooxygenase –1 (COX-1) inhibitors through their binding nature with proteins. Other anti-inflammatory models are recommended to corroborate the findings from this research as well as determine the exact mechanism(s) of action of the extracts and its active compound(s).

### **Conflict of Interest Statement**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of research reported.

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