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# PHYTOCHEMICAL EVALUATION AND ANTI-INFLAMMATORY ACTIVITY OF THE LEAF, STEM AND ROOT BARK EXTRACTS OF *STROPHANTHUS SARMENTOSUS* DC

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## ABSTRACT

*Strophanthus sarmentosus* DC commonly called arrow poison or spider tresses is a very popular plant among traditional medical practitioners in Africa and is used to treat arthritis and wound infections. This study was carried out to qualitatively and quantitatively determine the phytochemical composition and the anti-inflammatory activities of different solvent extracts of *S. sarmentosus.* The anti inflammatory activity was studied using egg albumin-induced inflammation of rat paw oedema. The crude extracts of the leaf, root bark and stem extracts obtained using *n*-hexane, dichloromethane and methanol were administered at concentrations of 200 and 400 mg/kg body weight of the animals. The phytochemical analysis revealed the presence of alkaloids, flavonoids, saponins, tannins, terpenoids and glycosides in varying proportions. The anti-inflammatory activity of all the extracts revealed significant ($p > 0.05$) anti-inflammatory effect with maximum suppression occurring at the fifth hour. The root bark extract had the highest mean % inhibition at both concentration of 200 and 400 mg / kg body weight of the animals, with 63.59 and 79.30 mean % inhibitions, although at the highest dose of 400 mg / kg, the extracts were found to be lethal to the animals. Of the three partitioned stem fractions, the *n*-hexane

fraction had the highest mean % inhibition and a 100% inhibition at the fifth hour. From their mean % inhibitions, all the extracts had increased suppression of inflammation with increase in the concentration of administered dose from 200 to 400 mg/kg body weight. There was no noticeable suppression observed for the methanol fraction of the stem extract. The marked inhibitory effect on paw oedema shows that *Strophanthus sarmentosus* possesses significant anti-inflammatory activity, thus justifying the use of the plant in the treatment of various inflammatory diseases by traditional medical practitioners.

**KEYWORDS:** *Strophanthus sarmentosus* DC, phytochemical, inflammation, anti inflammatory, oedema, inhibition.

## INTRODUCTION

Both traditional and modern day medicine have found rich sources of drugs derived from natural products.[1] The use of plants in medical applications are inexhaustible and these are mainly due to the presence of several chemical constituents (secondary metabolites) and other active compounds.[2] A lot of scientific research and investigations have been going on to ascertain the actual potentials of these chemical constituents found in plants.[2] These various investigations have in more ways than one successfully proved that these secondary metabolites have pharmacological effects and are biologically active against many of the diseases encountered in day to day life, hence the reliance of modern day medicine on plants with such benefits.[3, 4] One of such medically active plants is *Strophanthus sarmentosus* DC, commonly known as "spider tresses" and "arrow poison" in English, although not commonly found, it is a plant widely distributed and popular for its ethnomedicinal values in Africa, particularly West Africa.[5] Used for the treatment of several diseases which includes eye infections, arthritis, rheumatism, veneral diseases and constipation, in Congo the stems and leaves are used to make steam baths and infusions against rheumatism.[2] The term inflammation is derived from the Latin word – Inflammare, which means burn. Inflammation can be defined as a condition in which part of the body becomes red, sore and swollen because of infection or injury. Any constraint, infection or injury to the human body can result in a series of chemical changes in the affected area and the body as a whole and to successfully take care of inflammatory diseases analgesic and anti inflammatory agents is required. Several plants possess anti inflammatory and analgesic properties and this has aided many traditional medical practitioners in the treatment of the diseases as well as different types of rheumatic diseases which are seen as a major and worldwide problem. *In vivo*

antitrypanosomal investigations by Onotu and others [6] into the methanolic extracts of the stem of *S. Sarmentosus* revealed the presence of glycosides and saponins as some of its phytochemical components. An annual review of anti-inflammatory medicinal plants by Gollapalli and others[7] listed *Strophanthus sarmentosus* DC as one of the medicinal plants used in the treatment of inflammations from the ethanolic extracts of the dried roots with flavonoids, tannins, glycosides, saponins and alkaloids as the phytochemical components present.[8] Provision of new drugs for the health sector is a continuous process and constitutes a major challenge for researchers of drug development. In the occurrence of epidemics, diseases tend to grow resistant to drugs which necessitate the need for continuous search for drugs in order to tackle such issues. Plants are good sources of raw materials for the discovery of drugs and this is highly utilized by researchers worldwide to search for newer bioactive agents from herbal origin. The present work is out to contribute to the search for newer compounds which can serve as precursors to drug development.

## MATERIALS AND METHODS

### Plant material collection, authentication and identification

The leaves, roots and stems of *S. sarmentosus* were collected from Edokota Forest along Bida-Zungeru road, Bida, Niger state, Nigeria in February 2014 and was taxonomically authenticated and identified at the Department of Biological Sciences, Herbarium Section, Ahmadu Bello University Zaria, Kaduna State, Nigeria by a taxonomist Mallam Muhammad Musa, where a voucher specimen (V/N:900/60) was deposited.

### Experimental animals

Adult albino rats of both sexes weighting between 120-210 g were used for the anti inflammatory activity. The rats were obtained from the Biochemistry Department of Federal University of Technology Minna, Niger State, Nigeria.

### Preparation and partitioning of crude methanol extracts of the leaf, stem and root bark of *S. sarmentosus*

The leaves, stem and root bark of *S. sarmentosus* were separately shade air dried for several days, each chopped into small pieces and pulverized to fine powder. Each of the pulverized materials were extracted by percolation methods (3 times) in aqueous methanol (70% w/v) in large stoppered bottles at room temperature for 72 h. Separation of organic compounds (metabolites) extracted into the solvent was done by suction filter and subsequently with Whatman No.1 filter filter paper. All the resulting filtrates from the three macerations were

pooled together and this was carried out for the three different *S. sarmentosus* plant parts. The solutions were collected separately and concentrated *in vacuo* using rotary evaporator (R E-600, Shendi, Shanghai) to obtain the crude extracts. The extracts were further dried in a water bath and then air dried, yielding sticky gelatinous extracts. The crude methanolic stem extracts of *S. sarmentosus* was separately dissolved in 70 % methanol (250 $cm^3$) and transferred into 500 $cm^3$ separatory funnel. This was then partitioned with 250 $cm^3$ *n*-hexane and dichloromethane to give *n*-hexane, dichloromethane and methanol fractions.[9] Each solvent soluble fraction was evaporated over a water bath and subsequently air dried and used for further analyses.

**Qualitative phytochemical analysis of crude methanol extract of the leaf, stem and root bark *S. sarmentosus***

Each of the crude extract was subjected to phytochemical analysis qualitatively for the presence of some secondary metabolites such as alkaloids, tannins, glycosides, steroids, terpenoids, flavonoids, saponins and resins, using standard methods. [9]

**Quantitative phytochemical analysis of crude methanol extract of the leaf, stem and root bark *S. sarmentosus***

Quantitative phytochemical analysis of the crude extracts was carried out to determine the composition in percentage of alkaloids, tannins, flavonoids and saponins present. [10]

**Anti inflammatory activity of the extracts from the leaf, stem and root bark of *S. sarmentosus***

The rats were randomly divided into 12 groups of three animals each (n = 3). Acute inflammation was induced by injecting 0.1ml of freshly prepared egg albumin into the sub-plantar region of the right hind paw of rats. Increase in the right hind paw diameter of the rats, induced by a phlogistic agent was used as the measure of acute inflammation. Thirty minutes before inducing inflammation, the groups were treated with the drugs intraperitoneally.[11, 12] The groups are as follows

Group 1: negative control
Group 2: *n*- hexane fraction (stem) 200 mg/kg
Group 3: methanol fraction (stem) 200 mg/kg
Group 4: dichloromethane fraction (stem) 200 mg/kg
Group 5: crude methanolic leaves extract 200 mg/kg
Group 6: crude methanolic roots bark extract 200 mg/kg

Group 7: *n*-hexane fraction (stem) 400 mg/kg
Group 8: methanol fraction (stem) 400 mg/kg
Group 9: dichloromethane fraction (stem) 400 mg/kg
Group 10: crude methanolic leaves extract 400 mg/kg
Group 11: crude methanolic roots bark extract 400 mg/kg
Group 12: standard drug (aspirin) 200 mg/kg

The linear paw diameter was measured using the cotton thread method before and after induction first after thirty minutes and subsequently at 1 hour intervals after treatment for five hours.[11, 12] The percentage inhibition of inflammation was calculated at different hours using the formula.

$$\text{inhibition (\%)} = \frac{\text{mean paw diameter(control)} - \text{mean paw diameter(treated)} \times 100}{\text{mean paw diameter (control)}}$$

**Weight determination**

The treated animals were monitored for a period of one week after which their weights were determined to check for any reduction in the weight of the experimental rats due to the effect of the treatment.

**Statistical analysis**

Results of the anti inflammatory test were expressed as mean ± standard error mean and statistically analyzed by the one-way Analysis Of Variance (ANOVA) to determine which of the groups showed the highest activity in relation to the control groups. Values were statistically significant when P values are less than 0.05 ($p < 0.05$). Computer statistical package SPSS software version 20; SPSS Inc., Chicago, IL, USA.

**Column Chromatographic separation of *n*-hexane fraction**

The *n*-hexane partitioned fraction of the stem showed the highest anti inflammatory activity; hence it was mounted on the column. Elution was carried out using gradient mixtures of solvent with the polarity gradually increased starting with 100 % *n*-hexane, *n*-Hex – Ethyl acetate , Ethyl acetate – Methanol and 100 % methanol. Eluents were collected in 20cm$^3$ bottles and monitored by TLC behavior.[9] Eluents exhibiting similar TLC behaviours were pooled together to give the derived fractions. Derived fraction $F_{900}$ obtained using 100 % methanol was further analysed using GC – MS technique.

**GC-MS analysis and identification of phytoconstituents**

The GC-MS analysis was carried out on the combined fraction $F_{900}$, at National Research Institute for Chemical Technology (NARICT) Zaria, Kaduna State, Nigeria, using GCMS model QP2010 PLUS (Shimadzu, Japan). Identification interpretation of mass spectrum GC-MS was carried out by comparing the mass spectrum of each compound with the mass spectral database of National Institute for Standard Technology NIST05.LIB Library.

## RESULTS

**Qualitative phytochemical composition of crude methanolic extracts of the leaf, stem and root bark of *S. sarmentosus***

The qualitative phytochemical screening of all the three crude extracts revealed the presence of alkaloids, glycosides, saponin, cardiac glycosides, coumarin glycosides, tannins, flavonoids and terpenoids in all three extracts while steroids were present in the stem and root bark extract but absent in the leaf extract (Table 1).

**Table I: Qualitative phytochemical analysis.**

| Phytochemical component | Plant part | | |
|---|---|---|---|
| | Leaf | Stem | Root bark |
| Alkaloids | + | + | + |
| Saponins | + | + | + |
| Cardiac glucosides | + | + | + |
| Coumarin glycosides | + | + | + |
| Resins | + | + | + |
| Flavonoids | + | + | + |
| Tannins | + | + | + |
| Steroids | - | + | + |
| Terpenoids | + | + | + |

*+ signifies presence, - signifies absence*

**Quantitative phytochemical composition of crude methanolic extract of the leaf, stem and root bark of *S. sarmentosus***

Quantitative phytochemical analysis of the crude extract was carried out to determine the composition in percentage of alkaloids, tannins, flavonoids and saponins present (Fig. 1). All three extracts indicated very high saponin content with a composition of 95.45 % for the leaf, 85.15 % for the stem and 78.25 % for the root bark. All three extracts possessed high flavonoids content with a composition of 60.4 % for the leaf, 52.43 % for the stem and 43.4 % for the root bark. The composition of tannins in all the three extracts and the alkaloids composition were relatively similar (Fig. 1) and the extract with the least composition of the

phytochemical constituents quantified was the root bark, although the tannins content in the root bark extract is slightly higher than in the stem while the leaf extracts have the highest composition of all the phytochemical components (Fig. 1).

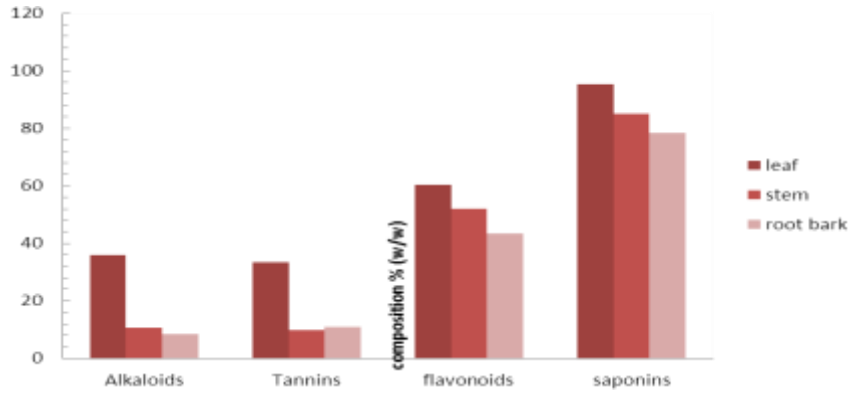


**Figure 1: Quantitative phytochemical composition of crude methanolic extracts of the leaf, stem and root bark of *S. sarmentosus***

**Anti-inflammatory activity of the extracts from the leaf, stem and root bark of *S. sarmentosus***

The effect of crude methanolic extracts of the leaves and roots bark and the dichloromethane, n-hexane and methanol extracts of the stem of *S. sarmentosus* on egg albumin induced inflammation of right hind paw in rats at an hourly interval for five hours, at the doses of 200 and 400 mg / kg body weight, indicated the extent of anti-inflammatory activity for the treated groups with reference to the control group. The fractions were found to show a significant ($p > 0.05$) anti-inflammatory effect in the rats at all the administered doses when compared to the negative control group. All extracts significantly exhibited dose-and time-dependent anti-inflammatory effect in the rats hind paw compared to the standard drug, aspirin. The standard drug (aspirin) had the highest anti-inflammatory effect on the animals with 100 % inhibition at the fifth hour, the *n*-hexane stem extract also suppressed the inflammation with a 100 % inhibition at the fifth hour. The mean % inhibition derived from the five hour monitoring time was calculated and the crude methanolic root bark extract gave the highest both at the concentration of 200 and 400 mg / kg, with 63.59 and 79.30 mean % inhibitions respectively. The crude methanolic leaf extract also proved very effective with a mean % inhibition of 61.18 and 69.69 at 200 and 400 mg / kg respectively. Of the three partitioned stem fractions, the *n*-hexane fraction had the highest mean % inhibition and a 100% inhibition at the fifth hour while the methanol fraction showed no noticeable increase in % inhibition even at the highest dose level. The result of the anti inflammatory activity is represented on Fig. 2.

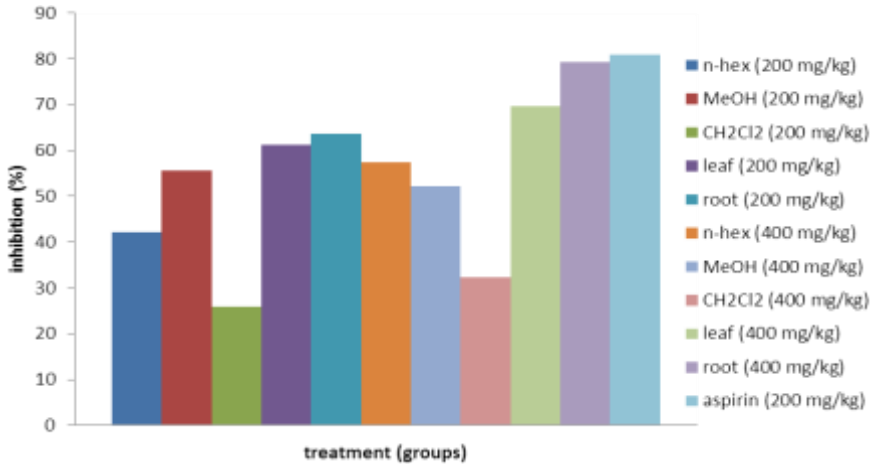


**Figure 2: Anti inflammatory activity of crude methanol extracts of the leaf, root bark and the dichloromethane, *n*-hexane and methanol fractions of the stem of *S. sarmentosus***

**Table 2: Chemical compounds deduced from GC – MS spectrum the derived fraction $F_{900}$**

| Line# | Retention time | Area% | Peaks | Molecular Formula | Molecular weight | Compound name |
|---|---|---|---|---|---|---|
| 1 | 16.3 | 4.15 | 30,41, (43),68,82,96, 109,123, 138,194 | 254 | $C_{16}H_{30}O_2$ | Trans-11-tetradecenyl acetate |
| 2 | 18.2 | 2.36 | 27,41, 43,57, (74),87, 109,127 | 158 | $C_9H_{18}O_2$ | Methyl 6-methyl heptanoate |
| 3 | 19.7 | 17.93 | 27,41, (43),60,73,85, 98,115,129, 157,176, 185,213, 256 | 256 | $C_{16}H_{32}O_2$ | Hexadecanoic acid (palmitic cid) |
| 4 | 21.2 | 8.57 | 27, (41),55, 69,83,97, 123,137, 264 | 282 | $C_{18}H_{34}O_2$ | 9-Octadecenoic acid (oleic acid) |
| 5 | 21.6 | 2.6 | 27,41, 43,57, (74),87, 109,115, 127 | 158 | $C_9H_{18}O_2$ | Methyl 6-methyl heptanoate |
| 6 | 22.4 | 46.67 | 27, (41),55, 69,83,97, 123,137, 264 | 282 | $C_{18}H_{34}O_2$ | 9-Octadecenoic acid (oleic acid) |
| 7 | 22.6 | 13.09 | 41,(43),60, 73,85,98, 115,129, 143,157, 171,185, 199,213, 227,241, 284 | 372 | $C_{22}H_{44}O_4$ | Octadecanoic acid, 2-(2-hydroxyethoxy)ethyl ester |
| 8 | 24.6 | 1.58 | 14,27, 41,(43),57,83, 85,111,126 | 296 | $C_{20}H_{40}O$ | Octadecyl vinyl ether |
| 9 | 26.3 | 3.06 | 27,41, (43),57,71,96, 109,123, 127,183, 222,240, 310 | 398 | $C_{26}H_{54}O_2$ | Hexadecanal diisopentyl acetal |

*Values in parenthesis represent the base peaks*

**GC–MS identification of compounds**

GC-MS analysis of the derived fraction $F_{900}$ (100 % methanol fraction) revealed the presence of nine (9) compounds. The identified compounds include trans-11-tetradecenyl acetate, methtyl 6-methylheptanoate, hexadecanoic acid (palmitic acid), 9-octadecenoic acid (oleic acid), octadecanoic acid 2(2-hydroxyethoxy)ethyl ester, octadecyl vinyl ether and hexadecanal diisopentyl acetal. These compounds are presented on Table 2 according to their retention time. The area %, fragmentation peaks, molecular formula and molecular weight of the compounds are also given.

**DISCUSSION**

Qualitative phytochemical screening of crude methanolic extracts of the leaf, stem and root bark of *Strophanthus sarmentosus* revealed the presence of alkaloids, saponins, flavonoids, coumarin glycosides, cardiac glycosides, resins, tannins, steroids and terpenoids, some of which were quantitatively determined. The presence of saponins and glycosides is in relation with the phytochemical screening of the methanolic stem extract of *S. sarmentosus* by Onotu *et al.* [6] which revealed the presence of glycosides and saponins. The quantitative phytochemical analysis gave the percentage composition of alkaloids, saponins, tannins and flavonoids in the extracts. Saponins have been confirmed to be present in the extracts of *S. sarmentosus*,[6, 8] Flavonoids are known to target prostaglandins, involved in late phase of acute inflammation and pain perception,[14, 15] tannins have been reported to have anti inflammatory effects,[16] hence there quantitative determinations. Both *in vitro* and *in vivo* experiments have shown that flavonoids, tannins, triterpenoids and other secondary plant metabolites possess analgesic and anti-inflammatory properties in various experimental animal models.[17] Abilities of all the extracts of the leaf, stem and root bark of *S. sarmentosus* to significantly ($p > 0.05$) reduce inflammation induced by egg albumin, at the doses of 200 and 400 mg / kg body weight after five hours of administration indicates their potential to suppress the inflammation at the latter phase, with the maximum suppression occurring at the fifth and final hour. Although at the high dose of 400 mg / kg, the root bark extracts were found to be lethal to the animals which developed some deformities after treatment. About two of the animals died a few days later, but at the lower dose of 200 mg / kg, though the treatment initially caused some adverse reactions in the animals. The extracts were found to be highly effective against inflammation with the highest mean % inhibitions of 63.59% and 79.30% both at 200 and 400 mg / kg after five hours. The dried ethanolic root extract have been successfully investigated to show dose – dependent anti inflammatory

activity at the doses of 50, 100 and 200 mg / Kg with the highest percentage inhibition (51.2 %) at the dose of 200 mg / Kg. [8] The anti inflammatory activity observed by the root bark extract in this present study concurs with the works of Agbaje and Ajidahun [8] proving the anti inflammatory activity of the roots extract to also be dose and time dependent due to the increase in the % inhibition of the extract with increase in the administered doses. The phlogistic agent, egg albumin, triggers the release of mediators like histamine, serotonin, prostaglandins, which are indicators of inflammatory activity. [16] Development of induced inflammation in the hind paw of rat is believed to be a two-phase process. [18] The first phase begins within minitues of injecting the phlogistic agent and last for the first 1 – 2 hours due to the release of histamine or serotonin, while the second phase is due to the release of prostaglandins and lysosome. [19, 20] The differences in the anti inflammatory activities of the partitioned stem fractions, based on the extracting solvents depends on the differences in the polarity of these solvents as different compounds having different bioactive potentials are extracted by them. GC-MS analysis conducted on semi purified fractions from the *n*-hexane stem extracts of *S. sarmentosus*, the derived fraction $F_{900}$, revealed the presence of some useful fatty acids such as cis-9-octadecanoic acid (oleic acid) and hexadecanoic acid (palmitic acid). Oleic acid being the chief fatty acid found in olive oil is known for its therapeutic properties is believed to be good for human health in lowering the blood levels of cholesterol and have been shown to slow the development of heart disease and also promote the production of antioxidants. Moderate levels of palmitic acid in diets also display antioxidant properties.

## CONCLUSSION

The presence of the identified chemical components in *S. sarmentosus* as confirmed by the findings of this study, justifies its use in traditional medical applications in the treatment of inflammatory diseases and many other diseases associated with inflammation. This study has also proved that extracts of *S. sarmenthosus* contains biologically active components which are responsible for the observed activities and these compounds can be used in the treatment of inflammatory diseases and other related diseases such as oxidative stress as indicated from the GC-MS analysis of the phytoconstituents.

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