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Phytochemical evaluation and cytotoxic potential ofthe leaf, stem and root bark of *Strophanthus sarmentosus* DC

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ABSTRACT: *Strophanthus sarmentosus* DC(Apocynaceae) also called arrow poison or spider tresses in English is a very popular plant among traditional medical practitioners in Africa, it is used for the treatment of several diseases which includes arthritis, rheumatism, stomach disorders and wound infections. This study was carried outto evaluate thequalitative and quantitative phytochemical components, and the cytotoxic potential of crude methanolic extracts from the leaf, stem and root bark of *S. sarmentosus*.The cytotoxicity test was carried out using the brine shrimp lethality bioassay of each of the extracts at varying concentrations of 1000, 100, 10 and 1 mg / L respectively. The test was carried out in triplicates, the % mortality of the brine shrimps determined and the data collected were subjected to Finney's probit analysis to determine the LC_{50} of each extract.Qualitative phytochemical analysis revealed the presence of secondary metabolites such as alkaloids, saponins, tannins, flavonoids, steroids, glycosides, resins and terpenoids. The quantitative phytochemical analysis gave the compositions of some of these components.The cytotoxic potentials of each extract was evaluated from theLC_{50}value, as extracts havingLC_{50}less than 250 µg/cm^3 are regarded as cytotoxic. The percentage mortality of the brine shrimpsdecreased with decreasing concentration for all the three extracts.The LC_{50} for the leaf, stem and root bark are 57.12, 25.47 and 119.35 µg/cm^{3}respectively, indicating the cytotoxic potential of all the three extracts. The cytotoxicity result also indicated that the stem extract was the most cytotoxic of the three crude extracts. The results obtained in this study is an indication that *Strophanthus sarmentosus*has excellent cytotoxic potentials and contains potent bioactive compounds with possible anti cancer properties worthy of further investigation.

Key words: *Strophanthus sarmentosus*DC,secondary metabolites,cytotoxicity, % mortality, bioassay, LC_{50}

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].Traditional medicines have proved very effective in the fight against some of the world's most stubborn ailments. *Strophanthus sarmentosus* (Apocynaceae) is commonly known as "spider tresses" and "arrow poison" in English, it is a plant widely distributed and popular for its ethnomedicinal values in Africaand used in West Africa for the treatment of several diseases which includes eye infections, arthritis, rheumatism, cardiac arrest, veneral diseases and constipation[3]. The phytochemical constituents of *Strophanthus sarmentosus* includes, glycosides, saponins, alkaloids [4]. *In vivo* antitrypanosomal investigations by Onotuand others[5] into the methanolic extracts of the stem of *S.Sarmentosus* revealed the presence of glycosides and saponins as some of its phytochemical components.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

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development.Toxicity is the degree to which a substance can damage an organism and cytotoxicity is a process that is harmful to cells. Cytotoxic refers to the toxicity of a substance to cell or a process which results in cell damage or cell death. Cytotoxic drugs are drugs that prevent cells function, they are drugs that cause damage to cells, they include drugs that are used to treat cancer. They have an effect of preventing the rapid growth and division (mitosis) of cancer cells.

MATERIALS AND METHODS

Plant material collection, authentication and identification

The leaf, stem and root 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.

Preparation 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 two weeks, 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 [6].

Qualitative phytochemical analysis of crude methanol extractsof 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 [6].

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

Quantitative phytochemical analysis of the crude extracts were carried out to determine the composition in percentage of alkaloids, tannins, flavonoids and saponins present[7].

Alkaloids determination: From each of the extracts, 0.2 g was weighed into a 250 cm^3 beaker, 8 cm^3 of 10 % acetic acid in ethanol was added and covered and allowed to stand for 4 h. These were filtered and the filtrates were concentrated on a water bath to one quarter of their original volumes. Concentrated NH_4OH was added drop wise to the extract until the precipitation was completed and washed with dilute ammonium hydroxide and then filtered. Each residue was dried, weighed and recorded as mass of alkaloid. The percentage alkaloid were calculated using the formula of Kumar and Bhardwaj *et al.,* as reported by Mann *et al.*, 2014 [7].

$$\%\, Alkaloid = \frac{Mass\, of\, Alkaloid}{Mass\, of\, Sample} \times 100$$

Or

$$\%\, Alkaloid = \frac{(weight\, of\, filter\, paper + alkaloid) - (weight\, of\, filter\, paper)}{weight\, of\, sample}$$

Tannins determination: From each of the samples, 0.1 g was weighed into 50 cm^3 plastic sample bottles, 10 cm^3 of distilled water was added and shaken for 60 minutes in a mechanical shaker. These were filtered into 50 cm^3 volumetric flasks and made up to the mark. About 5 cm^3 of each filtrate was transferred into a test tube and mixed with 2 cm^3 of 0.1 M $FeCl_3$ in 0.1 M HCl and 0.008M potassium ferrocyanide. The absorbances were taken with U-V spectrophotometer at 720nm within 10minutes. The measurements were repeated and the average absorbances were taken for each extract. Tannic acid was used as standard and the solution was prepared by dissolving 0.1g of tannic acid to 100cm^3 with distilled water.

The % tannins were calculated as follows

$$\%\,\text{tannins} = \frac{An}{As} \times C \times \frac{100}{W} \times \frac{Vf}{Va}$$

Where An: Absorbance of test sample

As: Absorbance of standard solution

C = concentraton of standard Solution

Vf = Total volume of sample used

Va = volume of extract used

W = weight of sample used.

Flavonoids determination: From each of the samples, 0.5 g was weighed and extracted with 10 cm^3 of 8% aqueous methanol at room temperature. The mixtures were them filtered using Whatman no. 1 filter paper. The filtrates were transferred into 250 cm^3 beakers and evaporated to dryness on a water bath and weighed.

The percentage flavonoids were calculated as follows:

$$\%\,flavonoids = \frac{weight\ of\ flavonoids}{weight\ of\ sample} \times 100$$

Or

$$\%\,flavonoids = \frac{(weight\ of\ beaker + flavonoids) - (weight\ of\ empty\ beaker)}{weight\ of\ sample} \times 100$$

Saponins determination: From each of the samples, 2 g was weighed into a 250 cm^3 conical flasks and 10 cm^3 of 20% ethanol was added. Each mixture was heated over a hot water bath for 4 hours with continuous stirring at about 55 °C. The mixtures were then filtered and the residues each re-extracted with 20 cm^3 of 20% ethanol. The combined extracts were concentrated to about 16cm^3 over a water bath at about 90%. The concentrated extracts were then transferred into 250 cm^3 separatory funnels and 20 cm^3 of diethyl ether was added to each extract. The aqueous layers were recovered while the diethyl ether layers discarded and the purification process was repeated. About 60 cm^3 of n-butanol was then added to each sample and the combined n-butanol were washed with 10 cm^3 of 5% NaCl. The remaining solutions were then heated on a water bath to evaporate to dryness and the residues were then weighed. The percentage saponins were calculated as follows

$$\%\,saponins = \frac{weight\ of\ saponins}{weight\ of\ sample} \times 100$$

Cytotoxicity using Brine Shrimp Lethality Bioassay

The methods of Mclaughlin*et al*.[8]; Meyer's *et al*. [9] and Solis *et al*. [10] were used with some modifications. An improvised hatching chamber made of 1000cm^3 conical flask was filled with natural sea water from bar beach, Lagos. Brine shrimps were added into the chamber. The hatchery with the eggs was placed under fluorescent light for 48 h for the brine shrimp eggs to hatch. Thirty six sample vials (12 sample vials per extract) of the same size were used for the test. Ten brine shrimp larvae were counted into each sample vile containing 4.0 cm^3 of sea water with the aid of a dropping pipette. The stock solution was prepared by separately dissolving 0.02 g of each extract in 2 cm^3 of methanol. To 0.2 cm^3 of each stock solution, 1.8 cm^3 of sea water was added to give 1,000 mg / L solution. Subsequent concentrations of 100, 10 and 1 mg / L were obtained from this for all three sample extracts. From these concentrations, 0.5 cm^3 of each were added into the sample vials containing 10 brine shrimp larvae in 4.0 cm^3 of sea water, and made up to 5 cm^3 with sea water. The tests were carried out in triplicates. A controlled solution containing 5 cm^3 of Sea water and ten brine shrimp larvae were set up. The tests were maintained at room temperature for 24 h under light after which the number of surviving and dead larvae were counted and recorded.The data collected were subjected to Finney's probit analysis [11] to determine the LC_{50} of each extract. The cytotoxicity is expressed by the LC_{50} which is defined as the concentration of the sample that kills 50 % of the larvae within 24 h. Percentage mortality was calculated as follows:

$$\%\,mortality = \frac{No.\,of\ dead\ larvae}{initial\ no.\,of\ live\ larvae} \times 100$$

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 [6]. 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 AND DISCUSSION

Qualitative phytochemical analysis 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 I). The presence of saponins and glycosides supports the result of Onotu *et al.* [5] in the phytochemical screening of the methanol stem extract of *S. sarmentosus*which revealed glycosides and saponins as some of the phytochemical components present. Investigations by Agbaje and Ajidahun [4] also confirmed the presence of alkaloids in the stem of *S. sarmentosus.*

Quantitative phytochemicalanalysis 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 (Figure II). All three extracts indicated a very high saponin content with a composition of 95.45 mg / g for the leaf, 85.15 mg / g for the stem and 78.25 mg / g for the root bark. All three extracts also possess high flavonoids content with a composition of 60.4 mg / g for the leaf, 52.43 mg / g for the stem and 43.4 mg / g for the root bark. The composition of tannins in all the three extracts and the alkaloids composition are relatively similar (Figure II) and the extract with the least composition of the phytochemical constituents quantified is 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 (Figure II). Saponins have been confirmed to be present in the extracts of *S. sarmentosus* [4, 5], Flavonoids are known for their ability to reduce inflammation and to act against diseases associated with inflammation [12, 13], tannins have been reported to have anti inflammatory effects [14], alkaloids are also used as anti cancer agents, hence the quantitative determinations of these phytochemical components.

Cytotoxicity test

Brine shrimp lethality bioassay is used to indicate the cytotoxic effects of plant extracts. The cytotoxicity test carried out on the crude methanol

Table I
Qualitative phytochemical analysis

S. No	*Chemical component*	*plant part* *Leaf*	*Stem*	*Root bark*
1	Alkaloids	+	+	+
2	Saponins	+	+	+
3	Cardiac glucosides	+	+	+
4	Coumarin glycosides	+	+	+
5	Resins	+	+	+
6	Flavonoids	+	+	+
7	Tannins	+	+	+
8	Steroids	-	+	+
9	Terpenoids	+	+	+

+ signifies presence, - signifies absence

Figure I: The fruits and leaves of *Strophanthus sarmentosus*

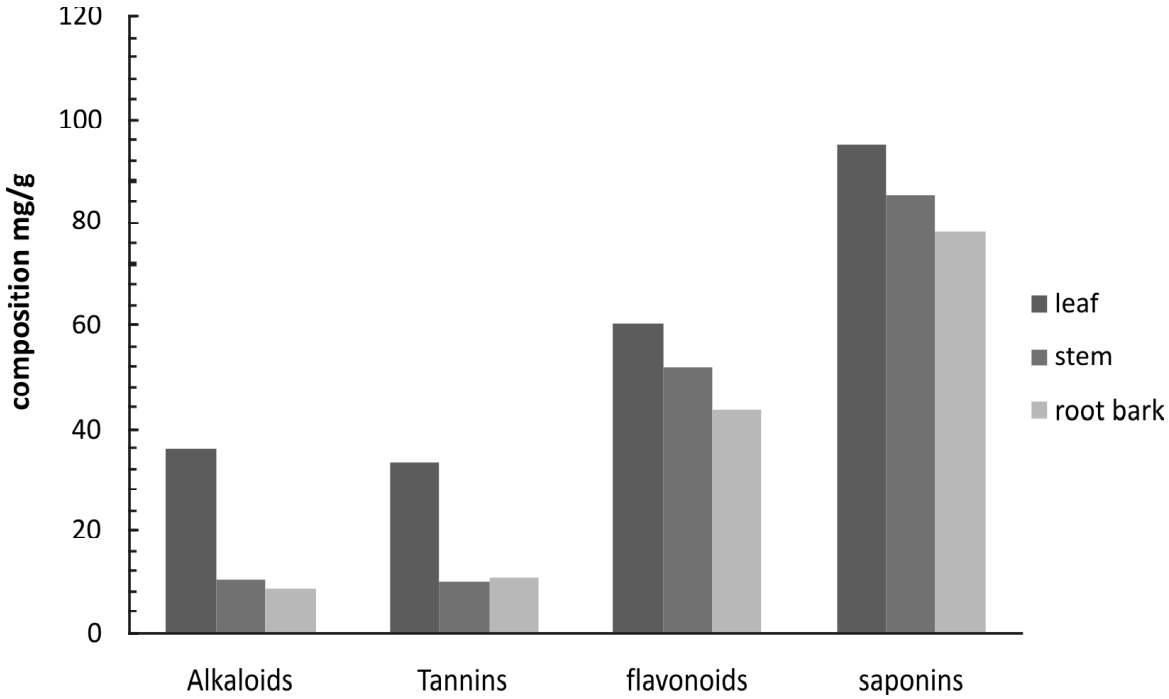

Figure II: Quantitative phytochemical analysis of crude methanolic extracts of the leaf, stem and root bark of *S. sarmentosus*

extracts of the three parts of *S. sarmentosus* plant is based on their brine shrimp lethality. The results showed a considerable brine shrimp larvicidal activity by all the extracts, which proves the cytotoxic effects of these plant parts. At 1000 mg / L, the high % mortality of the brine shrimp larvae indicates the toxicity of all the three extracts to the brine shrimps, at 100 mg / L, there is a decrease in the brine shrimp mortality with 50%, 46.7% and 50 % mortality for the leaves, stem and roots bark extracts respectively. The mortality of the brine shrimps tend to decrease with decrease in the concentrations of the extracts and at the lowest concentration of 1 mg / L, the mortality is very low with the roots bark extracts having no mortality at all while the stem extracts had the

highest at 23.33%. Meyer *et al.* [9]classified extracts and pure substances having LC_{50} value < 1000 µg / cm^3 as toxic and those with LC_{50} value > 1000 µg / cm^3 as non toxic, while extracts resulting in LC_{50} values less than 250 µg / cm^3 are considered to have significant cytotoxic effects[15].Therefore the LC_{50} values of all the three extracts is also an indicationof their toxicity to the brine shrimps.The stem extracts showed the highest toxicity of the three extracts with LC_{50} value of 25.47 µg / cm^3 but also the most cytotoxic (Table II).Brine shrimp lethality bioassay is used for testing the bioactivity and cytotoxicity of plant extracts based on their LC_{50} values, the result in most cases correlates with the cytotoxic and antitumor properties of the plants and this serves as a pre-screening tool for anti-tumor drug research[15]. Natural products are used in chemotherapy for cancer[16, 17].Most anti cancer agents are derived from plant based materials either directly or as models for synthetic compound[18]. The LC_{50} values of the crude extracts from the leaf, stem and root bark of *S. sarmentosus* indicated that they possess considerable cytotoxic potentials, particularly to the brine shrimps. The cytotoxicity shown by all the three crude extract was is a proof of the excellent cytotoxic potentials of *S. sarmentosus*.The results of this study shows that *Strophanthus sarmentosus*contains potent bioactive compounds with possible anti cancer properties. The anti cancer properties can be attributed to the presence of alkaloids as indicated by the phytochemical screening which are also used as anti cancer agents.

Table II
Brine shrimp lethality bioassay of crude methanol extract of the leaf, stem and root bark of S. sarmentosus

Plant part	*Conc (mg /L)*	*no. alive*	*no. dead*	*% mortality*	*LC_{50} (µg / cm^3)*
Leaf	1000	1, 0, 1	9, 10, 9	93.33	57.12
	100	4, 4, 7	6, 6, 3	50.00	
	10	6, 7, 7	4, 3, 3	33.33	
	1	8, 9, 10	2, 1, 0	10.00	
Stem	1000	1, 0, 0	9, 10, 10	96.67	25.47
	100	6, 6, 4	4, 4, 6	46.67	
	10	8, 6, 8	2, 4, 2	26.67	
	1	8, 8, 7	2, 2, 3	23.33	
Root bark	1000	2, 0, 3	8, 10, 7	83.33	119.35
	100	3, 6, 6	7, 4, 4	50.00	
	10	7, 8, 7	3, 2, 3	26.67	
	1	10, 10, 10	0, 0, 0	0.00	

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 III according to their retention time. The area %, fragmentation peaks, molecular formula and molecular weight of the compounds are also given.

Table III
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	(43),30,41, 68,82,96, 109,123, 138,194	254	$C_{16}H_{30}O_2$	Trans-11-tetradecenyl acetate
2	18.2	2.36	(74),27,41, 43,57,87, 109,127	158	$C_9H_{18}O_2$	Methyl 6-methyl heptanoate
3	19.7	17.93	(43),27,41 60,73,85, 98,115,129, 157,176, 185,213, 256	256	$C_{16}H_{32}O_2$	Hexadecanoic acid (palmitic acid)
4	21.2	8.57	(41),27,55, 69,83,97, 123,137, 264	282	$C_{18}H_{34}O_2$	9-Octadecenoic acid (oleic acid)
5	21.6	2.6	(74),27,41, 43,57,87, 109,115, 127	158	$C_9H_{18}O_2$	Methyl 6-methyl heptanoate
6	22.4	46.67	(41),27,55, 69,83,97, 123,137, 264	282	$C_{18}H_{34}O_2$	9-Octadecenoic acid (oleic acid)
7	22.6	13.09	(43),41,60, 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	(43),14,27, 41,57,83, 85,111,126	296	$C_{20}H_{40}O$	Octadecyl vinyl ether
9	26.3	3.06	(43),27,41, 57,71,96, 109,123, 127,183, 222,240, 310	398	$C_{26}H_{54}O_2$	Hexadecanal diisopentyl acetal

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