

PHYTOCHEMICAL, ANTIOXIDANT, CYTOTOXICITY, ANTIPROLIFERATIVE AND ANTIMICROBIAL STUDIES OF *SECURINEGA VIROSA* AERIAL PARTS EXTRACT

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Abstract

Cancer and infectious diseases are leading causes of death, globally. *Securinega virosa* is locally used for the management of cancerous tumour and infectious diseases. This study was designed to investigate the phytochemical constituents of the plant's aerial parts and determine the antioxidant, antiproliferative and cytotoxic properties of the extract using standard laboratory procedures. The plant was collected and the aerial parts was extracted with methanol. The extract was found to contain alkaloids, flavonoids, tannins, saponins and cardiac glycosides. It displayed potent antioxidant activity with an IC₅₀ of 0.018±0.002 mg/mL compared to ascorbic acid (0.008±0.001 mg/mL) and was non-toxic to brine shrimp. The extract displayed potent antiproliferative activity with an IC₅₀ value of 1.50±0.037 mg/mL compared to the value for cyclophosphamide, 0.55±0.043 mg/mL in *Allium cepa* root growth inhibitory assay. The extract displayed potent activities (IC₅₀ = 11.31±0.18 and 7.23±0.01 µg/mL) on RD and Hep-2C cancer cells, respectively, compared to cyclophosphamide (IC₅₀ = 0.32±0.18 and 2.56±0.13 µg/mL). The extract also displayed some antibacterial and anti-fungi activities with up to 10 and 14 mm zones of inhibition against *Escherichia coli*, *Citrobacter ferundii* at 0.625 mg/disc, respectively. *Securinega virosa* extract displayed antioxidant, antiproliferative, cytotoxic and antimicrobial activities and the plant maybe considered a viable candidate for discovery of chemotherapeutic agent(s).

Keywords: *Securinega virosa*, Cancer cell lines, Antioxidant, Antiproliferative, Antimicrobial

INTRODUCTION

Cancer and chronic infectious diseases are leading causes of death and over 60% of all approved anticancer and 75% anti-infective agents currently in use are derived from plant sources (1). Herbal medicines consisting of either single or combination of medicinal plants are commonly recommended either by a herbalist, herb sellers or elderly community members for the treatment of various ailments (2, 3). Interestingly, over 80% of the world population are directly or indirectly dependent on plant derived medicines for their primary health care needs (4). Despite the promising biological efficacy and phytochemical diversity of several medicinal plants used in traditional medicine, many of the plants have not been

scientifically evaluated for both safety and efficacy (5). Hence, the desire for new medicine and chemical diversity is the main drive for the continuous interaction between scientific and ethno-medicinal communities.

Securinega virosa is a shrub that belongs to Phyllanthaceae family. It is commonly used in African traditional medicine and locally called "Iraje" in the South-western part of Nigeria, where it is claimed to be effective in the management of cancer and other swallowed or

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tumour growth. In other parts of Africa, it is used mainly to treat gastritis, ulcers, pain, uterine diseases, menstrual and infertility problems, venereal diseases, hernia, and intestinal complications. The plant had been reported to possess interesting biological activities including anti-inflammatory and analgesic (6), anti-diarrhoeal (7), sedative (8, 9) and hypoglycaemic (10) effects. Hence, the goal of this study is to evaluate the anti-proliferative and anti-microbial activities of *S. virosa* extract with the aim of identifying medicinal plants that can be used in the discovery new chemotherapeutic agent(s).

METHODS

Collection and Preparation of Plant Extraction

Fresh aerial parts of *S. virosa* were procured at Oje market in Ibadan, Oyo State, Nigeria and later identified and authenticated (voucher number UILH/003/1273) at the herbarium unit of Department of Plant Biology in University of Ilorin, Ilorin, Nigeria. The aerial parts were shade-dried, pulverized and stored in an air and moisture tight container at room temperature prior to extraction. Methanol extract of *S. virosa* was obtained by macerating 200 g of the powder exhaustively with distilled methanol in four successions of 72 h maceration each. The extract thus obtained was concentrated *in vacuo* at 40 °C and the concentrated extract was stored at 4 °C in the refrigerator until needed for analysis.

Qualitative Phytochemical Analysis

Methanol extract of *S. virosa* aerial part was evaluated for the presence of different classes of secondary metabolites such as alkaloids, phenolics and glycosides using methods of phytochemical screening described by Trease and Evans (11).

In vitro DPPH radical scavenging assay

The antioxidant capacity of the extract was

accessed by the ability of the extract to scavenge 2, 2-diphenyl-1-picryl hydrazyl (DPPH) free radicals. A modified method described by Singh and co-worker (12), was utilized to access the antioxidant activity of the extract. Briefly, six different concentrations (0.200, 0.100, 0.050, 0.025, 0.013 and 0.006 mg/mL) of the extract, 100 µL each, were prepared by dissolving 2 mg of the extract in distilled methanol in a 96 well-plate and 150 µL of DPPH in methanol (0.00004 mg/mL) was added to each well. The negative control wells contained only DPPH solution without extract, while varying concentration of ascorbic acid (0.200, 0.100, 0.050, 0.025, 0.013 and 0.006 mg/mL) were used as positive control. The micro-plate was immediately covered with aluminium foil and incubated in the dark at RT (25°C) for 30 min. Thereafter, the absorbance of the resulting solution was measured at 517 nm using the UV spectrophotometer (Paul Bucher Analytik und Biotechnologie, Switzerland). The experiment was carried out in three replicates. The results were expressed as percentage inhibition and deduced from the following formula:

$$\text{Inhibition (\%)} = \frac{A-B}{A} \times 100 \quad \text{----- (equation 1)}$$

Where: A = the mean optical density of control wells.

B = the mean optical density of the wells with plant extracts.

Brine Shrimp lethality (BSL) Assay

The preliminary cytotoxicity potential of the extract was determined by the ability of the extract to kill the nauplii of *Artemia salina*. The nauplii of *A. salina* were obtained following a 48 h incubation of the *A. salina* eggs in natural sea water. The BSL assay was done using a modified method described by Krishnaraju and co-worker (13).

Antiproliferative Assays

***Sorghum bicolor* Radical Growth Inhibitory Assay**

Two hundred (200) mg of *S. virosa* extract was dissolved in 20 mL of 5% DMSO (Sigma-Aldrich, Germany) to achieve 10mg/mL stock concentration. Thereafter, 10 mL of 2.50, 0.63, 0.16 and 0.04 mg/mL were prepared by 1 in 2 dilutions of 10mL of the stock solution. The same concentration as the extract was prepared for cyclophosphamide (positive control). Different concentrations of the extract (10 mL) were added into different Petri-dishes already lined with cotton wool and filter paper. Ten viable *Sorghum bicolor* seeds were spread on the filter paper in each Petri-dish. Thereafter, the plates were incubated in a dark cupboard at room temperature and the lengths of the radicle emerging from the seeds were measured after 96 h incubation. The negative control test group contained seeds treated with 10 mL of 5% DMSO in distilled water (14). The experiment was repeated in three replicates for all concentrations and controls. The radicle lengths were measured and recorded in millimeter.

***Allium cepa* Root Growth Inhibitory Assay**

Allium cepa root growth inhibition was determined using a method described by Akinboro and Bakare (15). Clean bulbs (50 ± 10 g) were incubated at room temperature in the dark for 24-36 h until the roots have grown to approximately 2-3 cm length. Two hundred (200) mg of *S. virosa* extract was dissolved in 20 mL of 5% DMSO to achieve 20 mL of 10 mg/mL. Thereafter, 10 mL of 2.50, 0.63, 0.16 and 0.04 mg/mL were prepared by 1 in 2 dilutions of 10 mL of 10 mg/mL. The same concentration as the extract was prepared for cyclophosphamide (positive control). The different concentrations were poured into different Petri-dish and the base of each of three *A.*

cepa bulbs were placed on a Petri-dish containing each extract (0.04-10.00 mg/mL). The same concentrations as above were prepared for cyclophosphamide (positive control), while the negative control bulbs were treated with 20 mL of 5% DMSO in distilled water. The root lengths were measured at 0 and after 96 h for each concentration of extract and control. The percentage root growth inhibition after treating with extract/cyclophosphamide at 96 h was determined (15).

The MTT Colorimetric Assay

The cytotoxicity of the extract was determined using the MTT colorimetric assay, a method described by Tolosa and coworkers (16). The extract was dissolved in DMSO to achieve a concentration of 10 mg/mL. From the stock, 0.1 mL was dissolved in 0.9 mL of maintenance medium to obtain a concentration of 1 mg/mL and designated as “neat”. Serial ten-fold dilutions of the extracts were prepared from the “neat” using maintenance medium as diluent to obtain different concentrations. A volume of 50 µL of each extract dilution was dispensed into 96-well microtitre plates already seeded with monolayer of Rhabdomysarcoma (Rd) and laryngeal carcinoma (Hep-2c) human cancer cell lines and a normal cell line (Vero) from African green monkey kidney. The plates were incubated at 37°C in a CO₂ environment and the cells observed under microscope after 72 h.

Supernatants were removed from all wells after the 72 h incubation. Then, 25 µL of 2 mg/mL yellow solution of MTT in Phosphate Buffer Saline (PBS) was added to each well and the plates were incubated for additional 2 h at 37°C. Thereafter, 125 µL of DMSO was added into the wells to solubilize the purple colour formazan crystals formed by viable cells. The plates were placed on a shaker for 15 min to ensure that the formazan crystals were well dissolved and dispersed. Thereafter, the absorbances of the plates were read at a wavelength of 492 nm using a multi-well spectrophotometer (Paul

Bucher Analytik und Biotechnologie, Switzerland). Experiments were performed in triplicates.

The percentage cytotoxicity was calculated as:

$$\% \text{ cytotoxicity} = \frac{A-B}{A} \times 100 \quad \text{-----} \quad (\text{equation 2})$$

Where: A = the mean optical density of untreated cells.

B = the mean optical density of cells treated with plant extracts/ fractions.

The selective index (SI) of the two extracts and cyclophosphamide was estimated using the Vero cells. Data were expressed as the means $\pm$ SEM. Selective Index (SI) greater than 1 is indicative of selectivity.

$$\text{Selective index (SI)} = \frac{CC_{50} \text{ on Vero cells}}{CC_{50} \text{ on cancer cells}} \quad \text{-----} \quad (\text{equation 3})$$

Determination of Antimicrobial Activity

Test Organisms

Three clinical isolates of bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, *Citrobacter ferundii*) and one clinical isolate of fungi (*Candida albicans*) were used as test organisms to determine the antimicrobial potential of *S. virosa* extract. The organisms were diluted to achieve optical densities corresponding to 2.0×10^6 colony forming units (CFU/mL) for bacterial and 2.0×10^5 spores/mL for fungal strain when needed.

Antimicrobial Susceptibility Assay

Antimicrobial assay was performed using disc diffusion technique (17). The bacterial test strains were spread over the nutrient agar plates by using separate sterile cotton buds. The fungal strain was spread over the potato dextrose agar plates. Four concentrations (50.00, 25.00, 12.50 and 6.25 mg/mL) of the extract were prepared by serial dilution. Thereafter, discs (5 mm diameter) impregnated with 100 μ L of 50.00, 25.00, 12.50 and 6.25 mg/mL of extract to achieve 5, 2.5, 1.25 and 0.63 mg/disc concentrations, were mounted on inoculated agar and incubated. All bacterial plates were

incubated at 37°C for 24 h and fungal plates at 25°C for 72 h. Moreover, filter paper discs (5 mm diameter) containing standard antibiotics; gentamicin (0.04 mg/disc) and fluconazole (0.05 mg/disc) were used as positive controls. The zones of inhibition were recorded in millimeter (mm) as the diameter of growth-free zones around discs. The extract and standard antibiotics were independently tested in three replicates and the results presented as mean $\pm$ SEM. Extract with concentration greater than ≥ 10 mm is considered active (18). An estimated zone of inhibition at concentration of control (E-ZIC) was obtained from a linear regression curve by GraphPad Prism. Activity index (AI) which compares the antimicrobial activity of the extract to the positive control was estimated using the following formula:

$$AI = \frac{E-ZIC}{ZOI} \quad \text{-----} \quad (\text{equation 4})$$

AI = Activity Index

E-ZIC= Estimated Zone of Inhibition at Concentration of Control

ZOI = Zone of Inhibition of positive control

Data Analysis

Data obtained were analyzed by Graphpad prism computer program. The concentration with 50% inhibition (IC₅₀) in DPPH scavenging assay, 50% lethality (IC₅₀) in BSL assay, 50% growth inhibition (IC₅₀) in *Sorghum bicolor* radicle growth inhibitory assay, 50% growth inhibition (IC₅₀) in *Allium cepa* root growth inhibitory assay and IC₅₀ cytotoxicity in MTT colorimetric assay were estimated from a dose-response inhibition curve using a non-linear regression curve data analysis. The results are displayed as mean $\pm$ SEM of three replicates. Statistical significance was evaluated using student's t-test and results with $p < 0.05$ were considered significant.

RESULTS

Qualitative Phytochemical composition of *S. virosa*

The dried aerial part powder of *S. virosa* yielded 18.35 g of green extract following extraction with distilled methanol. Qualitative phytochemical screenings of *S. virosa* extract revealed the presence of various secondary metabolites including alkaloids, flavonoids, tannins, saponins and cardiac glycosides. Tannins were found to be more abundant in the extract. However, free and combined anthraquinones were not detected (Table 1).

Antioxidant Effect of *S. virosa* Extract

The extract as well as the positive control (Ascorbic acid) displayed concentration - dependent DPPH radical scavenging activity. The positive control ($IC_{50} = 0.008 \pm 0.001$ mg/mL) displayed higher DPPH radical scavenging than the extract ($IC_{50} = 0.018 \pm 0.002$ mg/mL) as shown in Table 2.

Brine Shrimp Lethality Effect of *S. virosa* Extract

Toxicity of the extract was assessed using lethality of the extract on brine shrimps. Both the extract and positive control showed concentration - dependent brine shrimp lethality. However, the extract ($LC_{50} = 1.07 \pm 0.001$ mg/mL) displayed very weak brine shrimp lethality compared to the positive control ($LC_{50} = 0.05 \pm 0.01$ mg/mL) implying that the extract may be non-toxic (Table 3).

Antiproliferative effects of *S. virosa* Extract

The extract and positive control (cyclophosphamide) displayed concentration-dependent radicle and root growth inhibition after 96 h of growth. The extract displayed antiproliferative activity with IC_{50} of 4.15 ± 0.040 and 1.50 ± 0.037 mg/mL compared to cyclophosphamide (with IC_{50} of 1.75 ± 0.053 and 0.55 ± 0.043 mg/mL) in *S. bicolor* radicle

growth (SBRG) and *A. cepa* root growth (ACRG) assays, respectively.

Cytotoxicity of *S. virosa* extract on cancer and normal cell lines

The extract and positive control (cyclophosphamide) displayed cytotoxicity on different cell lines as shown in Table 5. The extract displayed less cytotoxic activity on the three cell lines than cyclophosphamide. The extract ($IC_{50} = 7.70 \pm 0.11$ μ g/mL) displayed less toxicity on normal cell line compared to cyclophosphamide ($IC_{50} = 3.19 \pm 0.07$ μ g/mL). On the two cancer cell lines (RD and Hep-2C), the extract displayed potent activities ($IC_{50} = 11.31 \pm 0.18$ and 7.23 ± 0.01 μ g/mL respectively) but were less compared to cyclophosphamide ($IC_{50} = 0.32 \pm 0.18$ and 2.56 ± 0.13 μ g/mL). The extract displayed varied selectivity indices (SI) as shown in Table 5. The extract displayed higher SI for Hep-2C (SI=3.00) than RD (0.68).

Antimicrobial Effects of *S. virosa* extract

The extract showed concentration-dependent anti-microbial activity as shown in Table 6, with significant effect against *E. coli* and *C. ferundii* even at 0.625 mg/disc. Activity index (AI) estimation indicated that the extract has the highest activity, compared to control, in the order: *E.coil* (0.79) > *C. ferundii* (0.76) > *S. aureus* (0.64) > *C. albican* (0.32).

DISCUSSION

Extraction of *S. virosa* plant material (100 g) yielded 18.35 % w/w of dark green extract and qualitative phytochemical test of the extract revealed the presences of alkaloids, flavonoids, tannins, saponins and trace cardiac glycoside. Different types of secondary metabolites have been reported to display several biological activities including, antioxidant, cytotoxicity, antiproliferative and antimicrobial. Tannins and flavonoids have been reported to display antioxidant activity (19). Interestingly,

Table 1: *In vitro* Phytochemical Analysis of the *Securinega virosa* aerial part Extract

Bioactive Constituent	Chemical Test	Inference/Result
Alkaloids	Wagner's	++
	Meyer's	++
Flavonoids	Lead acetate	+
Tannins	FeCl ₃	+++
Saponins	Frothing	++
	Emulsifying	++
Anthraquinone	Borntrager's	-
	Modified Borntrager's	-
Cardiac glycoside	Kedde	+

Keys:

-, denotes absence of component; + denotes trace presence of component;

++ denotes moderate amount of component, +++ denotes copious amount of component

Table 2: Percentage inhibition and IC₅₀ of DPPH Free Radical by *S. virosa* aerial part Extract

Conc. (mg/mL)	Percentage (%) inhibition	
	<i>S. virosa</i> Extract	Ascorbic Acid
0.400	79.350±0.610	84.630±0.320
0.200	72.890±0.600	83.230±0.120
0.100	71.290±0.080	82.630±0.320
0.050	64.100±0.570	80.000±0.000
0.025	54.750±0.800	76.860±0.070
0.013	42.500±1.370	62.130±0.060
0.006	38.380±0.970	35.190±0.090
IC ₅₀ (mg/mL)	0.018±0.002	0.008±0.001

Table 3: Percentage Lethality and LC₅₀ of *S. virosa* Extract on Brine Shrimps

Conc. (mg/mL)	Percentage (%) Lethality	
	SVE	CTZ
1.000	50.00±0.00	100.00±0.00
0.500	23.33±3.33	70.00±0.00
0.100	10.00±0.00	50.00±0.00
0.010	0.00±0.00	36.67±3.33
0.001	0.00±0.00	6.67±3.33
LC ₅₀ (mg/mL)	1.07±0.01	0.05±0.01

Keys:SVE: *Securinega virosa* Extract

CTZ= Cyclophosphamide

Table 4: Antiproliferative effect of *S. virosa* Extract on *Sorghum bicolor* Radicle and *Allium cepa* Root

Conc. (mg/mL)	SBRG Inhibitory Assay		ACRG Inhibitory Assay	
	SVE	CTZ	SVE	CTZ
10.000	94.04±0.034	100.00±0.000	100.00±0.000	100.00±0.000
5.000	83.23±0.184	100.00±0.000	98.03±0.034	100.00±0.000
2.500	22.16±0.124	97.04±0.023	90.03±0.032	97.97±0.311
1.250	19.09±0.633	50.28±0.239	76.15±0.100	95.27±0.080
0.625	10.18±0.138	46.98±0.033	64.87±2.134	87.02±0.693
IC ₅₀ (mg/mL)	4.15±0.040	1.75±0.053	1.50±0.037	0.55±0.043

Keys:SVE: *Securinega virosa* Extract

CTZ= Cyclophosphamide

Table 5: Cytotoxicity of *S. virosa* Extract on Two Cancer and One Normal Cell lines and Selective indices

Cell Lines	Normal Cell line		Cancer Cell lines			
	Vero		RD		Hep-2C	
Sample	SVE	CTZ	SVE	CTZ	SVE	CTZ
IC ₅₀ (µg/mL)	8.47±0.110	3.19±0.073	11.31±0.184	0.32±0.176	7.23±0.012	2.56±0.133
Selectivity Index (SI)			0.749	9.968	1.172	1.246

Keys:SVE: *Securinega virosa* Extract

CTZ= Cyclophosphamide

Table 6: Zones of Inhibition of *S. virosa* aerial part Extract

Conc. (mg/disc)	Zone of Inhibition (mm)			
	<i>E. coli</i>	<i>S. aureus</i>	<i>C. ferundii</i>	<i>C. albicans</i>
5.000	20±1.000	14±0.330	24±1.330	15±1.333
2.500	18±0.000	11±0.000	21±0.000	11±0.000
1.250	15±0.000	8±0.000	18±0.000	10±0.000
0.625	10±0.000	3±0.000	14±0.000	7±0.000
E-ZIC	19.100	12.740	22.830	7.020
Positive Control	24±1.333 ⁺	20±3.333 ⁺	30±1.667 ⁺	22±0.000 ⁺⁺
Activity Index (AI)	0.79	0.64	0.76	0.32

Keys:

E-ZIC= Estimated Zone of Inhibition at Concentration of Control

⁺ = Zone of Inhibition of gentamicin at 0.04 mg/disc⁺⁺ = Zone of Inhibition of fluconazole at 0.005 mg/disc

many alkaloids and saponins have been reported to possess significant anticancer activity (20, 21).

Free radicals have been implicated in cancer, diabetes, cardiovascular diseases, Alzheimer's disease and many other degenerative diseases (22). Some plants are known to contain secondary metabolites that neutralize free radicals thereby preventing many degenerative diseases. Interestingly, the extract displayed comparable DPPH radical scavenging activity (38.38 ± 0.97 % inhibition) to ascorbic acid (35.19 ± 0.09 % inhibition) at 0.006 mg/mL. Dickson and co-workers earlier reported that chloroform extract of *S. virosa* root displayed some DPPH radical scavenging activity with IC_{50} of 0.003 mg/mL (23). The antioxidant activity of *S. virosa* may be attributed to the presence of tannins and flavonoids. This report suggests that *S. virosa* methanol aerial part extract possesses significant antioxidant activity. Tannins and flavonoids have polyhydroxyl functional groups that are capable of donating protons to free radicals (24). This is the first report of the antioxidant activity of methanol extract of *S. virosa* aerial part.

Brine shrimp lethality (BSL) assay is very often used as a rapid and inexpensive bench top assay for testing samples for bioactivity which in most cases, correlates with cytotoxicity and anti-tumour property (13). Higher BSL suggests higher toxicity and /or cytotoxicity, while lower BSL is suggestive of non-toxicity and non-cytotoxicity. Pagmaja and co-worker classified BSL of extracts as follows; $LC_{50} \geq 1$ mg/mL are nontoxic, $LC_{50} = 0.5-1$ mg/mL are weakly toxic, $LC_{50} = 0.1-0.5$ mg/mL are moderately toxic, and $LC_{50} \leq 0.1$ mg/mL are strongly toxic. Interestingly, methanol extract of *S. virosa* displayed mild toxicity in BSL assay with $LC_{50} > 1$ mg/mL suggesting that the extract is non-toxic. This is the first report on BSL of *S. virosa* and may support the safe use of *S. virosa*, traditionally.

Antiproliferative assays including SBRG and ACRG inhibitory assays are simple bench top assays that are predictive of important biological activities such as herbicidal, allelopathic or antitumour properties of plant extracts. Here, an extract with antiproliferative activity inhibits proliferation of mitotic cells of *S. bicolor* radicle and *A. cepa* root either by interfering with the biochemical system and/or other growth-related systems, like DNA division (14). Methanol extract of *S. virosa* displayed concentration-dependent SBRG and ACRG inhibition. The extract interestingly, displayed potent SBRG and ACRG inhibition with IC_{50} values of 4.15 ± 0.040 and 1.50 ± 0.037 mg/mL respectively, comparable to cyclophosphamide with IC_{50} of 1.75 ± 0.053 and 0.55 ± 0.043 mg/mL, respectively. This is the first report of the antiproliferative activity of *S. virosa*.

The *in vitro* cytotoxic assessment outcomes of methanol extract of *S. virosa* on the two cancer cell lines further support the antiproliferative activity observed above. Here the extract demonstrated significant cytotoxicity on the cancer cell lines with $IC_{50} < 50$ μ g/mL though, less than that of cyclophosphamide. The extract showed better cytotoxicity ($IC_{50} = 7.23 \pm 0.01$ μ g/mL) and selectivity (SI=1.17) for Hep-2C than RD human cancer cell lines. The findings here corroborate an earlier report by Tatematsu and co-worker where the ethanol extract of *S. virosa* was found to be active against P-388 cell line and two cytotoxic alkaloids namely virosecurinine and viroallosecurinine from the ethanol extract of *S. virosa* (25). However this is the first report of the cytotoxic activity of methanol extract of *S. virosa* aerial part on skeletal muscle cancer, laryngeal carcinoma and normal cell lines.

Antimicrobial drug resistance has rendered many antimicrobial agents ineffective, thereby creating a global healthcare problem (26). Hence, there had been huge search for new

sources of antimicrobial agents and medicinal plants are considered as viable source for new antimicrobial agents. The antimicrobial activity of the extract was assessed by challenging one Gram +ve (*S. aureus*), two Gram –ve (*E. coil*, *C. ferundii*) and a fungus (*C. albicans*) with varying concentrations of the extract. The extract demonstrated varying degrees of activity across the different micro-organisms. The extract had the following order of zones of antibacterial inhibition against the organisms: *C. ferundii* > *E. coil* > *S. aureus* similar to positive control (gentimicin). The activity of the extract was theoretically comparable to that of the positive control. The extract was found to exhibit superior activity comparable to gentimicin on Gram -ve bacteria (AI of *E. coil* and *C. ferundii* = 0.79 and 0.76, respectively) to Gram +ve (AI of *S. aureus* = 0.64). However, the extract had low activity against *C. albicans* (0.32), comparable to that of fluconazole. The extract demonstrated higher antibacterial activity than antifungal activity. This is the first report of antimicrobial activity of *S. virosa* extract.

CONCLUSION

The methanol extract of *S. virosa* showed the presence of various secondary metabolites including alkaloids, flavonoids, tannins, saponins and cardiac glycosides. The extract displayed antioxidant activity and was non-toxic on brine shrimps. The extract displayed potent antiproliferative and cytotoxic effects. Interestingly, the extract also showed broad spectrum antibacterial and some anti-fungal activities. The findings of the study support the traditional use of *S. virosa* in management of cancer and various infectious diseases. *Securinega virosa* may be considered as a candidate for discovery of chemotherapeutic agent(s).

Contributions

KM Salawu and EO Ajaiyeoba conceived and designed the work. KM Salawu and EO Ajaiyeoba wrote and edited the manuscript.

KM Salawu collected the plant sample, performed DPPH antioxidant and MTT colorimetric assays. KM Salawu and AA Oyerinde performed *in vitro* phytochemical analysis, brine shrimps lethality, *Allium cepa* root growth inhibitory assay, *Sorghum bicolor* radicle growth inhibitory assay and antibacterial analysis. All the authors read and approved the final manuscript.

Conflict of interests

The authors declare that they have no competing interests.

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