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SECURINEGA VIROSA LEAF AND ROOT BARK EXTRACTS: A COMPARATIVE ANTI-CANCER STUDY AGAINST HUMAN BREAST (MCF-7) AND LUNG (NCI-H460) CANCER CELL LINES

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ABSTRACT

Securinega virosa (Roxb. ex.Willd.) is a medicinal plant with folkloric use in the treatment of cancer and other diseases and has earlier shown significant cytotoxic and growth-inhibitory prospect in our past work. The methanol extracts of the leaves and root barks obtained by cold maceration were screened for anti-cancer properties on breast (MCF-7) and lung (NCI-H460) cancer cells at 1-250 µg/mL adopting the SRB assay. The active leaf extract was partitioned into aqueous and chloroform fractions which were further tested at 1-100 µg/mL on the cell lines. The leaf and root bark extracts exhibited higher sensitivities on MCF-7 than NCI-H460 cell lines. At 100 µg/mL the leaf extract showed cytotoxicity and growth-inhibitory activities of –1.09 and +61.35 % on MCF-7 and NCI-H460 cell lines which later increased to –18.67 and +77.13 %, respectively at 250 µg/mL with GI 50 and TGI of 42 and 63 µg/mL on MCF-7 as well and 98.01 and 132.50 µg/mL on both cells. At the maximum concentration, the root bark extract recorded cytotoxicity of –2.60 % on MCF-7, and a growth-inhibitory activity of +57.9 % on NCI-H460 cell lines. Fractionation of the leaf extract improved its activity at GI_{50}, TGI of 28.11, 34.22 µg/mL on MCF-7 and 42 and 52.69 µg/ mL against NCI-H460 cells, respectively. The traditional use of this plant in the treatment of tumor ailment has further been justified by these results.

Keywords : *Securinega virosa*, anti-cancer, fractionation, MCF-7, NCI-H460, cytotoxic, growth-inhibitory

INTRODUCTION

The application of herbal remedy to control several diseases is a general usage in developing nations, where most people depend on herbal drugs as the main method of therapy (WHO, 2004). Although tumors have been handled with herbal drugs as practised in folkloric medicine, there is really inadequate scientific proof on the potency of such herbs, since ethnomedicinal information is usually kept in secrecy by traditional herbalists, making it hard to handle any scientific investigations. It is, therefore, necessary to conduct vital assays on such herbs to ascertain their pharmacological efficacy as well as potentially toxic side-effects. This approach may

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also reveal novel bioactive molecules with anti-cancer potential (Damery *et al.*, 2011).

The emergence of drug-resistance, which is a major barrier hampering an effective cancer chemotherapy (Kuete and Efferth, 2015; Efferth *et al*., 2008), has led to a constant quest for new anti-cancer remedies. Medicinal plants over the years have served as sources of anti-cancer drugs such as vinblastine and vincristine and have been used for the formulation of modern drugs (Takimoto and Calvo, 2008). Over the years, various African medicinal plants have reportedly demonstrated cytotoxic properties against drug-resistant cell lines (Choumessi *et al*., 2012; Kuete *et al*., 2014). In Nigeria, the cancer burden for the entire country is unclear; however, most practising physicians have reported an increase in cancer cases among patients visiting local health facilities (Jedy-Agba *et al.*, 2012). Surgery, radiotherapy and chemotherapy remain gold standards in effective cancer management. However, the evolution of chemo- and radio-resistance could be a principal agent for high recurrence rates and mortality. Furthermore, it is difficult for scientifically not-trained traditional practitioners to diagnose cancer (Arruebo *et al.*, 2011).

Researchers such as Cordell *et al*. (1991) and Popoca *et al*. (1998) reported that in the screening and selecting of anti-cancer plants, other disease conditions (e.g viral, bacterial, inflammatory and parasitic diseases) which are linked to symptoms of cancer should be taken into account.

The plant Securinega virosa is popularly called "cure all" because of the medicinal values of all the morphological parts. The plant which usually grows in tropical Africa, India, Malaya, China and Australia (Dalziel, 1936) is locally known in parts of Nigeria as "Tsuwaawun kare" (Hausa), "Iranje" (Yoruba) and "Njisi nta" (Igbo) (Neuwinger, 1996). The plant parts, particularly the root and leaf, have been reported to be used in the management of epilepsy and mental illness in Northern Nigeria (Neuwinger, 1996).

Our previous work has reported the comparative cytotoxic, growth-inhibitory as well as spectrophotometric quantification of phytochemicals of the two morphological parts. However, there is a need to further evaluate this plant scientifically for other unnoticed potentials it might possess. The present study, therefore, took another look at pre-screening the extract and fractions of the leaf of *S. virosa* for some biological activities using bench-top methods as well as assessing their anti-tumor potentials against breast and lung cancer cell lines.

MATERIALS AND METHODS

Collection and Identification of *Securinega virosa*

The fresh leaves and root barks of *Securinega virosa* were obtained from the previous site of collection (University of Benin Teaching Hospital sporting playground) and were properly identified. The plant specimens were further authenticated by Mr. Odewo Akinniyi Samuel of Forestry Institute of Nigeria, Jericho, Ibadan where a voucher number (FHI 112300) was obtained.

Preparation and fractionation of plant extract

A total of 1.2 kg of the powdered samples of the leaves and root barks were extracted separately by cold maceration using 80 % methanol for 3 days after which they were filtered and concentrated using a rotary evaporator.

Cell Lines and Cell Cultures

The MCF-7 (Breast) and NCI-H460 (lung) human cancer cell lines were obtained from PCMD Molecular Bank, University of Karachi, Pakistan. The cells were grown and kept in a suitable medium, pH 7.4, accompanied with 10% fetal calf serum, glutamine (2 mM), penicillin (100 units/mL) and streptomycin (100 μg/mL). The cell cultures were grown in a carbon dioxide incubator (Heraeus, GmbH, Germany) at 37°C with 90% humidity and 5% CO_2 (Ikpefan *et al*, 2019).

Anti-cancer studies of leaf and root bark extract of *Securinega virosa*

The growth-inhibitory activities of methanol extracts of *S. virosa* were tested using human breast cancer cell lines (MCF-7) and lung cancer (NCI-H460). The stock solutions of plant extracts (40 mg/mL in DMSO) and the control doxorubicin (1 mM in distilled water) were prepared. The corresponding dilutions were prepared in RPMI-1640 comprising gentamicin (50 µg/mL) and the monolayer trypsinization, cell viability determination as well as cell counting from a confluent flask (75 cm^2) were carried out. The cells (10,000 cells/well/100 µL for MCF-7 and NCI- H460) were seeded for monolayer formation and incubated in a CO_2 incubator at 37°C for 24 h. The various concentrations of methanol extracts of *S. virosa* (1, 10, 50, 100, 200, and 250 µg/mL) and fractions (1, 25, 50, 75 and 100) were added (100 µL/well) in appropriate wells, followed by incubation for 48 h. Appropriate controls and blanks (drug and extract) were also prepared. At the end of 48 h, time zero-1 (Tz 1 plate) and time zero-2 (Tz 2 plate) cells (10,000 cells/well/100 µL for MCF-7 and NCI-H460) were seeded for monolayer formation and incubated in a CO_2 incubator at 37°C for 24 h. These were then left at room temperature for 30 min, washed (3x), and dried overnight. At the end of 48 h, the experimental plates were also fixed in a similar manner. The air-dried fixed plates were stained with 100 µL of sulforhodamine solution (0.4% wt/vol made in 1% acetic acid) for 10 min, accompanied by washing (5 times) with 1% acetic acid to eliminate excess stain, and were air-dried. Lastly, tris base solution (100 µL with pH 10.2, 10 mM) was combined to solubilise the protein-bound stain and the corresponding absorbance was recorded at 545 nm using a microplate reader. All the experiments were carried out in replicates of three and results were presented as GI_{50}, TGI and LC_{50} (µg/mL) values.

Fractionation of the leaf extract

Part of the resulting extract of the leaf (45 g) was suspended in equal amount of water and methanol (1:1, 400 mL) and subjected to solvent-solvent partitioning using chloroform. The aqueous and chloroform fractions were collected, concentrated and weighed.

Statistical analysis

The data obtained were evaluated using GraphPad Prism 7.0. One-way analysis of variance (ANOVA) was used in data analysis and were represented as Mean ± Standard Error of Mean (SEM).

RESULTS

Effects of the leaf extract on MCF-7 and NCI-H460 cell lines

The leaf extract recorded both cytotoxic and growth-inhibitory effects on MCF-7 and NCI-H460 cell lines in varying levels. At 1-50 µg/mL, growth-inhibitory activities of 21, 56 and 92 % were recorded against MCF-7. The activity became cytotoxic at 100-250 µg/mL with GI_{50} and TGI of 42 and 63 µg/mL with LC_{50} of >250 µg/mL recorded. Also, against NCI-H460 cell lines, the leaf extract recorded growth-inhibitory effects at all concentrations with a GI_{50} and TGI of 78.3 and 106.11 µg/mL as well as LC_{50} and TGI greater than 250 µg/mL (Table 1).

Table 1: Sensitivities of the breast (MCF-7) cancer cell lines to the methanol extracts of the medicinal plants.

Test agent	(µg/mL)	% Growth-inhibition/ cytotoxicity on MCF-7 cell lines	GI_{50}	LC_{50}	TGI
			(µg/mL)		
Leaf extract	1	+21.17± 1.26	42.16 ± 0.06	>250	63.33±0.68
	10	+57.51± 0.85			
	50	+92.40± 1.88			
	100	–1.09± 0.34			
	200	–8.24 ± 0.65			
	250	–18.67 ± 1.45			
		% Growth-inhibition/ cytotoxicity on NCI-H460 cell lines			
Leaf extract	1	0.00± 0.00	78.33 ± 7.42	>250	106.11 ± 4.06
	10	+7.82± 1.02			
	50	+50.57 ± 4.58			
	100	+61.35 ± 3.12			
	200	+69.05 ± 3.00			
	250	+77.13 ± 1.29			

Control absorbance (Blank) in MCF-7 at 545 nm = 1.9 ± 0.1 while for NCI-H460 in 545 nm = 2. 0± 0.1, value represents % mean ± SEM of 3 independent experiments as compared to control. The "+" implies growth-inhibition, "-" cytotoxicity, "GI_{50} "and "TGI" denotes concentration of drug causing 50% and 100 % growth inhibition of cells, while "LC_{50}" is concentration of drug that killed 50% cells.

Effect of the root bark extract on MCF-7 and NCI-H460 cell lines

As observed in the leaf extract, the root bark extract was observed to be less sensitive to NCI-H460 cell lines and more on MCF-7 cell lines. For example, against MCF-7, the extract exhibited growth-inhibitory activities at concentrations between 1-50 µg/mL which later became cytotoxic between 100-250 µg/mL with GI_{50} and TGI of 51 and 97.3 µg/mL, respectively (Table 2). However, against NCI-H460 cell lines and at all concentrations, growth inhibitory activities were recorded as well as GI_{50} and TGI of 98.01 and 132.50 µg/mL (Table 2).

Table 2: Sensitivities of the breast (MCF-7) and lung (NCI-H460) cancer cell lines to the root bark extracts of *S.virosa*.

Test agent	Conc. (µg/mL)	% Growth-inhibition/ cytotoxicity on MCF-7 cell lines	GI_{50}	LC_{50}	TGI
			(µg/mL)		
Root bark extract	1	+6.09± 0.18			
	10	+15.11± 1.02	51 ± 0.02	>250	**97. 33±6. 77**
	50	+52.18± 0.99			
	100	+68.04± 0.34			
	200	+84.73 ± 2.10			
	250	–2.60 ± 0.62			
		% Growth-inhibition/ cytotoxicity on NCI-H460 cell lines			
Root bark extract	1	0.00± 0.00			
	10	+0.00± 0.00			
	50	+7.98 ± 2.12	98.01 ± 4.06	>250	132. 50 ± 3. 98
	100	+19.73 ± 0.18			
	200	+42.12 ± 1.00			
	250	+57.92 ± 2.19			

The control absorbance (Blank) in MCF-7 at 545 nm = 1.9 ± 0.1 while for NCI-H460 in 545 nm = 2. 0± 0.1. The "+" implies growth-inhibition, "-" cytotoxicity, "GI_{50} "and "TGI" denotes concentration of drug causing 50% and 100 % growth inhibition of cells, while "LC_{50}" concentration of drug that killed 50% cells.

Effect of the leaf extract partitioned fractions of *S. virosa* on MCF-7 and NCI-H460 cell lines.

The aqueous fraction recorded little or no activity on both cell lines as the chloroform fraction recorded higher sensitivities on MCF-7 than NCI-H460 cell lines. At 1 and 10 µg/mL, the chloroform fraction gave cytotoxicities of -4.80 and -9.5 % on MCF-7 cell lines which later increased to -32.71% at the maximum concentration of 100 µg/mL (Table 3). Also, the chloroform fraction exhibited growth-inhibitory activities of +48.2, +71.5 and +98.7 % on NCI-H460 cell lines at concentrations between 1-50 µg/mL. However, cytotoxicity of -3.18 % was recorded at 100 µg/mL with GI_{50} and TGI of 42 and 52.69 µg/mL, respectively (Table 3).

Table 3: Effect of the partitioned fractions on breast (MCF-7) and lung cancer cell lines (NCI-H460).

Test agent	(µg/mL)	% Growth-inhibition/ cytotoxicity on MCF-7 cell lines	GI_{50} (µg/mL)	LC_{50} (µg/mL)	TGI (µg/mL)
Chloroform fraction	1	-4.80 ± 0.16	28.11 ± 0.62	>100	34.22±0.18
	10	-9.50 ± 1.62			
	50	-14.62 ± 2.64			
	100	–32.71± 4.10			
Aqueous fraction	100	>100	>100	>100	**>100**
		% Growth- inhibition/ cytotoxicity on NCI-H460 cell lines			
Chloroform fraction	1	+48.16± 0.82	42.00± 3.61	>100	52.69 **± 5.07**
	10	+71.50± 2.64			
	50	+98.72 ± 1.27			
	100	-3.18 ± 0.62			
Aqueous fraction	100	>100	>100	>100	>100

DISCUSSION AND CONCLUSION

The treatment of cancer involving the use of surgery or radiotherapy is valuable, especially at the early stages of the disease. However, at the advanced stage, chemotherapy which is a systematic drug-base system with low efficacy and numerous side-effects as well as having non-selective potentials on cells, has been suggested as a better form of treatment especially at the advanced stage of the disease (Baskar *et al.*, 2012). The need to develop new drugs with high efficacy and with high selectivity for cancer cells and low side-effects has become necessary (Denny and Wansbrough,1995). Also, the increasing interest in medicinal plants for treatment of cancer could be as a result of their multiple chemical composition which could help as a lead for modern drug discovery (Newman and Cragg, 2007). Medicinal plants have a long history of folkloric use in the treatment of various forms of diseases including cancer. They have served as a rich source of therapeutic agents to man (Sofowora *et al.*, 2013).

Our previous work on the leaf and root bark extracts of *S. virosa* showed a higher cytotoxic and growth- inhibitory effects of the leaf extract over the root bark extract towards young tadpole (*Raniceps raninus*) and germinatinig radicles of *Sorghum bicolor*, respectively (Ikpefan *et al.*, 2015a, 2015b). Our studies also showed the presence of various phytochemical groups such as flavonoids, alkaloids, tannins, glycosides, terpenes, etc in varying intensities. The higher activities exhibited by the leaf over the root bark extract may be due to the abundance of one or more of these phytochemicals , some of which act as anti-radicals in the body thereby preventing carcinogenesis. Comparing the activity of the leaf extract of *S.virosa* with that of the root bark, it was observed that the leaf extract exhibited higher growth-inhibitory and cytotoxic activities against MCF-7 and NCI-H460 cell lines. This difference in activities could be attributed to the time of collection of the different morphological parts of the plant, which might have affected the translocation of the secondary metabolites. Partitioning of the leaf extract was occasioned by the higher activities it portrayed over the root bark extract and this was observed to increase the activity when compared with the crude extract. For example, the chloroform fraction at 100 µg/mL produced -32.71 and -3.18 % cytotoxicities as well as GI_{50} / TGIs of 28.11/34.22 µg/mL and 42/ 32.69 µg/mL against the breast and lung cancer cell lines, respectively. The higher sensitivities of the chloroform fraction over the crude extracts could be as result of diffusing power of chloroform fraction into the cells, hence interfering more with some biomolecular process in cell division such as stimulation of apoptotic process and transmembrane depolarization of mitochondria.

Previous studies by Sulistyani and Nurkhasanah (2006) as well as Osman *et al.* (2016) have shown significant activities of the chloroform fraction of leaves of *Elephantopus scaber* and *Citrus limon* against breast cancer (T47D) and human gastric cancer cell line (AGS) which is in line with our findings.

This study has shown the antiproliferative potentials of the crude leaf extract of *S. virosa* with its chloroform fraction among other fractions exhibiting the highest activities. The results of the present study have further validated the folkloric history of the plant in the treatment of cancer.

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