



## Comparative cytotoxic and spectrophotometric quantification of phytochemicals of the methanol extracts of the leaf and root bark of *Securinega virosa* (Roxb ex. Willd) Baill (Euphorbiaceae)

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Received 5<sup>th</sup> July 2015; Accepted 31<sup>st</sup> July 2015

### Abstract

The comparative cytotoxic and spectrophotometric quantification of phytochemicals of the methanol extracts of the leaf and root bark of *Securinega virosa* was carried out. Phytochemical screening and spectrophotometric quantification of total flavonoids and phenolics of the extracts were carried out using standard reported methods. Phytochemicals detected in the extracts include alkaloids, cardiac glycosides, flavonoids, steroids, tannins, terpenoids and saponins. The total flavonoids and phenolic contents were observed to be more on the leaves than the root bark. The cytotoxic effects of the methanol extracts were evaluated between 10-400 µg/ml over a period of 24 hr using the tadpole mortality assay. At 100 µg/ml, the methanol extract of the leaf produced 73.30 ± 3.33% mortality which increases to 100 % at 200 and 400 µg/ml while the root barks produced 26.78 ± 4.45 and 56.70 ± 6.67 % mortalities respectively at the same concentrations. The significant cytotoxic effects observed coupled with the presence of phytochemicals such as phenolics supports the ethnomedicinal claim of this plant in treating cancer in Ogun State.

**Keywords:** Cytotoxicity; Phytochemicals, *Securinega virosa*; Phenolics; *Raniceps raninus*; Spectrophotometric

### INTRODUCTION

Cancer is one of the leading causes of death worldwide (Murray *et al.*, 2000). Nationwide data for cancer patients in Nigeria is not accessible; however, the data from the Nigerian Cancer Registry indicate that in general, cancer has been on the rise (Curado *et al.*, 2009). According to the World Health Organization (WHO), the use of herbal remedies throughout the world exceeds that of the conventional drugs by two to three times

(Evans, 1994). In spite of technological advancement in modern medicine, many people in the developing countries still rely on traditional healing practices and medicinal plants for their daily healthcare needs (Ojewole, 2004).

A number of medicinal plants are presently use in the management of cancer in parts of Nigeria. One of such plant is *Securinega virosa* the greatest usually described as a true “cure all”, of which all

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parts are used as remedies (Neuwinger, 1996). It is a dense, low branching, many branched shrub, sometimes a small spreading tree up to about 6 meters high of the family Euphorbiaceae. It is widely distributed throughout tropical Africa, also in India, Malaya, China and Australia (Dalziel, 1936). It is commonly referred to in local Nigerian languages as “tsuwaawun karee, gussu, gwiiwar karee” (Hausa), “iranje” (Yoruba), and “njisinta” (Ibo). The decoction of the leaf of *Securinega virosa* with some other plants is used in northern Nigeria for the treatment of mental illness (Neuwinger, 1996). The leaf and barks have been reported to be use in the treatment of cancer in Ogun State of Nigeria (Soladoye et al., 2010).

The present study was therefore carried out to ascertain the probable cytotoxic activity of the methanol extracts of the leaf and root bark of *S. virosa* using tadpoles of *Raniceps raninus* and quantify the total phenols and flavonoid contents using standard methods. Cytotoxicity screening models are the preliminary methods for selection of active plant extracts against cancer (Cardellina et al., 1999).

## EXPERIMENTAL

**Collection and preparation of plant materials.** The two morphological parts (leaves, and root barks) of *Securinega virosa* were collected in November 2013 at a play ground behind the University of Benin Teaching Hospital, Benin City. The identity of the plant was authenticated at Forest Research Institute of Nigeria (F.R.I.N.) Ibadan where a herbarium specimen FHI 108339 was deposited. The parts of the plant were air dried for 3 days. The plant materials were further dried in the oven maintained at 40°C (for the leaves) and 60°C (for the root barks) to remove moisture. After this treatment, each material was ground to powder form using a laboratory electric milling machine (Chris Norris, England).

Each powdered material was separately kept in an airtight container for subsequent use.

**Extraction.** About 300g each of the plant materials (leaf and root barks of *Securinega virosa*) were exhaustively extracted by Soxhlet apparatus with absolute methanol at 70°C. The total methanol extract was reduced under vacuum at 40°C using rotary evaporator (Eyela, Tokyo Rikikai Co. Ltd, Japan).

**Phytochemical screening.** Phytochemical screening was carried out using standard methods Khandelwal (2006), Evans (2002) and Sofowora (1993).

**Determination of cytotoxic effects of the aqueous and chloroform fractions using tadpoles (*Raniceps ranninus*).** Newly hatched tadpoles were scooped from ponds at Olomo Beach in Uhonmora village in Owan West Local Government Area of Edo State and were properly identified in the Department of Animal and Environmental Biology, Faculty of Life Science, University of Benin. Ten tadpoles of similar sizes (five days old) were selected with the aid of a broken Pasteur pipette into different beakers containing 30 ml of the natural water from the habitat of tadpoles. This was made up to 49 ml with distilled water. The mixture was made up to 50 ml with 20, 40, 100, 200 and 400 µg/ml of the chloroform fraction in 5% DMSO (Obuotor and Onajobi 2000). The experiment was repeated for the aqueous fraction and it was carried out in triplicates for all concentrations and controls. Similar procedure was repeated for subsequent chromatographic fractions.

**Spectrophotometric quantification of phytochemicals of the methanol extracts of leaf and root barks of *Securinega virosa*.**

*Quantification of flavonoids.* The total flavonoid content was determined according to the aluminum chloride colorimetric method (Lin and Tang, 2007). 0.5 mL (100mg) plant extracts was mixed with 0.5 mL of methanol,

0.1 mL of 10% aluminum chloride hexahydrate ( $\text{AlCl}_3$ ), 0.1 mL of 1 M potassium acetate ( $\text{CH}_3\text{COOK}$ ) and 2.8 mL of deionized water reagents. After incubation at room temperature for 40 min, the absorbance of the reaction mixture was measured at 415 nm on a UV-visible spectrophotometer (model UV-1601; Shimadzu®, Kyoto, Japan) and compared with the absorbance of deionized water as the control. Flavonoid contents were calculated on the basis of the calibration curve of Quercetin standard (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one with 98% purity; Reg. 317313 Sigma, St. Louis, Missouri, USA). Data was expressed as milligrams Quercetin equivalents (QE)  $100\text{mg}^{-1}$  plant extract.

**Quantification of total phenolics.** In order to measure the total phenolic content, Folin-Ciocalteu method described by Singleton and Rossi (1965) was used. 1 mL (100mg) of the extract fraction was mixed with 2.0 mL of 7.5% sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) and 2ml of Folin-Ciocalteu reagents. After incubation using water bath at  $40^\circ\text{C}$  for 45 min, the absorbance of the reaction mixture was measured at 765 nm on a UV-visible spectrophotometer (model UV-1601; Shimadzu®, Kyoto, Japan). Tannic acid was used as standard. The calculation of total

phenol content was based on the calibration curve of the tannic acid standard and the data was expressed as microgram tannic acid equivalents (TAE)  $100\text{mg}^{-1}$  plant extract

## RESULTS

**Phytochemical screening.** The results of the phytochemical screening (Table 1) shows the presence of phytochemicals such as alkaloids, cardiac glycosides, flavonoids, steroids, tannins, terpenoids, saponins. Anthraquinones were however absent.

**Effects of the extracts on tadpole mortality.** The methanol extracts of the leaves and root bark elicited a concentration dependent effects on the tadpole. However, the methanol extract of the leaves produced the highest activities as it imparted 100% mortality on the tadpoles at a concentration of 200 and  $400\mu\text{g/ml}$  compared to  $26.78 \pm 4.57$  and  $56.70 \pm 6.7\%$  mortality produced by the root barks respectively (Figure 1).

**Spectrophotometric analyses of total flavonoids and phenolics:** Quantitative analysis of total flavonoids and phenolics in the methanol extracts of the leaves and root bark of *S. virosa* revealed higher concentrations on the leaves than the root bark (Figure 2).

**Table 1:** Phytochemical screening of the methanol extract of the leaf and root bark of *Securinega virosa*

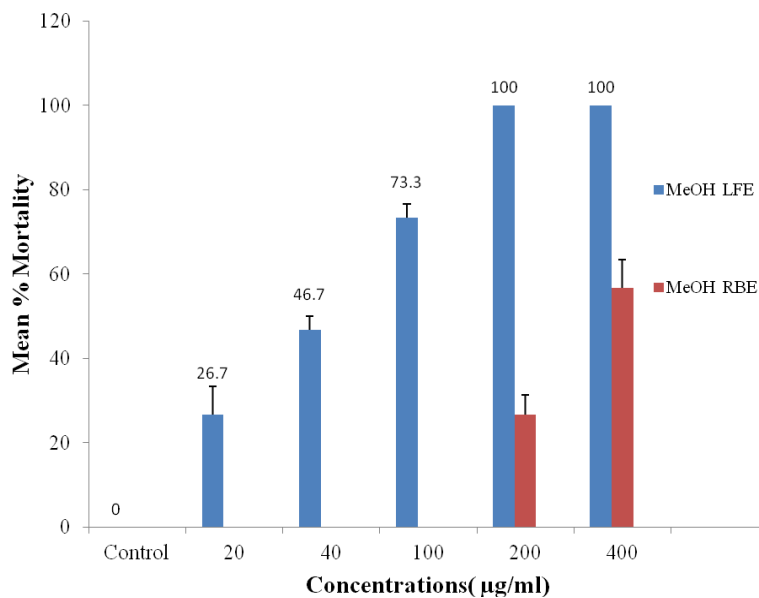
Phytochemical group	Leaf	Root bark
Alkaloids	+++	+
Anthraquinones	-	-
Cardiac glycosides	+++	+
Flavonoids	+++	+
Saponins	+++	++
Steroids	-	-
Tannins	+++	++
Terpenes	+	++

+++ : appreciable amount; ++ : moderate amount; + : minute amounts; - : not detected

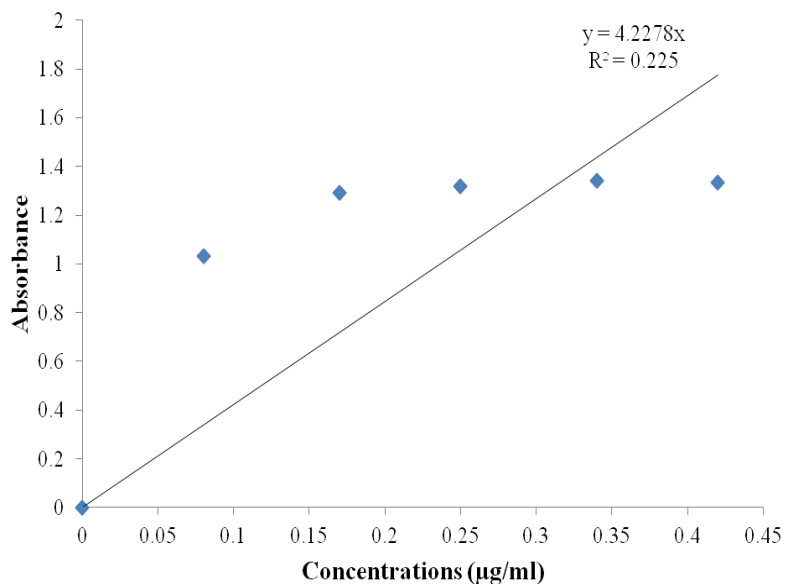
**Table 2:** Quantitative analysis of flavonoids and total phenolics of the leaves and root bark of *Securinega virosa*

S/N	Methanol Extracts	Flavonoid (mg/100 $\mu\text{g}$ )	Phenolics (mg/100mg)
A	Leaves	$0.32 \pm 0.02$	$0.36 \pm 0.01$
B	Root bark	$0.26 \pm 0.01$	$0.33 \pm 0.00$

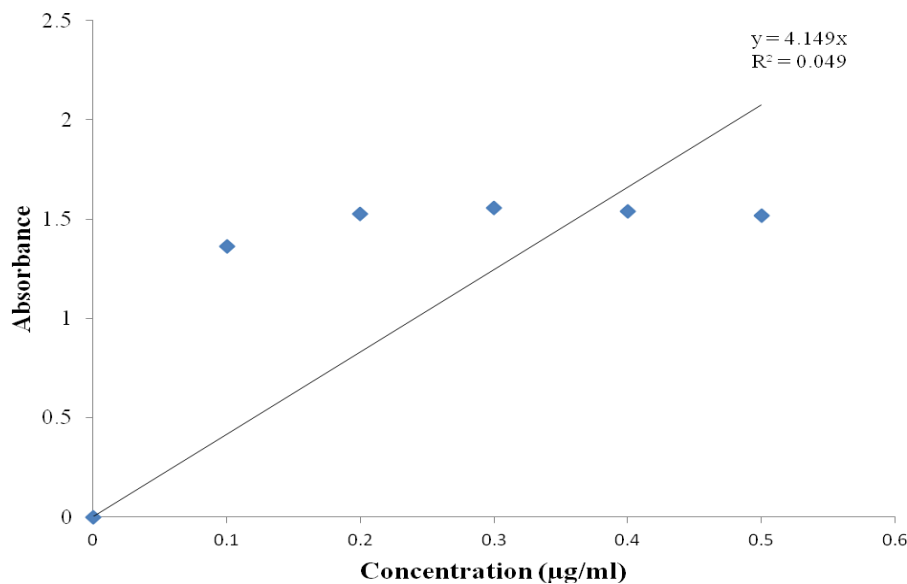
All values are means of triplicate determinations  $\pm$  standard deviation (SD)



**Fig. 1:** Comparative cytotoxic effects of the methanol extract of the leaf and root bark of *Securinega virosa* on tadpoles. Values are Mean  $\pm$  S.E.M, n = 10.\*Significant between Meth leaf and control. P<0.05 MeOH LF=Methanol leaf extract; MeOH RB = Methanol root bark extract



**Fig. 2:** Graph of absorbance (415nm) against concentration of flavonoids



**Fig. 3:** Graph of absorbance (415nm) against concentration of phenolics

## DISCUSSION

Preliminary phytochemical screening of methanol extracts of the leaf and root bark of *S. virosa* revealed the presence of alkaloids, cardiac glycosides, flavonoids, steroids, tannins, terpenoids and saponins. These phytochemicals were found to be present more on the leaves and fairly distributed in the root barks. This could be due to the fact that as at the time of collection, the secondary metabolites were more concentrated on the leaves awaiting translocation to other parts of the plant.

Flavonoids and other phenolics are known for their important medicinal properties, especially in the prevention of cancer and heart diseases (Kähkönen *et al.*, 1999). These compounds have been identified as natural antioxidants that may reduce oxidative damage to the human body (Namiki, 1990). They are very effective in preventing the destructive processes caused by oxidative stress (Zengin, *et al.*, 2011). Our study shows that the total phenol contents of the leaves and root bark of *S. virosa* expressed as microgram tannic acid equivalents of dry sample (standard plot:  $y = 4.1491x$ ,  $R^2=0.0491$ ) were found between 0.16 to 0.36

µg tannic acid equivalent /g. A critical look at the values shows that the methanol extract of the leaves contains more phenolic compounds than the root bark. Similarly, using the standard plot of Quercetin equivalents ( $y = 4.2278x$   $R^2 = 0.225$ ), the leaves were also found to contain more flavonoids (0.32 µg) than the root barks (0.26 µg).

The cytotoxic effects of the extracts were observed to be time and concentration dependent. The methanol extract of the leaf was observed to be more potent than that of the root and the stem bark. The mortality of the tadpoles was indicated by their complete motionless and submergence in water. The methanol extract of the leaf was observed to produce  $73.30 \pm 3.33$  % mortality at a concentration of 100µg/ml. This eventually increased to 100 % at a concentration of 200 and 400µg/ml achieved within 16 and 7 minutes of treatment respectively. However, at this maximum concentration (400 µg/ml), the methanol extract of the root bark produced  $56.67 \pm 6.67$  % mortality.

The cytotoxic study of the two morphological parts of the plant revealed the leaves to be most active. The result also shows that the leaves contain more phenolics

than the root bark. Hence, it can be inferred that *S. virosa* particularly the leaves may probably have effects on tumor-producing cells as stated in ethnomedicine. However, further research work need to be carried out to confirm this.

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