



A publication of College of Natural and Applied Sciences, Fountain University, Osogbo, Nigeria.

Journal homepage: [www.fountainjournals.com](http://www.fountainjournals.com)

ISSN: 2354-337X (Online), 2350-1863 (Print)

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## Gas Chromatography-Mass Spectrometry and Cytotoxicity of *Securidaca longepedunculata* (polygalaceae) Root Bark Extract

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### Abstract

*Securidaca longepedunculata* is a savannah shrub found growing in tropical Africa. It is reputed to have more than a hundred medicinal uses and is a major component of anticancer decoctions in Nigeria. Despite its reported use as an anti-cancer plant, there is a dearth of information on the anti-cancer potential and mechanism of its anticancer activity. The present study is to identify the chemical constituents present in *Securidaca longepedunculata* by quantitative Gas Chromatography-Mass Spectrometry (GC-MS) analysis and determine the cytotoxic effect of the plant on Ehrlich ascites carcinoma cells. Secondary metabolites in the extract were predicted using GC-MS. *In vitro* cytotoxic activity was determined using the trypan blue dye exclusion assay by incubating Ehrlich ascites carcinoma cells with various concentrations of *S. longepedunculata* aqueous extract. Analysis by GC-MS revealed the presence of the following compounds: bis (2-ethylhexyl) phthalate (90.99%), 1-decanol (4.17%) and cyclododecane (1.86%), phenol, 2, 2<sup>1</sup>-methylene bis (1.32%), cyclopenten-4-one, 3-hydroxy-1, 2, 3, 5, 5<sup>1</sup>-pentakis (trimethylsilyloxy)- (0.89%) and phenol, 2, 4-bis (1, 1-dimethylethyl)(0.78%). *Securidaca longepedunculata* aqueous extract (SL) was cytotoxic to Ehrlich ascites carcinoma cells *in vitro*. The IC<sub>50</sub> of SL on EAC cells was 67 µg/ml. The aqueous root-bark extract of *S. longepedunculata* contains bioactive agents and was cytotoxic to Ehrlich ascites carcinoma cells *in vitro*.

**Keywords:** phthalate, Ehrlich, carcinoma, medicinal, plants

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### Introduction

The increase in the use of medicinal plants in the treatment and diagnosis of ailments in Africa and other parts of the world has been documented (Shoeb, 2006; Saluja *et al.*, 2011). *Securidaca longepedunculata* (Polygalaceae) is a medicinal plant found growing in some countries including Nigeria. It is used in the treatment of variety of ailments in Nigeria hence the name *Uwar Magunguna* (Mother of all drugs) by the Hausas in Northern Nigeria (Dapar *et al.*, 2007).

It has been reported as an essential part of anticancer decoctions in South-West Nigeria (Soladoye *et al.*, 2010). The constituents of *S. longepedunculata* were investigated and four novel highly oxygenated xanthenes, named muchimangins A-D were reported (Dibwe *et al.*, 2012b). The plant also displayed potent preferential cytotoxicity when tested on a human pancreatic cancer PANC-1 cell line in a nutrient-

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deprived medium (Dibwe *et al.*, 2012a). Several cancer cell lines are employed in testing for cytotoxic compounds amongst which is Ehrlich ascites carcinoma (EAC) cells - spontaneous murine mammary adenocarcinoma adapted to ascites form and carried in outbred mice (Ozaslan *et al.*, 2011). There is currently a dearth of information on the *in vitro* potential of *Securidaca longepedunculata* on mammary cancer cell lines. Hence, the objective of this research is to investigate the cytotoxic activity of aqueous root-bark extract of *Securidaca longepedunculata* using EAC cells from mice peritoneal cavity and determine the bioactive components in the extract.

## Methodology

### Plant collection

*Securidaca longepedunculata* root barks were collected from Osogbo, South-West, Nigeria in January, 2010. Plant material was identified and authenticated by Mr Odewo in the Department of Botany, University of Lagos in January, 2010. A voucher specimen was deposited in the University Herbarium, University of Lagos, Lagos, Nigeria with voucher number: LUH 3593.

### Preparation of aqueous extract of *Securidaca longepedunculata*

The plant materials were shade dried for 3 days and pulverized into powder. Aqueous extract of the coarsely powdered material was prepared by macerating 1kg of root bark in 1 L of distilled water for 72 hours. The macerate was filtered and the filtrate was concentrated using the Rotary Evaporator (Rotavapor® R-300, BUCHI, Switzerland) and further concentrated to constant weight *in vacuo* using a lyophilizer (Lyotrap, LTE, England).

### Gas chromatography-mass spectrometry (GC-MS)

The modified method of Botes *et al.* (2008) was used for the characterization of the secondary metabolites of the aqueous root-bark extract of *Securidaca longepedunculata*. The analysis was carried out with Agilent 6890 GC coupled to a 5973 MS detector (California, U.S.A). For the acquisition of an electron ionization mass spectrum, an ion source temperature of 200 °C and electron energy of 70 eV was used. Helium (1

mL/min) was used as the carrier gas. The oven temperature programme for analyzing the aqueous extract utilized an initial oven temperature of 40°C, maintained for 2 min, followed by a steady climb to 350°C at a rate of 5°C/min.

### Short term Cytotoxicity study

*In vitro* cytotoxic activity was carried out using the Trypan Blue dye exclusion method. Briefly, aqueous extract of *Securidaca longepedunculata* in Phosphate buffered saline (1000, 100, 10, 1 and 0.1 µg/ml) were incubated with EAC cells at 37°C for 25 h after which cells were stained with trypan blue dye (Sigma-Aldrich, St. Louis, USA). Ascitic tumour cell counts were done in a Cedex Cell Counting machine (Roche, California) in which viable cells were unstained and damaged cells were stained blue. Results were expressed as percentage cell viability (Saluja *et al.*, 2011). % Mortality = 100 - % cell viability.

### Statistical Analyses

This was carried out using SPSS v 17.0 statistical package. Results are expressed as Mean ± standard error of the mean. Student's t-test was used to test for the differences in mean values between the groups and control. The IC<sub>50</sub> was calculated using the Finney Probit Analysis method (Finney, 1947).

### Results

The results of GC-MS for *Securidaca longepedunculata* are shown in Table 1. More than 6 compounds were detected in six peaks for the aqueous extracts of *S. longepedunculata*. The results revealed that bis (2-ethylhexyl) phthalate (90.98%) was found as the major compound while 1-Decanol (4.17%) and Cyclododecane (1.86%) was the other compound with relatively high composition. Other compounds detected were phenol, 2, 2<sup>1</sup>-methylene bis (1.32%), cyclopenten-4-one, 3-hydroxy-1, 2, 3, 5, 5<sup>1</sup>-pentakis (trimethylsilyloxy) - (0.89%) phenol, 2, 4-bis (1, 1-dimethylethyl) (0.78%). *Securidaca longepedunculata* at concentrations of 0.1, 1, 10, 100, 1000 µg/ml caused mortalities of 7.3, 10.6, 27.9, 45.1 and 82.5 % respectively in Ehrlich ascites carcinoma cells. The death rate of Ehrlich

Table 1: GC-MS Peaks and components of aqueous extract of *S. longepedunculata*

Peak	RT (mins)	Library/ID	Mol. Formula	MW	Area (%)	Compound Nature
1	6.3281	Cyclopenten-4-one,3-hydroxy-1,2,3,5,5-pentakis (trimethylsilyloxy)-	C <sub>20</sub> H <sub>46</sub> O <sub>7</sub> Si <sub>5</sub>	538	0.89	Ketone compound
2	11.8961	1-Decanol	C <sub>10</sub> H <sub>22</sub> O	158	4.17	Alcohol
3	12.1688	Phenol, 2, 4-bis (1,1-dimethylethyl)-	C <sub>14</sub> H <sub>22</sub> O	206	0.78	Phenolic compound
4	13.4418	Cyclododecane	C <sub>12</sub> H <sub>24</sub>	168	1.86	Hydrocarbon Phenolic
5	26.1234	Phenol, 2,2'-methylenebis	C <sub>13</sub> H <sub>12</sub> O <sub>2</sub>	200	1.32	compound
6	27.1824	bis (2-ethylhexyl) ester	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	390	90.98	Ester Compound

R.T. - Retention time, M.W. - Molecular weight

ascites tumour cells *in-vitro* increases with increasing concentration of *S. longepedunculata* root bark extract (Table 2). The IC<sub>50</sub> (i.e. the lethal concentration that kills 50% of cells) was calculated to be 67 µg/ml.

Table 2: *In vitro* cytotoxic effect of aqueous extracts of *Securidaca longepedunculata* on Ehrlich Ascites Carcinoma cells.

Concentration (µg/ml)	Mortality (%)
1000	82.5 ± 5.02 <sup>d</sup>
100	45.1 ± 1.56 <sup>c</sup>
10	27.9 ± 0.29 <sup>b</sup>
1	10.6 ± 0.40 <sup>a</sup>
0.1	7.3 ± 0.35 <sup>a</sup>

\*Results are expressed as Mean of 3 determinations ± Standard error of the mean

## Discussion

Gas, Chromatography-Mass Spectrometry is an analytical technique which combines the features of gas-chromatography and mass spectrometry to identify different substances with a potential to quantify within a test sample

(Al-Doush *et al.*, 2012). In this study, Gas Chromatography-Mass Spectrometry analysis of *S. longepedunculata* root bark extract led to the identification of 6 compounds; bis (2-ethylhexyl) phthalate, 1-decanol, cyclododecane, phenol, 2, 2'-methylene bis, cyclopenten-4-one, 3-hydroxy-1, 2, 3, 5, 5'-pentakis (trimethylsilyloxy) and phenol, 2, 4-bis (1, 1-dimethylethyl). According to Lahousse *et al.* (2006), phthalates are chemicals majorly used as softening agents in plastic-containing products. The phthalates are not covalently bound to the matrix. They therefore leach out of the plastic materials after some time and result in environmental contamination. Phthalate exposure inhibited follicle stimulating hormone (FSH)-stimulated cAMP production and suppressed basal and FSH-stimulated Sertoli cell proliferation (Heindel and Chapin, 1989; Li *et al.*, 1998). In rodents, high-dose phthalate exposure targets the fetal and pubertal testis, leading to alterations in endocrine and spermatogenic functions (Boekelheide *et al.*, 2004). Habib and Karim (2009) confirmed the cytotoxic activity of bis (2-ethyl hexyl) phthalate. Joo *et al.* (2010) reported that white rose flower extract,

containing a high amount of cyclododecane, strongly scavenged free radicals in a dose-dependent manner and also inhibited the growth of *E. coli*. In a study by Wang *et al.* (2012), Eryngiolide A (I), - C20 diterpenoid derived from a cyclododecane core isolated from edible mushroom *Pleurotus eryngii* was found to exhibit moderate cytotoxicity against two human cancer lines, HeLa and HepG2, *in vitro* using the MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyl tetrazolium bromide) method. Phenol, 2, 4-bis (1,1-dimethylethyl)- was recognized as the compound responsible for the high antibacterial activity of Mango kernel against gram positive bacteria (Abdullah *et al.*, 2011). A study by Prakash and Suneetha (2014) showed that *Pinus granatum* rind extract containing phenol, 2, 4-bis (1,1-dimethylethyl)- exhibited a better antioxidant activity than L - ascorbic acid. The antimicrobial activity of phenol, 2, 4-bis (1,1-dimethylethyl)- appeared to correlate with the antioxidative activity associated with this compound, because it was able to inhibit the ROS production in both *Aspergillus* and *Phytophthora cinnamomi* hence regulating hydrogen peroxide production (Romero-Correa *et al.*, 2014). Among the identified phytochemicals, 2, 2<sup>1</sup>-methylenebis(6-(1,1-dimethylethyl)-4-ethyl-phenol (an analogue of phenol 2, 2<sup>1</sup> methylenebis) belongs to a class of compounds with powerful antimicrobial and antiseptic properties which further confirms the cytotoxic potential of the extract (Celis *et al.*, 2011). Marko *et al.* (2002) reported that 3-hydroxy-4-[(E)-(2-furyl) methylidene] methyl-3-cyclopentene-1,2-dione (an intensively coloured Maillard reaction product) also inhibited tumor cell growth and microtubule assembly.

In the US National Cancer Institute plant screening programme, a crude extract is generally considered to have *in vitro* cytotoxic activity if the IC<sub>50</sub> value (Inhibitory Concentration that causes a 50% cell kill) in carcinoma cells, following incubation between 48 and 72 hours, is less than 20 µg/ml, while it is less than 4 µg/ml for pure compounds (Boik, 2001). The trypan blue exclusion assay is a direct, simple and inexpensive method of determining the relative amount of damaged cells. The trypan blue exclusion assay used in the *in vitro* cytotoxic study relies on the exclusion of intact and viable cells by the negatively charged trypan

blue dye which is taken up by damaged and dead cells (Choudhury *et al.*, 2010). A relatively high number of cells remained unstained in the tube that had no extract while the number of stained cells was high in the tubes to which extracts were added. This indicates a direct cytotoxic effect of the extract on Ehrlich ascites carcinoma cells. This effect was observed to increase with concentration of *Securidaca longependunculata* root bark extract suggesting that the quantity of the cytotoxic agent increased with concentration of *Securidaca longependunculata* root bark extract. The low IC<sub>50</sub> of 67 µg/ml recorded in this study suggests that the aqueous root bark extract of *S. longependunculata*, which has been shown to possess agents, such as Phenol, 2,2'-methylenebis and phenol, 2, 4-bis (1,1-dimethylethyl), implicated in cell-lysing possesses significant *in vitro* cytotoxic activity.

### Conclusion

Aqueous root-bark extract of *Securidaca longependunculata* contained cytotoxic bioactive agents which are thought to be responsible for its cytotoxic activity against Ehrlich ascites carcinoma cells *in vitro*.

### Acknowledgements

The authors will like to thank the authorities of the University of Lagos, Lagos, Nigeria for permission to conduct the research in the Molecular Biology Laboratory, Department of Biology, University of Gaziantep, Gaziantep, Turkey and Dr. F.K Lawal for co-sponsoring the trip.

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