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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, 2^1 -methylene bis (1.32%), cyclopenten-4-one, 3-hydroxy-1, 2, 3, 5, 5^1 -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_{50} of SL on EAC cells was 67 μ g/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

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 xanthones, 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-

Corresponding author: +2348056036852 Email address: alawal@unilag.edu.ng 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 $40^{\circ}C$, maintained for 2 min, followed by a steady climb to $350^{\circ}C$ at a rate of $5^{\circ}C$ /min.

Short term Cytotoxicity study

In vitro cytotoxic activity was carried out using the Trypan Blue dye exclusion method. extract aqueous of Securidaca longepedunculata in Phosphate buffered saline (1000, 100, 10, 1 and 0.1 μ g/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 \pm 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 IC₅₀ 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, 21-methylene bis (1.32%), cyclopenten-4-one, 3-hydroxy-1, 2, 3, 5, 5^1 -pentakis (trimethylsilyloxy) - (0.89%) phenol, 2, 4-bis (1, 1dimethylethyl) (0.78%). Securidaca longepedunculata at concentrations of 0.1, 1, 10, 100, 1000 μ g/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

			Mol. Formula	l		Compound
Peak	RT (mins)	Library/ID		MW	Area (%)	Nature
1	6.3281		C ₂₀ H ₄₆ O ₇ Si ₅	538	0.89	Ketone
		Cyclopenten-4-one,3- hydroxy-1,2,3,5,5-pentakis (trimethylsilyloxy)-				compound
2	11.8961	1-Decanol	C ₁₀ H ₂₂ O	158	4.17	Alcohol
3	12.1688	Phenol, 2, 4-bis (1,1-dimethylethyl)-	C ₁₄ H ₂₂ O	206	0.78	Phenolic compound
4	13.4418	Cyclododecane	C ₁₂ H ₂₄	168	1.86	Hydrocarbon Phenolic
5	26.1234	Phenol, 2,2'-methylenebis	C ₁₃ H ₁₂ O ₂	200	1.32	compound
6	27.1824	bis (2-ethylhexyl) ester	C ₂₄ H ₃₈ O ₄	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_{50} (i.e. the lethal concentration that kills 50% of cells) was calculated to be $67\mu g/ml$.

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 ^d		
100	45.1 ± 1.56°		
10	27.9± 0.29 ^b		
1	10.6± 0.40°		
0.1	7.3 ± 0.35^{a}		

^{*}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, 21methylene bis, cyclopenten-4-one, 3-hydroxy-1, 2, 3, 5, 51-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 (1,1dimethylethyl)- 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 containing phenol, 2, 4-bis extract 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, 21-methylenebis(6-(1,1-dimethylethyl)-4-ethyl-phenol (an analogue of phenol 2, 21 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 3hydroxy-4-[(E)-(2-furyl) methylidene] methyl-3cyclopentene-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 IC50 value (Inhibitory Concentration that causes a 50% cell kill) in carcinoma cells, following incubation between 48 and 72 hours, is less than 20 μ g/ml, while it is less than 4 μ g/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 longependuculata root bark extract suggesting that the quantity of the cytotoxic agent increased with concentration of Securidaca longependuculata root bark extract. The low IC₅₀ of $67\mu g/ml$ recorded in this study suggests that the aqueous root bark extract of S. longepedunculata, 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 longepedunculata contained cytotoxic bioactive agents which are thought to be responsible for its cytotoxic activity against Ehrlich ascites carcinoma cells in vitro.

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