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Evaluation of the cytotoxic potential of *Securidaca longepedunculata* on human breast adenocarcinoma (MCF-7) cells

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ABSTRACT: The prevalence of cancer has increased interest in the role of complementary and alternative medicine, employing the use of plant products. *Securidaca longepedunculata* (SL) is an example of plant product which serves as a major component of anticancer decoctions in Nigeria. In other to scientifically ascertain this claim, this study was carried out to evaluates the cytotoxic potential of the crude extract and fractions of SL root bark against human breast adenocarcinoma (MCF-7) cell line. The root back of SL was pulverized and extracted with 80% methanol to yield a crude extract which was then submitted to liquid–liquid fractionation with dichloromethane (DCM) and butanol (BUT). The extract and fractions were subjected to Gas Chromatography-Mass Spectrometry (GC-MS). MCF-7 cell line was treated with graded concentrations (9.77 – 5000 µg ml^{-1}) of the aqueous methanol crude extract as well as the DCM and BUT fractions for 24 or 48 hours. Cell viability was thereafter measured by XTT proliferation assay. The inhibitory activities of the aqueous methanolic crude extract was found to be both dose-dependent and time independent with IC_{50} values of 2241 µg ml^{-1} and 1808 µg ml^{-1} after 24 and 48 hours respectively. The DCM and BUT fractions were significantly ($p < 0.05$) cytotoxic with IC_{50} values of 86.27 µg ml^{-1} and 12.08 µg ml^{-1} respectively after 48 hours of treatment. The study provide evidence that BUT fractions of SL has the highest potential to significantly show anticancer properties.

Keywords: *Securidaca longepedunculata*, GC-MS, MCF-7, Cytotoxicity

Introduction

Scientists all over the world are now turning to phytochemicals from plants for new drugs as a result of their potency and less side effects compared to synthetic chemical compounds. Plant-based natural constituents can be derived from any part of the plant like barks, leaves, flowers, roots, fruits, seeds, etc (Gordon, 2001). Nigeria as one of the countries found in the tropics is rich with medicinal plants that have been found to possess different potential biological activities; and there is an increasing awareness in linking the phytochemical compounds and their biological activities (Robertson, 2005). An example of medicinal

plants that have been found to possess the potentials for these biological activities is *Securidaca longipedunculata.*

Securidaca longipedunculata is a small tree up to 6 m high with a pale grey, smooth bark and oblong, more or less hairless alternate leaves that are variable in size and shape and crowded towards the stem tips (Van Wyk *et al*., 2009). In Nigeria, *S. longepedunculata* is commonly used for the treatment of inflammatory conditions and as laxative. An oral administration of a decoction of the root has been shown to produce a sedative effect, mainly attributed to the presence of oleanolic acid glycoside (Sofowora, 1980). Extracts of the plant have been reported to possess both gastrointestinal, trypanocidal and antimicrobial effects (Olajide *et al.*, 1999; Atawodi *et al*., 2003; Ajali and Chukwurah, 2004). The root barks of *S. longepedunculata* have been reported to have a very high antioxidant and anti-inflammatory properties (Muanda *et al*., 2010). It is one of the plants used traditionally in treating cancer (Alawode, 2013).

Cancer is a multifactorial disease involving abnormal cell growth with the potential to invade or spread to other parts of the body (Klug *et al*., 2010). Cancer has become a major source of morbidity and mortality globally (Sylla and Wild, 2011). There are over 100 different types of cancer, and each is classified by the type of cell that is initially affected, including cervical cancer, skin cancer, leukemia, lung cancer, prostate cancer, breast cancer etc. (Ozbay and Nahta, 2011). Breast cancer, the most frequently diagnosed life-threatening cancer in women, refers to cancers originating from the breast tissue, most commonly from the inner lining of milk ducts of the lobules (Sharma *et al.*, 2010). It is the leading cause of cancer death among women, accounting for 25.1% of all cancers (Ghoncheh *et al*., 2016).

Breast cancer is currently being managed by a combination of synthetic drugs such as cepecitabine, carboplatin, raloxifene, lapatinib which are associated with a wide range of side effects such as fatigue, headaches, dental issues, heart problems, new cancers, bone loss and osteoporosis, cataracts etc. (Wolff, 2014). While Chemotherapy, hormone therapy and targeted therapies are current approaches in management of breast cancer, several drawbacks resulting from accumulation of these synthetic drugs in the body system have been reported (Piedrola *et al.*, 2001). Also, some medicinal plants being used in the management of breast cancer do not have scientific authentication yet. This present study was therefore aimed at investigating the cytotoxicity potential of *S. longipedunculata* root bark extract and fractions on the human breast adenocarcinoma (MCF-7) cells.

Materials and Methods

Plant material

Securidaca longepedunculata root barks were collected from Osogbo, South- West, Nigeria. The plant material was identified and authenticated by Mr. Odewo in the Department of Botany, University of Lagos. A voucher specimen (LUH 3593) was deposited in the University Herbarium, University of Lagos, Nigeria.

Preparation of aqueous methanolic extract

The root bark of *Securidaca longepedunculata* were shade-dried for 3 days and pulverized into powder. 300g of coarse powdered material was extracted by maceration in 80% methanol for 72 hours. The filtrate was concentrated using the Rotary Evaporator at reduced pressure and freeze dried to a dry mass. The crude yield was determined to be 26.33g.

Phytochemical screening

The extract were screened for the presence of phytochemicals like alkaloids, tannins, cardiac glycosides, terpenoids, flavonoids, and steroids as described by (Edeoga *et al*., 2005).

Solvent partitioning

The crude methanolic extract was subjected to liquid–liquid partitioning in dichloromethane and butanol to yield dichloromethane (DCM) and butanol (BUT) fractions respectively.

Gas chromatography–mass spectrometry (GC-MS)

Identification of the bioactive compounds present in methanolic crude extract, DCM and BUT fractions of *Securidaca longepedunculata* root bark was carried out using GC-MS technique using Agilent Technologies 7890A gas chromatograph coupled to an Agilent Technologies 5975 C Mass Spectrometer and Helium, employed as mobile phase and a 5% phenyl, 95% dimethylpolysiloxane non-polar column represented the stationary. The chemical components of the extracts were identified by comparing the retention time of the chromatographic peaks with that of authentic compound using the database of National Institute Standard and Technology having more than 62,000 patterns. The spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

Preparation of extract and fractions

One hundred milligrams of crude extracts and fractions were mixed with 100 µL of Dimethylsuflfoxide (DMSO) and quantity sufficient to 10mL with Phosphate buffered saline (PBS) to make a stock concentration of 10000 µg ml^{-1}. The stock extract was filter sterilized into sterile airtight bottles and stored at 2-8^{0}C until needed.

Cell culture and condition

Growing human breast adenocarcinoma (MCF-7) was propagated ad maintained in EMEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin and streptomycin, and incubated at 37^{o}C in a 5% CO_2 incubator. The growth medium was changed twice weekly. Cells grown to 75-85% confluence were washed with phosphate buffered saline (PBS), trypsinized with 0.25% trypsin/0.53mM EDTA, suspended with fresh medium and counted by hemocytometry.

Cell proliferation assay

MCF-7 cells were seeded in a 96-well plate at a density of 10^4 cells/well in 100µL of culture medium (EMEM) and incubated for 24 hours to allow the cells attach. A 100 µL aliquot of the aqueous methanolic extract or fractions of *S. longepedunculata* was diluted in fresh medium to constitute a working concentration of 5000 µgmL^{-1} and initiated into well plate in a 2-fold dilution. The cells were incubated in a 5% CO_2 incubator for 24 hours or 48 hours. After the incubation time points, 50 µL of XTT test solution was added to each well and further incubated for 3h. Absorbance was measured at test and reference wavelengths of 490 nm and 650nm respectively using ELISA plate reader. The percentage viability was determined using the equation:

$$\text{Cell viability (\%)} = \frac{\text{OD (test)} - \text{OD (blank)}}{\text{OD (negative control)} - \text{OD (blank)}} \times 100$$

Statistical analysis

Data are presented as mean ± S.E.M for at least three replicates. Statistical analysis was performed using two-way ANOVA with Sidak multiple comparison test with significant level set at $P<0.05$. The IC_{50} values were obtained by nonlinear regression analysis GraphPad Prism (Version 7.00). All p-values less than 0.05 were considered to be significant.

Results and Discussion

Table 1 shows the result obtained for the qualitative phytochemical screening of the methanolic extract of *S. longepedunculata* root bark. The phytochemical screening revealed that flavonoid, cardiac glycoside,

phenol, alkaloid, saponin and reducing sugar were present while tannin, phlobatanin, terpenoid and steroid were absent. The quantitative phytochemical screening of selected phytochemicals present in the methanolic extract of *S. longepedunculata* is shown in Table 2. Alkaloid was found to be present in the highest amount while saponin was found to be in the lowest amount. This suggests that the phytochemicals present in extract of *S. longepedunculata* root bark possess compound with antioxidant and anticancer properties. Antioxidant activity in higher plants has often been associated with phenolic compounds (Akinmoladun *et al*., 2010). Flavonoids are the largest group of phenolics (Liu, 2004). They have been identified in fruits, vegetables, and other plant parts and linked to reducing the risk of major degenerative diseases (Liu, 2004). The cytotoxicity of polyphenols on a range of cancer cells has been demonstrated and their antioxidant properties determined (Azmi *et al*., 2006; Siriwantanmetanon *et al.*, 2010; Heo *et al*., 2014). Polyphenols are thought to have apoptosis inducing properties showing anticancer properties. Polyphenols are thought to kick start apoptosis by controlling the movement of copper ions which are bound to chromatin causing DNA fragmentation (Azmi et al., 2006). However, data from this present work, as shown in Table 2, indicates that *S. longepedunculata* root bark extract is low in phenol but high in flavonoid.

Table 1: Qualitative phytochemical profile of methanolic extract of *S. longepedunculata* root bark

Flavonoid	Phenol	Alkaloid	saponin	reducing sugar	cardiac glycoside	Tannin	Steroid
+	+	+	+	+	+	-	-

KEY
(+) = Presence of phytochemical
(-) = Absence of phytochemical

Table 2: Quantitative phytochemical profile of methanolic extract of *S. longepedunculata* root bark

S/N	Phytochemical	Quantity (mg/100g)
1	Flavonoid	18.98± 0.255
2	Phenol	8.625± 0.163
3	Alkaloid	32.98± 0.057
4	Saponin	6.55± 0.042
5	Reducing sugar	19.415±0.318
6	Cardiac glycoside	16.255±0.347

Results are expressed in mean ± SEM

The GC-MS total ion chromatogram of the crude extract and fractions were resolved into several peaks indicating the corresponding compounds. The crude extract revealed the presence of 48 bioactive compounds shown in Table 3, while six and four phytoconstituents were identified in the BUT and DCM fractions respectively as shown in Tables 4 -5 respectively.

Table 3: Compounds Identified in the Methanolic Extract of *S. longepedunculata* in GC-MS

RETENTION TIME	COMPOUND NAME	MOLECULAR FORMULA	MOLECULAR WEIGHT (g/mol)	PEAK AREA %	% QUALITY
3.842	1-Butene, 3-(2-butenyloxy)	$C_8H_{14}O$	126	0.70	10
4.243	Isobutanol, TMS derivative	$C_4H_{10}O$	74	1.45	64
4.489	Furfural	$C_5H_4O_2$	96	1.33	80
4.729	2-Furanmethanol	$C_5H_6O_2$	98	0.90	90
5.336	Thiazole, 4,5-dihydro-2-(methylthio)-	$C_4H_7NS_2$	133	0.43	16
5.719	1-Pyridineacetic acid	$C_7H_9NO_2$	139	0.54	59
6.388	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	144	1.07	80
6.486	Benzene, 1-ethyl-3-methyl-	C_9H_{12}	120	0.55	90
7.310	Benzyl alcohol	C_7H_8O	108	0.50	55
8.340	Thymine	$C_5H_6N_2O_2$	126	1.34	50
9.021	2-Propanamine, N-methyl-N-nitroso-	C_3H_9N	59	0.40	38
9.169	4H-Pyran-4-one	$C_5H_40_2$	96	2.81	62
9.547	Heptanediamide, N,N'-di-benzoyloxy	$C_{21}H_{22}N_2O_6$	398	1.42	50
9.753	Dodecane	$C_{12}H_{26}$	170	0.94	60
9.850	Methyl salicylate	$C_8H_8O_3$	152	2.51	95
10.302	m-Guaiacol	$C_7H_80_2$	124	0.43	93
10.531	5-Hydroxymethylfurfural	$C_6H_6O_3$	126	10.28	83
10.760	Benzeneacetic acid	$C_8H_8O_2$	136	0.38	64
11.258	Tridecane	$C_{12}H_{28}$	184	0.66	95
11.395	1H-Indene, 1-ethylidene-	C_9H_8	116	0.45	95
11.532	Salicylic acid	$C_7H_6O_3$	138	0.84	93
11.630	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	1.13	90
12.368	Hexadecane, 7,9-dimethyl-	$C_{18}H_{38}$	254	0.92	70
12.494	Decahydro-1,1,4a,5,6-pentamethylnaphthalene	$C_{15}H_{28}$	208	0.47	98
12.688	Tetradecane	$C_{14}H_{30}$	198	0.61	94
12.917	Vanillin	$C_8H_8O_3$	152	0.61	97
13.243	Naphthalene, 2,6-dimethyl-	$C_{12}H_{12}$	156	0.87	95
13.484	Methyl 6-hydroxy-o-anisate	$C_9H_{10}O_3$	166	3.44	96
13.541	Phenol, 2-methoxy-4-(1-propenyl)-	$C_{17}H_{19}NO_4$	301	1.67	97
14.039	Pentadecane	$C_{15}H_{32}$	212	2.18	95
14.948	Dodecanoic acid	$C_{12}H_{24}O_2$	200	0.98	92
15.120	2,4,7(1H,3H,8H)-Pteridinetrione	$C_6H_4N_40_3$	194	4.78	38
15.389	Hexadecane	$C_{16}H_{34}$	226	0.57	95

RETENTION TIME	COMPOUND NAME	MOLECULAR FORMULA	MOLECULAR WEIGHT (g/mol)	PEAK AREA %	% QUALITY
19.812	Benzoic acid, 2-hydroxy-, phenylmethyl ester	$C_{14}H_{12}O_3$	228	1.09	95
19.955	Nonadecane	$C_{19}H_{40}$	269	0.57	93
20.447	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	6.61	97
20.625	3,4-Dimethoxycinnamic acid	$C_{11}H_{12}O_4$	208	1.20	64
22.484	Oleic Acid	$C_{18}H_{24}0_2$	282	0.49	86
22.810	Cyclic octaatomic sulfur	S_8	256	0.76	87
23.205	Methanone, [1,1'-biphenyl]-4-ylphenyl-	$C_{19}H_{14}O$	258	10.36	52
23.343	8,11-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	0.69	94
23.451	trans-13-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	1.66	99
23.760	Phenylmethanesulfonylacetic acid	$C_9H_{10}O_4S$	212	6.03	47
24.189	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	0.50	99
24.693	Octadecanoic acid	$C_{18}H_{36}O_2$	284	1.53	96
24.991	Benzene, (2,2,2-triethoxyethyl)	$C_{14}H_{22}$	238	0.61	43
27.382	Benzophenone, 2,4,4'-trihydroxy-2'-methyl-	$C_{14}H_{12}O_4$	244	0.37	47
28.315	4-Chloro-3H-1,2,3-benzotriazole	$C_6H_4ClN_3$	154	1.77	27
28.704	Eicosane	$C_{20}H_{42}$	283	0.42	86

Table 4: Compounds Identified in the BUT fraction of *S. longepedunculata* in GC-MS

RETENTION TIME (RT)	COMPOUND NAME	MOLECULAR FORMULA	MOLECULAR WEIGHT (g/mol)	PEAK AREA %	% QUALITY
4.065	3-Butenenitrile, 2-methyl-	C_5H_7N	81	2.00	59
18.221	Bicyclo[3.1.1]heptane-2-carboxaldehyde, 6,6-dimethyl-	$C_{10}H_6O$	152	9.54	87
18.891	Oxacycloheptadec-8-en-2-one	$C_{16}H_{28}O_2$	252	2.95	58
19.663	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	270	39.35	96
22.553	9-Octadecyne	$C_{18}H_{34}$	250	20.50	55
22.942	Heptadecanoic acid, 16-methyl-, methyl ester	$C_{19}H_{38}O_2$	298	25.66	96

Table 5: Compounds Identified in the DCM fraction of *S. longepedunculata* in GC-MS

RETENTION TIME (RT)	COMPOUND NAME	MOLECULAR FORMULA	MOLECULAR WEIGHT (g/mol)	PEAK AREA %	% QUALITY
19.698	Hexadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	256	23.79	96
22.559	9,17-Octadecadienal, (Z)-	$C_{18}H_{32}O$	264	18.05	95
22.959	Methyl Stearate	$C_{19}H_{38}O_2$	298	24.61	95
31.468	9,12-Octadecadienoic acid, (Z,Z)-	$C_{18}H_{32}O_2$	280	33.54	99

The chromatograms of the crude extract and fractions are shown in Figures 1 -3. Some bioactive compounds found in medicinal plant has been reported to have antiploriferative potential (Kandasamy, *et al*., 2012). In the present study, the compound found in the BUT fraction identified as 3-Butenenitrile, 2-methyl-(2.00%), Bicyclo[3.1.1] heptane-2-carboxaldehyde, 6,6-dimethyl-(9.54%), Oxacycloheptadec-8-en-2-one (2.95%), Pentadecanoic acid, 14-methyl-, methyl ester (39.35%), 9-Octadecyne (20.50%), and Heptadecanoic acid, 16-methyl-, methyl ester (25.66%) with the longest RT of 22.942. Heptadecanoic acid, 16-methyl-, methyl ester was shown by, to be highly potent against skin cancer than a standard drug "dyclonidine" (Kandasamy, *et al*., 2012).

The DCM fraction contains bioactive such as Hexadecanoic acid (23.79%), 9,17-Octadecadienal (18.05%),MethylStearate (24.61%), and 9,12-Octadecadienoic acid (33.54%). Hexadecanoic acid identified in the DCM fraction has also been reported to have antibacterial, antioxidant, and antitumor properties (Moonjit and Himaja, 2014). Harada *et al*. (2002) reported that palmitic acid, which contains n-Hexadecanoic acid, isolated from a marine alga may be a lead compound of anticancer drugs. Also, Octadecadienoic acid (Z, Z), which is one of the compounds identified in the DCM fraction, had earlier been reported to have an anti-inflammatory property (Rani *et al*., 2009). This suggests that the extract *S. longepedunculata* root bark contains bioactive compound which have potential for anticancer properties

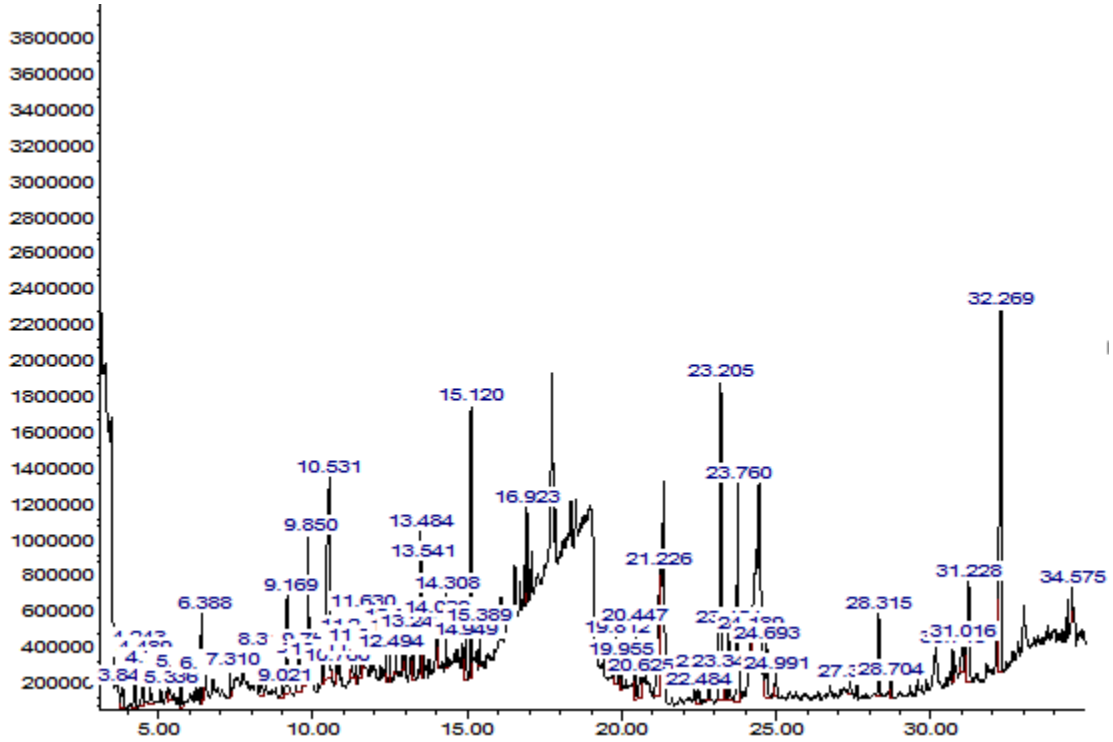

Figure: 1 GC-MS Chromatogram of methanolic extract of the root bark of *S. longepedunculata*

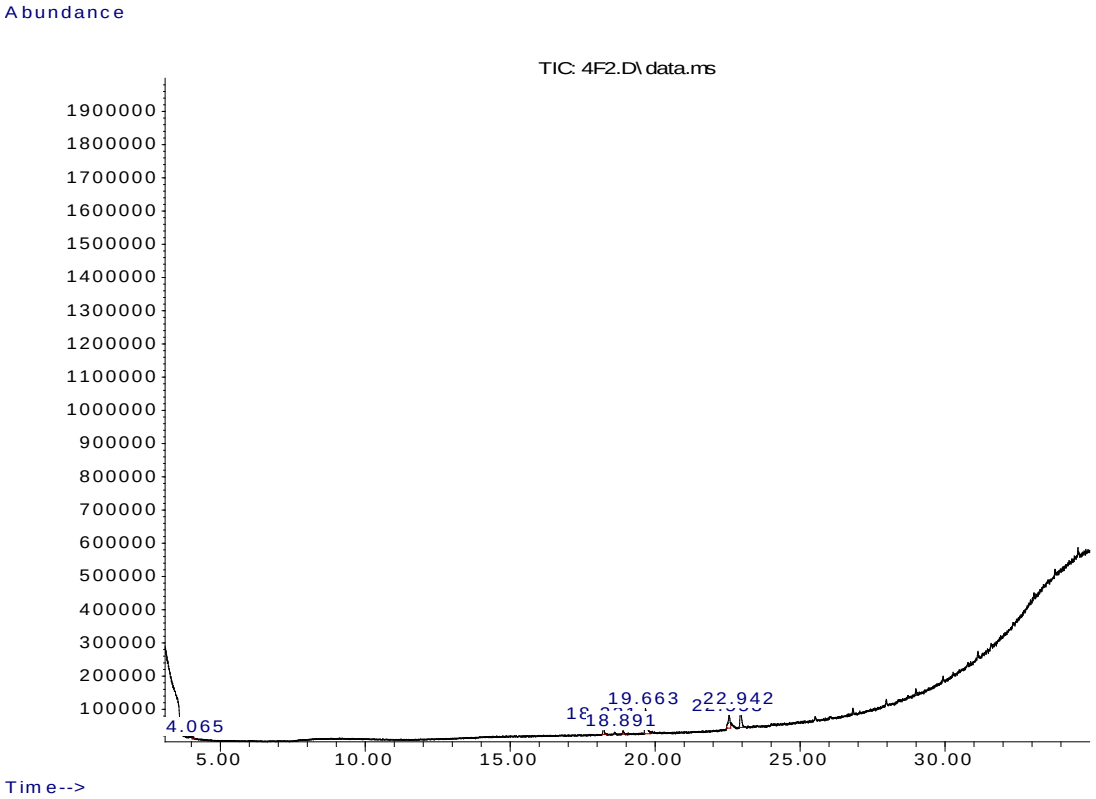

Figure: 2 GC-MS Chromatogram of BUT fraction of the root bark of *S. longepedunculata*

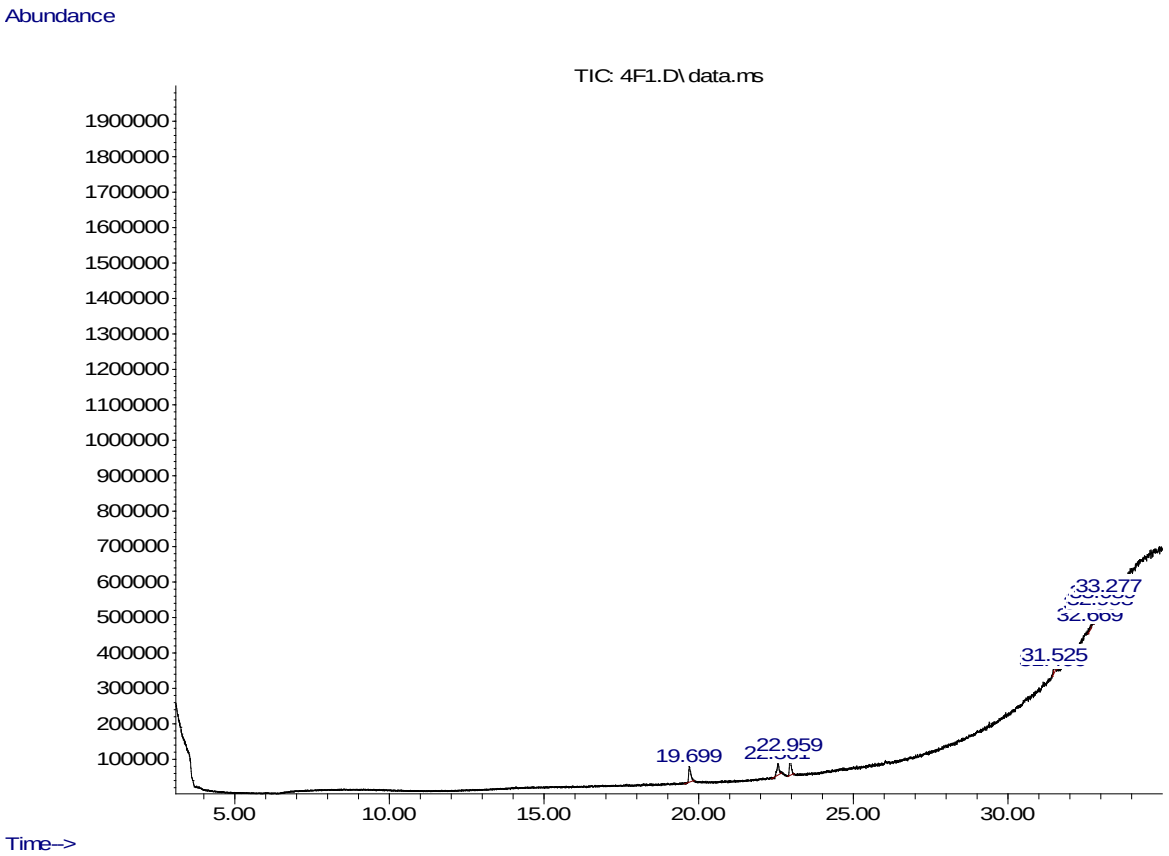

Figure: 3 GC-MS Chromatogram of DCM fraction of the root bark of *S. longepedunculata*

Human breast adenocarcinoma (MCF-7) cells were incubated with different concentrations of methanolic crude extract, DCM and BUT fractions of *S. longepedunculata* root bark (9.77 to 5000 µg mL^{-1}) for 24 h or48 h. After incubation, cell viability was determined using the XTT assay. The cytotoxic efficacy of *S. longepedunculata* root bark extracts was investigated using the XTT assay. Data generated from this assay strongly suggest that the crude extract *S. longepedunculata* root bark has a mild cytotoxic effect on MCF-7 cells even at high doses of exposure. Also, DCM fraction showed a moderate cytotoxic effect. The response of the MCF-7 cells to treatment with aqueous methanolic crude extract (Figure 4) for 24h or 48h demonstrated a dose-dependent and time independent activity. More so, the activities of BUT and DCM fractions against MCF-7 after 48 hours of treatment were found to be dose-dependent and dose-independent respectively (Figure 5). According to the National Cancer Institute (NCI) guideline, an extract and/or a compound with IC_{50} values <20 μg/mL is considered cytotoxic (Boik, 2001). The significant difference ($P<0.05$) in the IC_{50} values of the crude extract (1808 µg/ml), DCM fraction (86.27 µg/ml) and BUT fraction (12.08 µg/ml) as presented in Table 6 may be due to the masking of biological activity owing to the presence of some inhibitory compounds in the crude extract and DCM fraction (Nasrin et al., 2015). It may also be attributed to differences in docking energy of the bioactive molecules to receptor molecules on MCF-7 cells. Previous study by Kandasamy et al. (2012) had reported heptadecanoic acid, 16 methyl, methyl ester; the major component of BUT fraction of *S. longepedunculata* to have docking score of −11.4592 Kcal/mol, while 9,12-Octadecadienoic acid, the major component of DCM fraction of *S. longepedunculata* as having the docking score of −9.286 Kcal/mol. The lesser the docking score, the more the binding capacity of the ligand. Therefore, the IC_{50} value of BUT fraction evidences that it may be considered as a promising anticancer agent.

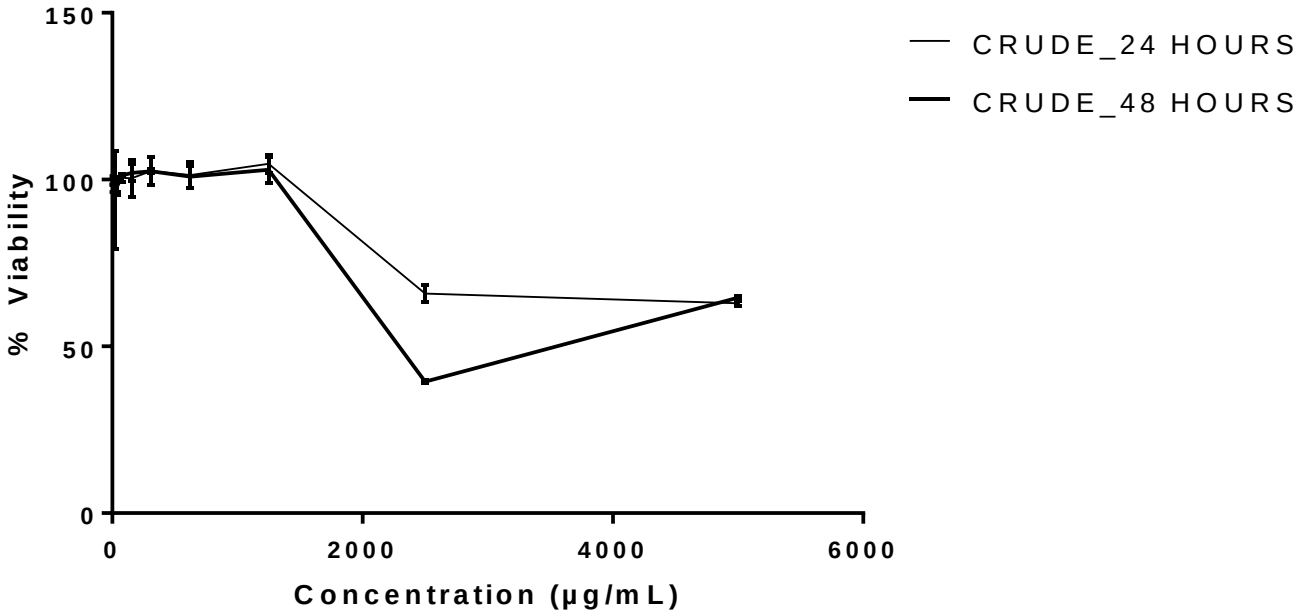

Figure 4: Percentage (%) cell viability of MCF-7 cells at different concentrations of the methanolic crude extract of *S. longepedunculata.*

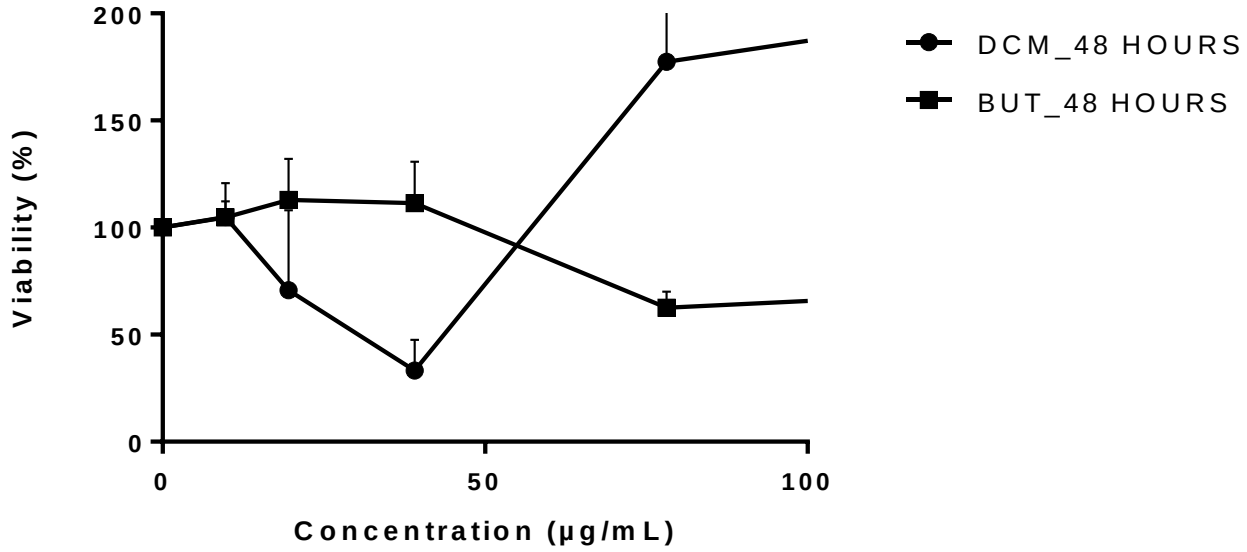

Figure 5: Percentage (%) cell viability of MCF-7 cells at different concentrations of DCM and BUT fractions of *S. longepedunculata.*

The study lends credence that methanolic extract, DCM and BUT fractions of *S. longepedunculata* may contain lead agent with anticancer properties and that informed its use in ethnomedicine.

Table 6: IC_{50} values of *S. longepedunculata* crude extract and fractions

S. longepedunculata	IC_{50} after 24hours	IC_{50} after 48hours
Crude extract	2241 µg/ml	1808 µg/ml
DCM fraction	-	86.27 µg/ml
BUT fraction	-	12.08 µg/ml

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