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METABOLITE PROFILING OF ETHYL ACETATE STEM EXTRACT OF *SOLANUM ERIANTHUM* FOR POTENTIAL ANTICANCER COMPOUNDS

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ABSTRACT

Cancer continues to be associated with high mortality despite the various therapeutic options available for its treatment. *Solanum erianthum* is used in traditional medicine for the treatment of cancer. This study evaluates the potential of the ethylacetate extract of the stem of the plant as an anticancer agent by screening it against the hepatocellular carcinoma (HepG2) cell line using the 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide **(**MTT) assay. The extract was screened for activity at concentrations ranging between 1 and 1,000 µg/ml (in triplicates). The half-maximal inhibitory (IC_{50}) value was obtained from a plot of percentage cell viability against extract concentrations. The extract was then derivatized into trimethylsilyl and methyl ester forms and subsequently analyzed using Gas Chromatography Mass Spectrometry (GCMS) technique. Cell viability decreased with increasing extract concentration (values were significantly different at all test concentrations). The extract was moderately active against the HepG2 cell line, with IC_{50} value of 125µg/mL. The compounds identified in the extract include Nobiletin, Friedelan-3-one, Stigmasterol and n-hexadecanoic acid. From literature reports, these compounds possess anticancer activities against a broad range of human cancer cell lines, and could be partly responsible for the anti-proliferative properties of the extract against the HepG2 cell line. This study has provided some justification for the use of *S. erianthum* for cancer treatment by traditional healers.

KEYWORDS: **Cancer; HepG2; MTT; GCMS; Derivatization.**

INTRODUCTION

Cancer is still a major public health burden around the world. More than 19 million new cancer cases will be reported globally by 2020. In Nigeria, 124,815 new cancer cases were reported in 2020 [1]. Cancer treatment typically involves the use of surgery, chemotherapy, and radiotherapy. Despite these treatment options, cancer is still associated with a high mortality rate. Furthermore, many of the anticancer drugs available in the market have serious side effects including neurotoxicity, cardiotoxicity, and nephrotoxicity [2]. These factors have necessitated a search for anticancer drugs with higher efficacy and fewer adverse effects.

Several plants used in traditional medicine contain compounds with anticancer properties. For example, vinblastine and vincristine were isolated from *Catharanthus roseus* [3]; paclitaxel was isolated from *Taxus brevifolia* [3]. In addition, epipodophyllotoxin, an epimer of podophyllotoxin, was isolated from the roots of *Podophyllum* species and used to synthesize docetaxel, etoposide, and teniposide [4].

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In the separation and characterisation of natural products from plants, the bioassay-guided technique has been widely used. However, the approach frequently results in the isolation of well-known compounds of low pharmacological importance. As a result, approaches that can identify novel from known natural compounds at an early stage are needed to save time and money. Hyphenated approaches, for example, High Performance Liquid Chromatography coupled with UV photodiode array detection (LC-DAD-UV), High Performance Liquid Chromatography coupled with mass spectrometry (LC-MS or LC-MS-MS), and Gas Chromatography coupled with Mass Spectrometry (GC-MS) combine separation and spectroscopic technologies with online database searches to identify constituents of a plant extract prior to isolation[5].

Traditional medicinal practitioners in south-western Nigeria utilize *Solanum erianthum* to treat cancer. Previous reports indicate that the ethyl acetate extract of the leaves of the plant has mild antibacterial activity [6]. The root of the plant contain β-sitosterol, Stigmasterol, (-)-Solavetivone, Solanerianones A and B, (+)-anhydro-rotunol, solafuranone, lycifuranone A, N-trans-feruloyltyramine, palmitic acid, and acetovanillone.(-)-Solavetivone is fungitoxic and has a low cytotoxicity [6]. The essential oil from the leaves inhibited the growth of human breast (Hs 578T) and prostate (PC-3) tumor cells, respectively [7]. Currently, there is no literature report validating the anticancer properties of the stem of *S. erianthum*. Also, metabolite profiling of the stem of the plant has not been carried out. This study aims to evaluate the anticancer potential of the stem of *S. erianthum* by screening its ethyl acetate extract for activity against the hepatocellular carcinoma (HepG2) cell line; and to identify potential anticancer compounds from the stem of the plant using GCMS.

MATERIALS AND METHODS

Extraction

Samples of the stem of *S. erianthum* were collected from the Botanical Gardens of the University of Ibadan. After identification, a voucher specimen was deposited at the University herbarium. The deposited sample was assigned the voucher number, UIH-22441. The samples were dried, ground and defatted using hexane. The defatted sample was extracted using ethyl acetate and concentrated to dryness.

Anticancer Screening of Selected Extracts in HepG2 Cell Line

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay was used to test the extract for anticancer activities. Cell culture (2 x 10^3 cells/ml) was prepared and plated into 96-well plates at a rate of 100µl/well. The wells were filled with various extract concentrations (ranging from 1 µg/ml to 1000 µg/ml) and incubated for 72 hours. MTT solution was applied to the cells after the samples had been incubated. Afterwards, the mixture was incubated for another 3 hours. The formazan crystals formed were dissolved in dimethyl sulfoxide (DMSO), and the optical density measured at 570 nm with an ELISA reader. The percentage of viable cells was calculated using the following formula:

$$Cell\ viability = \frac{Absorbance\ of\ sample\ (mean)}{Absorbance\ of\ Control\ (mean)}\ x\ 100\ \%$$

Graphs of the percentage of cell viability against the different concentrations were plotted. The cytotoxicity of the extracts was recorded as the IC_{50} value (the concentration required to kill 50 % of the cancer cells) [8].

GC-MS Analysis of Extracts

Derivatization of Extracts into Trimethyl Silyl (TMS) forms

A 3 mg portion of the extract was mixed with 60 µL of dry pyridine and 100 µL of bis(trimethylsilyl)trifluoroacetamide. The mixture was heated at 70°C for 30 minutes. An aliquot (1 µL) of the resulting solution was analyzed using GCMS [9].

Derivatization of Extracts into Fatty Acid Methyl Ester (FAME) Derivatives

The extracts (5 mg) were introduced into test tubes containing $CH_3OH/H_2SO_4/CHCl_3$ (1.7:0.3:2.0 v/v/v, 4 mL). The mixture was heated for 90 minutes at 90°C. After cooling, water (1 mL) was added and shaken vigorously. The lower layer ($CHCl_3$) was carefully removed using a Pasteur pipette and transferred into GC vials [10].

GC-MS Analysis of Derivatized Extracts

The derivatized samples were analyzed on a Gas Chromatograph (Agilent 7890) coupled to a mass selective detector (Agilent 5975C) operating in electron impact mode. The capillary column (Chrompack CP-Wax 52 CB) had a length of 30 m, an internal diameter of 0.32 mm, and a film thickness

of 0.25 µm. The diluted sample (1.0 µL) was injected into the Chromatograph at a temperature of 250˚C. Helium, with a flow rate of 5 ml/min and inlet pressure of 12.936 p.s.i., was used as the carrier gas. The column oven temperature was raised gradually from 50˚C to 240˚C at a rate of 8˚C/minutes, with a final hold time of 5 minutes. The identification of the sample constituents was done by matching the mass spectra of the eluted compounds with mass spectra fragmentation patterns of compounds stored in the National Institute of Standards and Technology (NIST) Mass Spectral Library (NIST 14L) of the GCMS. A similarity index of ≥95% was used as a criterion for acceptance.

Statistical Analysis

The assays were carried out in triplicates, and the absorbance was recorded as a mean (± standard deviation). A t-test was used to determine if there was a significant difference ($p<0.05$) between the means obtained at the different test concentrations.

RESULTS

Table 1 shows the absorbance values and the percentage cell viabilities of HepG2 cells exposed to different concentrations of the most active extracts after 72 hours exposure.

Figure 1 shows the plot of the percentage of cell viability against the corresponding extract concentrations. From the graph, the IC_{50} value (the concentration that caused 50% inhibition of cell growth) was determined as 125 µg/ml.

The compounds identified in the extract are presented in Tables 2 and 3. The major compounds identified in the derivatized extracts are Nobiletin, Squalene, Friedelan-3-one, Hexadecanoic acid, and Stearic acid.

DISCUSSION

From Table 1, it could be observed that the absorbance values are significantly different at all the test concentrations (at $p < 0.05$). Furthermore, as shown in Figure 1, the percentage of cell viability decreased with increasing extract concentration. This result conforms to previous findings from similar studies. For example, Thusyanthan *et al*. [11] reported that the viability of HepG2 cell line decreased as the concentration of different extracts increased.

The ethylacetate stem extract of *S. erianthum* showed some activity against the HepG2 cancer cell line. However, the activity obtained is relatively low since only extracts with IC_{50} values less than 30 µg/ml are considered anti-proliferative agents [12]. The low activity obtained could imply that the active principles are present in low concentrations in the extract. Also, extracts from several plant species exhibit differing selectivity against several human cancer cell lines. For example, leaves and seeds extracts of *Artemisia absinthium* and *Plantago major* showed different selectivity against human lung carcinoma (A-549), human colorectal carcinoma (CCC-221), human leukemic carcinoma (K-562), human breast carcinoma (MCF-7) and human prostatic carcinoma (PC-3) cell lines [13]. The ethyl acetate extract of *S. erianthum* may be selectively more active against other cancer cell lines that have not been part of this investigation.

Though limited, the anti-proliferative property observed provided a hint that some anticancer compounds are present in the extract. To confirm this, the extract was derivatized and subsequently subjected to GCMS analysis. Derivatization was carried out before analysis because GCMS can only analyze volatile and thermally stable compounds. Many hydroxyl and amino compounds regarded as non-volatile or unstable at 200 – 300 °C have been analyzed successfully in GC after silylation. The silylated derivatives are more volatile and more stable and thus yielding narrow and symmetrical peaks [14]. Some amino acids and organic acids produce relatively unstable silylated derivatives. Therefore, the fatty acid constituents of the extract were derivatized into the methyl ester forms [14].

The inhibitory activities some of the compounds detected in the ethyl acetate extract against a variety of human cancer cell lines have been widely described in the literature. For example, Nobiletin showed antiproliferative activity against human lung carcinoma cells (A549), squamous cell carcinoma (HBT43), leukemia (HL-60), T-cell leukemia (CCRF-HSB-2), and B16 melanoma cells [15]. Squalene demonstrated cytotoxicity against HCT-116 and MCF-7 [16]. Friedelan-3-one, is a triterpenoid, has been reported to have anti-tumor activities against various cancer types, including human malignant melanoma A375, human cervical tumor HeLa, human macrophage tumor THP-1, and mouse lung epithelial tumor L929 cells [17]. n-hexadecanoic acid showed significant activity against Human Colorectal carcinoma (HCT-116) cells (IC_{50} value of 0.8 µg/ml) [18]. The mild anticancer activity shown in the HepG2 cell line could be attributed to these compounds. The presence of these compounds in the extract suggests that it may have greatly increased anticancer activity against other cancer cell lines.

Table 1: Absorbance values and percentage of cell viability obtained for HepG2 cells exposed to various concentration of SESEE

Concentration (µg/ml)	Absorbance (Mean ± SD)	% Viability
1000	0.142± 0.012[a]	13.990
500	0.164± 0.004[b]	16.157
200	0.366 ±0.001[c]	36.059
100	0.578 ± 0.014[d]	56.946
50	0.616± 0.001[e]	60.700
20	0.715± 0.002[f]	70.443
10	0.743 ± 0.001[g]	73.202
1	0.776 ±0.005[h]	76.453
Control	1.015±0.001[i]	

Concentrations with different superscripts are significantly different for each extract ($p < 0.05$)

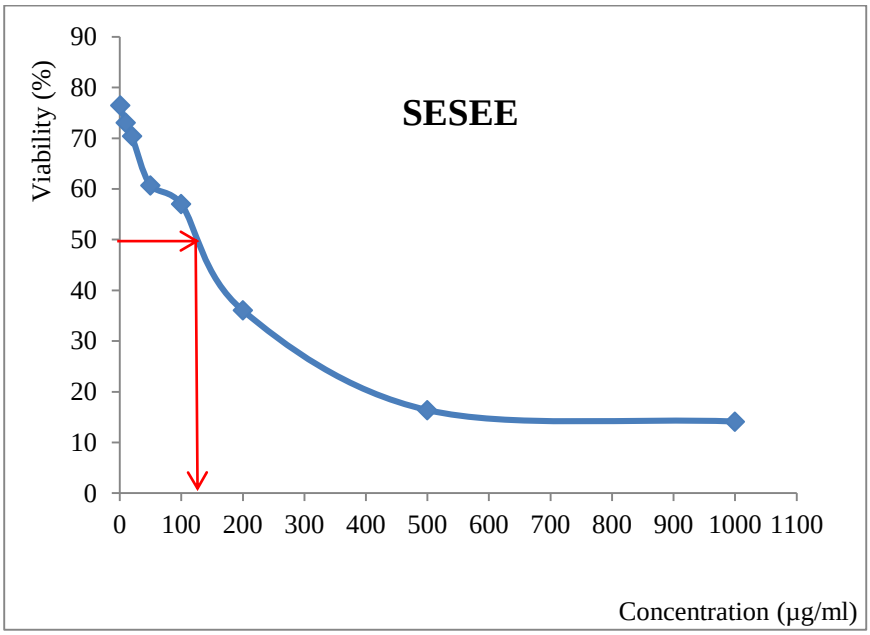

Figure 1: Effect of *S. erianthum* stem ethyl acetate extract on HepG2 cells after 72 hours exposure.

Table 2: Compounds detected in TMS-derivatized SESEE

t_R(min.)	Peak area (%)	Name of Compound	NIST matching (%)
17.844	1.78	Hexadecanoic acid methyl ester	97
20.299	1.39	Beta-Sitosterol	98
22.244	30.23	3',4',5,6,7,8-Hexamethoxyflavone (Nobiletin)	96
24.453	25.21	Squalene	99
26.106	23.18	Friedelan-3-one	99

Table 3: Fatty acids in FAME-derivatized SESEE (detected as methyl esters)

Pk no	t_R(min.)	Peak area (%)	Name of Compound	NIST matching (%)
1	13.787	0.86	Tetradecanoic acid	99
3	15.979	20.23	Hexadecanoic acid	99
4	16.156	6.32	Hexadecanoic acid	96
5	16.356	4.90	n-Hexadecanoic acid	99
6	16.637	0.57	Hexadecanoic acid, ethyl ester	97
11	17.947	13.46	Stearic acid	99
13	18.508	0.37	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	98
14	19.560	3.81	10-oxo-Octadecanoic acid	83
15	19.738	2.16	Eicosanoic acid	99
16	21.414	3.25	Docosanoic acid	99
18	22.965	1.31	Tetracosanoic acid	99
19	24.367	2.35	Cholesta-3,5-diene	99

CONCLUSION

The ethyl acetate stem extract of *Solanum erianthum* possesses moderate anticancer properties against HepG2 cancer cell line. Some of the extract constituents (identified by GCMS) demonstrated anticancer activity against several human cancer cell lines. The extract may therefore exhibit better activity against other cancer cell lines. This should be confirmed through further studies. This study provided partial justification for the plant's use in cancer treatment by traditional medical practitioners in Nigeria.

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