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Original Research Article

UHPLC-DAD-MS^3 FINGERPRINTING ANALYSES AND CARCINOGENESIS PREVENTIVE POTENTIALS OF *CYPERUS BULBOSUS* VAHL. (CYPERACEAE) BULB EXTRACTS ON RHABDOMYOSARCOMA AND BREAST CANCER CELL LINES

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ABSTRACT

Despite tremendous progress in cancer treatment, cancer remains a worldwide health burden with a rapidly increasing incidence and death rate. Although conventional cancer medications have demonstrated notable therapeutic success, patients are nonetheless subjected to unfavourable side effects. Compounds produced by nature are being investigated in the quest for substitute anti-cancer medications. Nature continues to be the primary source of bioactive molecules, notwithstanding recent developments in chemically synthesized pharmacologically - active compounds s. This work aims to evaluate the UHPLC-DAD-MS^3 fingerprinting analyses and antiproliferative activity of *Cyperus bulbosus* Vahl. (Cyperaceae) on rhabdomyosarcoma and breast cancer cell lines. The dry bulb of *Cyperus bulbosus* was successively extracted using n-hexane, ethyl acetate, and methanol for 72 h, respectively. The cytotoxicity assay of the plant extracts against human cancer cells was assessed using MTT (3-(4,5-dimethyl thiazole-2yl)-2,5-diphenyl tetrazolium bromide) assay. Cytotoxicity concentration (CC_{50}) and selectivity index were evaluated. Methanol extract showed the highest cytotoxicity effect on RD (Rhabdomyosarcoma) cell line (CC_{50} =7.83±0.06 µg/mL). Ethyl acetate (ECB) extract showed the highest cytotoxicity effect (CC_{50} =7.74±0.01 µg/mL) on MCF-7 (breast cancer cells) at 1000 µg/mL when compared to Vincristine (CC_{50} =0.34±0.17 µg/mL) at 100 µg/mL. The ethyl acetate extract has the highest nitric oxide inhibitory activity (IC_{50}=53.85±0.08 µg/mL) at 800 µg/mL when compared to ascorbic acid (IC_{50}=83.39± 0.14 µg/mL) at 100 µg/mL. UHPLC-DAD-MS^3 analyses for ethyl acetate revealed the presence of 33 compounds. Some molecular ion peak (+MS) of compounds identified are represented (+MS 401.15, RT 80.2 mins, Area% 100), (+MS 425.20, RT 106.9 mins, Area% 61.99), (+MS 282.10, RT 105.1 mins, Area% 51.88), (+MS 403.15, RT 95.9 mins, Area % 45.35), (+MS 433.29, RT 92.5 mins, Area% 26.29). Components in extracts of *Cyperus bulbosus* have antiproliferative activity on rhabdomyosarcoma and breast cancer cell lines.

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INTRODUCTION

Cancer is one of the leading causes of death worldwide and is characterized by uncontrollable cell proliferation that invades other body components [1]. Cancer is caused by harmful molecular changes that occur in normal cells found in many

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bodily tissues, which can result in fatal illnesses [2-5]. The latest estimates released by the World Health Organization's International Agency for Research on Cancer, predict a 77% global increase in new cancer cases in 2050, up from the 20 million estimated in 2022. As a result, the number of cancer deaths worldwide is expected to double by 2050 to an estimated 18.5 million compared to 9.7 million in 2022. According to the estimates, Africa is the region expected to see the greatest percentage increase in cancer cases in 2050, rising nearly 140% to 2.8 million compared to an estimated 1.2 million cases in 2022. Current estimates from the WHO's Global Cancer Observatory show lung, female breast and colorectal cancers combined accounted for more than 6.7 million, or one-third, of all new cases globally in 2022, with those cancer types making up an estimated 35% of the 9.7 million cancer-related deaths that year [6]. On the other hand, rhabdomyosarcoma is an extremely aggressive cancer that mostly develops from skeletal muscle cells and can manifest at many anatomic locations across the body. It is the most prevalent soft-tissue sarcoma in children, accounting for 5% of all pediatric malignancies while being very uncommon [7].

In addition to the treatment resistance displayed by certain cancer stem cells, the use of traditional therapeutic modalities such as surgery, chemotherapy, radiation, and immunotherapy alone or in combination is not without significant adverse effects [8-14]. Thus, an alternative medicine strategy for cancer drug discovery is required. The diversity of flowers in West Africa's tropical rainforest is astounding [15-16]. Interestingly, due to a lack of high-level instruments and facilities, the great bulk of their biological activities and therapeutic potentials as used in Nigerian ethno-medicine are still not extensively studied for scientific validation. However, these possibilities are threatened by the disastrous loss of biodiversity; current projections indicate that one in five plant species on Earth may go extinct [17-20]. Thus, it is crucial to record and investigate the bioactivity of medicinal plants utilized in diverse ethnobotany and to establish a connection between the application of medicinal plants in traditional medicine and the creation of novel medications for the treatment of cancer disorders in this area [21-22].

Cyperus bulbosus Vahl (Cyperaceae) is a species of sedge found across Africa, the Middle East, the Indian subcontinent, Southeast Asia, and Australia [23]. In Australia, it is commonly called Nalgoo or (Australian) bush onion [24] or "wild onion", but is not related to the onion or other Alliaceae. Typically green to straw in colour, the slender plant propagates via rhizomes (horizontal, underground stem extensions), ending at bulbs. Approximately, 20-40 cm in height (approx. 8-16 inches), it has fragments (or culms) as long as its leaves (which are typically 1 – 2 mm wide).

Natural agents can increase the cytotoxic efficiency of anticancer drugs in tumour cells; they can also cause cell death by up-regulating pro-apoptotic targets; they can promote DNA damage; or they can control the expression of both altered and unaltered drug targets. Therefore, this study aims to evaluate the carcinogenesis preventive potentials of *Cyperus bulbosus* extracts on breast and rhabdomyosarcoma human cancer cell lines.

MATERIALS AND METHODS

Solvent/reagents

Acetonitrile, methanol and formic acid (Merck, Darmstadt, Germany). Water (Bedford, MA, USA), n-hexane, ethyl acetate, methanol (analytical grade, sigma USA), sodium nitrite, Phosphate buffer saline, Sodium nitroprusside, metaphosphoric acid, N-(1-naphthyl) ethylenediamine (NNED), Sulphanilamide, Griess reagent, disodium hydrogen orthophosphate dehydrate, tetrazolium dye (MTT), DMSO, Sodium pyruvate, penicillin-stretomycin, Vincristine, Human Rhabdomysarcoma (RD) cancer cell line, Human breast cancer cell line (MCF-7).

Equipment

Rotary evaporator (Buchi), UHPLC-3000 RS system (Dionex, Germany), AmaZon SL ion trap mass spectrometer with an ESI interface (BrukerDaltonik GmbH, Germany), Diode Array Detector were the features of the UHPLC-3000 RS system (Dionex, Germany), UV Spectrophotometer (SPECTRAmax GEMINI XS, Molecular Devices, US).

Plant Collection and Preparation of the Extract

Dried bulbs of *Cyperus bulbosus* plants were collected at Orba in Udenu local government area of Enugu State on April 8th, 2023 and identified by a taxonomist Mr Felix Nwafor of the Department of Pharmacognosy and Environmental Medicine, University of Nigeria, Nsukka. The plant sample was thoroughly cleaned and then pulverized using a laboratory mill. The ground sample was weighed to note the actual weight of the sample and then stored in an air-tight container for laboratory analyses.

Plant Extraction Technique

The powdered plant sample (1450 g) was macerated using successive extraction methods based on the increasing polarity of the solvents (n-hexane, ethyl acetate, methanol). The powdered plant was first macerated with n-hexane using a macerating jar at room temperature for 72 h with occasional stirring every 8 h before it was filtered and the filtrate was stored for further use. The Marc was air-dried and macerated again for another 72 h using ethyl acetate and with occasional stirring every 8 h. On the expiration of the 72 h, it was filtered to collect the filtrate and the Marc was air dried for another extraction using methanol for 72 h. The filtrates obtained were concentrated using a rotary evaporator at 40°C to obtain the different extracts. The extracts obtained were then placed in sample vials for phytochemical screening and biological assays.

MTT Assay

Cell Culture

The cancer cell line was supplied by the Centre for Disease Control (CDS), Atlanta, Georgia, and maintained in the WHO

Polio Laboratory, Department of Virology, University of Ibadan, Nigeria. The Human Rhabdomyosarcoma (RD) cancer cell line was obtained from the Virology laboratory stock of the Department. The cells were cultured in modified essential medium Eagle (EMEM), which was supplemented with 1% Penicillin-Streptomycin, 2 mM L-glutamine, and 10% heat-inactivated Foetal Bovine Serum (FBS). Also added to the DMEM was 0.01 mg/mL of sodium pyruvate. The cells were passaged every two weeks and kept at 37°C in a humidified incubator with 5% CO_2.

Splitting of Cancer Cell Line

Liquid media was emptied into the waste container; the T flask was slightly swirled with 2 mL Phosphate Buffer Saline (PBS) and emptied into a waste container. The flask was slightly swirled with 1 mL of trypsin and incubated for 3 mins until the cell detached. The flask was removed from the incubator; the side of the flask was tapped repeatedly to ensure lumps of cells were dispersed. This was viewed under the microscope to ascertain if the cells had detached from the surface of the flask. The cells were then resuspended in freshly prepared EMEM media (growth medium) ensuring even distribution by swirling media. Finally, 100 µL of the mixture was seeded into a 96 microwell-plate and incubated for 24 h.

Procedure

The MTT (3-(4,5-dimethyl thiazole-2yl)-2,5-diphenyl tetrazolium bromide) test was used to measure the cytotoxicity of the plant extracts against human cancer cells. 96-well microtiter plates were seeded with 100 µL of RD and MCF-7 cells. After being redissolved in DMSO, each extract had a concentration of 10 mg/mL. A dilution of 1000 µg/mL was obtained by adding 0.1 mL of the stock to 0.9 mL of maintenance medium containing antibiotics. This was referred to as "neat." To achieve varying concentrations, the extracts were serially diluted 10 times from the neat using a maintenance medium as a diluent. To 96-well microtiter plates that had already been seeded with a monolayer of RD and MCF-7, 50 µL of every extract dilution was added. The plates were then incubated in triplicate at 37°C with 5% CO_2 in a humidified environment. After 72 h period, the cells are examined under a microscope [25]. After incubating for 72 h, 37.25 µL of a 2 mg/mL (Sigma) solution in Phosphate Buffer Saline (PBS) was added to each well to dissolve the MTT crystals. The supernatants were then collected from each well. The plates were shaken for fifteen mins, and then a multi-well spectrophotometer was used to measure the absorbance at 492 nm (TitertekUniskan). The MTT colourimetric test was employed to assess how much the extracts reduced the viability of cell cultures both in their presence and absence. The tetrazolium dye (MTT), which is converted into an insoluble, coloured formazan product by the mitochondrial enzymes of live (surviving) cells, was used to assess the cytotoxic potential of the plant extracts. The quantity of healthy, live cells in each well of the 96-well microtiter plate determines the amount of metabolism that takes place there. Vincristine was used as a standard. The percentage cytotoxicity is calculated using Equation 1.

$$\%\ Cytotoxicity = (A - B) \times \frac{100}{A} - - - Equation\ 1$$

Where A = the mean optical density of untreated cells

B = the optical density of cells treated with plant extracts.

Selectivity Index (SI)

The SI was measured using the normal African green monkey kidney epithelial cell line (Vero). The ratio of the cytotoxic effect on a normal cell line to the cytotoxic effect on cancer (RD) and MCF-7 cell lines was used to compute the SI of the most active fraction.

Antioxidant assay

Nitric Oxide Inhibitory Assay

The plant extracts' nitric oxide inhibitory effects were determined using the Griess reagent methods [26], with minor modifications. The Griess reaction generates a pink azo-product with maximal absorbance at 540 nm using a straightforward colourimetric process involving nitrite, sulphanilamide, and N-(1-naphthyl) ethylenediamine. The data were represented as µg/mL and the concentrations were calculated using a sodium nitrate standard curve.

Standard Sodium Nitrite Curve

Sodium nitrite is an inorganic salt that generates nitrite ions in solution, NO_2. A standard sodium nitrite curve is required to estimate the amount of nitric oxide remaining in the nitric oxide inhibition assay. A stock solution of 10 mM sodium nitrite ($NaNO_2$; m.w=68.995 g/mol) solution was prepared by weighing 6.9 mg of sodium nitrite into 10 mL of sterile distilled water which resulted in 0.69 mg/m of $NaNO_2$ (equivalent to 10 mM). The working solution used in the experiment was a 1:50 dilution of 10 mM of the working solution of $NaNO_2$. Briefly, 100 µL of 0.2 mM $NaNO_2$ solution was dispensed into the well in a 96-well plate while 50 µL of distilled water was dispensed into the subsequent wells. Serial dilution was achieved by transferring 50 µL of the solution in the first well into the next well to the sixth well. Thereafter, 50 µL of the freshly prepared Griess reagent was dispensed into the wells and the absorbance was recorded at 540 nm using a UV Spectrophotometer (SPECTRAmax GEMINI XS, Molecular Devices, US).

Preparation of Extracts and Standard Drug

Twenty milligrams (20 mg) of extracts were weighed and dissolved in 2 mL of DMSO, making a stock solution of 10 mg/mL concentration. The concentration was reduced to 1000 µg/mL as the starting concentration by diluting with phosphate buffer saline and 2-fold serial dilution was done subsequently. Similarly, the standard drug, Ascorbic acid (20 mg/mL) was

diluted to a starting concentration of 100 µg/mL and serial dilution was carried out in the test tubes. Thereafter, Nitroprusside was added and incubated at 29°C for 3 h. A 100 µL of supernatant was then transferred into a 96-well plate and a 100 µL of freshly prepared Griess reagent was added and incubated for 10-15 mins. Absorbance was measured at 540 nm. The percentage inhibition is calculated using Equation 2

$$\%\ inhibition = (Average\ of\ test\ agents) /(Average\ of\ control) \times 100 - - - - Equation\ 2$$

UHPLC-DAD-MS3 Analyses

Plant Material and Preparation of Extracts

Ten milligrams of each extract, precisely weighed, were dissolved in methanol. The tube was then centrifuged at ambient temperature for three mins at RCF 9503. A 0.45 µm syringe filter made of PVDF was used to filter the supernatant. Until UHPLC analysis, which required no more than 72 h from the time of preparation, the samples were maintained at 4°C.

UHPLC-DAD-MS3 Qualitative Analyses

The process for utilizing UHPLC-DAD-MS3 to evaluate extracts was as follows. Three microliters of each extract were separated using a Kinetex XB-C18 (150 mm × 2.1 mm × 1.7 µm, Phenomenex, Torrance, CA, USA). The elution gradients were 0 min–1% B, 60 min–26% B, and 120 min–95% B for the mobile phases, which were 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). Before each injection, the column was cleaned for seven mins using the mobile phase's original composition (1% B). There was a 0.3 mL/min flow rate. The range of 190–800 nm was used to obtain the UV–Vis spectra. Compounds were characterized using the maxima observed in UV-Vis spectra, MS spectra, and comparison with published data.

Statistical Analyses

All experiments were conducted in triplicate and values were expressed as mean ± standard deviation. The quantitative results for each treatment were transformed into a cytotoxicity percentage. To determine the cytotoxic concentration needed to cause a 50% reduction in cell viability, a non-linear regression analysis was performed (CC_{50}).

RESULTS

Cytotoxic Assay

The cytotoxicity effect of n-hexane extract (HCB), ethyl acetate extract (ECB) and the methanol extract (MCB) of *Cyperus bulbosus* bulb was evaluated on both the breast cancer cell lines (MCF-7) and Rhabdomysarcoma cell lines (RD). Fifty percent (50%) cytotoxicity concentrations (CC_{50}) which is the concentration of test extracts required to reduce cell viability by 50% were used to determine the cytotoxicity effect of the extracts. The cytotoxicity effect of extracts on RD (Rhabdomysarcoma cell) were CC_{50} values of 14.17±0.12 µg/mL, 11.52±0.19 µg/mL and 7.83±0.06 µg/mL for HCB, ECB, and MCB, respectively at 1000 µg/mL when compared to vincristine (CC_{50} 0.03±0.15µg/mL) at 100 µg/mL. Methanol extract showed the highest cytotoxicity effect on the Rhabdomyosarcoma cell line. This shows that the methanol extract may be a potent drug for the treatment of Rhabdomyosarcoma (Table 1). The cytotoxic effects of various extracts on breast cancer (MCF-7) were CC_{50} values of 13.80±0.01µg/mL, 7.74±0.01 µg/mL and 34.18±0.05µg/mL for HCB, ECB and MCB, respectively when compared to vincristine (CC_{50} 0.34±0.17 µg/mL). Ethyl acetate extract of *C. bulbosus* has the most cytotoxic effect on breast cancer cells (MCF-7) (Table 2).

Selectivity Index

The selectivity index of n-hexane extract of *Cyperus bulbosus* (HCB) on RD was 6.98 µg/mL, for ethyl acetate extract of *Cyperus bulbosus* (ECB) on RD was 2.17 µg/mL, for methanol extract of *Cyperus bulbosus* (MCB) it was 15.14 µg/mL, while the standard, vincristine has 2.33 µg/mL. This show that the methanol extract (MCB) has a higher selectivity index for RD than any of the other extracts of the bulb of *Cyperus bulbosus* which indicates that MCB was more toxic to the cancer cell line (RD) compared to normal cells (Vero), while HCB has the better selectivity for breast cancer at 7.17 µg/mL compared to ECB (3.23 µg/mL) and MCB (3.47 µg/mL) when compared to standard (Vincristine) with 0.21 µg/mL (Table 3).

Nitric Oxide Inhibitory Effect

From our study, it was seen that the extracts were able to inhibit nitric oxide at different concentrations. Ethyl acetate extract (ECB) had the highest nitric oxide inhibitory activity (IC_{50}=53.85±0.08 µg/mL), followed by n-hexane extract (HCB) (IC_{50}=73.71±0.03 µg/mL) at 800 µg/mL when compared to Ascorbic acid (IC_{50}=83.39± 0.14 µg/mL) at 100 µg/mL concentration. The methanol extract (MCB) has the lowest nitric oxide inhibitory activity (IC_{50}=151.10±0.11µg/mL) (Table 4).

UHPLC-DAD-MS Analyses

UHPLC-DAD-MS^3 analyses revealed the presence of thirty-three (33) compounds with fragmentations from ethyl acetate extract of *Cyperus bulbosus* bulb. The compounds identified are (+MS, 401.15; RT 80.2 mins, Area% 100), (+MS, 425.20; RT 106.9 mins, Area% 61.99), (+MS, 282.10; RT 105.1 mins, Area% 51.88), (+MS, 403.15; RT 95.9 mins, Area % 45.35), (+MS, 433.29; RT 92.5 mins, Area% 26.29), (+MS, 505.41; RT 99.0 mins, Area% 17.41), (+MS, 453.29; RT 37.9 mins, Area % 13.96), (+MS, 170.91; RT 73.6 mins, Area% 9.61), (+MS, 441.22; RT 103.0 mins, Area % 5.34) (Table 5, Figure 1).

Table 1: Cytotoxic effect of extracts of *Cyperus bulbosus* bulb on RD (Rhabdomyosarcoma cells)

Conc. (µg/mL)	% inhibition by HCB (µg/mL)	% inhibition by ECB (µg/mL)	% inhibition by MCB (µg/mL)	Conc. (µg/mL)	Vincristine
1000	87.39 ± 0.00	85.51 ± 0.01	89.13 ± 0.01	100	45.12 ± 0.02

100	74.53 ± 0.01	78.02 ± 0.00	73.22 ± 0.01	10	43.71 ± 0.03
10	33.84 ± 0.00	39.83 ± 0.01	49.09 ± 0.02	1	42.14 ± 0.03
1	14.13 ± 0.04	5.48 ± 0.10	33.76 ± 0.01	0.1	42.06 ± 0.046
0.1	4.67 ± 0.02	3.37 ± 0.05	13.16 ± 0.01	0.01	38.85 ± 0.011
0.01	0.02 ± 0.025	0.49 ± 0.02	9.98 ± 0.012	0.001	33.76 ± 0.02
CC_{50} (µg/mL)	14.17 ± 0.12	11.52 ± 0.19	7.83 ± 0.06		0.03 ± 0.15

Keys: HCB (n-hexane extract), ECB (ethyl acetate extract), MCB (Methanol extract) of *Cyperus bulbosus* bulb.

Table 2: Cytotoxic effect of extracts of *Cyperus bulbosus* bulb on MCF-7 (breast cancer cells)

Conc. (µg/mL)	**% inhibition by HCB (µg/mL)**	**% inhibition by ECB (µg/mL)**	**% inhibition by MCB (µg/mL)**	**Conc. (µg/mL)**	**Vincristine**
1000	57.23 ± 0.00	48.04 ± 0.00	57.53 ± 0.01	100	64.34±0.02
100	48.95 ± 0.01	47.89 ± 0.02	37.80 ± 0.02	10	53.01± 0.02
10	19.72 ± 0.00	34.79 ± 0.00	13.10 ± 0.01	1	48.95±0.04
1	13.86 ± 0.00	0.00 ± 0.01	11.14 ± 0.00	0.1	40.97±0.03
0.1	3.31 ± 0.01	0.00 ± 0.06	9.34 ± 0.01	0.01	36.50±0.04
0.01	0. 00 ± 0.05	0.00 ± 0.04	0.00± 0.01	0.001	26.05±0.03
CC_{50} (µg/mL)	13.80 ± 0.01	7.74 ± 0.13	34.18 ± 0.05		0.34±0.17

Keys: HCB (n-hexane extract), ECB (ethyl acetate extract), MCB (Methanol extract) of *Cyperus bulbosus* bulb.

Table 3: Selectivity Index of extracts of *Cyperus bulbosus* bulb

Sample(s)	**IC_{50} (µg/mL,n=3)**				
	RD	SI	MCF-7	SI	VERO
HCB	14.17	6.98	13.80	7.17	98.90
ECB	11.52	2.17	7.74	3.23	25.03
MCB	7.83	15.14	34.18	3.47	118.60
Vincristine	0.03	2.33	0.34	0.21	0.07

Keys: HCB (n-hexane extract), ECB (ethyl acetate extract), MCB (methanol extract) of *Cyperus bulbosus*, SI: selectivity index, RD: rhabdomyosarcoma cells, MCF-7: Breast cancer cells, VERO: Normal African green monkey kidney epithelial cell line.

Table 4: Nitric Oxide Inhibitory effect of extracts of *Cyperus bulbosus* bulb

Conc. (µg/mL)	**% inhibition by HCB (µg/mL)**	**% inhibition by ECB (µg/mL)**	**% inhibition by MCB (µg/mL)**	**Conc. (µg/mL)**	**Ascorbic acid**
800	76.73 ± 0.00	66.61 ± 0.03	60.34 ± 0.01	100	81.35 ± 0.01
400	73.40 ± 0.00	59.51 ± 0.01	57.09 ± 0.00	50	78.46 ± 0.01
200	68.57 ± 0.01	57.99 ± 0.02	45.83 ± 0.03	25	75.26 ± 0.02
100	59.43 ± 0.01	54.75 ± 0.01	39.94 ± 0.04	12.50	72.59 ± 0.05
50	43.57 ± 0.01	47.34 ± 0.01	35.33 ± 0.02	6.25	67.71 ± 0.01
25	24.91 ± 0.00	17.58 ± 0.01	29.14 ± 0.00	3.13	62.79 ± 0.01
IC_{50} (µg/mL)	73.71± 0.03	53.85± 0.08	151.10 ± 0.11		83.39± 0.14

Keys: HCB (n-hexane extract), ECB (ethyl acetate extract), MCB (Methanol extract) of *Cyperus bulbosus* bulb.

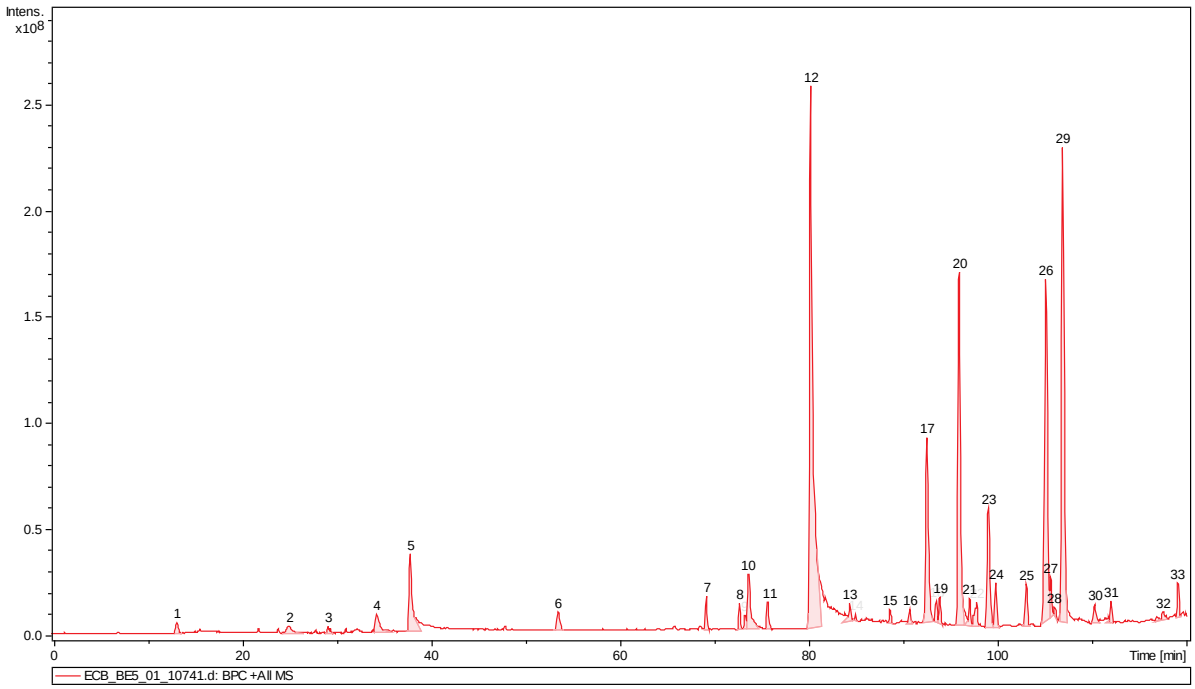

Figure 1: UHPLC-DAD-MS3 Chromatogram of ethyl acetate extract of *Cyperus bulbosus* bulb

Table 5: UHPLC-DAD-MS³ analyses data on ethyl acetate extract of *Cyperus bulbosus* bulb

Peak	Retention Time (min)	Chromatogram Area	Area (%)	+MS	Fragmentation MS
5	37.9	935025088	13.96	453.29	225.98, 336.14, 435.2
10	73.6	643994048	9.61	170.91	260.80, 277.05, 299.9
12	80.2	6698711552	100.00	401.15	200.79,329.15,365.06,383.1
17	92.5	1761381760	26.29	433.29	155.80, 313.18, 456.2
20	95.9	3037796352	45.35	403.15	180
23	99.0	1166329216	17.41	505.41	144.83,214.05,289.05
25	103.0	357532928	5.34	441.22	146.80,170.78,188.8,314.98
26	105.1	3475585024	51.88	282.10	134.99,265.05
29	106.9	4152202752	61.99	425.20	–
33	119.0	219183984	3.27	441.26	170.68,203.93,314.97,371.9

DISCUSSION

Potential candidates for combating drug resistance include chemosensitizers, which could be natural substances like phenolic derivatives, flavonoids, alkaloids, carotenoids, terpenoids, quinones, saponins, and steroids that can accumulate preferably within the tumour site and potentially produce systemic conventional therapeutic effects [27-29]. Previous research indicates that plant constituents can increase the duration that conventional chemotherapy remains effective in tumour cells. They can also induce cell death by up-regulating pro-apoptotic targets, promote DNA damage, or control the expression of both altered and unaltered drug targets. Even in cells that have developed acquired resistance, these pathways promote a synergistic impact by amplifying the lethal effect of anticancer medicines [12, 28]. To corroborate our findings, chemical components from the genus Cyperus have been reported to have cytotoxicity effects on different cancer cell lines. The extracts of *Cyperus rotundus* rhizomes were shown to inhibit the triple-negative breast cancer cells (TNBC) cell proliferation, Michigan Cancer Foundation-7 (MCF-7) breast cancer cell lines [30] and human cervical cancer (HeLa) cell lines [31]. Al-Nuari *et al.* [32] also reported the cytotoxic effect of *Cyperus conglomeratus* extracts in combination with silver nanoparticles on breast cancer (MCF-7), also the benzoquinones isolated from *Cyperus* sp. root demonstrated its anticancer activity on adenocarcinoma gastric (AGS) and human gastric cancer cell lines [33].

Furthermore, using organoleptic evaluation, it was observed that *C. bulbosus* has a pungent smell like sulphur which may play a role in its cytotoxic activity because sulphur-containing molecules have been gaining more attention in recent years on the road to the discovery of innovative anticancer drugs. It has been shown that in many human cell culture models, allium extracts and the phytochemical components they contain can cause apoptosis. Two proteomic studies that demonstrate the close relationship between apoptotic pathways and organosulfide-sensitive proteins provide further credence to this finding [34-35]. Brassica's chemopreventive properties are

ascribed to isothiocyanates (ITC) molecules, which are distinguished by an N=C=S functional group that contains sulfur. As cancer chemopreventive agents, sulforaphane (SFN) from broccoli, phenethyl isothiocyanate (PEITC) from watercress, and allyl isothiocyanate (AITC) from cabbage are often researched [36]. Numerous studies have demonstrated that diallyl sulfide, diallyl disulfide, diallyl trisulfide, and ajoene decrease human malignant cell viability (neuroblastoma, prostate cancer, lung tumour, and leukaemia cells) while not affecting human normal cell viability (human primary neurons, normal lung fibroblasts, normal prostate epithelial cells, and healthy mononuclear blood cells) [37-40]. Notably, some substances containing sulfur were the subject of clinical investigations. In three human volunteers' peripheral blood mononuclear cells, a single dose of 68 g of broccoli sprouts (or roughly 105 mg of sulforaphane) dramatically lowered histone deacetylase (HDAC) activity. The impact was observed 3 and 6 h after consumption and persisted for 24 to 48 h [41]. Additionally, 0.4% ajoene (17 mM) cream was used to treat a group of 21 individuals who had basal cell carcinoma, a nonmelanoma type of skin cancer. In 17 patients, the size of the tumour shrank, and this was linked to a drop in the expression of the B-cell lymphoma 2 (Bcl-2) protein [42].

The selectivity index is used in cancer studies to assess the effectiveness of a particular drug or treatment in targeting cancer cells while minimizing harm to normal healthy cells. It is important as it reduces the potential side effects and allows for safer and more effective cancer treatments [43]. *In vitro* experiments are categorized as non-selective (toxic) if the SI value is less than 1, weakly selective if the SI value is between 1 and 10, and safe (non-toxic) if the SI value is greater than 10 [44-45]. According to these results, HCB and MCB may contain bioactive chemicals that are highly selective for cancer cell lines and barely harmful to normal cells.

CONCLUSION

The components of ethyl acetate extract of Cyperus bulbosus have antiproliferative activity on rhabdomyosarcoma and breast cancer cell lines and nitric oxide inhibitory potentials. We therefore recommend the isolation of the molecules from the extracts which has the potential to form a template in the production of safer drugs for clinical use in the management of human cancer.

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Nitric oxide (NO) plays a pivotal role in many body functions; however, its overproduction can lead to cytotoxicity [46-49]. Therefore, NO inhibitors may be considered as a potential anti-cancer agent [50]. This shows that the extracts of *Cyperus bulbosus* bulb contain molecules that can prevent overproduction of nitric oxide within the cells thereby preventing carcinogenesis. Santhanamari *et al*. [51] corroborated our findings when they reported the radical scavenging activity of tubers of *C. bulbosus* on DPPH, Hydroxyl, ABTS and superoxide radicals. More so, so many other species in the genus Cyperus have demonstrated radical scavenging activity which may be due to flavonoids, tannins, and coumarins present in them [52-53].

Ultra High-Performance Liquid Chromatography (UHPLC) with Diode Array Detection (DAD) and Mass Spectrometry (HPLC-DAD-MS) is a very powerful analytical technique used in the identification, separation and quantification of complex mixtures of compounds. The compounds were represented based on their retention time (RT) which represents the elution time of the compound from the column, and the molecular ion peak (+MS) which represents the ion produced when the molecule is ionised by loss of an electron from the molecule. The m/z of this ion corresponds to the molecular weight (MW) of the compound and the area (%) which gives information on the degree of abundance. Two sesquiterpenoids, caryophyllene oxide (+MS, 220 g/mol) and humulene oxide (+MS, 220 g/mol) have been isolated from the essential oil of *C. bulbosus* [54] and Tricin7-O-beta-D-glucoside (+MS, 492.4 g/mol), Luteolin 7-O-beta-D-glucoside (+MS, 448.38 g/mol) both glycosyloxyflavone and Apigenin (+MS, 270.05 g/mol) is a flavonoid belonging to the flavone structural class, Luteolin 7-glucuronide (+MS, 462.4 g/mol), a luteolin glucosiduronic acid have been isolated from the leaves of *C. bulbosus* [55-57].

AUTHORS' CONTRIBUTION

Onoja O. Joel designed the experiment, supervised the research, interpreted the UPHPLC-MS^3 data and wrote the first draft of the manuscript, Ogbonna Sandra Esomchi, collected and extracted the plant and Abu Thomas performed the bioassay *in vitro*. All authors read and approved the manuscript.

CONFLICT OF INTEREST

There are no conflicts of interest.

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