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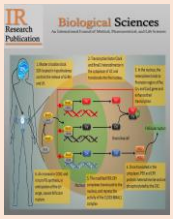

Anticancer Effect of Aqueous and Ethanol Extract of Pepperfruit (*Dennettia tripetala*) fruit on MCF-7 Breast Cancer Cell Line using Neutral Red Uptake Assay

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ABSTRACT

Breast cancer is the second leading cause of death in women. Natural products from plants and microorganism have provided the main source of folk medicine with a larger percentage of prescribed drugs containing compounds derived from plants, some being of great significance to cancer therapy. The significance of the study was to provide additional information on the potency of *Dennettia tripetala* against cancer. The study was aimed at evaluating the anticancer activity of aqueous and ethanol extract of *Dennettia tripetala* fruit against MCF-7 cell lines. About 150 g of the pulverized plant fruit was soaked in 1.5 litres of distilled water and alcohol to obtain aqueous and ethanol extracts. The extracts were concentrated to 3.125, 6.25, 12.5,25.0 and 50.0 µg/ml and used for experiment. Phytochemical analysis indicated presence of glycoside, saponins, steroids and triterpenes in the aqueous extract. All were present in the ethanol extract including tannins and excluding saponins. MCF-7 cell was cultured and then subcultured at confluence before treating with 5-fluorouracil drug as positive control, dimethyl sulfoxide as negative control and the various extracts of aqueous and ethanol extracts of *Dennettia tripetala*. The cytotoxicity assay was determined using neutral red uptake assay after 72 hours of treatment and the half inhibitory concentration was obtained from the linear regression equation after plotting a dose response curve of percentage cell inhibition. The result obtained from the cytotoxicity assay showed statistically significant increase ($P<0.05$) for percentage cell inhibition by the aqueous and ethanol extracts of *Dennettia tripetala* against the MCF-7 cell line at 25.0 and 50.0 µg/ml. The half inhibitory concentration was 37.42 and 61.74 µg/ml for aqueous and ethanol extract respectively. In conclusion, there is anticancer potency of *Dennettia tripetala* fruit against MCF-7 cancer cell line.

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Abrreviations: *5-FU: 5-fluorouracil; AS :Aqueous Extract; CAMRET: Centre for Advanced Medical Research and Training; D. tripetala: Dennettia tripetala; DMSO: Dimethyl sulfoxide; ES: Ethanol Extract; IC_{50}: Half-maximal inhibitory concentration; KMST-6: Non-tumorigenic immortalized human fibroblast cell lines; MCF-7: Michigan Cancer Foundation-7; MEM/EBSS: Eagle's Minimum Essential Media/Earle's Balanced Salt Solution; NCI: American National Cancer Institute; PBS: Phosphate buffer.*

Introduction

Cancer, a major public health challenge worldwide, has in recent years increased in its morbidity and mortality rate. Cancer is a class of diseases in which a group of cells display uncontrollable growth, invasion and sometimes metastasis [1-2]. American Cancer Society in 2007 estimated that 7.6 million people died from cancer [1-2].

Over 1 million new cases of breast cancer which constitutes a global major public health issue, is diagnosed annually and accounts for over 400,000 deaths annually [3]. About 4.4 million women are living with breast cancer, with 1 in 8 women being affected, making this the commonest cause of cancer mortality in women worldwide [3]. However, breast cancer can also occur in men. In Nigeria, there are about 100,000 new cases of cancer occurring every year [4]. Approximately, in 20% of Africa's population, and slightly more than half of the West Africa population, Nigeria contributed 15% to the estimated 681,000 new cases of cancer in Africa in 2008 [5]. Between 1990 to 2010 women at risk of breast cancer increased from about 24.5 million to 40 million and was projected to rise above 50 million in Nigeria by 2020 [6].

The risk of breast cancer has been associated with age, older women being more exposed, late menopause (after age 55), family history, especially close relative, early onset of menstrual period in children younger than age 12, being overweight or having dense breasts, hormone replacement therapy, birth control pill, prolonged exposure of estrogen in females, drinking alcohol and having children later than age 35 or not having children at all [3].

Nahata stated that "the use of plants as medicine is as old as human civilization" and may provide answer to the urgent need for cancer treatment and management [2]. Sixty percent of anticancer drugs consist of plants as a major source [2]. Natural products from botanical species like plants and microorganism have provided the main source of folk medicine and consist of a larger percentage in prescribed drugs with compounds derived from plants being of great significance to cancer therapy [2]. Between 1940 and 2002, 40% of drugs used for the treatment of cancer were natural products or derived from natural product with another 8% considered natural product mimics [7]. The World Health Organization stated that "herbal remedies are the most popular form of traditional medicines and result in billions of dollars of revenue worldwide" [8].

There is recognition of the potential treatment of cancer from plants from the scientific community with a vast body of work relating to possible treatments derived from plants [9-10]. Numerous studies and works are focused on plant derived compounds that have the potential to cure diseases and that have been used widely in traditional medicines [11].

Dennettia tripetala also known as pepperfruit belongs to the family Annonaceae [12-13]. *D. tripetala* is grown in the rain forest zones of Nigeria, some part of West Africa and sometimes in Savannah with its girth being about 0.6m, height ranging from 12-15m, the leaves 3-6 inches long and 1.5-2.5 inches broad [14-17]. Some of its local names includes; "Nmimi" in Igbo, "Igbere" in Yoruba and "Nkarika" in Efik [18]. The fruit has elliptic shape and is mainly made up of the seed and spicy flesh [13]. The fruit is usually green but appears red when it is ripe. The fruits, leaves, bark and root of the plant have a strong peppery and pungent taste. The fruit is edible and can be consumed in any form, fresh green, fresh ripened red, black dry fruit and dry seed [19]. Its fruits and leaves can serve as spice and seasoning for food e.g., soup, meat, local dishes [20-21]. The essential oil, from the fruit is effective in the preservation of grains such as cowpea and maize without negative effect on the grains [22].

In southern Nigeria, some communities use the leaves, roots, and fruits for medicinal purpose [14, 23-24]. Widely consumed by West Africans, *D. tripetala* is traditionally used to treat fever, diabetes, nausea, tooth ache and sore throat [13].

D. tripetala has been shown to possess properties consisting of anticancer [10]; analgesic/anti-inflammatory [21]; antifungal [25]; antimicrobial [25-27]; antihyperglycemic [28]; antioxidant [18, 29-30] and insecticidal effects.

Trace elements of minerals and water-soluble vitamins can be found in *D. tripetala* [17-31]. Phytochemicals from the ethanol extract show the presence of tannins, alkaloids, steroids, flavonoids, cardiac glycosides, saponins, and terpenoids [32]. Flavonoids in fruits and vegetables have been noted to decrease the risk of cancer [33-34]. Alpha-linoleic acid has been shown to reduce the risk of prostate cancer in men as well as cardiovascular diseases [35].

The aim of the study was to determine the anticancer effect of aqueous and ethanol fruit extracts of *Dennettia tripetala* on breast cancer cell, MCF-7.

Materials and methods

Plant material and authentication

Pepperfruit (*Dennettia tripetala*) fruit was obtained from Gosa Bush Market, Abuja, Nigeria and authenticated at the Herbarium, Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. The voucher number provided was ABU02737.

Preparation and concentration of plant extracts

The plant extraction was carried out at the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria. The fruit was washed to remove debris or dust and then air dried. The dried fruit was pulverized into fine powder and 150 g of the ground fruit was soaked in 1.5 L of distilled water and ethanol. The extracts obtained were concentrated serially in a 1:2-fold dilution method at the laboratory, Centre for Advanced Medical Research and Training (CAMRET), Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria, to obtain 3.125, 6.25, 12.5, 25.0 and 50.0 µg/ml solutions using distilled water and pure ethanol, respectively. The extracts were stored at 4°C until when needed. The concentration 50.0 µg/ml was adopted from Rohin et al. [36].

Phytochemical analysis

Phytochemicals were analyzed qualitatively and tests for secondary metabolites (alkaloids, anthraquinones, flavonoids, glycosides, saponins, steroids, triterpenes and tannins) were carried out using the methods described by Trease and Evans [37-38].

Test for anthraquinones (Modified Borntrager's test)

One gram of the extract was mixed with 5 mls of 10% hydrochloric acid for 3 minutes. The mixture was boiled and filtered. After cooling, the filtrate was extracted with 5mls of 99.9 % benzene. The benzene portion was pipetted off and, in a test, tube gently shook with 2 mls of 10% ammonium hydroxide. The production of pink-red colouration indicated presence of anthraquinones.

Test for alkaloids

Dragendoff's test

Half a gram of the powdered extract was dissolved in 10 ml of distilled water and 3 drops of Dragendoff's reagent were added to the solution. Redish brown precipitate indicated the presence of alkaloids.

Wagner's test

One gram of each portion of the extract was dissolved in 10 ml of distilled water in a test tube and 2 drops of Wagner's reagent were added to the solution. The presence of alkaloids was indicated by the presence of a whitish precipitate.

Test for flavonoids

Sodium hydroxide test

Half a gram of the powdered extract was dissolved in 5 ml of water in a test tube. Three drops of 5% sodium hydroxide were added to the solution. The presence of flavonoid was indicated by the appearance of yellow colouration.

Test for cardiac glycosides

Kella-Killiani test

Half a gram of the powdered extract was dissolved in 2 ml glacial acetic acid in a test tube. While holding the test tube at an angle of 45 degrees, 1ml of 99.9 % sulphuric acid was added down the side using a pipette. The appearance of a purple ring colour at the interface was indicative of the presence of cardiac glycosides.

Test for saponins (frothing test)

Half a gram of the powdered extract was dissolved in 10 ml of distilled water. For 30 seconds the mixture was vigorously shaken and stood for 30 minutes. The presence of saponins was noted by the formation of a honey-comb froth that persisted for more than 30 minutes.

Test for steroids triterpenes (Lieberman-Burchards test)

One mililitre of 99.9 % acetic anhydride was added to 1 ml of the extract. 1 ml of 99.9 % sulphuric acid was then added down the side of the test tube. Immediately after the addition of the acid, red, pink or purple colour observation indicated the presence of triterpenes while blue or blue-green colouration afterwards was indicative of the presence of steroids.

Test for tannins (Ferric chloride test)

One gram of the extract was dissolved in 10 ml of distilled water and the solution was filtered. Two drops of ferric chloride solution were added to the filtrate. The presence of hydrolysable tannins was indicated by a blue-black precipitate, while a green precipitate indicated that condensed tannins were present.

Cell line

Michigan Cancer Foundation-7, MCF-7 breast cancer cells and KSMT-6, non-tumorigenic immortalized human fibroblast cells were obtained from the laboratory where the research was carried out in the Centre for Advanced Medical Research and Training (CAMRET), Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria.

Cell culture

The cell line (MCF-7) was grown in a T-25 flask using MEM/EBSS supplemented with 2.00 mM L-Glutamine, 10% foetal bovine serum, Penicillin (100 IU/ml), and Streptomycin (100µg/ml) and incubated at 37°C in 5% CO_2 incubator. After 80-90% confluence, the cells were subcultured. Cell culturing was conducted according to the method described by Ramya et al. [39].

Cytotoxic assay using Neutral Red Reagent

KMST-6 and MCF-7 cells containing 2 x 104 cells/well were seeded into a 96-well culture plate and grown at 37˚ C in a humidified 5% CO_2 incubator. Following an overnight incubation, cells were treated with aqueous and ethanol extract of *D. tripetala* at different concentrations (3.125, 6.25, 12.5, 25.0 and 50.0 µg/ml). 5-fluorouracil at the concentrations of 3.125, 6.25, 12.5, 25.0 and 50.0 µg/ml was used as standard positive control and DMSO (1 µl/ml) was used as negative control. The samples were incubated for 72 hours. The medium was decanted and the plates rinsed with PBS (100 µl) to remove residue of the medium. Neutral red solution (100 µl) was added to the plate after centrifuging (5000 rpm for 5 minutes) and incubated for 3 hours. The dye was decanted and washed again with PBS (100 µl). Neutral red de-staining solution was added to extract the dye within the cells for 10 mins. Absorbance was measured at 630 nm using a microplate reader. Each test was done in triplicate [40].

% Cell Inhibition= (optical density of control cells-optical density of treated cells) / optical density of control cells x 100

Statistical analysis

Data obtained were expressed as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) followed by the Holm-Sidak method was used to compare differences using SigmaStat3.5 software (Systat Inc., Chicago, IL). $P < 0.05$ was considered statistically significant.

Results

Phytochemical analysis for *Dennettia tripetela* fruit extracts

The result for the phytochemical analysis for the aqueous and ethanol extracts of D. tripetela fruit is shown in Table 1. Alkaloids, anthraquinones and flavonoids were absent in both extracts. Glycosides, steroids and triterpenes were present in both the aqueous and ethanol extracts of *D. tripetala* fruit. Glycosides were in abundant amount in the ethanol than in the aqueous extract of the fruit. Saponins were present only in the aqueous extract of the fruit while tannins were present only in the ethanol extract.

Table 1. Phytochemicals in the aqueous and ethanol extract of *Dennettia tripetala* fruit.

Phytochemicals	Aqueous Extract Inference	Ethanol Extract Inference
Alkaloids	-	-
Anthraquinones	-	-
Flavonoids	-	-
Glycosides	+	++
Saponins	+	-
Steroids	+	+
Triterpenes	+	+
Tannins	-	+

- =Absent, += Present, ++= Present in abundant amount

The half-maximal inhibitory concentration

The half-maximal inhibitory concentration, IC_{50} (µg/ml) for the treatment of MCF-7 and KMST-6 cells were obtained from the linear regression equation after plotting a dose response curve of % cell inhibition 72 hrs after treatment with the 5-fluorouracil, aqueous extract and ethanol extract of D.

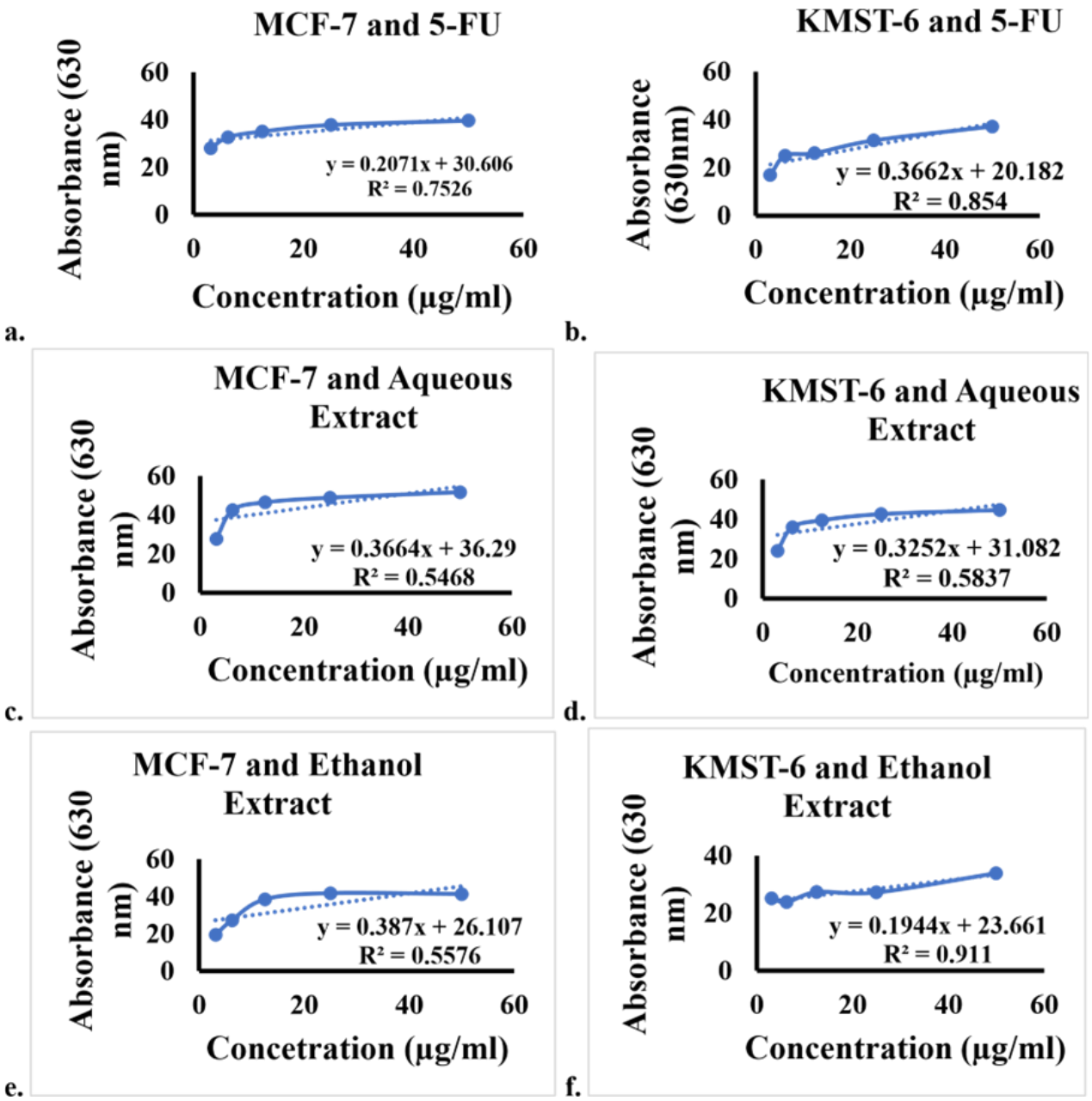

Figure 1. Dose response curve for % cell inhibition after 72 hrs of treatment, indicating increase in % inhibition proportional to concentration. a. MCF-7 cells to 5-fluorouracil b. KMST-6 cells to 5-fluorouracil c. MCF-7 cells to aqueous extract of D. tripetala d. KMST-6 cells to aqueous extract of D. tripetala e. MCF-7 cells to ethanol extract of D. tripetala f. KMST-6 cells to ethanol extract of D. tripetala. The halfmaximal inhibitory concentration (IC_{50}) was obtained from the linear regression equation, where y= % cell inhibition and x= concentration.

tripetala fruit. The dose response curves are shown in the figure 1a-f.

The result for IC_{50} is shown in Table 2. The lower the IC_{50}, the higher the cytotoxicity of the treatment agent. The treatment with the lowest IC_{50} for the MCF-7 cell line was the aqueous extract of *D. tripetala*, 37.42 µg/ml while the highest was the 5-fluorouracil with 93.65 µg/ml. The lowest IC_{50} for the KMST-6 cell line was the aqueous extract of *D. tripetala*, 58.17 µg/ml while the highest 135.49 µg/ml was from the ethanol extract of *D. tripetala*. The IC_{50} for the aqueous extract, was also lower than that of the 5-fluorouracil (81.43 µg/ml) for KMST-6 cells.

Cell cytotoxicity assay

MCF-7 and KMST-6 cell lines were treated with 5-fluorouracil, aqueous and ethanol extracts of *Dennettia tripetala*. After 72 hours of treatment, % cell inhibition was obtained using neutral red uptake (NRU) assay. Results for % cell inhibition for MCF-7 and KMST-6 cells for the treatments are shown in Tables 3 and 4, respectively.

Table 2. The half-maximal inhibitory concentration (IC_{50}) of treatments against MCF-7 and KMST-6 cell lines.

Cell lines	Treatment	IC50 (µg/ml)	Correlation coefficient(R^2)
MCF-7	5-Fluorouracil	93.65 µg/ml	0.75
	AS of *D. tripetala*	37.42 µg/ml	0.55
	ES of *D. tripetala*	61.74 µg/ml	0.55
KMST-6	5-Fluorouracil	81.43 µg/ml	0.85
	AS of *D. tripetala*	58.17 µg/ml	0.58
	ES of *D. tripetala*	135.49 µg/ml	0.91

AS= Aqueous Extract, ES= Ethanol Extract, MCF-7= Michigan Cancer Foundation-7, KMST-6= Non-tumorigenic immortalized human fibroblast cell lines.

Table 3. Percentage cell inhibition for MCF-7 cancer cell line after treatment for 72 hours.

Concentration (µg/ml)	5- Fluorouracil	Aqueous extract of *D. tripetala*	Ethanol extract of D. tripetala
3.125	28.00±1.47	27.62±0.61	19.39±0.10
6.25	32.65±0.87	42.41±0.27^{a}	27.24±0.88^{a}
12.5	35.05±2.11	46.42±0.23^{a}	38.40±0.16^{ab}
25.0	37.79±2.48^{a}	48.82±1.50^{ab}	41.71±0.40^{ab}
50.0	39.60±1.53^{a}	51.67±0.58^{ab}	41.29±0.27^{abc}

ANOVA followed by Holm-Sidak method; Result is expressed as mean ± standard error of mean. a, b, c, d and e indicate statistically significant difference when compared to 3.125, 6.25, 12.5, 25.0, 50.0 µg/ml respectively (P< 0.05).

Table 4. Percentage cell inhibition for KMST-6 fibroblast cell line after treatment for 72 hours

Concentration (µg/ml)	5-Fluorouracil	Aqueous extract of *D. tripetala*	Ethanol extract of *D.tripetala*
3.125	16.90 ±1.55	24.11±1.10	25.08±0.22
6.25	24.10±0.99^{a}	36.00±1.52^{a}	23.77±1.03
12.5	26.02±1.77^{a}	39.49±1.29^{a}	27.27±1.08
25.0	31.32±1.73^{ab}	42.61±0.076^{ab}	27.16±3.88
50.0	37.14±0.97^{abc}	44.70±1.86^{ab}	33.85±2.05^{b}

ANOVA followed by Holm-Sidak method; Result is expressed as mean ± standard error of mean. a, b, c, d and e indicate statistically significant difference when compared to 3.125, 6.25, 12.5, 25.0, 50.0 µg/ml respectively (P < 0.05).

There was significant increase in the % cell inhibition of MCF-7 cells treated with the aqueous extract of *D. tripetala* when other concentrations were compared to 3.125 µg/ml. Significant increase was also observed when 25.0, 50.0 µg/ml were compared to 6.25 µg/ml. For the ethanol extract, % cell inhibition increased significantly when all other concentrations were compared to 3.125 µg/ml. Also, there was significant increase when 12.5, 25.0 and 50.0 µg/ml were compared to 6.25 µg/ml and when 25.0 and 50.0 µg/ml were compared to 12.5 µg/ml. The 5-fluorouracil showed significant increase only when 25.0 and 50.0 µg/ml were compared to 3.125 µg/ml. The aqueous extract of *D. tripetala* showed higher % cell inhibition than the ethanol extract and 5-fluorouracil for MCF-7 cells. For instance, at 50.0 µg/ml, the aqueous extract of *D. tripetala* had an inhibition of 51.67 ± 0.58 percent while the ethanol extract and 5-fluorouracil were 41.29 ± 0.27 and 39.60 ± 1.53, respectively (Table 3).

For the KMST-6 cells, there was significant increase in the % cell inhibition when all other concentrations were compared with 3.125 for the aqueous extract of *D. tripetala* fruit. Significant increase was also observed when 25.0 and 50.0 µg/ml of the aqueous extract of *D. tripetala* were compared to 6.25 µg/ml. For the ethanol extract, % cell inhibition increased significantly when only 50.0 µg/ml was compared to 6.25 µg/ml. The 5-fluorouracil showed significant increase when all other concentrations were compared to 3.125 µg/ml. There was also significant increase when 25.0 and 50.0 µg/ml were compared to 6.25 µg/ml, and when 50.0 µg/ml was compared to 12.5 µg/ml. The aqueous extract of *D. tripetala* showed higher % cell inhibition than the ethanol extract and 5-fluorouracil for KMST-6 cells. At 50.0 µg/ml, the aqueous extract of *D. tripetala* had an inhibition of 44.70 ± 1.86 percent while the ethanol extract and 5-fluorouracil were 33.85 ± 2.05 and 37.14 ± 0.97, respectively (Table 4).

Discussion

One of the most common causes of cancer related death in women is breast cancer [41-42]. Breast

cancer can develop from mutation of genes influenced by exposure to radiation, hazardous chemicals or genetic factors [43-44], not excluding factors such as; food contamination, physical inactivity, late gestation (after 30 years of age), oral contraceptives, hormone therapy after menopause, and high intake of alcohol and tobacco [45]. Chemotherapy is one major way used in preventing and treating various cancers [39, 46-47].

The process of developing drugs targeting breast cancer cell without affecting the normal cells has been a challenging task [39]. Many conventional drugs have been produced to help combat this disease, and while successes have been recorded for some, increasing side effects as a result of the use of these drugs have been a major drawback [48]. Due to the limitations of clinical therapies including radiation, chemotherapy, immunomodulation and surgery in treating cancer, severe side effects such as bone marrow depletion, leukopenia, anaemia, alopecia, and hyperuricaemia, teratogenicity, carcinogenicity, and reduction in spermatogenesis in men, amenorrhea in women, the search for new cancer management is imperative [39].

The drug 5-fluorouracil (5-FU) is a known anticancer drug used for the treatment of solid tumours like breast cancer [49], which works by producing cytotoxic metabolites in the RNA and DNA and causing inhibition of thymidylate synthase to result in both the arrest of cell cycle and cancer cell death [50]. Chemotherapeutic drugs by interfering with the synthesis of DNA and mitosis process can lead to the death of cancer cells but, can end up resulting in the damage of normal healthy cells as the agents involved are non-selective to cells and possibly the adverse side effects associated with chemotherapeutic drugs [48].

In this study, 5-FU was used as a positive control to compare with the test agent, aqueous and ethanol fruit extract of Pepperfruit (*Dennettia tripetala*). Plants possess great potential to provide newer drugs [7]. The use of medicinal plant products to manage or arrest the carcinogenic process provides an alternative to the use of conventional allopathic medicine for treatment of the disease [9, 51-53].

Both the aqueous and ethanol extracts of *D. tripetala* fruit were found to contain steroid, triterpene and glycoside, however glycoside was more in the ethanol extract than in the aqueous. Steroids have good cytotoxicity potential towards cancer cells, with some even having the ability to cause DNA damage and apoptosis [54]. Triterpenes occur freely in plants and animals and possess anticancer properties capable to inhibit cell growth and proliferation, alter cell proteins to cause cytotoxicity, carcinogenesis inhibition and apoptosis [10, 54-55]. Metabolites like glycosides occurring naturally have demonstrated cytotoxicity against some cancer cell lines [56]. Saponin was found only in the aqueous extract of the plant fruit while tannin was in the ethanol extract alone. However, reports on phytochemical screening on *D. tripetala* fruits have indicated carbohydrate, tannins, alkaloid, sterol, terpenes, flavonoids, balsam and phenols [57] and specifically, the presence of alkaloids, flavonoids, tannins and saponins in its ethanol extracts and with the presence of phenols, flavonoids, saponins, tannins and alkaloids in both aqueous and ethanol extract of *D. tripetala* fruit [17, 58]. Studies have shown that factors like habitat, method of cultivation and presence of rainfall, can affect the presence or quantity of phytochemical component, resulting in variation of such components even in the same plants [59-60]. It is suggested that this may be a reason for the differences in these results from other studies. Also, the concentration of phytochemicals such as; phenols, saponins, tannins, flavonoids, and alkaloids in *D. tripetala* can undergo changes with ripening as reported earlier [17].

One of the most used cytotoxicity assays to test many biomedical and environmental applications is the neutral red uptake assay where viable cells incorporate and bind the neutral red dye as opposed to dead cells which cannot retain the dye [40, 61-62]. The neutral red dye was used to carry out cytotoxicity assay in this research. Following the assay, IC_{50} of the test drugs (aqueous and ethanol extract of *D. tripetala*) and 5-FU for both MCF-7 and KMST-6 cell lines were obtained from the dose-response curve of concentration on the X-axis to absorbance on the Y-axis. The IC_{50} known as the half-maximal inhibitory concentration is defined as the concentration that corresponds to 50% survival rate of cells in a well, and it is important for proper understanding of the pharmacological and biological characteristics of the chemotherapeutic agent undergoing study [63-66].

The aqueous extract of *D. tripetala* had the lowest IC_{50} for MCF-7 followed by the ethanol extract, suggesting that both extracts had more potency of cell inhibition on MCF-7 cancer cell than the 5-FU. For KMST-6 fibroblast cell, the aqueous extract of *D. tripetala* had a lower IC_{50} followed by 5-FU, indicating that it has more potential to be harmful to normal cells than both 5-FU and ethanol test extract. The ethanol extract of *D. tripetala* had the highest IC_{50} indicating less potency of cell inhibition. It is however important to note, that IC_{50} is dependent on some factors, one of which is the density of seeded cells in a well, the parameter and equation used to obtain the IC_{50}, can cause variation in the IC_{50} of a particular plant [66-67]. Moreover, as at the time this study was carried out not much work had been done to test *D. tripetala* extract on normal and cancer cell lines with the hope of ascertaining its IC_{50}, this limits our ability

to compare our findings with other studies. Onyancha et al. reported an IC_{50} of 38.8 ± 7.56 for HCC 1395 human breast cancer cell lines and 185.00 ± 75.0 for normal kidney epithelial cells from African green monkey (Vero E6) [68]. Akbari and Javar, also reported an IC_{50} of 1.3 µg/ml and 0.38 µg/ml after 24 and 48 hrs treatment of 5-FU against MCF-7 breast cancer cell line, respectively [67]. At 48 hrs after treatment, Hector et al., on transcriptional profiling of MCF-7 breast cancer cells in response to 5-flurouracil, reported the IC_{50} to be 10 µM, equivalent to 1.3 µg/ml [69]. Varying IC_{50} values have also been suggested to be as a result of the materials used, and the quantity of materials used, other than the number of cells seeded in each well [66-67].

The ethanol extract of *D. tripetala* having a lower IC_{50} than the 5-FU for MCF-7 and a higher IC_{50} than both the aqueous extract of *D. tripetala* and 5-FU for KMST-6, suggests that it can be a better treatment for breast cancer. This is because, the search for inexpensive drugs for cancer treatment, is not the only challenge. The challenge is finding inexpensive drugs that have minimal side effects to normal cells [39]. The American National Cancer Institute (NCI) guideline states that for IC_{50} of crude extracts to be considered to have high potential cytotoxic effect it should be ≤ 20 µg/ml after 48 to 72 hrs, to be considered to have moderate cytotoxic effect, it should range between 21-200 µg/ml and between 200-500 µg/ml for weak cytotoxic effect [70, 71]. The result for IC_{50} in this study for both aqueous and ethanol extracts on both cell lines, falls within the range of moderate cytotoxic effects.

The percentage of cell inhibition following the neutral red uptake for MCF-7 cancer cells indicated a statistically significant increase for the 5-fluorouracil, aqueous and ethanol extract of *D. tripetala* fruit at $P < 0.05$ when compared within groups. For the 5-FU, only the cells treated with 25.0 and 50.0 µg/ml show significant inhibition. The aqueous and ethanol extract of *D. tripetala* fruit showed better results of cell inhibition than the 5-FU. When a comparison was carried out between the three treatments, 5-FU, the aqueous and ethanol extract of *D. tripetala* fruit, there were also statistically significant differences. This thus supports the already known anticancer property of 5-FU on breast cancer [50, 72] and indicates the potency of the aqueous and ethanol extract of *D. tripetala* to inhibit viability of breast cancer, possibly as a result of the presence of triterpenes and alkaloids. A reviewed stated that 65% of the phytochemicals from West African plants possessing strong cytotoxicity against a panel of cancer cells included terpenes, steroids and alkaloids [54]. Similarly, for KMST-6 fibroblast cells, treatment with 5-FU, the aqueous and ethanol extract of *D. tripetala* fruit indicated cell inhibition as the dosage increased for all three treatments. However, statistically significant differences were noted with cells treated with only 50.0 µg/ml for the ethanol extract of *D. tripetala* fruit while for the aqueous extract of *D. tripetala* and 5-FU, statistically significant differences were observed for most concentrations.

Studies show that chemotherapeutic drugs like 5-FU may result in different conditions such as diarrhoea, stomatitis and gastrointestinal mucosal injury and if used for long can be toxic to normal healthy cells due to their non-specificity in protecting these cells [73-74]. The 5-FU has also been shown to cause myelotoxicity [75], and cardiotoxicity [76] and to act as a vasospastic agent in rare cases [77]. In a study carried out by Yusefi et al., almost all the nasopharyngeal normal (NP 460) and cancer (HONE-1) cells were killed by pure 5-FU at different concentrations [78]. Hence, this may suggest the reason 5-FU has the ability to bring about the inhibition of not only the MCF-7 cells but also the KMST-6 which are fibroblast cell lines and served as normal cell lines in this study. Aqueous and ethanol extract of *D. tripetala* increased inhibition of the MCF-7 and KMST-6. Although there is paucity of in vitro study to compare this work to, the level of inhibition for each extract was better on the MCF-7 then for KMST-6. Also, the ethanol extract of *D. tripetala* seemed to be mild on the KMST-6 cells, having a lower % cell inhibition at 12.5, 25.0 and 50.0 µg/ml than both the aqueous extract and 5-FU indicating that its impact on normal cell line, KMST-6, in comparison to both the aqueous extract and 5-FU was not as severe and may serve as a better treatment for breast cancer.

Conclusion

The aqueous and ethanol fruit extracts of Pepperfruit (*Dennettia tripetala*) possess phytochemicals with anticancer potentials on MCF-7 breast cancer cell line. There was increase in percentage inhibition as a result of cytotoxicity on the MCF-7 cells.

Contribution of authors

SID conceived the idea of the research and took part in the laboratory work. BD and ZMB supervised, proofread and provided linkage to the laboratory.

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Conflict of Interest

The authors declare there is no conflict of interest.

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