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Anticancer Effect of Aqueous and Ethanol Extracts of Pepper Fruit (*Dennettia tripetala*) on the Morphology of MCF-7 Breast Cancer Cell Line

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ABSTRACT

Cancer is a multifactorial disease caused by combined genetic and non-genetic changes. Breast cancer being the second leading cause of death in women. A large percentage of known anticancer medicines are derived from various plant species. *Dennettia tripetala* has been found to contain vitamins, minerals and phytochemicals that possess anticancer properties. The study was aimed at examining the anticancer activity of aqueous and ethanol extract of *Dennettia tripetala* fruit on the morphology MCF-7 cell lines. 150 g of the ground fruit was soaked in 1.5 L of distilled water and ethanol to produce aqueous and ethanol extracts respectively. Both extracts obtained were concentrated to 6.25, 12.5 and 25.0 µg/ml. Cell viability was determined using trypan blue and hemocytometer while morphological changes were examined, by using an inverted microscope. MCF-7 cell was cultured and subcultured at confluence before treating with, 5-fluorouracil drug as positive control, dimethyl sulfoxide as negative control and the extracts of *Dennettia tripetala*. Cell viability decreased significantly in a dose-time manner. Morphological observation showed distortion of the MCF-7 cell, after treatment with the extracts, especially at 25 µg/ml after 48 and 72 hrs. In conclusion, *Dennettia tripetala* fruit have potency to decrease the viability and result in morphological damage of MCF-7 cancer cells.

Keywords: *Dennettia tripetala*; cancer; cell viability; MCF-7 cell lines; trypan blue

INTRODUCTION

Cancer is a multifactorial disease caused by combined genetic and non-genetic changes induced by environmental factors that can trigger inappropriate activation or inactivation of specific genes leading to neoplastic transformations, or abnormal cell growth [1]. Estimation states that about 80% of cancers are due to environment or lifestyle and suggest that they are potentially preventable [2].

Breast cancer is the second most common cause of cancer related mortality in women worldwide but ranks fifth as cause of death accounting for about 23% (1.38 million) of the total new cancer cases and 14% (458,400) of the total cancer deaths in 2008 [3]. In Nigeria, 16% of all cancer related death is as a result of breast cancer [4]. Nigeria contributed about 15% to the estimated 681,000 new cases of cancer in Africa in 2008 [5]. Women at risk of breast cancer increased from about 24.5 million to 40 million between 1990 to 2010 and was projected to rise above 50 million in Nigeria by 2020 [6].

It has been reported that a large percentage of known anticancer medicines are derived from various plant species [7-8]. Herbal remedies are the most popular form of traditional medicines and result in billions of dollars of revenue worldwide [9]. The World Health Organization (WHO) states that consumption of fruits and vegetables can help prevent cancer, due to its composition with nutrients such as vitamins, minerals and fibre [10]. A vast body of work relating to possible treatments derived from plants has been recognized by the scientific community [11] with numerous studies and works focusing on plant derived compounds that have the potential to cure diseases and that has been used widely in traditional medicines [12].

Pepperfruit (*Dennettia tripetala*) is a medium sized tree commonly found in the tropical rainforest region of Nigeria and sometimes in savannah areas [13]. The leaves and fruits are known for its distinctive spicy taste [14]. In Igbo, the local name is "Nmimi", "Igbere" in Yoruba and "Nkarika" in Efik. The fruit has elliptic shape, usually green but appears red when it is ripe and is mainly made up of the seed and spicy flesh [15, 16]. The fruit is edible and can be consumed in any form, fresh green, fresh ripened red, black dry fruit and dry seed [17]. Its fruits and leaves can serve as spice and seasoning for food e.g., soup, meat, local dishes [18-19]. The essential oil, from the fruit is effective in the preservation of grains such as cowpea and maize without negative effect on the grains [20]. The fruits, leaves, bark and root of the plant have a strong peppery and pungent taste. In southern Nigeria, some communities use the leaves, roots, and fruits for medicinal purpose [21-23]. Widely consumed by West Africans, *D. tripetala* is traditionally used to treat fever, diabetes, nausea, tooth ache and sore throat [16].

Some properties *D. tripetala* has been shown to possess include anticancer, analgesic/anti-inflammatory, antifungal, antimicrobial, antihyperglycemic, antioxidant and insecticidal properties[11, 15, 19, 24, 25, 27-29]. *D. tripetala* contains trace elements of minerals and water-soluble vitamins and phytochemicals such as tannins, alkaloids, steroids, flavonoids, cardiac glycosides, saponins, and terpenoids in the ethanol extracts [13, 30, 31]. Studies show that flavonoids in fruits and vegetables have potential to decrease the risk of cancer [32-33]. Alpha-linoleic acid has been shown to reduce the risk of prostate cancer in men as well as cardiovascular diseases [34].

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

MATERIALS AND METHODS

Plant Material and Authentication

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

Preparation and Concentration of Plant Extracts

Preparation of the plants extract was carried out at the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The fruit was washed to remove debris or dust, air dried and then pulverized into fine powder. 150 g of the ground fruit was soaked in 1.5 L of distilled water and ethanol to produce aqueous and ethanol extracts respectively. Both extracts obtained were concentrated to 6.25, 12.5 and 25.0 µg/ml solutions and stored at 4°C until when needed.

Cell Line

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

Cell Culture

The MCF-7 cell line was grown in a T-25 flask using MEM/EBSS supplemented with 2.00 mM L-Glutamine, 10% fetal bovine serum, Penicillin (100 IU/ml), and Streptomycin (100 µg/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*.[35].

Experimental Design

MCF-7 cells were seeded in 6-well plates (3×10^4 cells/well) and treated following an overnight incubation under 5% CO_2 and at 37°C (Table 1). The experiment was done in triplicates.

Table 1: Experimental design

Well	Administration
1	MCF-7 cells + 2 µl DMSO (negative control)
2	MCF-7 cells + 6.25 µg/ml of 5-fluorouracil (positive control)
3	MCF-7 cells +12.5 µg/ml of 5-fluorouracil (positive control)
4	MCF-7 cells + 25.0 µg/ml of 5-fluorouracil (positive control)
5	MCF-7 cells + 6.25 µg/ml of aqueous *D. tripetala* fruit extract
6	MCF-7 cells +12.5 µg/ml of aqueous *D. tripetala* fruit extract
7	MCF-7 cells + 25.0 µg/ml of aqueous *D. tripetala* fruit extract
8	MCF-7 cells + 6.25 µg/ml of ethanol *D. tripetala* fruit extract
9	MCF-7 cells + 12.5 µg/ml of ethanol *D. tripetala* fruit extract
10	MCF-7 cells + 25.0 µg/ml of ethanol *D. tripetala* fruit extract

Cell Viability Assay

Cell viability was determined at 24, 48 and 72 hours using 0.4% Trypan blue staining and cell counting on a haemocytometer where non-viable cells stained blue and viable cells remain unstained. The method was adopted from Jagla[11].

Cell viability[36] =

$$\frac{\text{The number of unstained (viable) cells}}{\text{the total number of cells}} \times 100$$

Morphological Analysis

Morphological changes in the MCF-7 cancer cells after treatment was studied and observed under an inverted microscope (AmScope, USA) at 24, 48 and 72 hours. The method was adopted from Jagla[11]. The image background was adjusted using the AmScope software (AmScope, USA) during image acquisition, this was done to obtain a clear image of the cells for morphological examination.

Data Analysis

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

RESULTS

Cell Viability Assay

MCF-7 cells were treated with DMSO, 5-fluorouracil, aqueous and ethanol extract of *Dennettia tripetala*. After 24, 48 and 72 hours of treatment with 6.25, 12.5 and 25.0 µg/ml of all three treatments, cells were counted using 0.4 % trypan blue and a hemocytometer, in order to obtain % cell viability. Results for % cell viabilty for MCF-7 cells are shown in Fig. 1, 2 and 3.

MCF-7 cells treated with the 5-fluorouracil drug showed significant decrease in % viability at 24 hrs when 25.0 µg/ml was compared to DMSO and at 48 and 72 hrs when all three concentrations were compared to the DMSO. At 72 hrs after treatment there was significant reduction in cell viability when 12.5 and 25.0 µg/ml were compared to 6.25 µg/ml and when 25.0 µg/ml was compared to 12.5 µg/ml (Fig. 1a). Comparing the different time frame for each concentration, there was only significant difference when 72 hr was compared to 24 hr after treatment with 5-fluorouracil at 12.5 µg/ml (Fig. 1a). Other concentrations had no significant difference.

The aqueous extract of *Dennettia tripetala* had no significant decrease in cell viability at 24 hr except at 48 and 72 hr following treatment (Fig. 1b). At 48 hr, all three concentrations showed significant decrease in % viability when compared to DMSO and also when 25.0 µg/ml was compared to 6.25 µg/ml. At 72 hr, there was significant decrease in viability only when 25.0 µg/ml was compared to DMSO. However, there was no significant reduction when the differences in time was compared for all the three concentrations (Fig. 1b).

At 24 hr treatment with the ethanol extract of *Dennettia tripetala*, percentage viability of the MCF-7 cells decreased significantly when 12.5 and 25.0 µg/ml were compared to DMSO and when 25.0 µg/ml was compared to 6.25 µg/ml. There was significant decrease at 48 and 72 hr when all the concentrations were compared to DMSO. Cell viability also decreased significantly when 12.5 µg/ml was compared to 6.25 µg/ml at 48 hr, when treatment with

12.5 and 25 µg/ml was compared to 6.25 µg/ml at 72 hr and when 25 µg/ml was compared to 12.5 µg/ml at 72 hr (Fig. 1c). When comparing the time frame for all three concentrations (Fig. 1c), there was a significant reduction in percentage cell viability of the MCF-7 cells, when treatment after 48 and 72 hr were compared to 24 hr. There was also a significant reduction in the viability of the MCF-7 cells when treatment after 72 hr was compared to 48 hr for both 12.5 and 25 µg/ml of the ethanol extract of *Dennettia tripetala* fruit.

The decrease in cell viability for all treatment occurred in a time-dose dependent manner. The 5-fluorouracil (Fig. 1a) had the highest reduction in % cell viability, for the different time frames, next to it was the ethanol extract of *Dennettia tripetala* fruit (Fig. 1c). Dimethyl sulfoxide (DMSO), was used as negative control throughout the period of the test. Although, there was decrease in % cell viability, there was however, no significant difference.

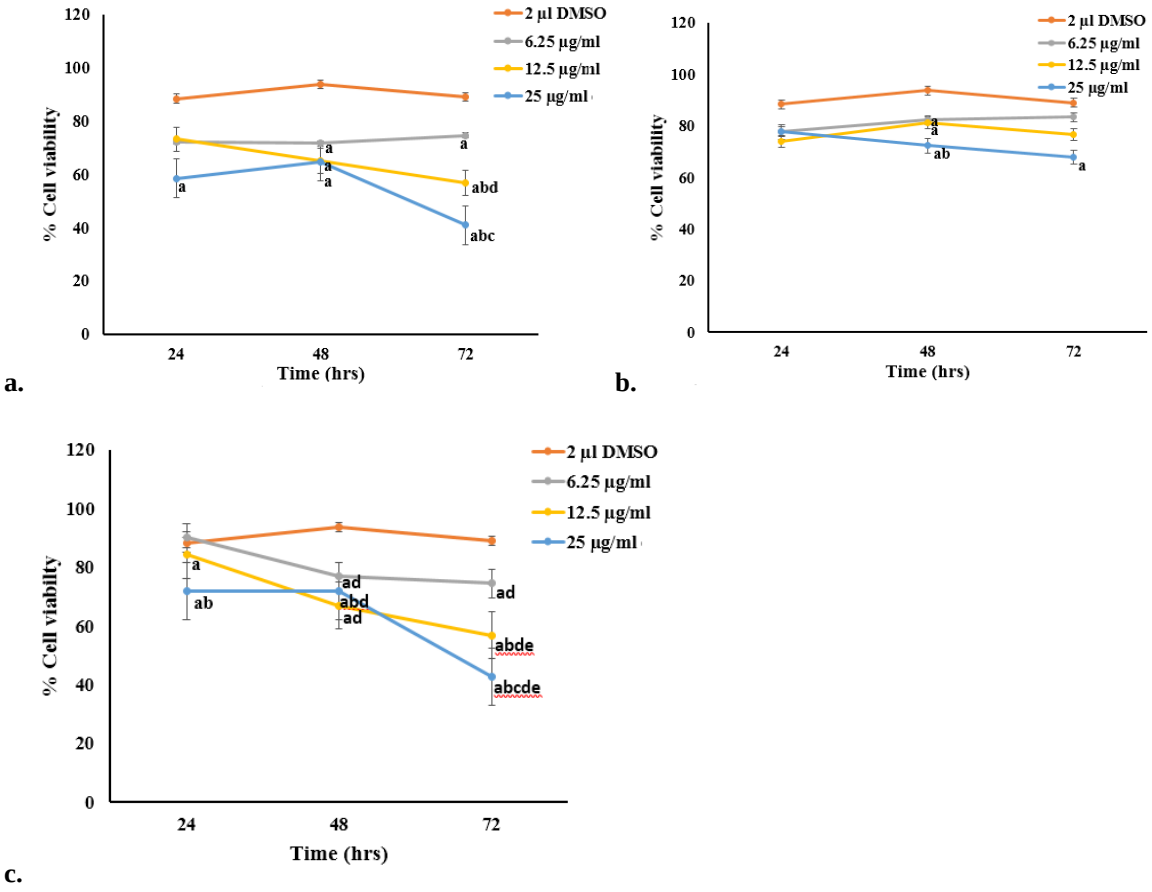

Figure 1: Percentage cell viability of Michigan Cancer Foundation-7 (MCF-7) cells treated with a. 5-fluorouracil b. aqueous extract of *D. tripetala* c. ethanol extract of *D. tripetala*

'a', 'b' and 'c', indicate statistically significant difference when compared to DMSO, 6.25 µg/ml, 12.5 µg/ml and 25.0 µg/ml respectively ($P < 0.05$). 'd' and 'e', indicate statistically significant difference within the group when compared with treatment after 24 and 48 hrs respectively ($P < 0.05$). Statistical differences were analysed by one-way ANOVA test followed by Holm-Sidak post hoc test.

Morphological Analysis

The changes in the morphology of the MCF-7 cells after exposure at 24, 48 and 72 hours to the negative control, dimethyl sulfoxide (DMSO), the positive control, 5-fluorouracil, the aqueous and ethanol extracts of *Dennettia tripetala* fruit are shown in Figure 2-5. The micrographs were taken using an inverted microscope with AmScope software, which adjusted the background for clear image observation. The morphological changes that occurred in the micrographs taken after exposure to all three treatments, were based on a time-dose dependent manner.

The micrographs taken for MCF-7 cells exposed to DMSO at 24, 48 and 72 hr, are shown in Figure 2a-c. Many cells retained their normal polygonal shape while distortion occur in few cells, where the cells lost their normal shape to become elongated or round. The presence of round cells indicated loss of membrane integrity, apoptosis and detachment for the culture plate.

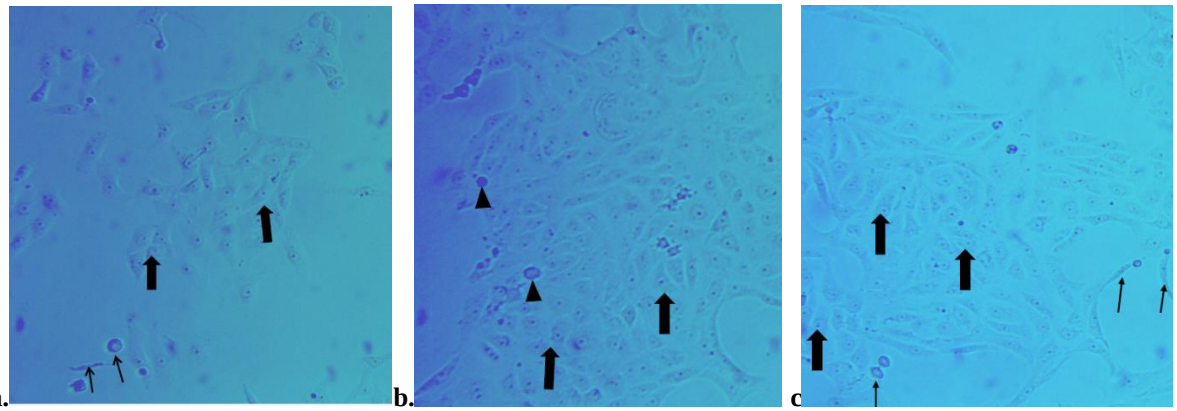

Figure 2: Michigan Cancer Foundation-7 (MCF-7) cells exposed to 2 µl of Dimethyl sulfoxide (DMSO) after a) 24 hrs b) 48 hrs c) 72 hrs (x100).

Many cells retained normal polygonal shape (thick arrows), distorted cells (thin arrows and arrow head). *Micrographs were taken using an inverted microscope with AmScope software*.

The exposure of MCF-7 to 5-fluorouracil is shown in Figure 3a-i. After exposing the cells to 6.25 µg/ml of 5-FU for 24 hr (Figure 3a), MCF-7 cells were greatly distorted and appeared scanty when compared to DMSO treated cells (Figure 2a-c). These distortions with presentations of elongation or change in shape to become round, increased with longer exposure at 48 and 72 hr (Figure 3b and 3c respectively). MCF-7 cells exposed to 12.5 µg/ml of 5-FU (Figure 5d-f) showed more cellular damage than 6.25 µg/ml (Figure 3a-c) at 24 hrs. The number of cells was more reduced and there was almost no trace of normal polygonal shaped MCF-7 cells. At 48 and 72 hr exposure (Figure 3e and 3f respectively), elongation of the MCF-7 cells, round apoptotic cells, detachment from the culture plate and floating of cells increased. At 24, 48 and 72 hr exposure to 25 µg/ml of the 5-FU (Figure 3g, 3h and 3i respectively), cellular damage to the MCF-7 cells was greater than at 12.5 µg/ml. The cells were scantier and floating at 72 hr after exposure. MCF-7 cells with normal polygonal shape were absent (Figure 3i).

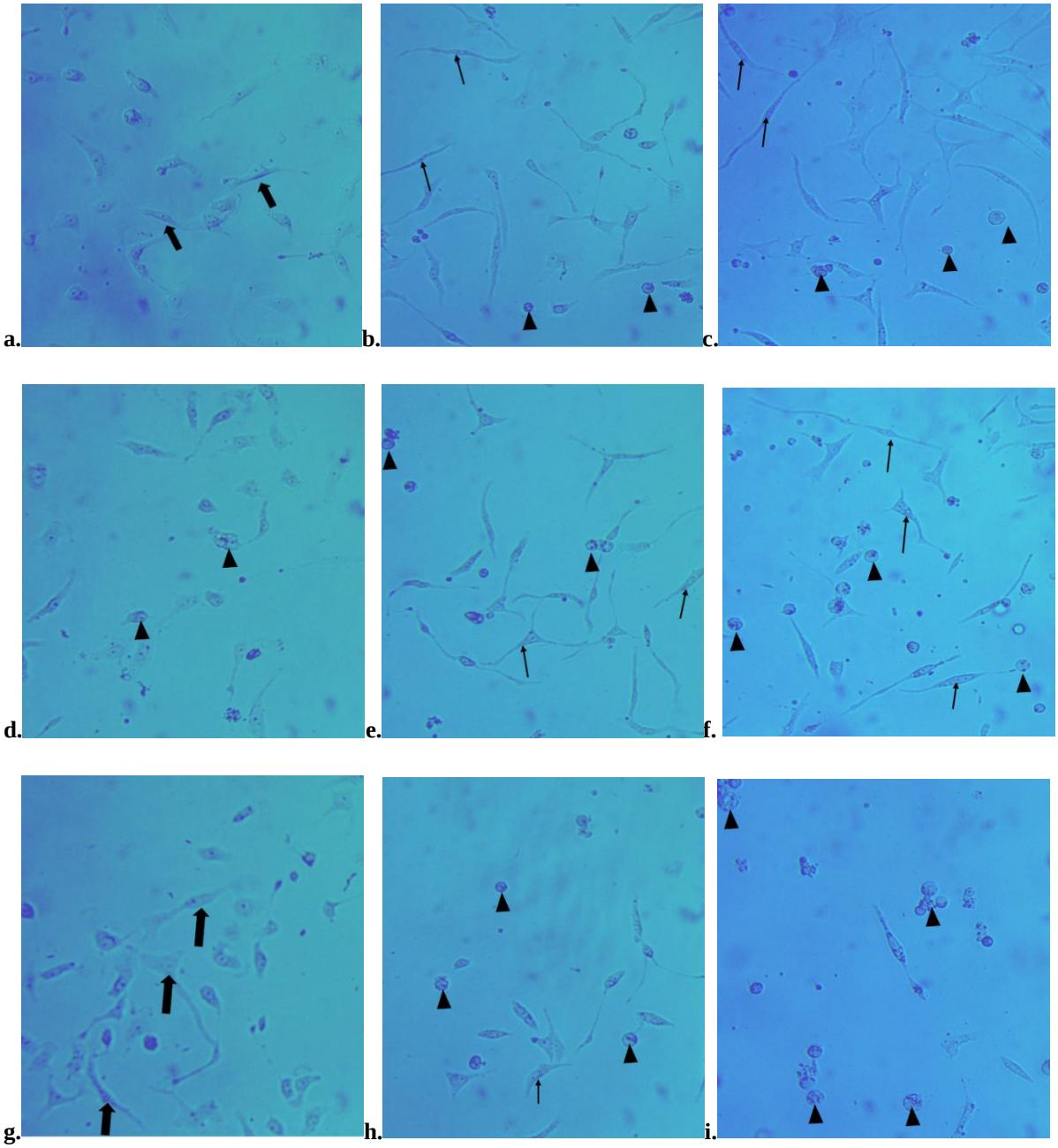

Figure 3: Michigan Cancer Foundation-7 (MCF-7) cells exposed to 5-fluorouracil (5-FU). a) 24 hrs b) 48 hrs c) 72 hrs of 6.25 µg/ml, d) 24 hrs e) 48 hrs f) 72 hrs of 12.5 µg/ml and g) 24 hrs h) 48 hrs i) 72 hrs of 25 µg/ml (x100).

Normal polygonal shape of cells was distorted (arrows). Arrowhead indicate apoptotic cells. Cells appeared scanty. *Micrographs were taken using an inverted microscope with AmScope software*.

The micrographs taken, following the exposure of MCF-7 cells to the aqueous extract *D. tripetala* fruit, are shown in Figure 4a-i. At 24 hr post-exposure to 6.25 µg/ml of the aqueous extract (Figure 4a), most cells retained their polygonal shape. However, after 48 and 72 hr (Figure 4b and 4c respectively), cellular damage was evident. The cellular damage increased over time with an increase in the concentration of the aqueous extract. Figures 4d, 4e and 4f exposed to 12.5 µg/ml of the aqueous extract, at 24, 48 and 72 hr respectively, showed that more of the MCF-7 cells had changed from polygonal shape to round shape with signs of cellular disintegration in Figure 4d and 4e. Exposure to 25 µg/ml of the aqueous extract after 24 hr showed presence of damaged cells (Figure 4g), with elongated and round apoptotic cells more evident at 48 and 72 hr post exposure (Figure 4h and 4i respectively).

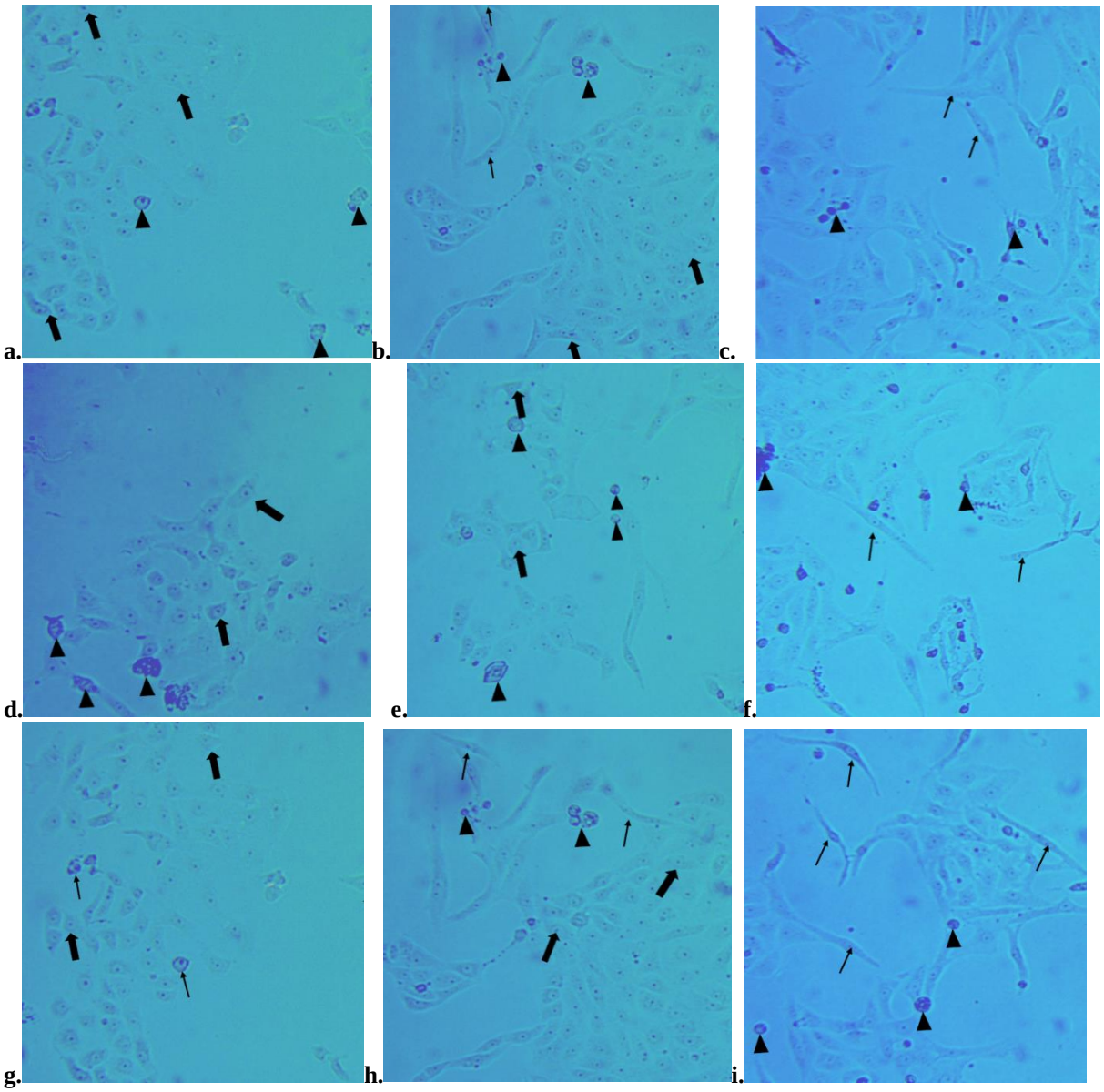

Figure 4: Michigan Cancer Foundation-7 (MCF-7) cells exposed to aqueous extract of *D. tripetala* fruit. a) 24 hrs b) 48 hrs c) 72 hrs of 6.25 µg/ml, d) 24 hrs e) 48 hrs f) 72 hrs of 12.5 µg/ml and g) 24 hrs h) 48 hrs i) 72 hrs of 25 µg/ml (x100).

Normal polygonal shaped cells (thick arrows), appearance of cellular damage, elongation of cells (thin arrows) and change in shape (arrow heads). *Micrographs were taken using an inverted microscope with AmScope software*.

The MCF-7 cells exposed to ethanol extract of *D. tripetala* fruit, have their micrograph shown in Figure 5a-c. Figure 5a exposed to 6.25 µg/ml of the ethanol extract for 24 hr, showed many cells retained their polygonal shape. Similarly, Figure 5b exposed to 6.25 µg/ml for 48 hr showed the same appearance but, with minimal distortion as cells appeared slightly stretched. There was increased distortion observed at 72 hr post exposure to the ethanol extract (Figure 5c). There was the presence of elongated and round apoptotic cells. The micrographs of MCF-7 treated with 12.5 µg/ml of the ethanol extract after 24 hr (Figure 5d) did not show much distortion. Only a few cells appeared round. However, after 48 and 72 hr exposure to the ethanol extract (Figure 5e and 5f respectively), there was a proportional increase in distortion based on time, with cells stretching and becoming round. At 24 hr post exposure of the MCF-7 cells to 25 µg/ml of the ethanol extract, there was already the presence of disintegrating cells (Figure 5g). At 48 and 72 hr post-treatment, stretched and round-shaped apoptotic cells were present (Figure 5h and 5i respectively).

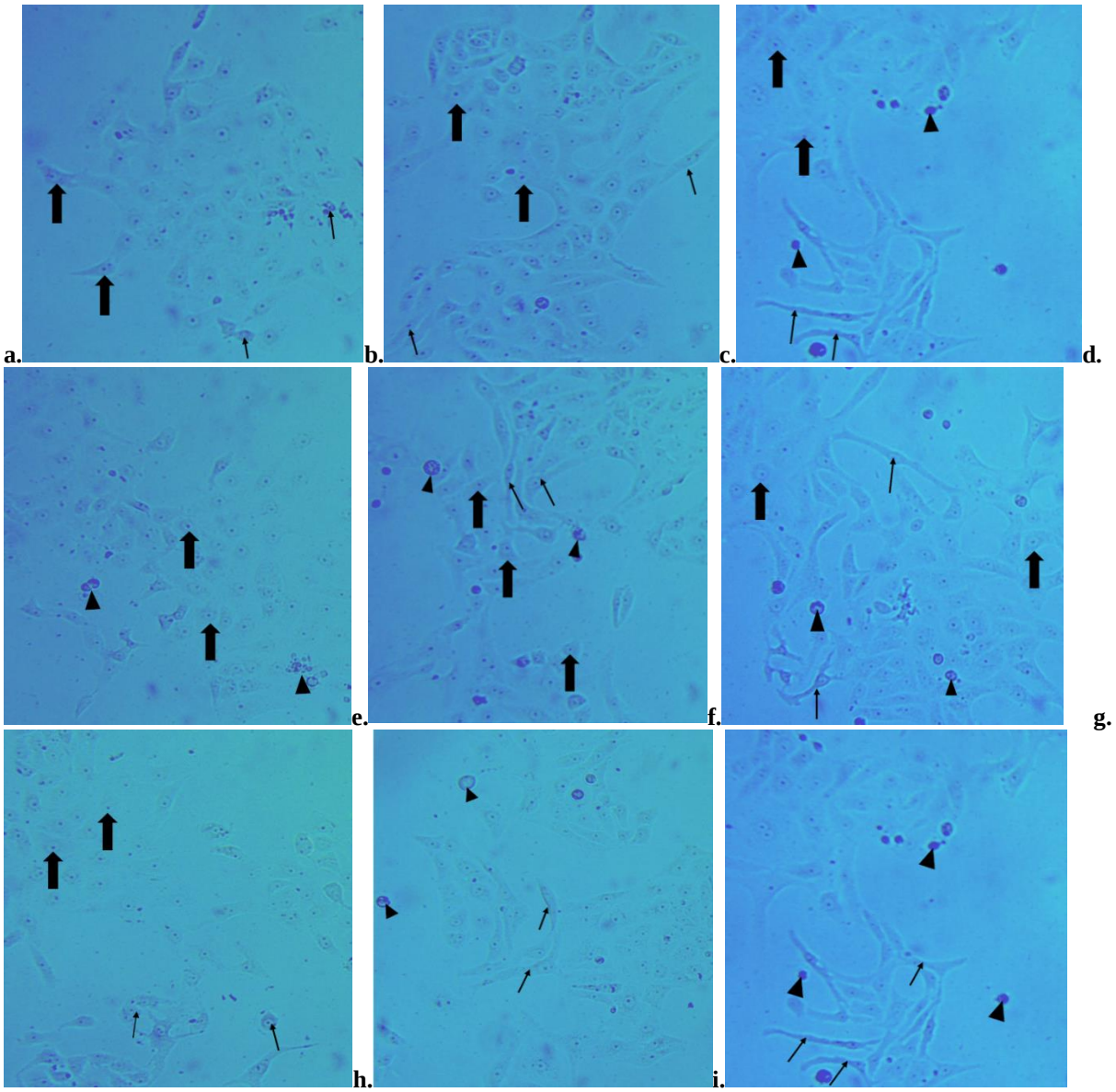

Figure 5: Michigan Cancer Foundation-7 (MCF-7) cells exposed to ethanol extract of *D. tripetala* fruit. a) 24 hrs b) 48 hrs c) 72 hrs of 6.25 µg/ml, d) 24 hrs e) 48 hrs f) 72 hrs of 12.5 µg/ml and g) 24 hrs h) 48 hrs i) 72 hrs of 25 µg/ml (x100).

Normal polygonal-shaped cells (thick arrows), the appearance of cellular damage, elongation of cells (thin arrows) and change in shape (arrowheads). *Micrographs were taken using an inverted microscope with AmScope software*.

DISCUSSION

Breast cancer being the second most common cause of cancer [3, 37]. Some factors influencing breast cancer development includes; physical inactivity, late gestation, oral contraceptives, hormone therapy after menopause, food contamination, high intake of alcohol and tobacco, exposure to radiation, hazardous chemicals or genetic factors [37-40]. 5-fluorouracil (5-FU), is an anticancer drug used for the treatment of breast cancer [41]. However, the major challenge of this drug as with many other anticancer drugs, is that it is non selective and can result in the damage of normal healthy cells [42]. The recommendation of medicinal plants to serve as an alternative to modern drugs in treating diseases like cancer have been in use for many years in Asian and African regions because they have great potential to provide newer drugs [43-45]. In this study, 5-FU served as a positive control to compare with the test agent, aqueous and ethanol fruit extract of Pepper fruit (*Dennettia tripetala*).

Studies have shown the presence of carbohydrate, tannins, alkaloid, sterol, terpenes, flavonoids, balsam

and phenols in *D. tripetala* fruits have phytochemicals like steroids have good cytotoxicity potential towards cancer cells, with some even having the ability to cause DNA damage and apoptosis [46, 47]. Triterpenes also present in freely in plants and animals have anticancer properties and can inhibit cell growth and proliferation, alter cell proteins to cause cytotoxicity, carcinogenesis inhibition and apoptosis [11.,47-48].

For both the % cell viability and the morphological studies, concentrations of 6.25, 12.5, 25.0 µg/ml of 5-FU, aqueous and ethanol extracts of the *D. tripetala* fruit were used. This was done to try to align the current work with the American National Cancer Institute (NCI) guidelines. The guideline says, for IC_{50} of crude extracts to be considered to have a high potential cytotoxic effect it should be ≤ 20 µg/ml after 48 to 72 hr, 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 [49-50].

The result obtained after the % cell viability assay was carried out showed there was statistically significant difference in the decrease of MCF-7 cells treated with 5-FU after 24 hr with 25.0 µg/ml, 48 and 72 hr following treatment with 6.25, 12.5 and 25.0 µg/ml when compared to the negative control (DMSO). However, statistically significant difference within the group was only observed at 12.5 and 25.0 µg/ml after 72 hr of treatment. The aqueous extract of *D. tripetala* fruit decreased cell viability significantly only after 48 hr of treatment for all concentrations and also at 25.0 µg/ml, 72 hr after treatment when compared to the negative control. Further observed within the group at 48 hr of treatment with 25.0 µg/ml. A statistically significant difference in reduction of cell viability at 24 hr following treatment with 12.5 and 25.0 µg/ml and at 48 and 72 hr treatment with 6.25, 12.5 and 25.0 µg/ml of the ethanol extract of *D. tripetala* were observed when comparison was made with the negative control, dimethyl sulfoxide (DMSO). There was also a difference within the group at 48 and 72 hr for 12.5 µg/ml and at 24 and 72 hr for 25.0 µg/ml.

A significant amount of decrease in cell growth following treatment and counting via Trypan blue was noticed when 100.0 µg/ml of *D. tripetala* seed extract was used to test cell viability on prostate cancer cell lines PC3 and LNCaP [11]. However, due to the shortage of information on works carried out using *D. tripetala* plant or specifically the fruit, the mechanism of action is yet to be explained. Comparing the decrease in % cell viability of MCF-7 cells over the time period of 24-, 48- and 72 hr, statistically significant decrease in % cell viability was only observable between 48 and 72 hr time period after treatment with 12.5 µg/ml of 5-FU. There was no significant difference between the time period for MCF-7 cells treated with the aqueous extract of *D. tripetala* fruit extract. The MCF-7 cells treated with the ethanol extract of *D. tripetala* fruit showed a statistically significant difference in the decrease of % cell viability between 24 and 48, 24 and 72 hr of treatment with 6.25, 12.5 and 25.0 µg/ml, and also between 48 and 72 hr of treatment with 12.5 and 25.0 µg/ml. This indicates that the effect of both the aqueous and ethanol extract on MCF-7 cells is both time and dose-dependent.

Morphological changes ranging from stretching of cells to altering their shape, to cells becoming round, indicating possible apoptosis were observed in wells containing MCF-7 cells treated with either 5-fluorouracil, the aqueous and ethanol extract of *D. tripetala* fruit at 6.25, 12.5, 25.0 µg/ml concentration for 24, 48 and 72 hrs. These changes in morphology occurred in a time and dose-dependent manner. At 6.25 µg/ml, 24 hr after treatment with 5-FU, cell damage and floating was noted with the most occurring at 72 hr following treatment with 25.0 µg/ml concentration. The 5-FU an anticancer drug is noted to have a mechanism of action that involves cytotoxic metabolites production involving RNA and DNA that can lead to cell cycle arrest and cell death via apoptosis [51]. Cellular morphological alteration was observed in cells treated with 6.25 µg/ml concentration after 72 hr, 12.5 µg/ml after 48 and 72 hr, and 25.0 µg/ml at 24, 48 and 72 hr of aqueous extract of *D. tripetala* fruit with the most alterations at 72 hr of treatment for both 12.5 and 25.0 µg/ml. The ethanol fruit extract of *D. tripetala*-treated cells showed alterations in cell morphology. Changes were observed at 6.25 µg/ml at 72 hr, at 12.5 µg/ml at 24 hr, 48 and 72 hr and finally at 25.0 µg/ml for 24, 48 and 72 hrs. Damage to cells treated with both aqueous and ethanol extract of *D. tripetala* fruit might have occurred as a result of some of the phytochemicals found in them [30, 52]. Jagla suggested the possibility of apoptotic pathway activation initiating programmed cell death as a result of compounds found in *D. tripetala*, although the work was carried out on the plant seed [11].

The drug 5-FU showed the most damage and alterations, morphologically on the MCF-7 cells. As a known standard drug for breast cancer inhibition, apoptotic activity has been observed in breast cancer cell lines treated with 5-FU [51]. Wells treated with 2 µl DMSO did not indicate much damage to the cell morphology. However, there was slight distortion to a few cells possibly as a result to prolonged treatment with DMSO. Some studies have shown that DMSO has the potential to alter the morphology of a cell [53-54].

Conclusion

The aqueous and ethanol fruit extracts of Pepper fruit (*Dennettia tripetala*) enhanced apoptosis and damage of the MCF-7 breast cancer cells. There was reduction

in the cell viability of the breast cancer cell line, MCF-7 and alteration of its morphology. *Dennettia tripetala* possess anticancer potentials.

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Conflict of Interest

The authors declare there is no conflict of interest.

REFERENCES

1. Kaplan W. Update on 2004 Background Paper 6.5 Cancer and Cancer Therapeutics. In: Kaplan W. Priority Medicines for Europe and the World. A Public Health Approach to Innovation; 2013.
2. Doll R, Peto R. The causes of cancer. Journal of the National Cancer Institute. 1981; 66:1191-1308.
3. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. International Journal of Cancer. 2010; 127:2893-2917. doi: 10.1002/ijc.25516.
4. Okobia MN, Bunker CH, Okonofua FE, Osime U. Knowledge, attitude and practice of Nigerian women towards breast cancer: A cross-sectional study. World Journal of Surgical Oncology. 2006; 4:11-15.
5. Sylla BS, Wild CP. A million Africans a year dying from cancer by 2030: What can cancer research and control offer to the continent? International Journal of Cancer. 2011; 130(2):245-50. doi: 10.1002/ijc.26333
6. Akarolo-Anthony SN, Ogundiran TO, Adebamowo CA. Emerging breast cancer epidemic: Evidence from Africa. Breast Cancer Research. 2010; 12:S4-S8.
7. Craig WJ. Phytochemicals: Guardians of our health. Journal of the American Dietetic Association. 1997; 97:s199-s204.
8. Craig W, Beck L. Phytochemicals: Health protective effects. Canadian Journal of dietetic practice and research: a publication of Dietitians of Canada. 1999; 60(2):78-84. PMID: 11551345.
9. World Health Organization. Traditional medicine fact sheet. Geneva: WHO. 2008.
10. Xiao H-J, Liang H, Wang JB, Huang C-Y, Wei WQ, Boniol M, et al. Attributable causes of cancer in China: fruit and vegetable. Chinese Journal of Cancer Research. 2011; 23(3):171-176.
11. Jagla SW. Effects of seed extracts from traditional Nigerian medical plants on prostate cancer cell growth. M.Sc thesis, University of Illinois, Chicago; 2013.
12. Shi M, Cai Q, Yao L, Mao Y, Ming Y, Ouyang G. Antiproliferation and apoptosis induced by curcumin in human ovarian cancer cells. Cell Biology International. 2006; 30(3):221-226.
13. Okwu ED, Morah NIF. Mineral and nutritive value of *Dennettia tripetala* fruits. Fruits. 2004; 59:437-442. doi: 10.1051/fruits:2005006
14. Keay RWJ. Trees of Nigeria. UK: Clarendon Press Oxford; 1989:19-30.
15. Adedayo BC, Oboh G, Akindahunsi AA. Changes in the total phenol content and antioxidant properties of pepperfruit (*Dennettia tripetala*) with ripening. African Journal of Food Science. 2010; 4(6):403-409.
16. Iseghohi OS. A review of the uses and medicinal properties of *Dennettia tripetala* (Pepperfruit). Medical Sciences. 2015; 3(4):104-111. doi:10.3390/medsci3040104
17. Bassey UE, Akpakpan EI, Etim OE, Akaka EUA, Ekanemesang UM. Effect of ethanol extract of ripe fruits of Dennettia tripetala on prostatic and testicular functions of male albino rats. The Journal of Medical Research. 2018; 4(2):89-92.
18. Ejechi BO, Akpomedaye DE. Activity of essential oil and phenolic acid extracts of pepperfruit (*Dennettia tripetala* G. Barker; Anonancea) against some food-borne microrganisms. African Journal of Biotechnology. 2005; 4(2):258-261.
19. Oyemitan IA, Iwalewa EO, Akanmu MA, Olugbada TA. Antinocieptive and anti-inflammatory effect of essential oil of *Dennettia tripetala* G. Baker (Annonacae) in Rodent. African Journal of Traditional Complementary and Alternative Medicine. 2008; 5(4):355-362.
20. Akinwumi FO. Evaluation of some plant materials for the control of smoked fish pest, *Dermestes maculatus degeer* (Coleoptera: *Dermestidae*) in *Clarias gariepinus Burchell* (Pisces: *Clariidae*). ARPN: Journal of Agricultural and Biological Science. 2011; (7):65-69.
21. Iwu MM. Food for medicine. In: Iwu M. (Ed.). Dietary plants and masticators as sources of biologically active substances. Ile Ife: Univ. Ife Press; 1989. p. 303-310.

22. Ikpi DE, Nku O. Effect of ethanolic extract of *Dennettia tripetala* fruit on haematological parameters in albino wistar rats. Nigerian Journal of Physiological Sciences. 2008; 23:13–17.
23. Nwosu CL, Adedire OC, Ogunwolu OE, Ashamo OM. Toxicological and histopathological effects of *Dennettia tripetala* seed used as grain protectant, food, and medicine. Food Quality and Safety. 2017; 1(3):211–220 https://doi.org/10.1093/fqsafe/fyx019.
24. Ejechi BO, Nwafor OE, Okoko FJ. Growth inhibition of tomato-rot fungi by phenolic acids and essential oil extracts of pepperfruit. Food Research International. 1999; 32(6): 395-399. doi: http://dx.doi.org/10.1016/ S0963-9969(99)00057-5
25. Anyaele OO, Amusan AAS. Toxicity of hexanolic extract of *Dennettia tripetala* (G. Baker) on larvae of *Aedes aegypti* (l). African Journal of Biomedical Research, 2003; 6: 49-53.
26. Nwachukwu E, Osuji J. Evaluation of plant extracts for antifungal activity against *Sclerotiumrolfsii*causing cocoyam cormel rot in storage. Research Journal of Agriculture and Biological Sciences. 2008; 6:784-787.
27. Anaga AO, Asuzu IU. Antihyperglycaemic properties of the ethyl acetate extract of *Dennettia tripetala* in diabetic rats. Journal of Complementary and Integrative Medicine. 2010; 7(1). doi:10.2202/1553-3840.1244.
28. Aderogba MA, Akinkunmi EO, Mabusela WT. Antioxidant and antimicrobial activities of flavonoid glycosides from *Dennettia tripetala* G. Baker Leaf Extract. Nigerian Journal of Natural Products and Medicine. 2011; 15:49-52.
29. Okolie NP, Falodun A, Davids O. Evaluation of the antioxidant activity of the root extract of Pepperfruit (*Dennettia tripetala*) and its potential for the inhibition of lipid peroxidation. African Journal of Traditional Complementary Alternative Medicine. 2014; 11(3):221–227.
30. Ihemeje A, Ojinnaka MC, Obi KC, Ekwe CC. Biochemical evaluation of Pepperfruit (*Dennettia tripetala*) and its use as substitute for ginger in zobo drink production. Academic Research International. 2013; 4(6):513-521.
31. Elekwa I, Okereke SC, Chukwudomo CS. Phytochemical screening and GC-MS analysis of the essential oil of *Dennettia tripetala* (Pepperfruit) seeds. ABSU Journal of Environment, Science and Technology. 2011; 1:93-98.
32. Neuhouser ML. Dietary flavonoids and cancer risk: evidence from human population studies. Nutrition and Cancer. 2004; 50(1):1-7.
33. Graf BA, Milbury PE, Blumberg JB. Flavonoids, flavones and human health: epidemiological evidence. Journal of Medicinal Food. 2005; 8(3):281-290.
34. Koralek DO, Peters U, Andriole GA. Prospective study of dietary α-linoleic acid and the risk of prostate cancer. Cancer Causes and Control. 2006; 17:783-791.
35. Ramya N, Priyadharshini PR, Dhivya R. Anticancer activity of *Trachyspermum ammi* against MCF7 cell lines mediates by p53 and Bcl-2 mRNA levels. The Journal of Phytopharmacology. 2017; 6(2):78-83
36. Segeritz C-P, Vallier L. Cell culture: Growing Cells as Model Systems *in vitro*: Basic Science Methods for Clinical Researchers. Elsevier; 2017.
37. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA: A Cancer Journal for Clinicians. 2005; 55:74-108.
38. Duncan JA, Reeves JR, Cooke TG. BRCA1, and BRCA2 proteins: roles in health and disease. Molecular Pathology. 1998; 51(5):237-247.
39. Weatherbee DS, Grenier KJ, Carrol BS. DNA to Diversity: Molecular Genetics and the Evolution of Animal Design. 2nd ed. Blackwell; Oxford; 2001:214
40. Gaffield ME, Culwell KR, Ravi A. Oral contraceptives and family history of breast cancer. Contraception. 2009; 80(4):372-380.
41. Chow LWC, Loo WTY. The differential effects of cyclophosphamide, epirubicin and 5-fluorouracil on apoptotic marker (CPP-32), pro-apoptotic protein (p21 $^{WAF-1}$) and anti-apoptotic protein (bcl-2) in breast cancer cells. Breast Cancer Research and Treatment. 2003; 80:239-244.
42. Senapati S, Mahanta KA, Kumar S, Maiti, P. Controlled drug delivery vehicles for cancer treatment and their performance. Signal Transduction and Targeted Therapy. 2018; 3:7
43. Newman DJ, Cragg GM, Snader KM. Natural products as sources of new drugs over the period 1981-2002. Journal of Natural Products. 2003; 66(7):1022-1037.
44. Schwartsmann G, Ratain MJ, Cragg GM, Wong JE, Saijo N, Parkinson DR. Anticancer drug discovery and development throughout the world. Journal of Clinical Oncology. 2002; 20:47S–59S.
45. Greenwell M, Rahman PKSM. Medicinal plants: Their use in anticancer treatment. International Journal of Pharmaceutical Sciences and Research. 2015; 6(10):4103-4112.

46. Egharevba HO, Idah EA. Major compounds from the essential oil of the fruit and comparative phytochemical studies of the fruits and leaves of *Dennettia tripetala* Barker F. Found in North Central Nigeria. International Journal of Pharmacognosy and Phytochemical Research. 2015; 7(6):1262-1266.
47. Sawadogo WR, Schumacher M, Teiten MH, Dicato M, Diederich M. Traditional West African Pharmacopeia, plants and derived compounds for cancer therapy. Biochemical Pharmacology. 2012; 84(10):1225-1240.
48. Chudzik M, Kornonek-Szlacheta H, Krol W. Triterpenes as potentially cytotoxic compounds. Molecules. 2015; 20:1610-1625.
49. Abdul-Hameed ES, Salih A, Bazaid SA, Shohayeb MM, El-Sayed MM, El-Wakil EA. Phytochemical studies and evaluation of antioxidant, anticancer and antimicrobial properties of Conocarpus erectus L. growing in Taif, Saudi Arabia. European Journal of Medicinal Plants. 2012; 2:93-112.
50. Sajjadi SE, Ghanadian M, Haghighi M, Mouhebat L. Cytotoxic effect of *Cousiniaver bascifolia bunge* against OVCAR-3 and HT-29 cancer cell. Journal of HerbMed Pharmocology. 2015; 4(1):15-19.
51. Longley DB, Harkin DP, Johnston PG. 5-Fluorouracil: Mechanism of action and clinical strategies. Nature Reviews Cancer. 2003; 3:330-338.
52. Omage OS, Orhue EJN, Omage K. Evaluation of the phytochemical content, in vitro antioxidant capacity, biochemical and histological effects of *Dennettia tripetala* fruits in healthy rats. Food Science and Nutrition. 2019; 7(1):65-75.
53. Pal R, Mamidi MK, Das AK, Bhonde R. Diverse effect of dimethyl sulfoxide (DMSO) on the differentiation potential of human embryonic stem cells. Archives of Toxicology. 2012; 86:651-661.
54. Yuan C, Goa J, Guo J, Bai L, Marshall C, Cai Z, Wang L, Xiao M. Dimethyl sulfoxide damages mitochondrial integrity and membrane potential in cultured Astrocytes. PLoS One. 2014; 9(9):e107447.