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Assessment of the Effects of Ripe *Carica papaya* Seed extracts on Mcf-7 Breast Cancer Cell Lines

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Abstract
BACKGROUND: *Breast cancer is the most frequently diagnosed cancer in women and leading cause of mortality worldwide. The most commonly used method of treatment is chemotherapy which has adverse side effects. Carica papaya fruit is consumed by most people in Nigeria. All parts of the fruit such as the leaves, stem bark, roots, e.t.c are used to cure various ailments by traditional doctors.*
AIM:*The aim of this study was to assess the effects of ripe Carica papaya seed extracts on MCF-7 breast cancer cell lines.*
METHOD:*The ripe fruits were obtained, and the seeds were removed, washed with distilled water, air dried. The aqueous and methanolic extracts were prepared using cold percolation method. Phytochemical analysis was performed and the phytocompounds in the methanolic extract were determined using GC-MS analysis. The effect of both extracts was determined by evaluating their cytotoxicity on MCF-7 breast cancer cell lines using cell titre glow luminescent cell viability assay.*
RESULTS:*The phytochemical profile of the crude aqueous and methanolic extracts indicated the presence of Phenols, Flavonoids, Alkaloids, Tannins and Saponins. The phytocompounds identified in the methanolic extract includes fatty acids and a total of six (6) compounds which includeBenzyl nitrate, 5-hydroxymethyl furfural, Thioxyanic acid, phynylmethyl ester, 1-dodecanolHexadecanoic acid methylester, and 9-octadecanoic acid methyl ester. The result also shows higher concentration of the extract (400u/ml) had very high inhibitory effect on the breast cancer cell lines.*
CONCLUSION:*In conclusion, this study shows that the aqueous and methanolic extract of Carica papaya seeds possessed phytocompounds which can inhibit the proliferation of human breast cancer cells. Hence, Carica papaya seeds can be used in the management of breast cancer cells.*
Key Words: *Carica papaya,MCF-7, phytocompounds, phytochemical, GC-MS.*

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I. Introduction

Papaya belongs to a small family, *Caricaceae*, having four genera in the world. The genus *Carica*, is represented by four species in India, of which *Carica papaya* is the most widely cultivated and the best known specie. It is commonly known as papaya melon tree, Papaya or papaw kapaya (Bhattachrjee, 2014). The taxonomical classification includes kingdom Plantae, order Brassicales, family Caricaceae, Genus*Carica* and species *papaya*. Papaya probably originated in Southern Mexico and Costa Rica, its subsequently got introduced in Australia, Hawaii, South Africa, India and all tropical and subtropical regions. It is grown both commercially and in home gardens (Marotta*et al.*, 2006).

A study conducted has documented that papaya possessed powerful anticancer properties and its impact on numerous lab-grown tumours.Medicinal uses of papaya seeds are carminative, anti-fertility agent in males, counter irritant, as a paste in the treatment of ringworm, liver cirrhosis and abortificient. Seed juice paste is used as antihelmintic and in stimulation of menstruation or abortion, studies hasshown that *Carica papaya* seeds possessed effective antiheliminitic properties against nematodes found in animals (Chota, 2010).

Chinoy*et al*. (2006) proved the anti-fertility, anti-implantation and abortifacient properties of extracts from papaya seeds. It has been established in males that the seeds of C*arica papaya* has potential anti-fertility drugs (Lohiya*et al*., 2005).

Breast cancer is cancer that develops from breast tissue. Signs of breast cancer may include a lump in the breast, a change in breast shape, dimpling of the skin, and fluid coming from the nipples, newly inverted nipples, or a red or scaly patch of skin. In those with distant spread of the disease, there may be bone pain, swollen lymph nodes, and shortness of breath or yellow Skin (Saunders *et al*., 2001).

The incidence of breast cancer had doubled in the past 3 years. Between 50 and 75 percent of breast cancer begins in the ducts, 10 to 15 percent begins in the lobules and a few begin in other breast tissues (Dillion*et al*., 2010). Due to high mortality rate from breast cancer, which has increased in recent years with reduced emphasis on early detection the side effects of orthodox medicine has led to the screening of several medicinal plants with potential anticancer activity(Eliassen*et al.,*2010). In view of these current trends, this study aims to assess the effect of ripe *Carica papaya* seed extracts on MC-7 breast cancer cell lines.

II. Materials And Methods

Sample Source and Collection

Ripe fruits of *Carica papaya*were obtained from Station market, Kaduna State. The market is a popular fruit market located in Kaduna south local government area of Kaduna state. The state is located in north-western geopolitical zone. It lies within latitude 10.609319 and longitude 7.429504, with an estimated population of about 760,084 (NPC, 2006). Ripe *C.papaya* fruits were purchased twice at different days from the market. The Michigan Cell Foundation-7 (MCF-7) cell line was sourced from DNA Labs Kaduna. Gas Chromatography Mass Spectrometry(GC-MS) analysis of the plant extracts was carried out at National Agency for Food and Drug Administration and Control (NAFDAC) at Sabo, Kaduna state,

Preparation of*Papaya* Seeds Extract

The pawpaw was washed with distilled water,blotted dry with paper towel,and cut into half with the aid of a sterile knife to access the seeds.The seeds were scrapped and washed properly with distilled water.The washed seeds were spread on plastic trays and left to air dry for 7days. The dried seeds were subsequently milled into fine powder with a blender, after which they were packed in a nylon bag to the laboratory for weighing and to carry out the extraction process.

Citizen scale weighing balance was used to weigh 50g of the ripe seeds powder.Cold percolation method of extraction was used. The plant extract wasprepared by dissolving 50g of the powdered sample into 500ml of methanol and water each in a separate conical flask. These were all allowed to soak for two (2) days, after which the supernatant was collected and the residues washed twice by suspending them again in the respective solutions, mixing, and placing on a shaker overnight. The collected supernatant was pooled together and the residues were discarded. The aqueous and methanolic extracts were dried by filtering and concentratingto dryness by the use of a rotary evaporator machineovernight to ensure removal of traces of the solvents. The dried extract was stored at -20^{o}C.

Cell Culture

The MCF 7 breast cells were cultured in DMEM (Dulbecco Modified Eagle Medium). The medium is made up of D-Glucose, L-Glutamine and sodium pyruvate, the media was supplemented with 10%Foetal Bovine Serum (FBS) and 1% penicillin and streptomycin. The cells were incubated in a humidified incubator at 37^{o}C with 5% CO_2. Media was changed every 3 days and cells weresub cultured when culturebecomes confluent.

Sterility Test of the Extracts

Purity of extract was determined by streak inoculation on freshly prepared blood agar plates which was then incubated at 37^{o}C for 24 hours. At the end of incubation period, plates were examined for growth.

Phytochemical Screening

The aqueous and methanolic extracts obtained were subjected to phytochemical analysis to screen for the presence of the active components such as tannins, alkaloids, flavonoid,phenol, glycosides, and Saponins.

Quantitative Analysis of Ripe *Carica papaya* Extracts Using Gas Chromatography Mass Spectrometry (GC-Ms) Technique

Gas Chromatography Mass Spectrometry(GC-MS) analysis of the plant extracts was carried out to ascertain the active secondary metabolites of the extracts using automated GC-MS machines (model GC-MS TQ8040 Germany). The methanolic ripe sample was injected at different times into the Gas chromatograph (GC),and then heated at 300^{o}C for 3minutes; the material was then volatized and separated into components as sample flows through the column which was within a special oven which controls temperature from -20^{o}C to 320^{o}C.The column surface was coated with a material which separated the various chemical compounds in the sample based on size and/or polarity, sample components that were more volatile and smaller in size travelled through the column more quickly than others.

The separated components flowed directly out of the column and into the MS through the ionization source, filtered and detected respectively, thereby counting the number of filtered ions.The information was then sent to a computer and a mass spectrum, the distribution of ions of different sizes was generated.

In-Vitro Anticancer Activity (CellTitre-Glo®)

The cytotoxicity of sample on MCF-7 was determined by cell titre-Glo® luminescent cell viability Assay. The monolayer cells were detached and single cell suspension was made using trypsinethylenediaminetetra-acetic acid (EDTA). A hemocytometer was used to count the viable cells and the cell suspension was diluted with a medium containing 5% FBS (foetal Bovine Serum) in order to obtain final density of 1x 105cells/ml. Ninety Six (96) wells micro titre plate with the density of 10,000cells/well were seeded with 100µ/l/well of cell suspension of the cell titre-Glo®2.0 reagent and incubated for all attachment for 18hrs at 37°C, 5% CO_2,95% air and 100% relative humidity.

Aliquots of 100µl of different concentrations of sample A,B,C and D extracts (25,50,100,200 and 400µg/ml respectively) dissolved in DMSO(1%) were added to the appropriate wells already containing the 100µl of media and then incubated for 48hrs at 37°C, 5% CO_2,95% air and 100% relative humidity. After which the plate and its contents was equilibrated to room temperature for approx. 30minutes,after which it was then mixed for 2minutes on an orbital shaker at 150rpm to induce celllysis and the plate was then incubated at room temperature for 15minutes to stabilize. The viable cells were determined at 450nm using a Luminometer (MODULUS, Promega UK).The medium without sample was used as control and the assay was carried out in triplicate for all concentrations.

The effect of the samples on the proliferation of MCF-7 was expressed as the % cell viability and of cell growth inhibition using the following formulae;

% cell viability = $\frac{Abs\ 450\ of\ treated\ Cell}{Abs\ 450\ of\ control\ cells}$ x 100%

% cell growth inhibition = $\frac{100-Abs\ (samples)}{Abs\ (control)} x \frac{100}{1}$

Where:

Abs – Absorbance of Treated cells/ Control cells

Samples – Ripe Pawpaw Seeds Extract (Aqueous or Methanolic)

Control – Untreated Cell Lines

Statistical Analysis

The data was analysed using SPSS version 21.0 Student T-test was used to determine the significance by comparing the effect of both aqueous andmethanolic extracts of ripe pawpaw (*C. Papaya*) seeds on MC-7 breast cancer cell line.

III. Results

Phytochemical Analysis of Ripe Papaya Extract

The results of the quantitative phytochemical analysis of ripe Papaya seed extracts are presented in Table 3.1

GC-MS Analysis of Ripe Methanolic Extracts of *Carica papaya*

GC-MS analysis of ripe methanolic seed extracts showed a total of six (6) compounds. The result is shown in table 3.2. Benzyl Nitrate recorded values of 61.34% (area), 41.11% (height) and 5.55% (ratio) in the ripe methanolic extracts. 5-Hydroxymethyl Furfural recorded values such as 3.09% (area), 3.11% (height) and 3.69% (ratio) in the ripe methanolic extracts. Thioxyanic acid, Phynyl-methylesterrecorded values of 31.01% (area), 49.95% (height), and 2.57% (ratio) for the ripe methanolic extracts. Other compounds(1-dodecanol, Hexadecanoicacid.methyl ester and 9-Octadecanoic acid.methyl ester) were also identified.

Effect of Aqueous and Methanolic Extracts on MC-7 Breast Cancer Cell Line

Extracts activity as shown in figure 3.2 indicates that between the two extracts aqueous extracts were higher in cell inhibition than the methanolic extracts at each concentration level. There is a significant difference in the mean action of the two extracts at $p<0.05$

Comparative Analysis of Aqueous and Methanolic Extracts of Ripe Seed on MC-7 Breast Cancer Cell Line

In the analysis of the aqueous and methanolicextracts of ripe seed of *Carica papaya*, it was observed that cells inhibition were highest at 400µg/ml and the lowest cells inhibition at 25µg/ml. There was significant difference between the aqueous and methanolic extracts at ($p<0.05$). The result is presented in figure 3.4. The aqueous extract performed better than themethanolic extract.

Table 3.1: Phytochemical Analysis of Ripe *Carica papaya* Seed Extracts

Constituents	Ripe *Carica papaya* Seed Extracts	
	Methanolic extract (mg)	Aqueous extract (mg)
Phenols	11.3	9.1
Flavonoids	5.1	4.0
Saponins	1.1	34.0
Alkaloids	1.4	1.1
Tannins	2.3	3.0

Table 3.2:Phyto-compounds in Ripe Methanolic Extracts of *Carica papaya* Seeds

S/NO	PHYTOCOMPOUNDS	RIPE METHANOLIC EXTRACTS		
		% AREA(A)	% HEIGHT(H)	A/H RATIO
1.	BENZYL NITRATE	61.34	41.11	5.55
2.	5-HYDROXYMETHYL FURFURAL	3.09	3.11	3.69
3.	THIOXYANIC ACID, PHYNYLMETHYL ESTER	31.01	49.95	2.57
4	1-DODECANOL	2.25	4.76	1.76
5	HEXADECANOIC ACID,METHYLESTER	1.23	3.16	1.45
6	9-OCTADECANOIC ACID, METHYL ESTER	1.08	2.92	1.38

FIGURE 3.1GC-MS ANALYSIS OF RIPE PAPAYA SEED

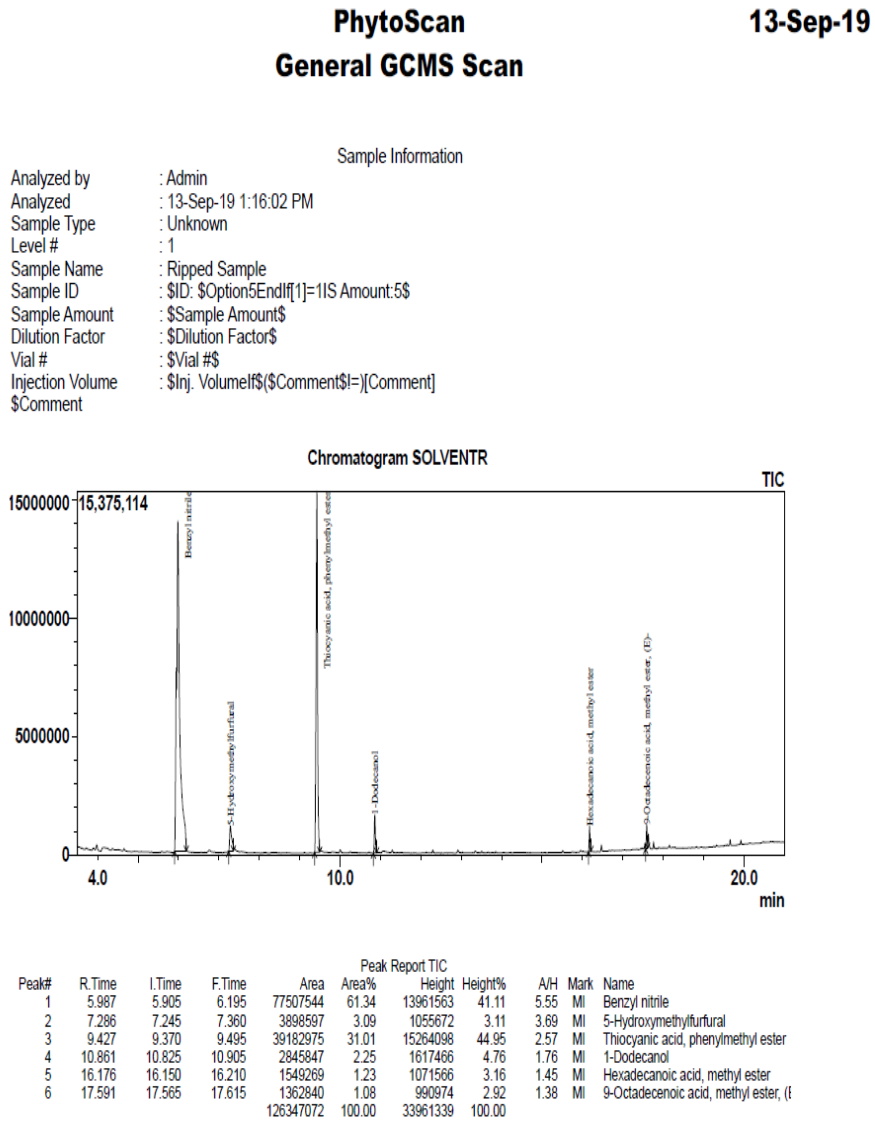


Figure 3.2: Inhibition of Aqueous and Methanolic Extracts on Cell Line

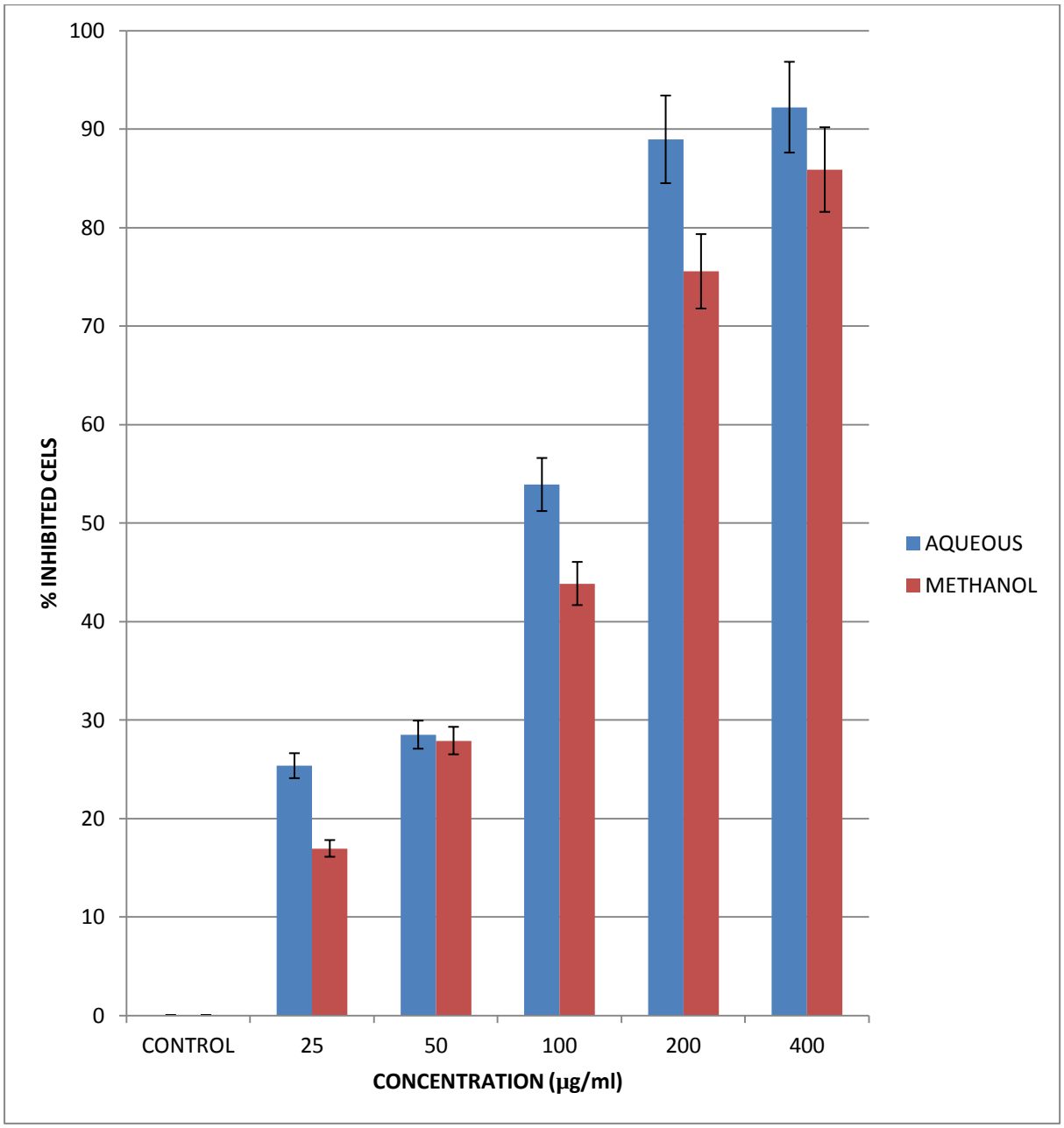
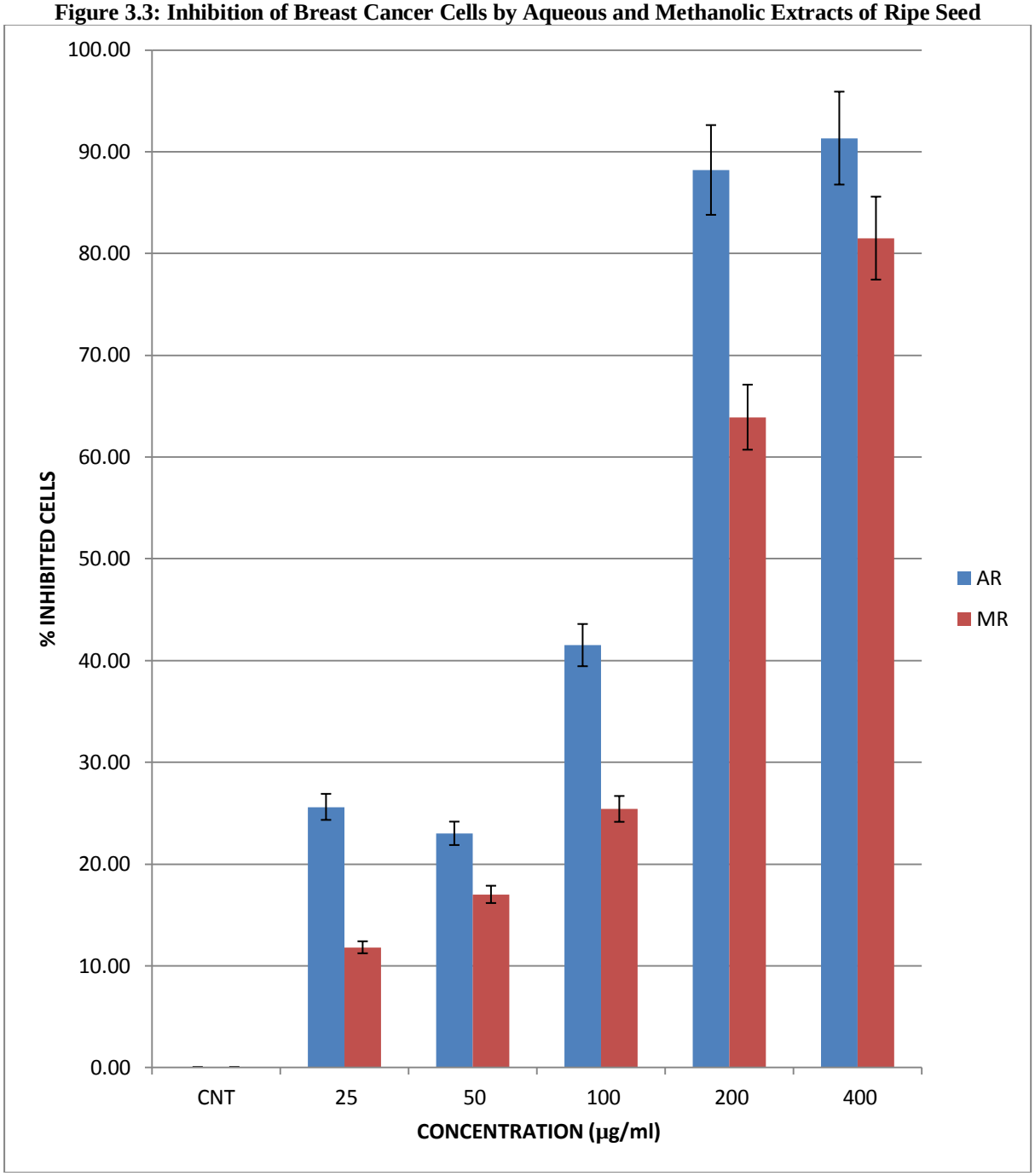

100
90
80
70
60
50
40
30
20
10
0
% INHIBITED CELS
CONTROL
25
50
100
200
400
CONCENTRATION (µg/ml)
AQUEOUS
METHANOL

Figure 3.3: Inhibition of Breast Cancer Cells by Aqueous and Methanolic Extracts of Ripe Seed



IV. Discussion

Carica papaya is well known for its nutritional and medicinal properties throughout the world. It is widely cultivated in the tropical and sub-tropical countries due to its multi-faceted properties. Its whole parts are utilised for their nutritional and medicinal properties. It contain enzyme Papain, Lycopene, Isothiocyanate, important minerals, vitamins, carbohydrates, carotenoids, and flavonoids. This important fruit feeds the body and boosts the immune system.

The phytochemical analysis of the methanolic and aqueous extracts of ripe papayaseedswas determined. Phenols, Flavonoids, Saponins, Tannins and Alkaloids were found to be present. Results show the highest phytochemical to besaponins in the aqueous extracts of ripe papaya seeds. On average, concentration of phytochemicals were found as follows; Saponins> Phenols > Flavonoids > Tannins > Alkaloids. Similar work of Udoh and Udoh (2005) showed that Phenol, Flavonoids, Saponins, Tannins and Alkaloids phytochemicals were also determined. In contrast, to the study carried out by Ikpeme*et al.* (2011) that reported the presence of Alkaloids, Saponins and Phenols but Flavonoids was not found.

The phytocompounds observed in the ripe methanolic extract include Benzyl Nitrite, 5-hydroxymethyl furfural, Thiocyanic acid, Phenylmethyl ester, 1-dodecanol, Hexadecanoicacid, methyl ester, and 9-octadecanoic acid, methyl ester. These phytocompounds could be the reason for the extract inhibiting breast cancer cells more than the others.

The seeds account for only 7% of*C.papaya* weight but are typically discarded. When unripe, papaya contains white seeds whereas the ripe papaya contains black seeds. The different colour of seeds may indicates that as papaya matures its compositions changes, and this could also be linked to the result of the differences observed in the phytochemical constituents of the ripe papaya seeds, and may differ from the unripe papaya seeds.

Potential of*C.papaya* seed extract as anti-proliferation of breast cancer cells is suspected due to the presence of bioactive compounds, Phenols, Saponins and flavonoids which are in very high concentrations as observed in the study. This supports the report of(Huang *et al*. 2009), that three bioactive compounds have considerable anticancer effects: phenolics, saponins and flavonoids. The results are in line with those of Lambert *et al.*(2005), who described an inhibitory effect of these compounds, particularly the effect of polyphenols on cancer cell proliferation.The plant's medicinal value depends on the presence of chemicals that showed positive pharmacological and physiological actions Yu*et al.* (1999).

Cell Titre- Glow assay was conducted to evaluate the growth inhibitory effects of aqueous and methanolic, ripe extract from*C. papaya* seeds on the cell viability on breast cancer cell lines (MCF-7), which is based on the reduction in the viability of cells at different concentrations (25, 50, 100, 200, and 400µg/ml). After 72hrs of treatment, aqueous crude extract of *Carica papaya* exhibited higher inhibitory effect against all tumor cells, with varying efficiencies and selectivity while others caused marginal cell inhibition. Study demonstrated that the aqueous extracts of both ripe and unripe papaya seeds had the best anti-proliferative activity at 400µg/ml against the human breast cancer cells (MCF-7),with a very low cell viability which is in relation to the control group with no intervention. The methanolic crude extract of both ripe and unripe papaya seed shows significant activity at maximum concentration of 400µg/ml which exhibited high cytotoxicity across all concentrations, and this is in accordance with the findings of Gul*et al.* (2013).

The effect of *Carica papaya* seed extract on cancer growth inhibition in this study is also in concordance with studies performed by Morimoto *et al.* (2008), who patented the extracts of different parts of papaya for the prevention, treatment, or improvement of many types of cancer. Data obtained from this current study indicate that aqueous extract of papaya seeds from ripe *Carica papaya* was effective in inhibiting cell proliferation of breast cancer cells.In contrast to their findings, this study found that the aqueous extract of ripe papaya seeds, surprisingly, inhibited breast cancer cell proliferation more than methanolic crude extract of ripe and unripe *Caricapapaya*seeds extract.

V. Conclusion

In conclusion, the data obtained from this study indicates that papaya seeds extract specifically reduced cell viability of human breast cancer cells. The preliminary photochemical screening showed that aqueous and methanolic extracts of ripe and unripe seeds of *caricapapaya* contains Alkahoid, Flavonoid, Saponins, Tannins and Phenols. However,Phenol and Saponins were present in excess and are known for their anti-cancer and anti-inflammatory activity. Data obtained from this study indicates that *papaya* seeds extract specifically reduced cell viability of human breast cancer cells MCF-7. However, the aqueous extract of ripe Papaya seeds were found to be more effective on the breast cancer cells (MCF-7) at a higher concentration (400µg/ml) with a higher cytotoxicity activity and a lower cell viability on the cells than the methanolic crude extract of the ripe papaya seeds. It was also found that the aqueous and methanolic extracts of papaya were more effective at higher concentration when compared to control. Results indicated a dose dependant inhibition of the MCf-7 cells. Thus, *Carica papaya* aerial parts could be helpful in cancer prevention and treatment. Therefore, papaya seed has anti-proliferative potentials and apoptotic induction on breast cancer cells (MCF-7).

VI. Recommendations

The findings of this study have produced many ideas for future research, and continuation of the exploration of bioactive compounds in *Carica papaya*.

1. Study of other parts of papaya plants, especially the edible parts in their various stages of ripening will be useful especially as a nutraceutical agent.
2. The observation concerning the effects of *Carica papaya* seed extract on the cytotoxic activity together with the variations in concentrations and time suggests further investigations of the effects of different factors such as temperature, pH dosage duration and pharmacokinetics of extracts.

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