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Research Article

Extract of Parkia Biglobosa (“Dawadawa”) Seed is A Rich Antioxidant Source Which Enhances Glucose Tolerance and Inhibits the Growth of Cancer Cells

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Abstract

Background: Diet is a major indicator in preventing and managing chronic diseases like cancer and diabetes. *Parkia biglobosa* seed, also known as “dawadawa” in most West African languages, is used in all parts of Ghana and West Africa to season traditional soups and stews and manage chronic diseases.

Objective: This study was aimed at evaluating the anticancer, antidiabetic, antioxidant, and phytochemical potential of fermented dawadawa extract (FDE) and unfermented dawadawa (UDE) aqueous seed extracts of Parkia biglobosa.

Methodology: Total antioxidant capacity was assessed using DPPH assay, and total phenolic was assessed using Folin Ciocalteu assay. The antidiabetic potential was investigated using the yeast cell glucose uptake assay. The anticancer screening was performed on the prostate (PC3 and LNCAP), breast (MCF7), liver (HEPG2) cancer cells and prostate normal cells (PNT-2). The Standard used was curcumin. The selectivity index was determined for each extract. Qualitative screening for terpenoids, saponins, tannins, and flavonoids was conducted. Gas chromatography-Mass Spectrometry analysis was performed on the methanolic extracts for the detection of major compounds.

Results: Unfermented *Parkia biglobosa* had the highest DPPH scavenging activity with an EC50= 0.54 mg/ml and fermented had an EC50 = 2.27 mg/ml. The FDE had the highest phenolic content (0.24 ± 0.01 mg/100gGAE) while the UDE had 0.18 ± 0.01 mg/100gGAE. The FDE had the highest total flavonoid content of 1178.91mg/100g QE per 10 mg of the extract while the UDE had 511.95mg/100g QE per 10 mg extract. The FDE showed the highest glucose uptake followed by UDE and metformin recorded the lowest uptake. All samples were cytotoxic against cancer cell lines except UDE on LNCAP. The highest activity in a FDE was recorded in MCF7 with an IC50 of 257.20 µg/ml and selectivity of 1.17. In the UDE sample, the highest activity was recorded in PC3 with an IC50 of 259.5µg/ml selectivity of 1.106. GC-MS analysis showed peaks of Cis-Vaccenic acid and 9-octadecenoic acid present in the methanolic extracts. Terpenoids and flavonoids were concentrated in both extracts.

Conclusion: The study showed that *Parkia biglobosa* seed possesses remarkable antioxidant, antidiabetic and anticancer properties.

Keywords: Antioxidant; Cancer; Diet; Diabetes; HEPG2; LNCAP; MCF7; *Parkia biglobosa*; PC3; PNT-2

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Abbreviations

FDE- Fermented “Dawadawa” Extract

UDE- Unfermented “Dawadawa” Extract

DPPH - 2, 2- diphenyl-1-picrylhydrazyl

GAE- Gallic Acid Equivalent

QE- Quercetin Equivalent

GC-MS- Gas Chromatography- Mass Spectrometry

RPMI- Roswell Park Memorial Institute Medium

FBS- Foetal Bovine Serum

DMSO- Dimethyl sulfoxide

SI- Selectivity Index

Background

Most African countries are burdened with communicable diseases, however, non-communicable diseases are the major burden both globally and regionally as a result of changing shifts in diet and lifestyle [1]. Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells. The disease is caused by both internal factors (inherited genetic mutations, immune conditions and

mutations that occur from metabolism) and external factors (nutritional deficiencies, infectious organisms, radiations and chemicals) that may act together or in sequence to initiate or promote its development [2]. Cancer is a major public health problem globally. It is the second leading cause of death in the United States with one in four deaths [3]. Research in this area has become paramount as understanding of the disease mechanism and susceptibility of individuals to it, will go a long way to aid in the search for a cure. In the search for a cure, diet is a major indicator in the prevention and management of chronic disease and cancer is no exception. Treatment options available for cancers are very expensive and these include chemotherapy, surgery, hormone therapy, radiotherapy, and cryotherapy, among others. The standard method of treating these people has been chemotherapy, which comes with numerous side effects – nausea, toxicity, and excessive hair loss, among others. Also, the non-selective nature of some chemotherapeutic agents makes them affect cancer and normal cells alike, often leading to undesirable side effects. These problems are also compounded by the high cost of Western anticancer drugs. Due to these, a large proportion of cancer patients in Ghana and other developing countries have resorted to the use of Complementary and Alternative Medicine either as an adjunct or to completely supplant the use of chemotherapy [4]. Particularly, herbal medicines are widely used, with about 80% of cancer patients in developing countries (particularly African and Asian populations) relying on this method for cancer treatment [5]. This has been the case as these medicinal plants such as *Parkia biglobosa.* They are considered to be cheaper, safer and with less side effects [4].

Parkia biglobosa is a perennial deciduous tree found in a wide range of environments in Africa. The fermented seeds of *P. biglobosa* are used in all parts of Ghana and West Africa to season traditional soups. Some tribes in Ghana use the seed and the fruit to treat stomach aches. However, the anticancer properties of the seed of *P. biglobosa* have not been scientifically validated although its traditional use is reported among these groups of people. *P. biglobosa* is less expensive and readily available than chemotherapeutic drugs hence the need to investigate the anticancer activities of the plant to serve as an alternative source of anticancer agents that will aid in the formulation of cheaper and safer anticancer drugs. Also, *P. biglobosa* is added as a spice in the preparation of food, hence knowledge about its, antioxidant, anticancer effect and antidiabetic effects will help in its promotion and consumption at the public health education level.

Materials and Method

Cell lines and reagents

Cell lines for the study were obtained from the Cell Bank of the Noguchi Memorial Institute for Medical Research (NMIMR), Legon. Cell lines used included HepG2 (Liver), MCF-7 (Breast), PC3 and LNCAP (Prostate), and PNT-2 (Prostate epithelial). Culture media used included Foetal Bovine Serum (FBS), supplemented with antibiotics (Glutamine, Streptomycin and Penicillin), Roswell Park Memorial Institute Medium (RPMI), Dulbecco's Minimum Essential Medium (DMEM), Phosphate buffered saline (PBS), Resazurin (7-Hydroxy-3H-phenoxazin-3-one 10-oxide), trypan blue solution, Potassium Ferricyanide (KCN), Trichloroacetate (TCA), Iron (III) Chloride ($FeCl_3$), Butylated hydroxytoluene (BHT), 2, 2- diphenyl-1-picrylhydrazyl (DPPH), Ascorbic acid, Sodium Carbonate (Na_2CO_3), Folin Ciocalteu, Pyro-Gallic Acid, Aluminium Chloride ($AlCl_3$), Quercetin, Commercial Yeast, Glucose and metformin. All reagents were analytical grade and were obtained from standard suppliers.

Plant material

The seed was collected from a farm in Bolgatanga in the Upper East Region of Ghana. They were identified and authenticated. They were identified as those of *P. biglobosa* at the Department of Herbal Medicine, KNUST, Kumasi, Ghana (with voucher no. KNUST/HM1/2018/S001). A portion of the seed was soaked in water and allowed to ferment. The fermented and unfermented samples were air-dried and milled.

Aqueous Crude Extract Preparation

In the aqueous extract of *P. biglobosa* seed sample preparation, 500 g of milled seed sample, both fermented and unfermented, was suspended in 500 mL of distilled water for 48 hours. The samples were centrifuged (Homef LC-30 centrifuge, LH) for ten minutes at a speed of 1106 ×g at room temperature in 50 mL Eppendorf tubes. The supernatants were taken up and the pellets were subsequently re-suspended with the same volume of distilled water and extracted again. All the supernatants were frozen at -20 °C and lyophilized using a vacuum freeze dryer (YK-118, Taiwan). The fermented extract was designated as FDE and unfermented as UDE.

Phytochemical Assessment of Crude Extracts of *P. Biglobosa*

The procedure used by [6], was adopted for the *in vitro* qualitative phytochemical investigation of extracts.

In Vitro Antioxidant Activity

DPPH (2,2-DIPHENYL-1-PICRYL HYDRAZYL) assay: The free radical scavenging activities of FDE and UDE were determined by DPPH assay with slight modification as described by [7].

Total Phenolic Content Determination: Total phenolic content was determined using the Folin-Ciocalteu assay as described by [8].

Total flavonoid content: In the total flavonoid assay used procedure described by [9]. In the total flavonoid content assay, quercetin was used as standard and a stock solution of quercetin was prepared by dissolving 1 mg of quercetin in 1 mL of absolute methanol into a 2 mL Eppendorf tube and then vortexed for 30 seconds to ensure dissolution. Sample concentration was obtained through ten-fold serial dilution in absolute methanol. A stock solution of Aluminium Chloride ($AlCl_3$) was prepared by dissolving 2 g of $AlCl_3$ in 100 mL of absolute methanol to make 2% $AlCl_3$. A stock solution of FDE and UDE was prepared by dissolving 20 mg of each sample in 1 mL of distilled water in a 2 mL Eppendorf tube. The samples were vortexed for 1 minute and sonicated for 5 minutes to ensure complete dissolution. Sample concentration of 10 and 5, mg/mL was obtained through two-fold serial dilution in absolute ethanol. Furthermore, 100 μL of the various concentration of sample and standard was pipetted in triplicates into the 96 well plate which was followed by the addition of 100 μL of 2% $AlCl_3$ into the well and sample incubated for 20 mins and absorbance read at 412 nm.

Glucose Uptake Assay- *In Vitro* Antidiabetic Assay

In the yeast preparation, 1 g of yeast was weighed into a falcon tube and 10 mL of distilled water was added and repeatedly washed via centrifugation at 3000 g for 5 mins. This was done repeatedly until

the supernatant was clean. Furthermore, 10% w/v of the supernatant was prepared with distilled water. In the preparation of the glucose solution, 18.016 mg of glucose was dissolved in 10 mL of distilled water. Metformin was used as a standard. In the metformin preparation, 100 mg of metformin was weighed into an Eppendorf tube and dissolved in 1 mL of distilled water and afterwards, 20% of the metformin solution was picked and run through nine-fold serial dilution to obtain concentrations of 20, 10, 5, 2.5, 1.25, 0.625, 0.3125 mg/mL. Stock solution of extracts of the sample was prepared by dissolving 20 mg of each sample in 1 mL of distilled water in a 2 mL Eppendorf tube. The sample was vortexed for 1 minute and sonicated for 5 minutes to ensure complete dissolution.

In the assay, 200 µL of the extract and metformin were added to 200 µL of the glucose solution in a 2 mL Eppendorf and incubated at 37^0C for 10 minutes. Furthermore, 20 µL of yeast suspension was added to each Eppendorf tube and vortexed for 30 seconds and afterwards incubated at 37^0C for 60 minutes. After incubation, the Eppendorf tube was centrifuged at 2500 x g for 5 minutes. The supernatant was collected after centrifugation and the absorbance was read at 720 nm to estimate glucose uptake.

Antiproliferative Assay

Cell Culture: Cells were cultured as described by [10]. Jurkat, LNCap, and PNT2 cells were cultured in the RPMI 1640 medium. MCF7 cell line was cultured in D-MEM medium. All culture media were supplemented with 1% PSG and 10% FBS. The cells were maintained in an incubator with 5% CO_2 concentration at 37 °C and passaged on reaching about 80% confluence.

Cell Viability Assay: The Alamar Blue assay or Resazurin-based Colorimetric Assay was used to determine the cytotoxicity of *P. biglobosa* on cancer and normal cell lines. The protocol used by [11] was used.

In the preparation of the stock solution, 100 mg of each extract was dissolved in 1 mL of distilled water. The solution was vortexed for 1 minute and afterwards, filter sterilized into cryotubes in a biosafety cabinet through 0.45 µm pore filters and subsequently, a twofold serial dilution was made of each extract to obtain four concentrations of 0.0625, 0.125, 0.25 and 0.5 mg/mL. Curcumin was used as a standard for the experiment and the concentration range used was 10-100 µg/mL in 1% DMSO.

A volume of 100 µL (1 × 10^5 cells/mL) of cells was seeded into 96-well plate. The cells were immediately treated with 10 µL of each extract dilutions in triplicates and incubated in an incubator with 5% CO_2 concentration at 37 °C for 72 hours. The untreated experiment which is the negative control was included in the plate. A blank of culture media on;ly was also included. And for each of the test extracts including the standard curcumin, a colour control plate was set up.

Resazurin solution was prepared by dissolving 0.15 mg of resazurin in 1 mL of PBS. The solution was vortexed for 1 minute and afterwards filter sterilized. After 72 hours of incubation at 37°C, under 5% CO_2, in a humidified atmosphere, 10 µL of the prepared resazurin solution was added to each well. The excitation and emission were measured at 560 nm and 590 nm respectively on a microplate reader.

Dose response curves were plotted as percentages of cell viability against concentration.

$$\% \text{ Cell Viability} = [(ODT_0-ODT_1)/(ODU_0-ODU_1)] \times 100$$

where ODT_0 is the average absorbance of wells treated with test extracts for all cell lines; ODT_1 is the average absorbance of wells with curcumin or test extract control; ODU_0 is the average absorbance of wells with untreated cells (negative control) for all cell lines; ODU_1 is the average absorbance of wells containing blank (culture media only).

Since the experiment was done in triplicates, the mean percentage of the cell viability was used to plot the dose-response curve and the inhibitory concentration at 50% was also determined using nonlinear regression. The inhibitory concentration at 50% IC_{50}) is the concentration of *P. biglobosa* extracts or Curcumin (the test substance) that induces 50% inhibition of the cell lines.

The selectivity Index (SI) which is the ratio of the IC_{50} of each sample (including standard drug curcumin) on normal cell line PNT-2 to the IC_{50} of each sample (including standard drug curcumin) on the cancer cell lines. According to [12], samples were said to have high selectivity towards cancer cells if their SI values were greater than 2.

Gas Chromatography-Mass Spectrometry (GC-MS)

The methanolic extracts were analysed using the GC-MS to identify the major compounds present using the PerkinElmer GC Clarus 580 Gas Chromatograph interfaced to a Mass Spectrometer (PerkinElmer Clarus SQ 8 S) equipped with ZB-5HTMS (5% diphenyl/95% dimethyl polysiloxane) fused capillary column (30 × 0.25 µm ID × 0.25 µm DF). The conditions of GC-MS were previously described [13]. The total GC-MS running time was 50 min respectively. Turbo-Mass (ver. 6.1.0) was used in this analysis and compounds were identified using the National Institute of Standard and Technology (NIST) database with over 62,000 patterns.

Statistical Analysis

Microsoft Excel (2013) was used for the calculation and plotting of mean and SD estimates in the graph. Mean EC_{50} and IC_{50} values were compared by one-way ANOVA using GraphPad prism (8.0) and values with $p < 0.05$ were considered statistically significant.

Results

Phytochemical constituents

Both the FDE and UDE were screened for phytochemicals namely: saponins, tannins, terpenoids and flavonoids (Table 1).

	Saponins	Tannins	Terpenoids	Flavonoids
Standard	+	+++	+++	+++
FDE	-	-	++	+
UDE	-	-	+	+

Table 1: Phytochemical constituent of aqueous extracts of fermented and unfermented *Parkia biglobosa*.

+++ means present in high concentration; ++Means present in moderate concentration; +Means present in low concentration; - means absent.

In vitro antioxidant assay

(2,2-DIPHENYL-1-PICRYL HYDRAZYL) DPPH Assay: The antioxidant effect was evaluated by calculating the $EC_{50.}$ All the extracts reduced DPPH to diphenylpicrylhydrazine and diminished the

absorbance at 517 nm. The UDE had the highest DPPH scavenging activity with an EC_{50}= 0.54 and while the FDE had an EC_{50}= 2.27 (Figure 1 & Table 2).

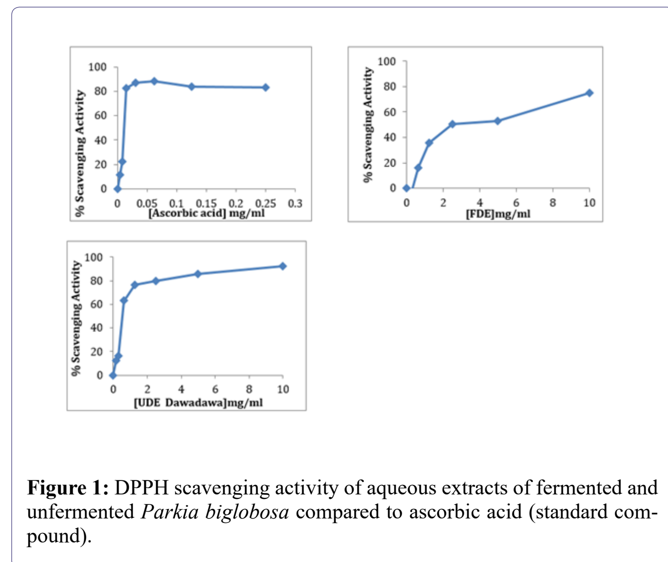

Figure 1: DPPH scavenging activity of aqueous extracts of fermented and unfermented *Parkia biglobosa* compared to ascorbic acid (standard compound).

Sample/Standard	EC_{50} value (mg/mL)	P VALUE
Ascorbic Acid (Standard)	0.01141	≤0.001
UDE	0.537177	≤0.001
FDE	2.274214	≤0.001

Table 2: DPPH scavenging activity of the fermented and unfermented Parkia biglobosa with their various EC-50 values.

Values are means ± standard deviation of three replicates

Total Phenolic Content

The results showed that the FDE had a higher concentration of phenols (0.24 ± 0.01) than the UDE (0.18 ± 0.01). Regarding the gallic acid equivalence, the total phenolic content of the FDE was 2408.96 GAE and the UDE was 1774.9 GAE. This was based on the Gallic acid standard curve (y=0.3927x + 0.0501, R^2=0.9934) (Figure 2).

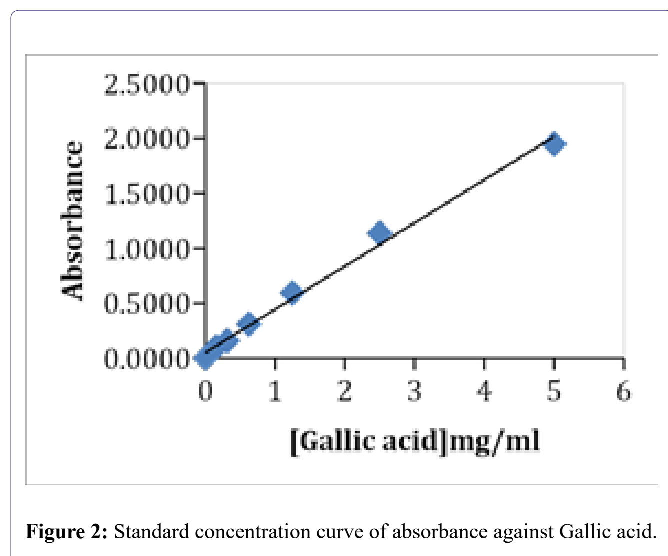

Figure 2: Standard concentration curve of absorbance against Gallic acid.

Total Flavonoids Content

The fermented *P. biglobosa* had the highest total flavonoid content of 812.36 mg/100g QE per 10 mg of the extract while the unfermented recorded 576.19 mg/100g QE per 10 mg extract. This was based on a standard Quercetin curve (y= 16.077x +0.082, R^2= 0.999) (Figure 3).

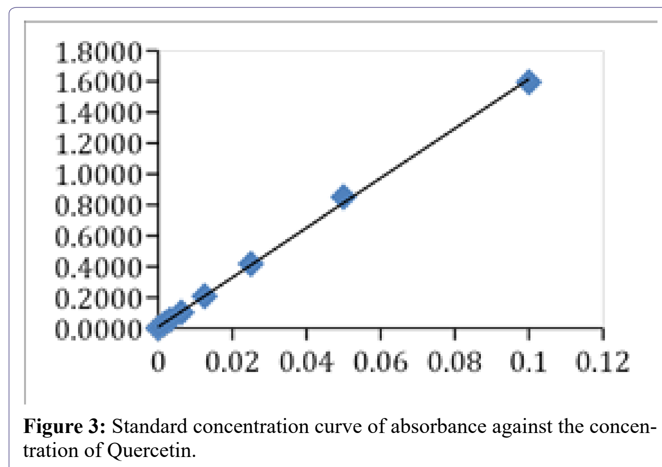

Figure 3: Standard concentration curve of absorbance against the concentration of Quercetin.

In-vitro antidiabetic assay (Yeast Cells Glucose Uptake Assay)

The glucose uptake at an initial concentration of 20mg/mL aqueous extracts of *P. biglobosa* which was reduced through a twofold serial dilution to obtain 6 different concentrations was comparable to standard drug metformin. However, the effect of unfermented seed and fermented seed aqueous extracts on the uptake of glucose by yeast cells was higher at all concentrations as compared to metformin. Fermented seed aqueous extracts showed the highest glucose uptake followed by unfermented and metformin recording the lowest uptake (Figure 4 & Table 3).

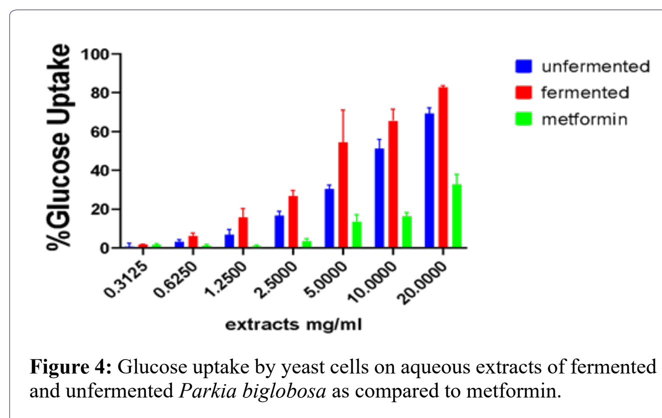

Figure 4: Glucose uptake by yeast cells on aqueous extracts of fermented and unfermented *Parkia biglobosa* as compared to metformin.

In vitro cytotoxic assay

The Fermented and unfermented *P. biglobosa* seed had a cytotoxic effect on liver cancer cell lines (HepG2), breast (MCF-7), prostate (PC3) and normal human prostate cells (PNT2). IC_{50} values were not obtained for the prostate cancer cell (LNCAP). UDE was most cytotoxic to PC3 and MCF 7. There was no IC_{50} value for the UDE on LNCAP. For the FDE, breast cancer cells MCF-7 had the highest activity (Figures 5-9 & Tables 4 & 5).

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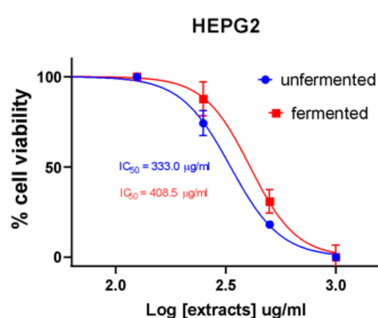
Concentration (mg/mL)	%Glucose uptake of sample/drug		
	Metformin	Fermented	Unfermented
20	32.7 ± 5.256	82.9 ± 0.969	69.3 ± 2.943
10	16.2 ± 2.189	65.6 ± 5.959	51.3 ± 4.742
5	13.6 ± 3.554	54.6 ± 16.543	30.4 ± 2.247
2.5	3.5 ± 1.251	26.7 ± 3.090	16.7 ± 2.433
1.225	0.9 ± 0.722	15.8 ± 4.599	6.9 ± 2.856
0.625	1.1 ± 0865	6.2 ± 1.705	3.2 ± 1.247
0.3125	1.7 ± 0.583	1.9 ± 0.217	0.8 ± 1.667

Table 3: Summary of percentage uptake of glucose by yeast cells on aqueous extracts of fermented and unfermented Parkia biglobosa as compared to metformin.

Values are means ± standard deviation of three replicates



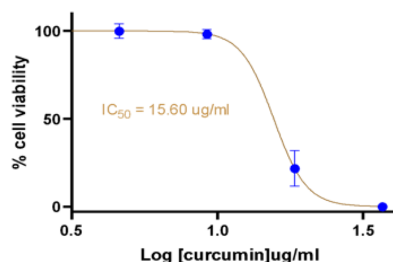


Figure 5: Cell viability curves showing cytotoxicity effect of FDE and UDE and the standard curcumin against liver cancer cell line (HepG2).

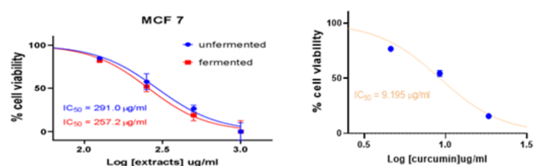
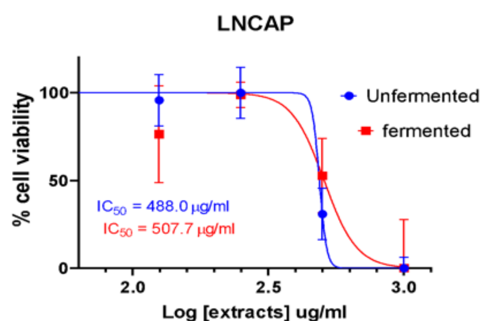


Figure 6: Cell viability curves showing cytotoxicity effect of of FDE and UDE and the standard curcumin against breast cancer cell line.



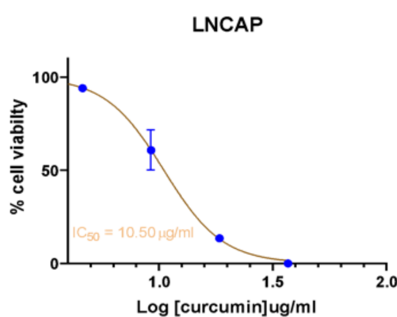
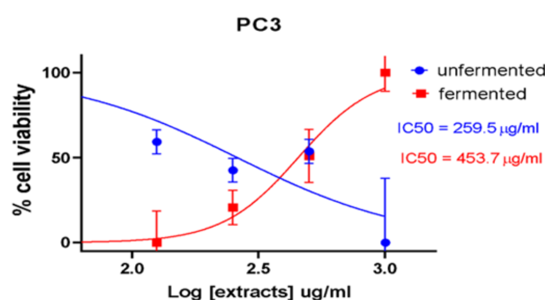


Figure 7: Cell viability curves showing cytotoxicity effect of of FDE and UDE and the standard curcumin against prostate cancer cell line (LNCAP).



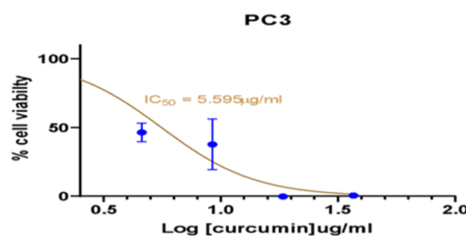


Figure 8: Cell viability curves showing cytotoxicity effect of FDE and UDE and the standard curcumin against prostate cancer cell line.

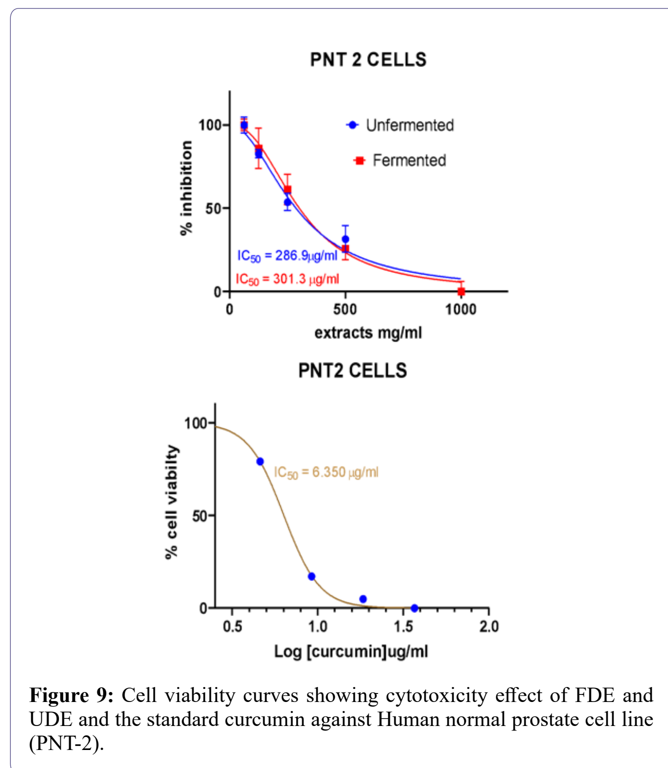


Figure 9: Cell viability curves showing cytotoxicity effect of FDE and UDE and the standard curcumin against Human normal prostate cell line (PNT-2).

Cell Lines	FDE	UDE	Curcumin	P-value
HepG2	408.5	333.0	15.60	≤0.001
MCF-7	257.2	291.0	9.195	≤0.001
LNCAP	507.7	>1000	10.50	≤0.001
PC3	453.7	259.5`	5.595	≤0.001
PNT-2	301.3	286.9	6.350	≤0.001

Table 4: Comparison of IC50 values of samples tested and curcumin standard on the various cell lines.

Values are means ± standard deviation of three replicates.

Selectivity Index

The **SI** of various extracts which is fermented, unfermented and curcumin was calculated by dividing the IC50 of the normal prostate cell line (PNT-2) by the IC_{50} of the cancer cell lines (HepG2, MCF-7, LNCAP, PC3) (Table 5).

Cell Lines	FDE	UDE	Curcumin
HepG2	0.738	**0.862**	0.407
MCF-7	**1.171**	**0.986**	0.691
LNCAP	**0..593**	**0.287**	0.605
PC3	**0.664**	**1.106**	1.135

Table 5: Selectivity indices of the aqueous extract of fermented and unfermented Parkia biglobosa.

GC-MS Analyses

Figure 10 shows the total ion chromatogram for the fraction which sums up the intensities of all mass spectral peaks. Also, some active compounds with their Retention Times (RT) in the fermented sample only are shown in table 6.

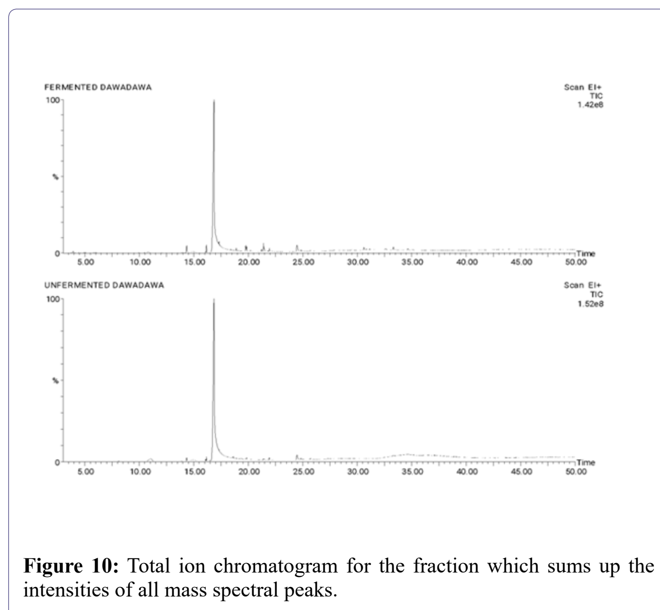


Figure 10: Total ion chromatogram for the fraction which sums up the intensities of all mass spectral peaks.

Discussion

The DPPH test is the oldest indirect method for determining the antioxidant activity based on the ability of the stable free radical 2, 2-diphenyl-1-picrylhydrazyl to react with hydrogen donors including phenols. DPPH, an N centered radical, has a characteristic absorbance at 517 nm, which decreases with the scavenging of the proton radical [14]. Phenolic compounds are also known to be potent antioxidants; flavonoids, tannins, etc. Total phenol content is measured by the Folin-Ciocalteu method at an absorbance of 750 nm [15]. Phenolic compounds have been suggested to induce the cellular antioxidant system by approximately 50% cellular glutathione concentration increment. Flavonoids are significant in the modulation of γ-glutamylcysteine synthase in both cellular antioxidant defences and detoxification of xenobiotics [16]. Among the extracts, the aqueous unfermented extract showed the strongest DPPH scavenging activity with an EC_{50} value of 0.54 followed by the aqueous fermented extract with an EC_{50} value of 2.27. The presence of phenolic compounds was also observed in all extracts, with the aqueous fermented extract recording the highest total phenolic content, 2408.964 mg/g plant extract (GAE) in crude extracts of *P. biglobosa* while the aqueous unfermented extract recorded a lower total phenolic content, 1774.892 mg/g plant extract (GAE). This suggests that the antioxidant activity of the *P. biglobosa* could be partly attributed to the presence of the various phenolic compounds in the extracts which may act through their ability to adsorb, neutralize, and quench free radicals. The result of the present investigation has shown that *Parkia biglobosa* possesses antioxidant activity and this property is predominantly found in the aqueous unfermented extract. In the total flavonoid assay, the fermented *P. biglobosa* had the highest total flavonoid content of 812.3613mg/100g QE per 10 mg of the extract and the unfermented showed a total flavonoid content of 576.1854mg/100g QE per 10mg extract. Therefore flavonoids were present in both aqueous fermented and unfermented extracts of *Parkia biglobosa* despite the disparity in the amounts. This suggests that the antioxidant activity of the *Parkia biglobosa* could be partly attributed to the presence of various phenolic compounds, particularly flavonoids. The extracts may act through their ability to adsorb, neutralize, and quench free radicals. The result of the present investigation has shown that *P. biglobosa* possesses antioxidant activity and this property is predominantly found in the aqueous fermented extract.

RF	Scan	Height	Area	Area %	Norm %	SI	COMPOUND
16.163	718	6,945,628	232,439.00	0.627	0.95	99.77 99.88 100.00	9-Octadecanoic acid (Z)-, methyl ester Trans-13-Octadecanoic, methyl ester 11-Octadecenoic acid, methyl ester
16.860	756	141,783,424	24,365,656.00	65.732	100	100.00 99.78 100.00	Cis-Vaccenic acid 9-Octadecenoic acid, (E)- cis- 13- octadecenoic acid
21.407	1004	7,871,788	509,356.80	1.374	2.09	97.03 96.87	Cyclopropaneoctanoic acid,2- [[2-[(2-ethyl-cyclopropyl) methylcyclopropyl] methyl]-, methyl ester 8, 11, 14 – Eicosatrienoic acid, (Z, Z, Z)- E, E- 1, 9, 17- Docasatriene

Table 6: Compounds Present in Extracts of Fermented seed of *P. biglobosa*.

This research was also aimed at evaluating the antiproliferative effect of this Ghanaian food spice *P. biglobosa*, using the aqueous extract of fermented and unfermented seeds. To date, no known study has been conducted or published investigating the in vitro anticancer activity of *P. biglobosa* seed extract. The In Vitro Cytotoxic assay was conducted for both the fermented and the unfermented *Parkia biglobosa* on four cancer cell lines which are LNCAP (prostate), MCF-7 (breast), HepG2 (liver), PC3 (prostate) and a normal human prostate cell line (PNT2). Curcumin was used as a positive control. The result from this study indicates that fermented and unfermented aqueous extracts of *P. biglobosa* had cytotoxic activity on Liver Cancer Cells (HepG2), breast cancer cells (MCF-7) and Prostate Cancer Cells (PC3). Aqueous extracts of fermented *P. biglobosa* seed had cytotoxic activity on Prostate Cancer Cells (LNCAP) but at the same time, no significant cytotoxic activity was recorded with aqueous extracts of unfermented on prostate cancer cells (LNCAP). The high amount of flavonoids and phenolics observed for the extracts might account for the promising antiproliferative activity observed in the various cancer cell lines as these secondary metabolites have proved to be good anticancer agents [17].

An IC_{50} was obtained for HepG2, MCF-7 and PC3 cells with respect to both fermented and unfermented seed extracts in a concentration-response curve. When sample extracts were tested against LNCAP cells, IC_{50} value was obtained only in the fermented sample as the unfermented sample showed IC_{50} greater than 1000. When the cytotoxic effects of various samples on cancer cells were compared, it was observed that in HepG2 and LNCAP cells, unfermented samples showed more pronounced effects than fermented samples. It was also observed that in MCF-7 and LNCAP cells the fermented sample showed a more pronounced cytotoxic effect than the unfermented sample. Also, it was observed that the most pronounced cytotoxic effect was observed in fermented samples against MCF-7 cells and the least cytotoxic activity was observed in unfermented samples against LNCAP cells in which case IC_{50} was greater than 1000. Furthermore, in the positive control test experiment in which Curcumin was used as the positive control, the most profound cytotoxic effect was observed in prostate cancer cell (PC3) followed by breast cancer cells (MCF-7), and then prostate cancer cells (LNCAP) with liver cancer cells (HepG2) showing the least profound cytotoxic effect. It is important to note that the half maximal inhibitory concentration (IC_{50}) as observed in the positive control sample that is curcumin against all the cancer cells had a relatively lower $IC_{50,}$ that is to say that curcumin had a more profound cytotoxic activity than both fermented and unfermented *Parkia biglobosa* seed extracts. Potential anticancer agents should exhibit cytotoxic specificity for cancer cells hence a factor called selectivity index is used to determine if a candidate is a good anticancer agent. Selectivity indexes greater than 2 make it a good candidate for further anticancer studies. It should be noted that none of the test samples had an SI of 2 including the positive control curcumin which is a very good anticancer agent showing distinctive antiproliferative activity like inhibiting proliferation, induction of apoptosis and invasion of tumours [18]. The highest selectivity index for all the test samples including the positive control curcumin obtained for breast cancer cells (MCF-7) for aqueous crude fermented sample followed by prostate cancer cell (PC3) for the positive control curcumin and then followed by prostate cancer cell (PC3) for aqueous crude unfermented sample. It can be inferred from this study that liver cancer cell (HepG2), breast cancer cell (MCF-7), Prostate Cancer Cell (PC3) are all susceptible to *Parkia biglobosa* seed while prostate cancer cell (LNCAP) was most resistant to the seed. This study has also shown that obtaining fractions of both the unfermented and the fermented sample should be obtained and this can increase the selectivity index which will help us make very good judgement.

In the GC-MS analysis prominent High peaks on the total ion Chromatogram showed the presence of Cis Vaccenic Acid and 9-octadecenoic acid present in both the fermented and the unfermented extracts. Preliminary Studies conducted by [19], showed that Cis-Vaccenic acid lowers risk of breast cancer. According to [20], Cis-Vaccenic acid present in the both extracts is associated with lower risk of coronary heart disease which subsequently leads to heart failure. 9-octadecenoic acid significantly decreased Reactive oxygen species (ROS) and Lipid Peroxidation (LPO) by raising the activity of antioxidant enzymes [21]. Hence the presence of 9-octadecenoic acid may account for the high antioxidant scavenging activity of the extracts. The presence of Cis-Vaccenic acid may account for the anti-cancer activity of the extracts. Fermented extracts showed prominent peaks of eicosatrienoic acid, docasatriene, cyclopropaneoctanoic acid which were absent in the total ion chromatogram of unfermented extracts. Fermentation plays a major role in the changes of the components in the secondary protein structure. Furthermore, a study showed that fermentation increased the total phenolic content of an extract [22]. The compound eicosatrienoic acid which was formed during fermentation as it was present only in the fermented extracts of *Parkia biglobosa* has proven to be a very promising compound which according to a

study conducted by [23], has proven to lower insulin resistivity. The formation of eicosatrienoic acid during fermentation may account for the increased antidiabetic activity in the fermented extracts. The study also proved that eicosatrienoic acid has anti-inflammatory properties hence the high total phenolics, total flavonoids and anticancer levels on breast cancer cells may be accounted for by the presence of eicosatrienoic acid in the fermented extracts [23].

In this study, both fermented and unfermented aqueous extracts of *Parkia biglobosa* were screened for the presence of phytochemicals. These included tannins, saponins, flavonoids, and terpenoids. In both extracts, only the presence of flavonoids and terpenoids were detected.Terpenoids, which plays an important role in wound and scar healing [24], treatment of skin disease [25]. Terpenoids are known to fight malaria and cancer [26], very active in wound healing [25]. From clinical studies, it is shown that terpenoids strengthen the skin, increase the concentration of antioxidants in wounds, and restore inflamed tissues by increasing blood supply. The observed antioxidant and anticancer activities in this study could partly be attributed to the presence of flavonoids, terpenoids as well as phenolic compounds.

Investigations revealed that both test samples, the aqueous fermented and unfermented *Parkia biglobosa* seed extracts, helped yeast cells to uptake glucose. The glucose uptake at a final concentration of 0.3125 mg/mL and an initial concentration of 20mg/mL of fermented and unfermented seed was comparable to standard drug metformin. However, the effect metformin on glucose uptake by yeast cells was lower compared to the two test samples. Of the two test samples, aqueous fermented *Parkia biglobosa* extract readily helped yeast cells to uptake glucose better than the unfermented. The antidiabetic properties present of *P. biglobosa* seed extracts can be attributed to bioactive compounds that were revealed during the phytochemistry and the antioxidant assays. Therefore, the presence of one of these compounds in the extract might help in the uptake of glucose by yeast cells in the present study.

Conclusion

The present investigation suggests that *Parkia biglobosa* seed possesses remarkable antioxidant, antidiabetic and anticancer properties and the chemical compounds detected by the phytochemical analysis and gas chromatography mass spectrometry analysis could be responsible for these properties.

Author's Contribution

Perez Frimpong Danso and Christopher Larbie conceived and designed the study. Perez Frimpong Danso and Adwoa Frema Amanfo, wrote the manuscript. Afua Ampem Gyenfi, Rosemond Serwaa Opoku helped in editing the manuscript. Regina Appiah-Oppong, Christopher Larbie Reviewed the manuscript and did the required editing.

Declarations

Competing interests

The Authors declare that they have no competing interest.

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