

Antioxidant and anti-prostate cancer activities of *Moringa oleifera*, *Phyllanthus amarus* and *Carica papaya*

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Abstract

Background: Globally, interest in herbal medicines is increasing. In Ghana, most herbalists use herbal medicines for treatment of various ailments including prostate cancer, although no empirical evidence on their efficacies exists.

Objective: The aim of the present study was to test for antioxidant and anti-prostate cancer activities of *Moringa oleifera*, *Phyllanthus amarus* and *Carica papaya*.

Methods: Plants parts used were air-dried, ground and sequentially extracted using solvents with increasing order of polarity (petroleum ether, dichloromethane, ethyl acetate, ethanol and aqueous). The 2, 2-diphenyl-1-picrylhydrazyl assay, Folin Ciocalteu method and tetrazolium-based calorimetric assay were used to determine total antioxidant capacities, total phenolic content of extracts and cytotoxicities of the extracts against LNCaP and PC3 prostate cancer cells, respectively.

Results: Ethanolic extract of *P. amarus* possessed the highest phenolic content while its aqueous extract showed the strongest antioxidant activity ($EC_{50} = 19.32 \pm 1.13 \mu\text{g/mL}$). Aqueous extract of *C. papaya* exhibited anti-prostate cancer activity with good selectivity towards PC3 cells [$IC_{50} = 45.68 \pm 1.16 \mu\text{g/mL}$, selectivity index (SI) = 18], whereas dichloromethane extract of *P. amarus* showed the strongest anticancer activity against LNCaP cells ($IC_{50} = 43.97 \pm 1.14 \mu\text{g/mL}$).

Conclusion: These findings lend pharmacological credence to the anecdotal evidence of the anti-prostate cancer property of the plants. Further studies must be performed to identify the active principles in the bioactive plant components.

Keywords: Anti-prostate cancer, *Carica papaya*, *Moringa oleifera*, *Phyllanthus amarus*

INTRODUCTION

Prostate cancer refers to the growth of malignant tumor in the prostate gland of the reproductive system in males [1]. Different reports indicate that, prostate cancer can be more aggressive and may advance to a castration-resistant disease in older people of African descent if it remains untreated [2,3,4]. Global prostate cancer occurrence accounts for about 899,000 new cases, and about 258,000 deaths each year [1]. In Ghana, Laryea et al. have reported on the prevalence of the disease (13.2%) while Globocan reported that the disease is the second leading cause of cancer related deaths in Ghanaian men [5,6]. A report by Pham-Huy et al. has revealed that free radicals and related reactive species generated from some lifestyle practices such as smoking, excessive alcohol intake, high intake of meat

and low intake of vegetables are the major causes of prostate cancer hence attracting a great deal of alertness for preventive and therapeutic strategies [7]. Currently, available treatments for prostate cancer are expensive, confronted with resistance, and have side effects. Hence, the search for alternative, affordable, effective, accessible and less toxic medicine is a major focus of researchers in modern cancer drug discovery. Ekor [8] has reported that there has been tremendous increase in the use of herbal products and supplements over the past 30 yr., with an estimated 80% of the world's population depending on them for some aspects of primary healthcare [8]. Use of herbal medicines as therapeutic remedies encompasses plant components such as seeds, fruits, roots, leaves, stem bark, or flowers for medicinal purposes. Previous studies have reported anticancer, anti-tumour and antioxidant properties of many medicinal plants [9,10,11]. In Ghana, many herbalists use medicinal plants for the management of prostate cancer without any scientific evidence of their effectiveness. A large number of consumers assume that herbal remedies are generally innately safe because they are

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derived from a natural source [12]. However, some of the well-known plant derived anticancer agents have been shown to have side effects such as cardiotoxicity, renal, and liver toxicities [13, 14]. Currently, scientific research is ongoing to support the use of *Croton membranaceus* and *Zanthoxylum xanthoxyloides* as anticancer agents, however research on the efficacies and toxicities of several other medicinal plants used for similar purpose has not yet been extensively done.

Carica papaya (local Twi name: Brofere) is an edible fruit, mostly grown in the tropics. It is one of the species often used as medication for the treatment of diseases such as dyspepsia and kindred ailments [15]. The seeds are usually chewed as dewormer. Also, the roots and juice are used for management and treatment of cough, bronchitis, and other respiratory diseases. Although, the leaves are normally discarded, they have been used in folk medication for the treatment of various conditions such as fever, gastric digestion problems, burns, asthma and amoebic dysentery [16,17]. The mixture of root and seed is taken as a purgative and may cause abortion, while the leaves have demonstrated anti-inflammatory activity [9]. Recently, anti-prostate cancer activity has been reported on black seeds of *C. papaya* [1].

Phyllanthus amarus (local Twi name: Bommaguwakyi) is a small herb belonging to the family *Euphorbiaceae*. This plant is mostly found in tropical countries. It is used traditionally in Nigeria and India to treat malaria associated symptoms and to ease genito-urinary system, stomach pain, liver, kidney, and spleen disorders [18]. Previous studies have reported cytotoxic activity of *P. amarus* extracts on several human cancer cell lines such as prostate cancer cell line DU145, colon cancer cell line Caco-2, lung cancer cell lines A549, breast cancer cell line MCF-7, and liver hepatocellular carcinoma HepG2 [11,19,20,21]. Kierner has indicated that extracts of *P. amarus* inhibited induction of interleukin (IL)-1 β , interferon- γ (IFN- γ), interleukin (IL)-10 and reduced tumor necrosis factor (TNF- α) production [22].

Moringa oleifera is a tree belonging to the family *Moringaceae*. Aqueous leaf extract of the plant is used in traditional medicine, to treat several maladies including swellings, genito-urinary ailments, hypertension, malaria, diseases of the skin, arthritis and diabetes [10,23]. The leaves have been shown to possess antioxidant compounds, which are of medicinal value [10]. *M. oleifera* leaves are rich in polyphenols, vitamins, saponins, flavonoids, carotenoids, alkaloids, tannins, isothiocyanates, and glucosinolates [24,25]. Interestingly, previous reports have indicated that different solvent extracts of moringa leaves contain phenolic compounds, hence it is a good source of polyphenols [24,25]. The widespread use of these plants coupled with their anecdotal evidence and pharmacological properties, led to the investigations on their anti-prostate cancer potential.

MATERIALS AND METHODS

Materials

Dimethyl sulfoxide (DMSO), methanol (MEOH), ethanol (ETOH), ethyl acetate (EA), dichloromethane (DCM), petroleum ether (PE) and isopropanol were obtained from Daejung (South Korea). Folin Ciocalteu reagent, butylated

hydroxyl toluene (BHT), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, Rosewell Park Memorial Institute 1640 (RPMI) culture medium and Fetal Bovine Serum (FBS) were obtained from Sigma-Aldrich (USA). All other reagents and supplies were obtained from standard suppliers.

Ethnobotanical survey and collection of plants

During the ethnobotanical survey, the names of plants used in the management of prostate cancer were provided by herbalists in Accra. The plants were collected early in the morning before 9 am (April 2017), authenticated by a taxonomist and voucher specimens were stored at the University of Ghana Herbarium. The fresh green leaves were cleaned, air-dried and ground into powder using an electric blender before use.

Extract preparation

Sequential extraction method was used to obtain crude extracts from powdered leaves of the plants using solvents with increasing polarity as described [26]. Each of the powdered plant leaves (50 g) were macerated three times with PE (total volume 500 mL) at room temperature (26°C) for 3 days. Air-dried residues were extracted sequentially with DCM, EA and ETOH following the above procedure. The filtrates of the same solvents were pooled together and evaporated using rotary evaporator, Rotavapor R-205 (Buchi, Switzerland) under low pressure at 50°C. Aqueous extracts were prepared by heating the powdered samples in distilled water (10% w/v) at 80°C for 1 h, cooling and filtering. The procedure was repeated with the pellet and supernatants were pooled, frozen and lyophilized in a freeze dryer (Lyotrap, United Kingdom). The percentage yield was calculated as (final weight of dried extract) / (Initial weight of plant powder) $\times$ 100.

Determination of antioxidant properties

Total antioxidant activity. The antioxidant activities of the extracts of each plant were determined using the DPPH assay with slight modification [27,28]. Two-fold serial dilutions of stock solution of BHT (1 mg/mL in methanol) which was used as positive control and plant extracts (1 mg/mL in ethanol) were separately prepared to obtain seven different concentrations. The aliquots of 100 μ L of each extract or BHT were transferred into 96-well plates and 100 μ L of 0.5 mM DPPH solution was added to each well to initiate the reaction. The plates were gently shaken to mix the content and incubated for 20 min before absorbance readings (Abs) were taken at the wavelength of 517 nm using a spectrophotometer (Tecan Infinite M200 Pro plate reader, Austria). Negative controls experiments were set up using the diluents (ethanol and methanol). Experiments were carried out in triplicate. Antioxidant activities were expressed as percentage free radical scavenging activity which was calculated as (Abs of blank solution - Abs of extract) / (Abs of blank solution) $\times$ 100

Absorbance. Effective concentrations at 50% scavenging activity (EC₅₀) was determined from a plot of percentage scavenging activity versus sample concentration.

Total phenolics content. Total phenolic content of the plant extracts was assessed using the Folin-Ciocalteu assay as described with slight modification [28]. Plant extract concentration of 5 mg/mL was prepared from each of the

extracts. The reaction mixture comprised 750 μ L distilled water, 10 μ L sample and 50 μ L of the Folin-Ciocalteu reagent. These were thoroughly mixed and incubated in the dark for 8 min. Subsequently, 150 μ L of Na_2CO_3 was added and the mixture was incubated for 2 h in the dark at room temperature (26°C). Absorbance was measured at a wavelength of 750 nm using the spectrophotometer. The total phenolic content was expressed as Gallic Acid Equivalents (GAE) in grams per 100 g dry weight of plant extract. All experiments were carried out in triplicate.

Cell culture

The PC3, LNCaP and PNT2 cells were cultured in RPMI culture medium supplemented with 10% FBS and 1% streptomycin-penicillin. The cells were incubated in a humidified incubator at 37°C with 5% CO_2 .

Cell viability assay. Cell viability was determined using the MTT assay as described [29]. The cells were seeded at 1×10^4 cells/well in 96-well plates and incubated for 24 h and treated with 10 μ L aliquots of different concentrations of each plant extract (0 - 1000 μ g/mL) and compound (0 - 37 μ g/mL). Final concentration of DMSO in each well was 1%. The plates were incubated for 72 h and subsequently 20 μ L of 2.5 mg/mL solution of MTT was added to the wells before re-incubation for 4 h. Aliquots of 150 μ L of acidified isopropanol were added to the wells to dissolve any formazan crystals formed and the plates were incubated at room temperature overnight. Absorbance was read at a wavelength of 570 nm using the spectrophotometer. Concentrations of extracts caused inhibition of 50% cell growth (IC_{50} value) were determined from plots of cell viability and extract concentrations.

Statistical analysis

Data obtained were expressed as means $\pm$ standard deviation (SD) using statistical software, Graphpad prism version 5 (GraphPad Software Inc. San Diego, CA, USA). Also, One-way ANOVA was used to determine the level of significance between the extracts and controls, followed by Tukey's multiple comparison test. The results were considered statistically significant at $p < 0.05$.

RESULTS

Fresh leaf samples of *C. papaya*, *M. oleifera* and *P. amarus* were collected for the study. Table 1 shows the plants, their families, parts used and voucher specimen numbers. The yield of each dried crude extract obtained are shown in Table 2. The highest yield was obtained from aqueous (AQ) leaf extract of *M. oleifera* and the lowest yield was obtained from ethyl acetate (EA) leaf extract of *C. papaya*. Total phenolic content (TPC) of extracts was determined from a calibration curve of the standard gallic acid ($y = 0.384x + 0.058$, $r^2 = 0.996$). All the extracts contained phenolic compounds with the highest recorded in the ETOH extract of *P. amarus* of 92.70 ± 3.62 (Figure 1).

Figure 2 shows that all the extracts exhibited antioxidant activities in a concentration dependent manner. The aqueous extract of *P. amarus* showed the strongest antioxidant activity ($\text{EC}_{50} = 19.32 \pm 1.13$ μ g/mL). The EC_{50} value of the positive control, BHT was 12.81 ± 1.07 μ g/mL. Generally, the ethanolic extracts had the highest levels of phenolics, whereas the aqueous extracts had stronger total antioxidant capacity. *M. oleifera*

Table 1: Ethnobotanical information on plant samples

Code	Plant	Family	Part used	Voucher specimen number
PA	<i>P. amarus</i>	Euphorbiaceae	Aerial parts	UGAJK-2017-005
CP	<i>C. papaya</i>	Caricaceae	Leaves	UGAJK-2017-001
MO	<i>M. oleifera</i>	Moringaceae	Leaves	UGAJK-2017-004

Table 2: Percentage yield of plant samples

Plant	Percentage yield of 50 g of plant sample				
	Petroleum ether	Dichloro methane	Ethyl acetate	Ethanol	Aqueous
<i>M. oleifera</i>	3.89	0.68	0.70	3.12	13.94
<i>C. papaya</i>	3.93	1.52	0.62	2.36	13.84
<i>P. amarus</i>	4.07	1.14	1.15	4.67	11.09

extracts showed weak antioxidant activity, thus EC_{50} values could only be obtained for the aqueous extract (250.20 ± 1.08 μ g/mL). Cytotoxicities of plant extracts and curcumin on prostate cancer cells, normal prostate cells and respective selectivity indices (SIs) are shown on Table 3. The median inhibitory concentrations, IC_{50} values, were derived from the log-concentration plots for the various crude plant extracts. Among the extracts, aqueous extract of *C. papaya* and DCM extract of *P. amarus* exhibited the strongest cytotoxic activity against PC3 ($\text{IC}_{50} = 45.68 \pm 1.16$ μ g/mL) and LNCaP ($\text{IC}_{50} = 43.97 \pm 1.14$ μ g/mL), respectively [32]. The petroleum ether extracts of *M. oleifera* and *P. amarus* were not cytotoxic against PC3 and LNCaP prostate cancer cells. Among the PA samples, the aqueous extract exhibited the strongest cytotoxic activity against PC3 cells ($\text{IC}_{50} = 48.20 \pm 1.20$ μ g/mL, SI = 5.92) and had good selective indices against PC3 and LNCaP cells with values 5.92 and 5.10, respectively. Although, DCM extract of PA exhibited moderate cytotoxic activities on LNCaP cells ($\text{IC}_{50} = 43.97 \pm 1.14$ μ g/mL), it had weak selectivity index (< 2).

DISCUSSION

We investigated the anti-prostate cancer activities and antioxidant properties of *P. amarus*, *C. papaya* and *M. oleifera*. Phenols are known to possess good antioxidant properties; therefore, we evaluated the phenolic content of the extracts. The ethanolic extracts possessed high amount of phenols indicating that most of the phenolic constituents were soluble in ethanol. However, these results do not confirm earlier results on studies done by Vuong [30] and Mandal [31] who found the converse to be true, with the highest amount of phenols present in aqueous extract of *C. papaya*. This could be due to the different extraction methods employed in both studies. In this study, ethanol extract of *P. amarus* possessed the highest amount of phenols indicating that ethanol is an ideal solvent for extracting the phenols. This was consistent with a recent study which indicated that

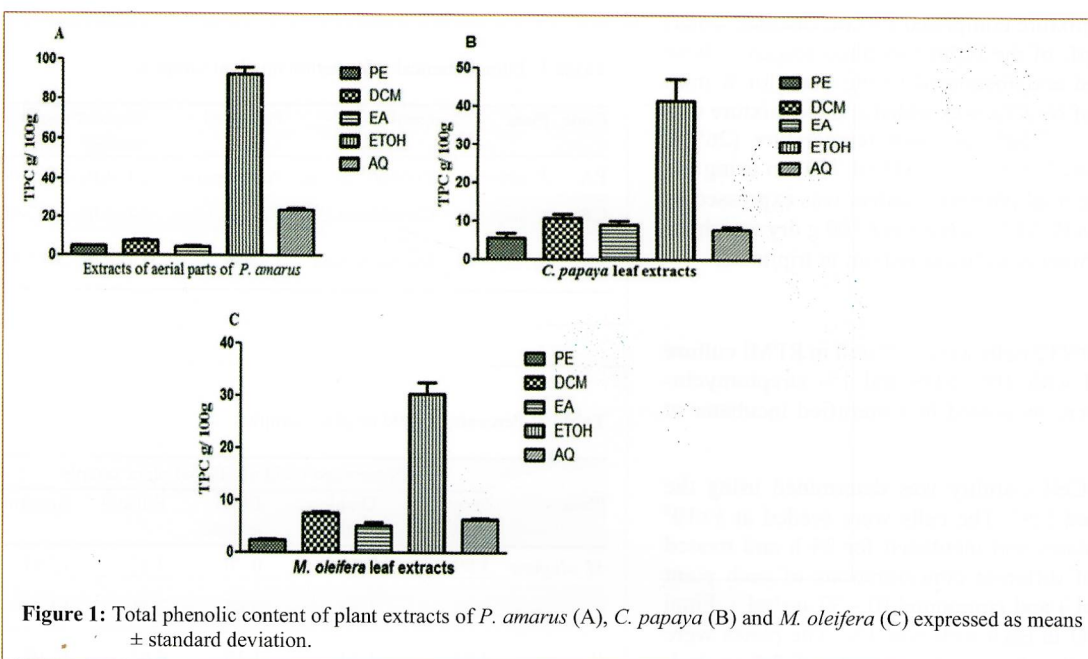


Figure 1: Total phenolic content of plant extracts of *P. amarus* (A), *C. papaya* (B) and *M. oleifera* (C) expressed as means $\pm$ standard deviation.

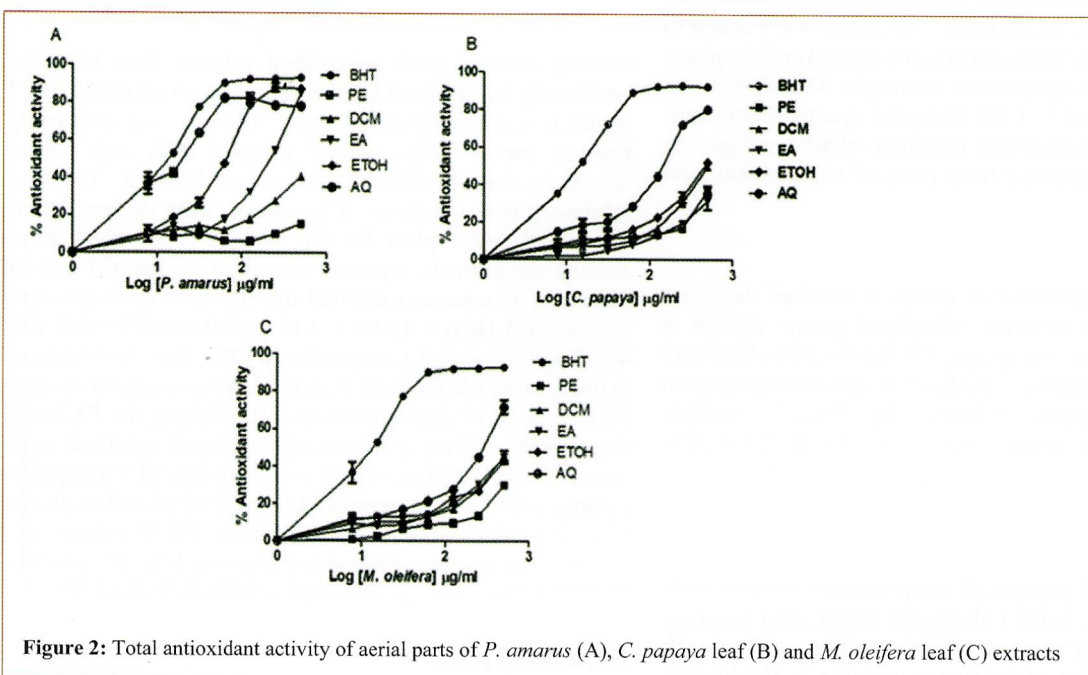


Figure 2: Total antioxidant activity of aerial parts of *P. amarus* (A), *C. papaya* leaf (B) and *M. oleifera* leaf (C) extracts

methanolic extract of *P. amarus* possessed more phenolic content than the aqueous extract [21]. All the extracts exhibited antioxidant activities with the aqueous extract showing the strongest. There appeared to be no correlation between the phenolic content of crude extracts and antioxidant activities. In this study there was no correlation between cytotoxic activity and the antioxidant activity of the extracts. The aqueous extract of *C. papaya* showed the strongest cytotoxic activity and selectivity towards PC3 cells ($IC_{50} = 45.68 \pm 1.16 \mu\text{g/mL}$, $SI = 25.5$). On the other hand, the dichloromethane extract was more selective towards LNCaP cells ($70.87 \pm 1.31 \mu\text{g/mL}$, $SI = 14.11$). Each of the extracts possessed various amounts of phenols. The ethanolic extracts had more phenols than the rest of the extracts.

These results confirm similar results from a previous the study [37]. Studies by Nguyen et al. have also indicated that aqueous and methanolic extracts of the *P. amarus* (whole plant) were cytotoxic to metastatic prostate cancer cell line DU145 with IC_{50} values of $26.71 \pm 8.10 \mu\text{g/mL}$ and $73.45 \pm 3.43 \mu\text{g/mL}$, respectively [21]. Nguyen et al. also reported that the methanolic extract had stronger antioxidant activity than aqueous extracts. This could be due to the presence of a very polar compound which possesses a stronger antioxidant activity and the different extraction methods employed. In addition, inhibition of cell cycle regulators responsible for cancerous growth has been reported on extracts of *P. amarus* [33]. Our results lend support to the earlier findings that *P. amarus* has anti-prostate cancer

Table 3: Cytotoxic effect of plant extracts on cells

Plant codes		PC3		LNCaP		PNT2 (CC ₅₀ , µg/mL)
		(IC ₅₀ , µg/mL)	SI	(IC ₅₀ , µg/mL)	SI	
PA	PE	465.10 ± 1.58 ^d	0.49	224.10 ± 1.43 ^c	1.02	229.70 ± 1.36 ^c
	DCM	98.82 ± 1.22 ^b	0.54	441.0 ± 1.14 ^a	1.22	53.54 ± 1.22 ^a
	EA	158.60 ± 1.41 ^c	0.61	70.20 ± 1.25 ^b	1.39	97.40 ± 1.30 ^b
	ETOH	473.10 ± 1.08 ^d	ND	>1000	ND	ND
	AQ	48.20 ± 1.20 ^a	5.92	55.50 ± 1.11 ^a	5.14	285.50 ± 1.12 ^c
MO	PE	>1000	ND	>1000	ND	ND
	DCM	163.80 ± 1.15 ^b	2.37	194.30 ± 1.24 ^c	2.00	388.50 ± 1.38 ^b
	EA	161.30 ± 1.20 ^b	1.38	129.70 ± 1.33 ^a	1.72	222.90 ± 1.20 ^a
	ETOH	846.60 ± 1.29 ^c	ND	>1000	ND	ND
	AQ	66.72 ± 1.15 ^a	14.99	148.90 ± 1.14 ^b	6.72	>1000
CP	PE	>1000	1.00	>1000	1.00	>1000
	DCM	70.87 ± 1.31 ^b	14.10	70.87 ± 1.31 ^a	14.10	>1000
	EA	336.00 ± 1.30 ^c	0.57	931.50 ± 1.26 ^b	0.21	192.20 ± 1.20 ^a
	ETOH	978.20 ± 1.21 ^d	0.89	>1000	0.87	868.80 ± 1.07 ^b
	AQ	45.68 ± 1.16 ^a	21.88	72.62 ± 1.13 ^a	13.77	>1000
	CUR	7.35 ± 1.27	0.73	2.84 ± 1.24	1.93	5.38 ± 1.12

*ND, not determined due to small sample size; SI, selectivity index. The SI values were calculated as the ratio of the concentration of extract compound to inhibit 50% of normal cell growth (CC₅₀) to IC₅₀. Different superscript alphabets (e.g., a,b,c) on IC₅₀ values of extracts in the same column of the same plant indicates significant differences ($p < 0.05$) between the values, whereas similar superscripts indicate no significant differences.

activity and could be useful for managing different types of prostate cancers, since it exhibited good selectivity towards four different prostate cancer cell lines. Previous studies have reported the presence of tannins, saponins and flavonoids, and suggested that they were responsible for the antioxidant and cytotoxic activities to cancer cells [33,21,34]. All the extracts of *C. papaya* showed antioxidant activities with the aqueous extract showing the strongest activity (EC₅₀ = 125.9 ± 1.08 µg/mL).

The antioxidant activity of *C. papaya* leaves has been reported to be associated with the phytochemical compounds such as flavonoid, tannin, anthraquinone, Vitamin A, Vitamin C, proanthocyanidins and flavonoids [35,36]. Other researchers have reported a strong anticancer activity of the aqueous extract of *C. papaya* leaves on Jurkat, Molt-4, CCRF-CEM and HPB-ALL leukemia cell lines [38]. This study lends support to the above report that the aqueous leaf extract possesses strong cytotoxic activity against prostate cancer cells. All extracts of *M. oleifera* tested in the present study had various amounts of phenols with the ethanolic extract having the highest amount. *M. oleifera* leaves have been reported to be rich in polyphenols, vitamins, saponins, flavonoids, carotenoids, alkaloids, tannins, isothiocyanates, and glucosinolate [24,25].

The aqueous extracts possessed the strongest antioxidant activity, and it also contained some amount of phenols which was five times lower than the amount present in the ethanolic extract. There was no correlation between the phenolic content of the fractions and their antioxidant activities. According to the US National Cancer Institute plant screening program, crude extracts with IC₅₀ values less than 30 µg/mL could be considered as potential anti-cancer agents for further development [39]. Plant extracts with IC₅₀ values between 30 µg/mL and 200 µg/mL have moderate potential to be developed into cancer therapeutic

agents whilst those above 200 µg/mL are unlikely to be selected for further development [40]. According to Boik classification, in this study, the aqueous extracts showed moderate cytotoxic activity against PC3 cells (IC₅₀ = 66.72 ± 1.15 µg/mL) and a good selective index (about 15). Although, the ethyl acetate fraction of MO appeared to be more cytotoxic to LNCaP prostate cancer cells, it possessed a lower selective index of 1.74 when compared to the aqueous extract.

Antioxidant action is a mechanism of action of some anticancer agents. Coppin et al. [41] have indicated that *M. oleifera* leaf extract is able to inhibit nitric oxide produced by macrophage cells which were treated with bacterial lipopolysaccharide. Moreover, anti-inflammatory and immunomodulatory effects of *M. oleifera* leaves have been reported in many *in vivo* and *in vitro* studies [42, 43]. In addition, ethyl acetate extract of *M. oleifera* leaves was reported to inhibit human macrophage cytokine production (TNF-α, IL-6 and IL-8) induced by extract of cigarette smoke [44]. In addition to previous studies that reported on anti-inflammatory and immunomodulatory effects, this study has shown that *M. oleifera* has anti-prostate cancer activity.

Conclusion

This study has shown that aqueous extract of *C. papaya* and dichloromethane extract of *P. amarus* possesses cytotoxic activities with good selectivity towards PC3 and LNCaP prostate cancer cell lines, respectively. All the plant crude extracts had antioxidant activity with the aqueous leaf extract of *P. amarus* exhibiting the strongest activity which was comparable with positive control. This lends support to its use in the management of oxidative stress related disease like prostate cancer. The bioactive compounds responsible for the anti-prostate cancer activity should be isolated and characterized. Also, toxicological

studies should be conducted to assess the safety of the bioactive extracts. Further work involving the determination of the targets of intracellular signaling pathways must be done.

DECLARATIONS

Ethical considerations

Ethical clearance for this study was obtained from the Institutional Review Board of Noguchi Memorial Institute for Medical Research (NMIMR). All procedures were in strict accordance with standard ethical protocols and approved by the NMIMR Institutional Review Board (NMIMR-IRB CPN 085/16-17revd. 2019).

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None

Competing Interests

No potential conflict of interest was reported by the authors

Author contributions

JA drafted the manuscript; JA, ED and AA contributed to the laboratory work and data analysis; AKN and RAO contributed to the study design, drafting of the manuscript and supervised the research; All the authors read and approved the manuscript.

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Availability of data

Data is available upon request to the corresponding author

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