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Anti-Cancer and Free Radical Scavenging Activity of Some Nigerian Food Plants *in vitro*

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ABSTRACT

This study was designed to screen different extracts of 15 commonly consumed Nigerian food plants for anti-cancer and free radical scavenging activities. Leaves, seeds or fruits of the plants were each successively extracted with n-hexane, dichloromethane, ethanol and water. The cytotoxic activity of each of the extracts was tested against human myeloid leukemia (HL-60), human hepatocellular carcinoma (SMMC-7721), human lung carcinoma (A-549), human breast adenocarcinoma (MCF-7) and colon cancer (SW480) cell lines using Cisplatin as standard. The free radical scavenging activities of the extracts against 1,1-diphenyl-2-picrylhydrazyl (DPPH) were also determined. The dichloromethane extract of *Vernonia amygdalina* leaves (VA-D) showed the strongest cytotoxic activity against all the cancer cell lines with IC_{50} range of 5.85-8.84 μg mL^{-1}. The dichloromethane extract of *Gongronema latifolium* leaves (GL-D) showed the highest activity against A-549 and MCF-7 with IC_{50} of 9.57 and 6.51 μg mL^{-1}, respectively, while *Piper guineense* leaves (PG-D) exhibited the highest activity against HL-60 with IC_{50} of 3.62 μg mL^{-1}. The other extracts were inactive against the cancer cell lines. The ethanolic extract of *Sesamum indicum* leaves (SI-E) and *Mucuna pruriens* seeds (MP-E) showed the highest free radical scavenging activity with SC_{50} of 6.8 and 7.3×10^{-2} mg mL^{-1}, respectively. Other extracts of some of the food plant samples showed varying free radical scavenging activities. The results from this study suggest that some of the food plants screened may possess anti-cancer and antioxidant properties.

Key words: Anticancer, free radical scavenging, food plant, chemoprevention, cancer

INTRODUCTION

Cancer is a public health problem worldwide affecting all categories of persons and a major cause of death in developed and developing countries (Jemal *et al*., 2010; Sylla and Wild, 2012). In Nigeria, 100 000 new cases of cancer occur every year, with a high case fatality ratio (Ferlay *et al*., 2010). Oxidative stress due to imbalance between the production of free radicals and antioxidants is one of the factors implicated in the etiology of cancer (Liu and Hotchkiss, 1995). This is because free radical induced oxidative stress results in damage to important biomolecules such as lipids, proteins and DNA leading to an increase in the risk of cancer via several mechanisms

(Ames *et al*., 1993). Cancer-induced oxidative damage might be prevented by dietary antioxidants in food plants. Phytochemicals in food plants amongst other mechanisms have the capability to prevent cancer and other chronic diseases through their ability to express anti-oxidant activity as a result of their free radical scavenging property (Liu, 2004; Sun *et al*., 2002). Plants have been widely employed in therapy and management of diseases including cancer (Kim and Park, 2002). A large percentage of orthodox drugs in clinical management of cancer have their roots in plants and other natural products (Cragg, 1998). In fact more than 80% of people in developing countries are estimated to depend on traditional herbal medicine (WHO) while more than 60% of patients are reported to use herbs in management and treatment of cancer (Madhuri and Pandey, 2008). The high failure rate of conventional treatment and management of cancer as well as its numerous adverse effects has generated more interest in chemoprevention using food plants as a viable strategy in the control of cancer. Epidemiological studies have shown that regular consumption of food plants reduces the risk incidence of chronic diseases including cancer (Liu, 2003). This is because they are good sources of natural antioxidants such as carotenoids, vitamins, phenolic compounds, flavonoids and endogenous metabolites that can scavenge oxygen free radicals and decompose peroxides (Arabshahi *et al*., 2007). There are several claims about the use of some Nigerian plants for management of several diseases including cancer (Ashidi *et al*., 2010; Sowemimo *et al*., 2009). The *in vitro* anti cancer activity of some Nigerian medicinal plants have been evaluated (Fadeyi *et al*., 2013). However, there is not much report on the anti-cancer and chemopreventive potential of Nigerian food plants. Nigerian food plants including vegetables, fruits and seeds elaborate an array of nutrient and non-nutrient substances with structural and chemical diversity and biological activity capable of conferring prevention against cancer and other diseases (Mensah *et al*., 2008). This study is intended to determine the free radical scavenging activity and anticancer potential of some Nigerian vegetables, seeds and fruits which are variously used as condiments, spices or thickening agents in soups and stews. Preliminary studies on some of these food plants have revealed their content of bioactive components capable of preventing carcinogenesis (Ameh and Eze, 2010). The discovery of new anti-cancer activities from the selected Nigerian food plants will contribute positively to boosting cancer treatment and control strategies.

MATERIALS AND METHODS

Chemicals and reagents: The n-Hexane, Dichloromethane (Rionlon, China) and all other solvents, chemicals and reagents used were of analytical grade.

Plant materials: Fifteen Nigerian food plants including leafy vegetables, seeds and fruit were selected for the study as shown in Table 1. Voucher specimens of each of the samples were deposited at the herbarium of Department of Biological Sciences, Covenant University, Nigeria.

Collection and preparation of plant materials: The selected plant samples were bought from a local market in Lagos, Nigeria and were authenticated by Dr. Conrad Omonhinmin, a plant biologist in the department of Biological Sciences, Covenant University, Ota, Nigeria. The plant samples were air-dried at tropical room temperature. The dried samples were coarsely powdered with a mechanical grinder and stored in tightly closed nylon bags.

Extraction of plant materials: Each powdered leaf (50 g) and seed/fruit (100 g) were successively extracted with 500 mL of n-hexane (H), dichloromethane (D), ethanol (E) and water

Table 1: List of food plant samples and their resources

Tag	Species	Family	Parts
GL	*Gongronema latifolium* benth.	Asclepiadaceae	Leaves
VA	*Vernonia amygdalina* del.	Asteraceae	Leaves
OG	*Ocimum gratissimum* L.	Lamiaceae	Leaves
GA	*Gnetum africanum* Welw.	Gnetaceae	Leaves
PM	*Pterocarpus mildbraedii* Harms	Papilionaceae	Leaves
PG	*Piper guineense* Schumach. and Thonn.	Piperaceae	Leaves
PS	*Piper guineense* Schumach. and Thonn.	Piperaceae	Seeds
SI	*Sesamum indicum* L.	Pedaliaceae	Leaves
BE	*Brachystegia eurycoma* Harms	Fabaceae	Seeds
DM	*Detarium microcarpum* Guill. and Perr.	Fabaceae	Seeds
MP	*Mucuna pruriens* (L.) DC.	Fabaceae	Seeds
XA	*Xylopia aethiopica* (Dunal) A. Rich	Annonacea	Seeds
IG	*Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) Baill	Irvingiaceae	Seeds
MM	*Monodora myristica* (Gaertn.)	Annonaceae	Seeds
TT	*Tetrapleura tetraptera* (Schumach. & Thonn) Taub.	Fabaceae	Fruit

(W) using a reflux apparatus. The extraction process was repeated three times for 3, 2 and 1 h, respectively. The resultant residue was dried each time before extracting with the next solvent. Extracts were then filtered and concentrated to the dry mass with the aid of rotary evaporator. The non polar solvent extracts were concentrated to dryness at 40°C in Rotary evaporator while the water extract was concentrated partially and then freeze dried. The yield of each extract was measured and residues were put in amber-coloured glass samples bottles and stored in the refrigerator for further analysis.

***In vitro* cytotoxicity assay:** A total of sixty *n*-hexane, dichloromethane, ethanolic and water crude extracts from the selected plant samples were evaluated for their cytotoxicity against human myeloid leukemia (HL-60), human hepatocellular carcinoma (SMMC-7721), human lung carcinoma (A-549), human breast adenocarcinoma (MCF-7) and colon cancer (SW480) cell lines. The cytotoxicity assay was performed according to the modified MTT (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) method by conducting colorimetric measurements of the amount of insoluble formazan precipitate formed in living cells based on the reduction of MTT (Alley *et al*., 1988). In this assay, MTS (3-(4,5-dimethylthiazol-2-yl)-5(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium), an analogue of MTT was used instead. Briefly, 100 μL of adherent cells was seeded into 96-well micro titer plates and allowed to adhere for 12 h before addition of crude plant extracts, whereas suspended cells were seeded immediately prior to plant extract addition, with an initial density of 1×10^5 cells mL^{-1}. Each tumor cell line was exposed to the crude extracts at various concentrations in triplicate for 48 h, using Cisplatin as positive control. After the treatment period, the absorbance of the lysate was measured at 490 nm in a 96-well micro plate reader (Bio-Rad 680). The cell viability was detected and plant extract concentrations inhibiting 50% of cell growth (IC_{50} values) were calculated as described in the literature.

***In vitro* free radical scavenging activity assay:** The DPPH free radical scavenging activity of the crude plant extracts were determined as described in a previous study (Wang *et al*., 2005). Five different concentrations including 375, 750, 1500, 3000, 5000 μg mL^{-1} of the crude hexane, DCM, ethanol and water extracts were prepared in ethanol. The reaction mixture was made up of

100 μL of each extract concentration, 100 μL of 200 μmol L^{-1} DPPH and 100 μL of methanol added into a 96-well plate and incubated at 25°C for 30 min. The absorbance at 517 nm was then monitored in a Microplate Reader (Molecular devices) The DPPH radical scavenging activity was then calculated from the equation:

$$\text{DPPH radical scavenging activity}(\%) = \frac{1-(A_{sample}-A_{blank})}{A_{control}} \times 100$$

where, A_{sample}, A_{blank} and $A_{control}$ are the absorbance of the crude extract with DPPH, the crude extract without DPPH and the DPPH (no crude extract), respectively. Ascorbic acid was used as positive control. The concentration of sample values required to scavenge 50% of DPPH radicals (SC_{50}) were evaluated using a linear regression analysis (Microsoft Excel DPS v3.01). The sample assays were done in triplicates.

RESULTS AND DISCUSSION

The results obtained indicated that not all the plant extracts showed cytotoxic activity. The extracts of plant samples that exhibited cytotoxic activities against the cancer cell lines are shown in Table 2. The samples include the dichloromethane extracts of *Gongronema latifolium* (GL-D) *Vernonia amygdalina* (VA-D), *Ocimum gratissimum* (OG-D), *Pterocarpus mildbraedii* (PM-D), *Piper guineense* (PG-D), *Piper guineense* seeds (PS-D), *Xylopia aethiopica* (XA-D), *Sesamum indicum* (SI-D) as well as the hexane extracts of *Piper guineense* (PG-H), *Piper guineense* seeds (PS-H) and *Xylopia aethiopica* (XA-H). Out of all the plant extracts, only the dichloromethane and ethanol extracts exhibited antioxidant activities with SC_{50} less than 10^{-2} mg mL^{-1} (Table 3). These include the dichloromethane extracts of *Gongronema latifolium* (GL-D), *Ocimum gratissimum*

Table 2: Cytotoxicities of plant extracts against cancer cell lines (IC_{50})

	Human myeloid leukemia	Human hepatocellular carcinoma	Human lung carcinoma	Human breast adenocarcinoma	Colon cancer
Plant extracts	HL-60	SMMC-7721	A-549	MCF-7	SW480
GL-D	4.14	32.34	9.57	6.51	10.24
VA-D	5.58	8.74	10.97	9.51	8.84
OG-D	4.52	39.56	35.77	37.05	>100.00
PM-D	10.57	35.56	31.18	32.61	91.80
PG-D	3.62	12.82	14.43	6.54	22.96
PS-D	35.02	53.35	33.81	28.16	56.65
XA-D	12.06	10.12	11.82	10.21	38.52
SI-D	37.75	37.82	45.34	34.85	34.12
PG-H	48.30	>100.00	67.97	30.24	35.76
PS-H	42.23	68.86	42.79	37.20	40.94
XA-H	14.93	11.09	13.06	11.92	26.23
Cisplatin	3.08	10.20	9.08	17.48	11.99

IC_{50}: Concentration of extracts (μg mL^{-1}) at which 50% of cancer cells are inhibited, GL-D: Dichloromethane extract of *Gongronema latifolium*, VA-D: Dichloromethane extract of *Vernonia amygdalina*, OG-D: Dichloromethane extract of *Ocimum gratissimum*, PM-D: Dichloromethane extract of *Pterocarpus mildbraedii*, PG-D: Dichloromethane extract of *Piper guineense*, PS-D: Dichloromethane extract of *Piper guineense* seeds, XA-D: Dichloromethane extract of *Xylopia aethiopica*, SI-D: Dichloromethane extract of *Sesamum indicum*, PG-H: Hexane extracts of *Piper guineense*, PS-H: Hexane extracts of *Piper guineense* seeds, XA-H: Hexane extracts of *Xylopia aethiopica*

Table 3: The DPPH free radical scavenging activities of the plant samples (SC_{50})

Plant extracts	SC_{50} (10^{-2} mg mL^{-1})[a]
GL-D	90.70±0.2
OG-D	55.70±0.3[b]
MM-D	54.50±0.3[b]
PS-D	93.70±0.1
SI-D	43.10±0.2[b]
VA-E	19.33±0.4
OG-E	11.70±0.1
PM-E	20.30±0.2
PG-E	36.30±0.2
MM-E	34.30±0.1
TT-E	12.70±0.1
BE-E	59.70±0.3
PS-E	30.30±0.2
XA-E	10.70±0.1
IG-E	15.30±0.1
DM-E	39.00±0.2
MP-E	7.30±0.2[a]
SI-E	6.80±0.5[a]
Ascorbic acid	0.39±0.1

SC_{50}: Concentration at which 50% of DPPH free radicals are scavenged, [a]Values represent Means±SD (n = 3), [b]Values with significant free radical scavenging activity, GL-D: Dichloromethane extract of *Gongronema latifolium*, OG-D: Dichloromethane extract of *Ocimum gratissimum*, MM-D: Dichloromethane extract of *Monodora myristica*, PS-D: Dichloromethane extract of *Piper guineense* seeds, SI-D: Dichloromethane extract of *Sesamum indicum*, VA-E: Ethanolic extract of *Vernonia amygdalina*, OG-E: Ethanolic extract of *Ocimum gratissimum*, PM-E: Ethanolic extract of *Pterocarpus mildbraedii*, PG-E: Ethanolic extract of *Piper guineense* leaves, MM-E: Ethanolic extract of *Monodora myristica*, TT-E: Ethanolic extract of *Tetrapleura tetraptera*, BE-E: Ethanolic extract of *Brachystegia eurycoma*, PS-E: Ethanolic extract of *Piper guineense* seeds, XA-E: Ethanolic extract of *Xylopia aethiopica*, IG-E: Ethanolic extract of *Irvingia gabonensis*, DM-E: Ethanolic extract of *Detarium microcarpum*, MP-E: Ethanolic extract of *Mucuna pruriens*, SI-E: Ethanolic extract of *Sesamum indicum*

(OG-D), *Monodora myristica* (MM-D), *Piper guineense* seed (PS-D) and *Sesamum indicum* (SI-D). It also included the ethanolic extracts of *Vernonia amygdalina* (VA-E), *Ocimum gratissimum* (OG-E), *Pterocarpus mildbraedii* (PM-E), *Piper guineense* (PG-E), *Monodora myristica* (MM-E), *Tetrapleura tetraptera* (TT-E), *Brachystegia eurycoma* (BE-E), *Piper guineense* seed (PS-E), *Xylopia aethiopica* (XA-E), *Irvingia gabonensis* (IG-E), *Detarium microcarpum* (DM-E), *Mucuna pruriens* (MP-E) and *Sesamum indicum* (SI-E). Studies have shown that the consumption of food plants offer protective effects against oxidative damage by inhibiting or quenching free radicals and reactive oxygen species in human and animal tissues and organs (Oboh *et al*., 2008). Food plants are known sources of natural antioxidants that can protect against oxidative stress and thus play an important role in the chemoprevention of diseases such as cancer that have their etiology rooted in reactive oxygen species (Dragland *et al*., 2003). Oxidative stress which refers to the imbalance between the generation of reactive oxygen species and the activity of the antioxidant defenses has been implicated in numerous human diseases including cancer, atherosclerosis, diabetes, malaria, iron overload, rheumatoid arthritis, Parkinson disease and in HIV infection and AIDS (Aruoma, 2003). Cancer has become a serious global problem that needs to be seriously tackled (ACS., 2007). In this study, the dichloromethane extract of *Vernonia amygdalina* showed the strongest activity against the growth of the five cancer cell lines especially hepatocellular carcinoma (SMMC-7721)

and colon cancer (SW480) with an IC_{50} of 8.74 and 8.84 μg mL^{-1}, respectively. The extracts of *V. amygdalina* has been reported to exhibit a cytotoxic action on human breast tumour, human hepatoblastoma (HepG2) and urinary bladder carcinoma (ECV-304) cell lines (Wong *et al.*, 2013; Oyugi *et al.*, 2009; Khalafalla *et al.*, 2009). The anticancer activity of *V. amygdalina* is primarily attributed to the actions of coumarins, flavonoids, sesquiterpene lactones and edotides. *Vernonia amygdalina* extracts may also suppress cancerous cells through the induction of apoptosis (Song *et al.*, 2005). This implies that *V. amygdalina* is a leafy vegetable with a high potential for prevention and treatment of cancer. The ethanol extracts of *V. amygdalina* also exhibited free radical scavenging activity. Different extracts of *V. amygdalina* have been shown to posses both *in vitro* and *in vivo* antioxidant properties (Iwalokun *et al.*, 2006; Farombi and Owoeye, 2011). The antioxidant activity of *V. amygdalina* is attributed to the presence of natural antioxidant phytochemicals such as flavonoids. In Nigeria, the leaves of *V. amygdalina* are used as soup condiments and folkloric treatment of a wide array of ailments (Ijeh and Ejike, 2011).

The dichloromethane extract of leaves of *Piper guineense* (PG-D) showed the highest activity against human myeloid leukemia (HL-60) cell line with an IC_{50} of 3.62 μg mL^{-1}. Similarly, the hexane extracts of both the seed and leaves showed varying cytotoxicities against all the cancer cell lines. The leaves of *P. guineense* have been reported to possess anticancer properties (Soladoye *et al.*, 2010). Also, the methanolic extract of the seed exhibited cytotoxicity against leukemia (CEM/ADR5000) cells (Kuete *et al.*, 2011). The cytotoxic properties of *Piper* plants are attributed to the chemical constituents which include amide alkaloids terpenes, steroids, piperolides, flavonoids and others. The ethanol extracts of *P. guineense* (PG-E) leaves and seeds also exhibited free radical scavenging activities. The *in vitro* and *in vivo* free radical scavenging activity of the extracts of leaves and seeds of *P. guineense* have been reported (Agbor *et al.*, 2007). The antioxidant property of *P. guineense* is a due to essential oils such as monoterpenes, benzoids and sesquiterpenes. Thus, Piper species can play a role in the modulation of free radical induced disorders such as cancer.

The dichloromethane extracts of leaves of *Ocimum gratissimum* (OG-D), suppressed the growth of the cancer cells particularly the human myeloid leukemia (HL-60). Its antitumor activity especially against breast tumor and human pulmonary adenocarcinoma cell (A549) has been reported (Chen *et al.*, 2010). Other species of Ocimum have showed activity against skin cancer and human breast cancer cells (Qamar *et al.*, 2010). The dichloromethane and ethanol extracts *O. gratissimum* (OG-D/OG-H) also exhibited free radical scavenging activity. A similar study showed that the methanolic extract of *O. gratissimum* posses a high free radical scavenging activities due to their high flavonoid content (Dev *et al.*, 2011). In Nigeria, *O. gratissimum* is a common ingredient in folk medicinal preparations, soups and stews. The free radical scavenging activity of *O. gratissimum* probably forms the scientific basis of its traditional use for the management of several ailments and hence its anticancer activity (Giron *et al.*, 1991).

Gongronema latifolium is primarily used as spice in soups and salads as well as employed in folk medicine. The dichloromethane extract of *G. latifolium* (GL-D) showed the strongest activity against human lung carcinoma (A-549) and human breast adenocarcinoma (MCF-7) with an IC_{50} of 9.57 and 6.51 μg mL^{-1}, respectively. The extract also exhibited free radical scavenging activity against DPPH. The ethanolic extract of *G. latifolium* leaves is reported to express antioxidant activity through several processes (Owu *et al.*, 2012). This vegetable is a reservoir of many natural antioxidants (Atangwho *et al.*, 2009). The presence of several phytochemicals including alkaloids, tannins, glycosides, polyphenols, saponins and flavonoids in the plant confers potential for prevention of diseases including cancer.

The dichloromethane and hexane extracts of *Xylopia aethiopica* (XA-D/XA-H) showed a strong activity against hepatocellular carcinoma (SMMC-7721) and human breast adenocarcinoma (MCF-7) cells. *Xylopia aethiopica* commonly used as a spice in Nigeria has several biological properties (Kuete, 2010). The extracts of *X. aethiopica* have demonstrable cytotoxic effects on a wide range of cancer cells (Kuete *et al*., 2013). The free radical scavenging activity of the ethanol extracts of *X. aethiopica* is a consequence of the antioxidant properties of its essential oils. Extracts and diets containing *X. aethiopica* have also been shown to have antioxidant effects (Adaramoye *et al*., 2011).

The dichloromethane extracts of leaves of *Sesamum indicum* (SI-D) showed varying medium activity against all the cancer cell lines. Also, the dichloromethane and ethanol extracts of *S. indicum* (SI-D/SI-E) exhibited the strongest free radical scavenging activities against DPPH with an SC_{50} of 6.8×10^{-2} mg mL^{-1}. *S. indicum* has quite a number of antioxidants such as sesamol, sesamolin sesamin, butylated hydroxytoluene, sesaminol triglucoside and sesaminol diglucoside. Sesame seeds have been used as a medicine since antiquity and are considered to have anticancer and antioxidant properties (Matsufuji *et al*., 2011).

Pterocarpus mildbraedii, a relatively unpopular plant food showed medium activity against all the cancer cell lines as well free radical scavenging property. This result is particularly important because of scanty information on the biological properties of this plant. However the leaves of this plant have been found to be rich in nutritional value and its antioxidant property has been reported (Odukoya *et al*., 2007). A further investigation of the anti-cancer property of this plant is progress.

The dichloromethane and ethanol extracts *Monodora myristica* (MM-D) was inactive against the cancer cell lines but exhibited free radical scavenging activity against DPPH. *M. myristica* seed is a popular spice and flavouring agent used in many Nigeria cuisine and folklore medicine. Its antioxidant properties have been reported and attributed mainly to its phenolic and flavonoids composition (Erukainure *et al*., 2012).

The ethanol extracts of *Brachystegia eurycoma*, *Irvingia gabonensis*, *Detarium microcarpum*, *Tetrapleura tetraptera* and *Mucuna pruriens* exhibited free radical scavenging activities with *Mucuna pruriens* showing the strongest activity with an SC_{50} 7.3×10^{-2} mg mL^{-1}. *B. eurycoma* has been reported to contain antioxidant phytochemicals such as flavonoids, tannins, terpenes, alkaloids, saponins and suggesting their potential use in the management of diseases associated with oxidative stress such as cancer (Igwe and Okwu, 2013; Igwe and Echeme, 2013). *Irvingia gabonensis* is reported to possess some antioxidant agents and the antioxidant activities of *Irvingia gabonensis*-supplemented diet has been shown to reduce lipid peroxidation and increase antioxidant enzymes (Uhegbu *et al*., 2013). *Detarium microcarpum* seeds have good nutritional quality and the functional properties which are needed to exhibit antioxidant effects. *T. tetraptera* contains potential antioxidant phytochemicals and have been reported to have strong radical scavenging and reducing capacities (Badu *et al*., 2012). In the present study, *M. pruriens* exhibited one of the stronger free radical scavenging activity and has been showed to posses significant *in vitro* antioxidant activity (Rajeshwar *et al*., 2005) this property may form the basis of its anticancer activity (Ibe *et al*., 2012).

CONCLUSION

In conclusion, the dichloromethane extract of *Vernonia amygdalina* leaves (VA-D) showed the strongest cytotoxic activity against all the cancer cell lines while that of *Gongronema latifolium*

leaves (GL-D) showed the highest activity against A-549 and MCF-7. Also the dichloromethane extract of *Piper guineense* leaves (PG-D) exhibited the highest activity against HL-60. The ethanolic extract of *Sesamum indicum* leaves (SI-E) and *Mucuna pruriens* seeds (MP-E) showed the highest free radical scavenging activity with SC_{50} against DPPH. This study reveals that several plant foods that are commonly consumed in Nigeria could have anti-cancer potential which could provide a plausible explanation for the apparently and comparatively lower incidence of cancer.

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