

*Full Paper***Preliminary qualitative screening for cancer chemopreventive agents in *Telfairia occidentalis* Hook.f., *Gnetum africanum* Welw., *Gongronema latifolium* Benth. and *Ocimum gratissimum* L. from Nigeria****Emeka E.J. Iweala**

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The leaves of four plant foods commonly consumed in Nigeria namely *Gnetum africanum* (Igbo name: Ukazi), *Gongronema latifolium* (Igbo name: Utazi), *Telfairia occidentalis* (Igbo name: Ugu), *Ocimum gratissimum* (Igbo name: Nchoanwu), were each screened for the presence of known potential chemopreventive agents using paper chromatography, thin layer chromatography and various chemical tests. All four vegetables showed the presence of phenolic compounds, flavonoids, phytosterols, tannins, saponins, chlorophyll and glycosides. Only *Telfairia occidentalis* and *Gnetum africanum* showed traces of alkaloids.

Keywords: *Gnetum africanum*, *Gongronema latifolium*, *Telfairia occidentalis*, *Ocimum gratissimum*, chemopreventive agents

Introduction

Cancer is one of the leading causes of death worldwide [1, 2]. Cancer control involves concerted actions aimed at reducing the incidence and death that is associated with it. Chemoprevention is one of the best strategies employed in cancer control and has evolved into one of the most exciting and promising area in cancer research [3]. Chemoprevention involves the use of natural and synthetic compounds to inhibit, reduce or reverse carcinogenesis [4]. A large number of potential chemopreventive agents have been identified from epidemiological surveys, experimental, preclinical and clinical observation and structural homology with known chemopreventive agents [5, 6].

In recent years, dietary constituents especially from plant foods have been found to protect against the occurrence of cancer [7, 8]. Some of these naturally occurring compounds include phenolic compounds, flavonoids, terpenoids, carotenoids, phytosterols, isothiocyanates and other phytochemicals [9, 10]. The mechanisms underlying chemoprevention involves anti-oxidant, anti-inflammatory, immune-enhancing, and anti-hormone effects. Other possible mechanisms include modification of drug-metabolizing enzymes, and influences on cell cycling and differentiation, induction of apoptosis, and suppression of proliferation and angiogenesis that play a role in the initiation and secondary modification of neoplastic development [11]. Plant foods including

vegetables and fruits are largely consumed by tropical subpopulations [12]. Tropical plant foods elaborate an array of nutrient and non-nutrient substances with structural and chemical diversity and biological activity capable of conferring prevention against cancer. This preliminary study is intended to identify possible potential chemopreventive agents in some commonly consumed Nigerian vegetables namely *Gnetum africanum*, *Gongronema latifolium*, *Telfairia occidentalis*, *Ocimum gratissimum* as a prelude to conducting more extensive studies on them.

Materials & Methods

Plant materials

The leaves of four locally consumed plant foods namely *Gnetum africanum*, *Gongronema latifolium*, *Telfairia occidentalis* and *Ocimum gratissimum* were bought from the local market at Nsukka town in Enugu State of Nigeria.

Preparation of plant materials

The leaves of the four plant food samples were picked to remove infected ones and air dried at room temperature. The dried leaves were then ground to a coarse powder and stored in amber bags at room temperature.

Phytochemical screening

Phytochemical screening for potential chemopreventive agents was done according to various screening methods [13, 14].

Test for phenolic substances

Plant material (5 g) was stirred with 50 mL of 2M HCl and heated in a water bath at 100 °C for 30 min, cooled and filtered. The filtrate was extracted into 30 mL of ether and the extract was concentrated to dryness. Residue was dissolved in few drops of chloroform and chromatographed one dimensionally on silica gel plate (20 cm x 20 cm) in acetone-chloroform (1:9). Phenolic substances

show a variety of colors including blue, gray, red and pink.

Test for flavonoids

Plant material (5 g) was hydrolyzed by heating in 2M HCl at 100 °C for 30 min, cooled and filtrate was extracted in 30 mL ethylacetate and the extract was concentrated to dryness. Residue was dissolved in few drops in Forestal-butanol-acetic acid-water (BAW) and phenol-water (pH-OH-H₂O) solvents. Flavones and glycosylflavones give varying yellow colours while biflavonyls give brown colours. Also, 1 mL of dilute ammonia solution was added to about 2 mL of the ethyl acetate extract and shaken. A yellow color at the lower ammonia layer is a positive indication for flavonoids.

Test for anthocyanadins and chalcones

Plant material (5 g) was hydrolyzed in HCl for 30 min at 100 °C. Hydrosylate was filtered and the filtrate was washed twice with ethyl acetate. The aqueous layer was heated at 80 °C to remove all the ethyl acetate and extracted into amyl alcohol. The extract was concentrated to dryness. Residue was dissolved in few drops of methanolic-HCl and chromatographed on silica gel and paper in BAW and water. Anthocyanidins give red, purple, and magenta colors while chalcones give red and brown colors.

Test for phytosterols

Plants tissue (5 g) was defatted three times with ether and extracted into hot methanol and concentrated to a small volume. Sample was chromatographed on silica gel in chloroform and detected with 50% H₂SO₄. Brown and violet colors are positive indicators.

Test for chlorophyll

All procedures are carried out in the dark. 2 g of fresh plant tissue was ground and extracted with 80% acetone and CaCO₃ (to prevent formation of pheophytin) until all the color was released from the tissue. The extract was made up to 100

mL with acetone and stored in an amber bottle in a refrigerator. The absorbance was read at 645 nm, 652 nm and 663 nm and the following calculation was made:

$$\text{Total Chlorophyll} = \frac{100 \times A_{652\text{nm}} \text{ or } 278 \times A_{652\text{nm}}}{36}$$

Test for tannins

Plant tissue (1 g) was extracted in 50 mL of distilled water and filtered. The filtrate was concentrated and chromatographed on silica gel in chloroform–acetone–formic acid (75:16.5:8.5). Also, 1 g of plant tissue was boiled in 10 mL of 45% ethanol for 3 min, cooled and filtered. Filtrate was divided into three portions. To one portion was added few drops of lead subacetate and observed for formation of gelatinous precipitate. To another portion was added 1 mL of bromine water and observed for pale brown portion precipitates. The last portion was diluted with distilled water and few drops of ferric chloride added and observed for a transient greenish to black color.

Test for saponins

Plant tissue (5 g) was extracted with 25 mL methanol for 5 min at 50 °C in a water bath. Filtrate was evaporated and mixed with 2.5 mL water and extracted with 15 mL of butanol and the butanol layer was then chromatographed on silica gel in chloroform–methanol–water. Also 1 g of plant tissue was boiled with 10 mL of distilled water for 5 min and decanted while still hot. 2 mL of the filtrate was diluted with 8 mL water and vigorously shaken and observed for a stable froth. To another 2 mL of filtrate was added 4 drops of olive oil, shaken and observed for formation of emulsion.

Test for alkaloids

Plant tissue (5 g) was mixed with 10 mL of 10% ammonia solution and extracted with 25 mL ethanol at 60 °C for 15 min. The extract was concentrated and chromatographed on silica gel in ethyl acetate–methanol–water (100:13.5:10). Blue or yellow fluorescence and brown or

orange spots with Dragendorff's reagent are positive. Also 1 g of plant tissue was boiled with 5 mL of 2% HCl and filtered. The filtrate was divided into four portions each of which is added 2 drops of Mayer's reagent, Dragendorff's reagent, Wagner's reagent and picric acid solution to observe cream coloured, orange coloured, reddish–brown and yellow precipitate respectively.

Test for glycosides

Plant tissue (5 g) was mixed with 50 mL of water and the solution heated for 5 min in a water bath, filtered and the filtrate was divided into two portions. Fehling solutions (1 mL) were added to 10 mL of the filtrate until alkaline and heated for 3 min. A brick red precipitate is positive. For cyanogenic glycosides, 5 g of tissue was mixed with 20 mL of water and heated in water bath with sodium picrate paper. A change from yellow to orange is positive. For cardiac glycosides, 5 g was extracted with 29 mL of water and filtered. The filtrate was evaporated and residue dissolved in 10 mL glacial acid with 3 drops of ferric chloride.

Results & Discussion

A summary of the screening results of the four local plant foods is presented in Table 1. The four plant foods showed the presence of phenolic substances. *G. latifolium* had the highest amount followed by *G. africanum*, *T. occidentalis* and *O. gratissimum* had the least. All the samples indicated the presence of flavonoids. The level of flavonoids was highest in *G. latifolium* and *G. africanum*. All the plant food samples showed the presence of phytosterol and chlorophyll. *G. latifolium* contained a higher level of phytosterol and chlorophyll than the other samples. Tannins were found to be present in all the samples, with *O. gratissimum* and *T. occidentalis* having higher levels than *G. africanum* and *G. latifolium*. All the samples showed the presence of saponins. Only *T. occidentalis* showed the presence of alka-

loids, while others showed slight traces of alkaloids. Generally, glycosides were

Table 1. Result of qualitative phytochemical screening for potential chemopreventive agents in *Gnetum africanum*, *Gongronema latifolium*, *Telfairia occidentalis* and *Ocimum gratissimum*.

Potential chemopreventive agents	<i>G. africanum</i>	<i>G. latifolium</i>	<i>T. occidentalis</i>	<i>O. gratissimum</i>
Phenolic compounds	++	+++	+	+
Flavonoids	++	++	+	+
Phytosterols	+	++	+	+
Chlorophyll	+++	+++	++	+
Tannins	++	+	+++	+++
Saponins	++	+	+++	++
Alkaloids	+	++	++	-
Glycosides	++	++	++	+
Anthocyanidins/chalcones	+++	+	+	+

+ indicates presence; - indicates absence

found to be present in all the samples with *O. gratissimum* showing very slight traces. All the samples were positive for cyanogenic glycoside while all except *G. latifolium* were seen to contain cardiac glycosides. Anthracene glycosides were absent in all the samples. All the samples showed presence of anthocyanidins/chalcones with *G. africanum* showing the highest level.

The concept of chemoprevention has assumed a global significance as a result of its acceptance in the management, prevention and treatment of a wide range of life threatening diseases such as cancer, diabetes and coronary diseases and in the maintenance of good health [15]. The enormous benefits to public health which the lowering of the incidence of cancer can generate are invaluable. A large number of chemopreventive agents identified in plants have considerably contributed to the concept of cancer prevention and control [16]. The chemical diversity of these naturally occurring substances increases the likelihood of their existence in tropical plant foods. There is an abundance of plant foods in the tropics especially Nigeria and their consumption by large tropical subpopulations can be correlated with the relatively and comparatively low incidence of cancers in the tropics.

The four plant foods investigated namely *Gnetum africanum*, *Gongronema latifolium*, *Telfairia occidentalis* and *ocimum gratissimum* are common tropical plant foods whose leaves are consumed especially in South Eastern Nigeria. The preliminary screening of these plant foods using simple chemical tests, paper chromatography showed that they contain some known potential cancer chemopreventive agents such as flavonoids, tannins, chalcones, anthocyanidins, phytosterols, chlorophyll, saponins, glycosides and alkaloids. The presence of these bioactive phytochemicals have been reported in several African plants [17]. Phytosterols which are terpenoid compounds have been reported to inhibit certain cancers in animals [18]. Flavonoids, anthocyanidins and chalcones are ubiquitous phytochemicals with pronounced bioactivity. Some of these compounds such as apigenin and licochalcone have showed chemopreventive properties against several cancers [19-21]. Saponins such as diosgenin have been found to have therapeutic and chemopreventive effects [22]. Tannins consumed in large quantities as fruits and vegetables on a daily basis such as ellagittannins are also effective against some cancers [23]. The chemopreventive prop-

erties of alkaloids and glycosides have also been reported [24, 25]. Chlorophyllin found in chlorophyll produces an anti-promoting effect on skin cancer in mice [26]. All the four plants almost had similar constituents of these substances except for alkaloids which was only prominent in *Telfairia occidentalis*. These chemopreventive agents are able to enhance host protective systems such as detoxification enzymes against carcinogens. Combinatorial effects are even more effective due to the synergistic actions of phytochemicals [27].

Accordingly, it would be anticipated that consumers of these tropical plant foods would have greater protection against specific carcinogenic compounds than non-consumers. This expectation is in accord with epidemiological studies that show inverse relationships between consumption of vegetables and fruits and occurrence of cancer. [28, 29]. Further investigations involving specific characterization of these potential chemopreventive agents and their effects in animal models are being carried out in our laboratory.

References

1. Ilson DH. Esophageal cancer chemotherapy: recent advances. *Gastrointest Cancer Res.* 2(2):85-92. 2008.
2. Tran E, Rouillon F, Loze JY, Casadebaig F, Philippe A, Vitry F, Limosin F. Cancer mortality in patients with schizophrenia: an 11-year prospective cohort study. *Cancer.*115(15):3555-62. 2009.
3. Greenwald P, Kelloff G.J. The role of chemoprevention in cancer control. *IARC Sci Publ.* 139:13-22. 1996.
4. Tsao AS, Kim ES, Hong, WK. Chemoprevention of cancer. *CA Cancer J Clin.*54:150-180. 2004.
5. Huang TH, Teoh AW, Lin BL, Lin DS, Roufogalis B. The role of herbal PPAR modulators in the treatment of cardiometabolic syndrome. *Pharmacol Res.* 60(3):195-206. 2009.
6. Rathee P, Chaudhary H, Rathee S, Rathee D, Kumar V, Kohli K. Mechanism of action of flavonoids as anti-inflammatory agents: a review. *Inflamm Allergy Drug Targets.* 8(3):229-35. 2009.
7. Wattenberg LW. Chemoprevention of cancer. *Cancer Res.* 45, 1-8. 1985.
8. Shukla S, Gupta S. Dietary agents in the chemoprevention of prostate cancer. *Nutr Cancer.* 53(1):18-32. 2005.
9. Lynn A, Collins A, Fuller Z, Hillman K, Ratcliffe B. Cruciferous vegetables and colo-rectal cancer. *Proc Nutr Soc.* 65(1):135-44. 2006.
10. Hayes JD, Eggleston IM. The cancer chemopreventive actions of phytochemicals derived from glucosinolates. *Eur J Nutr.* 47 Suppl 2:73-88. 2008.
11. Rabi T, Bishayee A. Terpenoids and breast cancer chemoprevention. *Breast Cancer Res Treat.*115(2):223-39. 2009.
12. Mensah JK, Okoli RI, Ohaju-Obodo JO, Eifediyi K. Phytochemical, nutritional and medical properties of some leafy vegetables consumed by Edo people of Nigeria. *African Journal of Biotechnology.* 7 (14), 2304-2309. 2008.
13. Harborne JB. *Phytochemical methods*, London. Chapman and Hall, Ltd. pp. 49-188. 1973.
14. Sofowara A. Medicinal plants and Traditional medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria. 1993.
15. Russo GL. Ins and outs of dietary phytochemicals in cancer chemoprevention. *Biochem Pharmacol.*74(4):533-44. 2007.
16. Baliga MS, Katiyar SK. Chemoprevention of photocarcinogenesis by selected dietary botanicals. *Photochem Photobiol Sci.* 5(2): 243-53. 2006.
17. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology* 4 (7), 685-688. 2005.
18. Yamaguchi C, In Y, Wada S, Yamada T, Tokuda H, Tanaka R. Cancer chemopreventive activity of oleanane-type triterpenoids from the stem bark of *Betula ermanii*. *Chem Biodivers.* 6(7):1093-100. 2009.
19. Bobe G, Peterson JJ, Gridley G, Hyer M, Dwyer JT, Brown LM. Flavonoid consumption and esophageal cancer among black and white men in the United States. *Int J Cancer.* 125(5):1147-54. 2009.
20. Lee CK, Son SH, Park KK, Park JH, Lim SS, Kim SH, Chung WY. Licochalcone A inhibits the growth of colon carcinoma and attenuates cisplatin-induced toxicity without a loss of chemotherapeutic efficacy in mice. *Basic Clin Pharmacol Toxicol.* 103(1):48-54. 2008.
21. Patel D, Shukla S, Gupta S. Apigenin and cancer chemoprevention: progress, potential and promise (review). *Int J Oncol.* 30(1):233-45. 2007.
22. Raju J, Mehta R. Cancer chemopreventive and therapeutic effects of diosgenin, a food saponin. *Nutr Cancer.* 61(1):27-35. 2009.
23. Larrosa M, Tomás-Barberán FA, Espín JC. The dietary hydrolysable tannin punicalagin releases ellagic acid that induces apoptosis in human colon adenocarcinoma Caco-2 cells by using the mitochondrial pathway. *J Nutr Biochem.* 17(9):611-25. 2006.

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24. Manoharan S, Balakrishnan S, Menon VP, Alias LM, Reena AR. Chemopreventive efficacy of curcumin and piperine during 7,12-dimethylbenz[a]anthracene-induced hamster buccal pouch carcinogenesis. *Singapore Med J.* 50(2):139-46. 2009.
 25. Lee JY, Woo E, Kang KW. Screening of new chemopreventive compounds from *Digitalis purpurea*. *Pharmazie.* 61(4):356-8. 2006.
 26. Kim J, Yook JI, Park KK, Jung SY, Hong JC, Kim KJ, Kim JA, Chung WY. Anti-promotion effect of chlorophyllin in DMBA-TPA-induced mouse skin carcinogenesis. *Anticancer Res.* 20(3A):1493-1498. 2000.
 27. de Kok TM, van Breda SG, Manson MM. Mechanisms of combined action of different chemopreventive dietary compounds: a review. *Eur J Nutr.* 47 Suppl 2:51-59. 2008.
 28. Thomasset S, Berry DP, Cai H, West K, Marczylo TH, Marsden D, Brown K, Dennison A, Garcea G, Miller A, Hemingway D, Steward WP, Gescher AJ. Pilot study of oral anthocyanins for colorectal cancer chemoprevention. *Cancer Prev Res (Phila Pa).* 2(7):625-633. 2009.
 29. Mann CD, Neal CP, Garcea G, Manson MM, Dennison AR, Berry DP. Phytochemicals as potential chemopreventive and chemotherapeutic agents in hepatocarcinogenesis. *Eur J Cancer Prev.* 18(1):13-25. 2009.