Advances in Biological Research 4 (6): 314-322, 2010
ISSN 1992-0067

Polyphenol Composition and *in vitro* Antioxidant Potential of Nigerian *Canarium schweinfurthii* Engl. Oil

S.E. Atawodi

Biochemistry Department, Ahmadu Bello University, Zaria, Nigeria

Abstract: Fruit mesocarp oil of *Canarium schweinfurthii* Engl used in the preparation of special delicacies by some ethnic nationalities in Nigeria and elsewhere in tropical west, central and east Africa, was analysed for its polyphenol composition using HPLC-UV, HPLC-MS and GC-MS techniques. Ten phenolic compounds, namely; catechol, p-hydroxybenzaldehyde, dihydroxyphenylacetic acid, tyrosol, p-hydroxybenzoic acid, dihydroxybenzoic acid, vanillic acid, phloretic acid, pinoresinol, secoisolariciresinol and some other peaks of unknown identity were detected by GC-MS following derivatization of the polyphenol-enriched methanol extract of the oil with N-methyl-N-(trimethylsilyl)-trifluoroacetamide (BSTFA). The methanol extract displayed very strong antioxidant and radical scavenging potential with IC_{50} values of 56 and 104 µl, respectively, when tested with the hypoxanthine/xanthine and 2-deoxyguanosine assay models, presumably as a result of its rich content of antioxidant polyphenols. These results strongly suggest that consumption of Fruit mesocarp oil of *Canarium schweinfurthii* might play a significant role in the chemoprevention of cancer and other oxidative-damage-induced diseases.

Abbreviations: ROS Reactive oxygen species · RNS · Reactive nitrogen species · BSTFA · N-methyl-N-(trimethylsilyl)-trifluoroacetamide · LC-ESI Liquid-Chromatography Electrospray-Ionization Mass Spectrometry · HPLC High performance Liquid chromatography GC-MS · Gas Chromatography-Mass Spectrometry

Key words: *Canarium schweinfurthii* · Polyphenol · Antioxidant potential · Chemoprevention · Vegetable oil

INTRODUCTION

Over the past few decades, an expanding body of evidence from epidemiological and laboratory studies suggest that some edible plants as a whole, or their identified ingredients with antioxidant properties have substantial protective effects on human carcinogenesis [1-6]. One major group of antioxidants that have received attention in cancer chemopreventive studies in the recent past, are the polyphenols [7-10].

Polyphenols are diverse group of compounds generally characterized with the presence of several hydroxyl groups attached to a ring or multi-ring structures and are thus classified as phenolic acids, stilbenes, lignans and flavonoids [10]. Polyphenol with several cancer chemopreventive potentials have been detected in several foods [11], including soy products [12-14], tea [7, 11, 15-17] and vegetables [18, 19]. This decade, evidence has accumulated to suggest that olive oil, an unique component of the Mediterranean diet may play some significant roles in the low incidence of certain cancers in the region [20].

Although, information about cancer incidence in Nigeria is rather scanty [21, 22], it is generally believed that there is low incidence of cancer in Africa, due to the nature of the diets and herbal medicaments, which are rich in anticancer constituents, especially, antioxidants [23, 24]. Therefore we investigated the polyphenol content and the antioxidant potential of *Canarium schweinfurthii* Engl. pulp oil, which is used in the preparation of special delicacies by some ethnic nationalities in the middle belt of Nigeria.

Canarium schweinfurthii, Engl. (Family: *Burseraceae*), commonly referred to as 'African canarium' or 'African olive' is respectively called 'Atile' and 'Odah' by the Hausa and Igala ethnic groups of northern and

Corresponding Author: Sunday Atawodi, Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria.
Tel: +234 (0) 8033 850 613, E-mail: atawodi_se@yahoo.com.

central Nigeria. The tree, which grows to a height of up to 150 ft with straight cylindrical bole to 90 ft, is widely distributed in the tropical west, east and central Africa. The fruits, stems and barks are used for treating coughs, venereal diseases and exudates. When parboiled, the mesocarp of the fruits are eaten and also used in the preparation of oil [25-28], which is utilized in the preparation of special delicacies by some ethnic groups in Nigeria and, probably, elsewhere in Africa.

The fruit mesocarp oil of *Canarium schweinfurthii* which constitutes 68% free-flowing lipid and 14% bound lipid [25] is reported to contain monoterpene hydrocarbons and linalool. The oil has an acid value of 0.68%, a saponification number of 196.35 and a peroxide value of 7.80 [27, 28]. Within this decade, a report appeared on phenolic metabolites from the seeds of the plant [29], but there are no reports on the polyphenol profile and antioxidant potential of its fruit oil and hence the focus of this investigation.

Sample: Fruit mesocarp oil of *Canarium schweinfurthii* Engl. was obtained from a local processor in Jos, Plateau State, Nigeria. It was stored at room temperature in glass bottle until transportation to laboratory of analysis in Heidelberg, Germany in August, 2004.

Reagents: Acetic acid, ethylene diaminetraacetic acid (EDTA), hypoxanthine, methanol, xanthine and xanthine oxidase were obtained from Merck (Darmstadt, Germany). K_2HPO_4 and KH_2PO_4 were obtained from Serva (Heidelberg, Germany). Formic acid, salicylic acid and $FeCl_3 6H_2O$ were obtained from Aldrich Chemie (Steinheim, Germany). N-methyl-N-(trimethylsilyl)-trifluoroacetamide (BSTFA) was obtained from Fluka (Buchs, Switzerland), while tetrabutylammonium hydroxide was obtained from Sigma Chemie (Deisenhofen, Germany). Standard phenolic compounds were obtained from laboratory stock, acquired from commercial sources or isolated, purified and characterized from natural sources. All solutions were made in double-distilled water.

Extraction of Phenolic Compounds: The phenolic compounds in the oil were extracted from the oil, basically as described by Owen and co-workers [30], but with minor modifications. The oil (10g) was vortexed for 2 min. at maximum speed in 50 ml polyphylene bottles with 3 X 2 ml methanol. The mixture was centrifuged at 4000 rpm for 30 min and methanol layer collected into graduated 20 ml glass test tubes. Pooled methanol fractions were dried under nitrogen, taken up in 1 ml acetonitrile and lipid

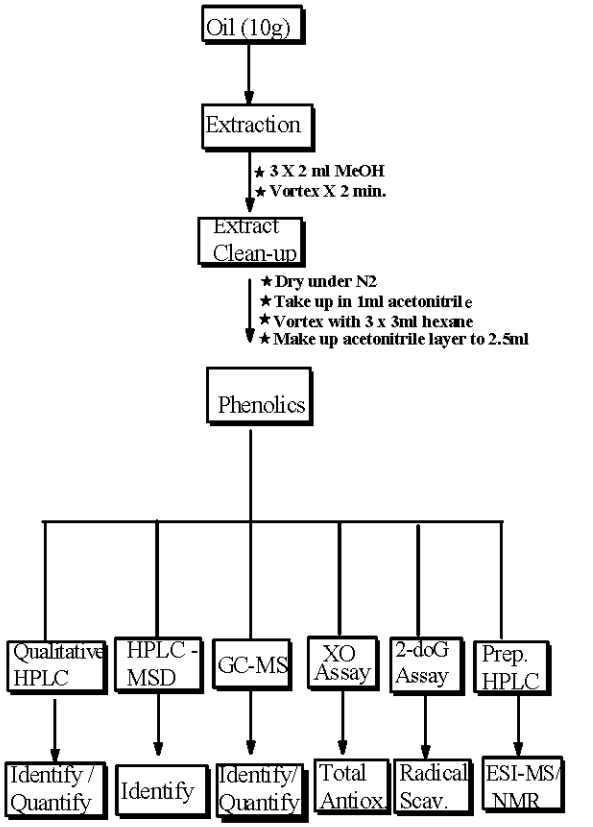

Fig. 1: Analytical scheme for determination of polyphenols in *Canarium schweifurthii* oil

contaminants removed by vortexing with 3 ml hexane (3 times). The mixture was centrifuged at 3000 rpm for 15 minutes each and the hexane layer discarded. The acetonitrile layer was made up to 2.5 ml and used for subsequent analysis (Fig.1).

Analytical High Performance Liquid Chromatography (HPLC): Analytical HPLC was conducted on a Hewlett-Packard (HP) 10980 Liquid chromatograph fitted with a C-18, reverse phase (5um) column (25 cm X 4 mm I.D) Latex, Eppelheim, Germany). For separation of individual compounds in the extract, 2% acetic acid in water (solvent A) and methanol (solvent B) were used as mobile phase when 20µl of the extract was injected.

The solvent gradient consisted of 95% A for 2 min, 75%A in 8 min, 60 % A in 10min, 50A in 10min and 0%A until completion of the run at 45 min [20, 30]. The flow rate of the mobile phase was maintained at 1ml/min and phenolic compounds in the eluate were detected with a UV dual-array detector (HP 1040M) set at 278 and 340 nm. Instrument control and data handling was by means of a HP Chemstation operating in the Microsoft Windows software environment.

Hypoxanthine/ Xanthine Oxidase Assay: To assess the total antioxidant potential due partly to the scavenging of reactive oxygen specie and the inhibition of the enzyme, xanthine oxidase, the Hypoxanthine/Xanthine oxidase assay system was utilized. In this assay, the extent of diphenol (2,5 dihydroxybezoic acid and 2,3 dihydroxybenzoic acid) produced by hydroxyl radical (HO*) attack on salicylic acid was measured from standard curves of their respective diphenols [20,30]. The assay involves the re-suspension of different dried extract residues, prepared in duplicates (by drying 0-500 µl of extracts in vacuum evaporator) in 1ml phosphate buffer (pH6.6). After addition of 5µl xanthine oxidase 20mu/1.09ml), the tubes were incubated at 37 °C for 3 hrs, following which reaction was stopped by addition of 5 µL of concentrated hydrochloric acid. Where necessary, the reaction mixture was centrifuged at 10,000 rpm for 2 min. in a Fico Biofuge, Hareaus Instruments) and 20 µL of the mixture was analysed by HPLC using the mobile phase and gradient condition earlier mentioned. The hydroxylation of hypoxanthine was monitored at 278nm, while the hydroxylation of salicylic acid was monitored at A325nm. The end products of the enzyme or free radical reaction were quantified against standard curves measured at the same wavelength.

Deoxyguanosine Assay for Radical Scavenging Potential: To evaluate the radical scavenging capacity of the extract, the 2-deoxyguanosine-assay model was adopted. The buffer system is similar to that of the hypoxanthine/xanthine oxidase system, except that salicylic is replaced with 2-deoxyguanosine (2mM). The generation of ROS was initiated by addition of ascorbic acid (500 µM). Dried residues, prepared in duplicates (by drying 0-500 µl of extracts in vacuum evaporator) in 1ml phosphate buffer (pH6.6). were re-suspended in buffer and incubated at 37 °C for 24 hrs. The assay of the 8-oxo-2-deoxyguanosine resulting from the ROS attack on 2-deoxyguanosine was analysed using an isocratic system consisting of 5% methanol and 95% aqueous buffer (5mM tetrabutlyammonium hydroxide, adjusted to pH 4.3 with 6% formic acid). The UV detector was set at A293nm [20, 30].

Determination of IC_{50}: The amount of extracts producing 50% inhibition of oxidation (IC50) using the hypoxanthine/xanthine oxidase model system as well as the 2-deoxyguanosine assay methods were determined using the Table Curve Program (Jandel Scientific, Chicago, IL, USA).

Gas Chromatography-Mass Spectrometry: Analyses were performed on a HP 5973 mass spectrometer coupled to a HP 6890 gas chromatograph. Prior to GC-MS analysis, dried methanolic extracts (1µl) were derivatized by addition of BSTFA (100 µl) at 37°C for 30 min. Separation of the analytes was achieved using a HP 5MS capillary column, (30 m X 0.25 mm I.D., 0.25 um film thickness). Helium was used as the carrier gas with a linear velocity of 0.9 ml/s. The oven temperature program was: initial temperature 100 °C, 100-270 °C at 4 °C/min and maintained at 270°C for 20 min. The GC injector temperature was maintained at 250 °C; the transfer line temperature was held at 280 °C. The mass spectrometer parameters for EI mode were: ion source temperature: 230°C; electron energy: 70 eV; filament current; 34,6 uA; electron multiplier voltage: 1200V [20, 30].

Liquid-Chromatography Electrospray-Ionization Mass Spectrometry (LC-ESI): LC-ESI was conducted on an Agilent 1100 HPLC coupled to an Agilent LC/MSD (HP1101). Chromatographic separation was conducted using a C-18, reversed phase (5 um column (25 cm X 2mm I.D.; Latex Eppelheim, Germany) utilizing the same mobile phase and gradient as described for analytical HPLC, except that the flow rate was maintained at 0.5ml / min. The analyses were conducted in the negative ion mode under the following conditions: dry gas (nitrogen) flow rate 10l/min.; nebulizer pressure= 30psi, drying gas temperature= 350°C; capillary voltage = 2500V; fragmenter voltage=100V; mass range=50-3000D

RESULTS

The phenolic constituents of fruit mesocarp oil of *Canarium schweinfurthii* Engl are presented on Table 1, while the GC-MS chromatogram for the total ion count (TIC) and ion 179, one of the most characteristic fragments of phenolic compounds, are presented in Fig. 2. HPLC analysis revealed the presence of catechol, while GC-MS analysis following derivatization with BSTFA revealed the presence of ten phenolic compounds, including catechol, p-hydroxybenzaldehyde, dihydroxyphenylacetic acid, tyrosol, p-hydroxybenzoic acid, dihydroxybenzoic acid, vanillic acid, phloretic acid, pinoresinol, secoisolariciresinol and some other peaks of unknown identity (Fig. 2). The structures of the identified phenolic compounds *Canarium schweinfurthii oil* are presented in Figure 3. The GC-MS data, including the molecular ions and the abundance of the characteristic fragment ions of these phenolic compounds are also presented on Table1.

Table 1: GC-MS data (EI mode) for TMS derivatives of identified phenolic constituents of fruit mesocarp oil of Canarium schweinfurthii

S/No	Compound	Ret. Time (minutes)	TMS groupsa	M+ (calc.)	M+obs.	Fragment ions
1	Catechol	8.64	2	254.11	254(30)	239(9), 73*(100), 45(10).
2	p-Hydroxybenzaldehyde	9.86	1	194.12	194(82)	179(100), 151(72), 73(57).
3	Tyrosol	15.24	2	282.17	282(23)	267(13), 193(12), 179(100), 180(18), 147(14), 103(15), 75(31), 73(74), 59(8), 44(14).
4	p-Hydroxybenzoic acid	16.72	2	282.12	282(25)	267(100), 268(23), 223(66), 193(51), 126(12), 73(44).
5	Dihydroxyphenylacetic acid	17.06	2	296.18	296(55)	281(56), 263(14), 252(58), 217(39), 179(100), 164(41), 149(21), 147(63), 133(27), 103(25),
6	Phloretic acid	20.24	2	310.18	310(28)	295(10), 193(15), 192(76), 180(17), 179(100), 163(6), 147(9), 117(6), 73(62), 45(11)
7	Vanillic acid	20.42	2	312.15	312(66)	297(100), 282(27), 267(68), 253(43), 223(45), 193(18), 179(7), 147(9), 126(24), 75(15), 73(58)
8	Dihydroxybenzoic acid	21.97	3	370.12	370(65)	355(33), 311(22), 281(12), 267(7), 223(12), 193(100), 165(8), 147(6), 137(6), 73(65), 45(10)
9	Secoisolariciresinol	47.31	4	650.42	650(3)	560(7), 261(17), 209(50), 179(12), 173(16), 147(32), 131(11), 129 (22), 103(19), 75(30), 73(100), 44(26).
10	Pinoresinol	58.07	2	402.39	502(86)	487(30), 277(16), 235(79), 223(100), 209(68), 194(45), 179(30), 166(19), 73(92), 44(70).

[a]TMS groups (-Si(CH3)3, MW.73) adds 72 to the original molecular weight for each hydroxyl proton it displaces.
[b]Abundance of molecular ions and fragment ions relative to base ions are provided in percentages (%).
[c]Base peaks are written in bold numbers.

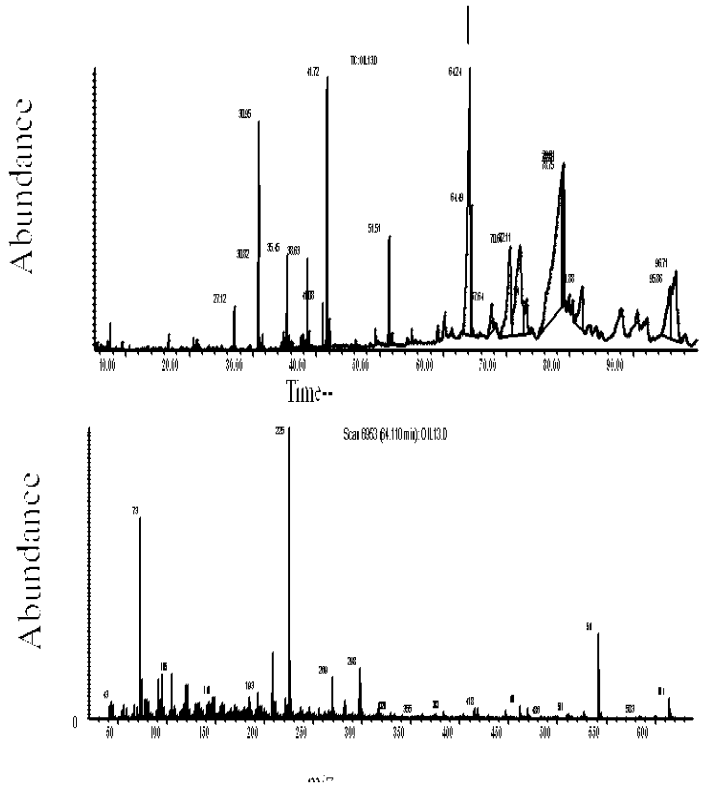

Fig. 2: TIC (A) and Ion 179 chromatogram of fruit mesocarp oil of *Canarium schweinfurthii* following GC-MS of BSTFA-derivatized extract; The identified phenolic compounds are: 1,Catechol; 2, p-hydoxybenzaldehyde; 3, tyrosol; 4, hydroxybenzoic acid; 5, dihdroxyphenylacetic acid; 6, phloretic acid; 7, vanillic acid; 8, didroxybenzoic acid; 9,secoisolariciresinol; pinoresinol

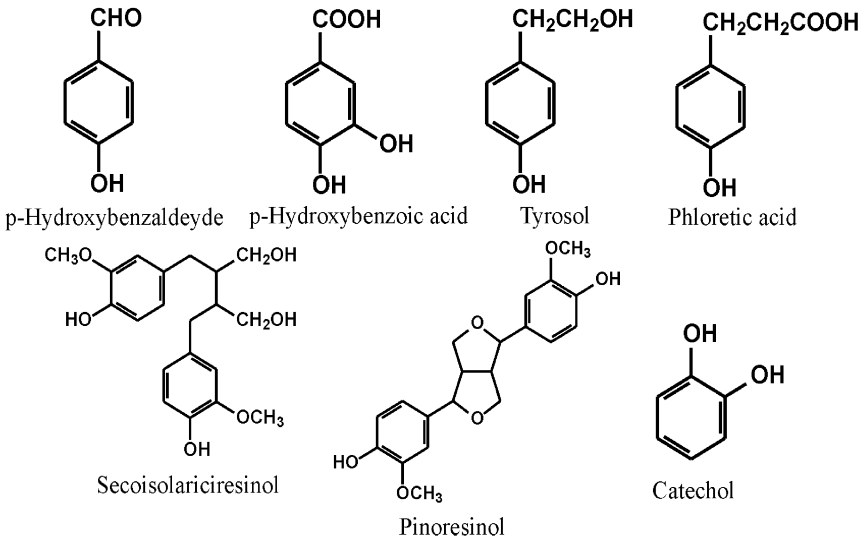

Fig. 3: Structure of identified Phenolic Compounds Detected in Fruit Mesocarp oil of *Canarium Schweinfurthii*

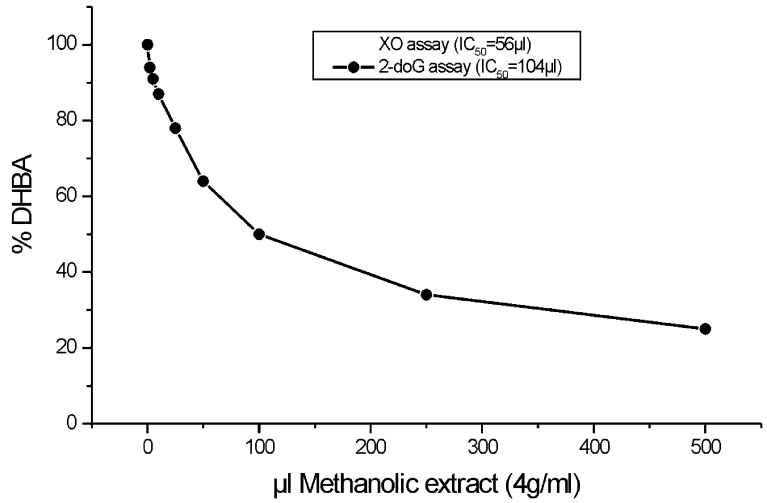

Fig. 4: *In vitro* antioxidant potential of *Canarium schweifurthii* fruit mesocarp oil using the hypoxanthine/xanthine oxidase and the 2-deoxyguanosine assay models

The result for the antioxidant assay/radical scavenging capacity is presented in Fig.4.The oil showed a very strong antioxidant potential (IC_{50}=56ul) and a very promising radical scavenging capacity (IC_{50}=104), when assayed by xanthine oxidase and 2-deoxyguanosine method, respectively.

DISCUSSION

Qualitatively, fruit mesocarp oil of *Canarium schweinfurthii* is only similar to olive oil by containing tyrosol and pinoresinol. Apart from these, catechol, p-hydroxybenzaldehyde, p-hydroxybezoic acid, phloretic acid, secoisolariciresinol which are present in canarium oil (Table1, Fig. 2) are not present in olive oil, neither are hydroxytyrosol, acetoxypinoresinol, secoridoid1 and secoridoid 2 and lignans found in olive oil [20, 30], detected in *Canarium oil* (Table 1; Fig.2).

The antioxidant activity compares very favourably with that of extra virgin olive oil [20, 30], which, perhaps, is the most celebrated cancer chemopreventive component of the Mediterranean diet [20, 30]. The high antioxidant and radical scavenging activity of *Canarium* oil (Fig. 3) is consistent with its polyphenol profile (Fig. 2; Table 1) and the fact that oils of other plant species, notably, olive [20, 30, 31] have been demonstrated to display high *in vitro* and to some extent, *in vivo* antioxidant activity.

Dietary phenolic may have physiological antioxidant properties, quenching reactive oxygen and nitrogen species (ROS and RNS, respectively) and hence potentially modifying pathological mechanisms relevant to cardiovascular diseases [32] and other diseases like gastrointestinal disorders and cancers [5, 9, 20, 30, 33-35]. The significantly lower incidence of breast and colorectal cancer in Mediterranean countries like Greece, Italy and Spain has been mainly attributed to the olive oil content of their diet. Like canarium oil, olive oil is known to contain tyrosol and pinoresinol, among others [20, 30].

Tyrosol is reported to possess strong antioxidant activity with IC_{50} value of 2.5 mM when tested with the hypoxanthine/xanthine oxidase system [20]. A derivative of tyrosol, hydroxytyrosol, the major representative phenolic compound of virgin olive oil is reported to protect human erythrocytes against oxidative damage [31]. Besides, both tyrosol and hydroxytyrosol have been shown to significantly inhibit autooxidation [31, 36, 37], production of isoprostanes and other markers of lipid oxidation [38] and oxidative stress induced by hydrogen peroxide and xanthine oxidation [31]. In addition, Owen and co-workers have demonstrated that tyrosol, secoisolariciresinol and pinoresinol, which were also detected in *Canarium* oil, possess significantly higher antioxidant potential than the classical antioxidant, Trolox, the synthetic analogue of vitamin E [30].

The hydroxybenzoic acid content of edible plants is generally very low, with the exception of certain red fruits, black radish and onions, which have concentration of central tens of mg/Kg fresh weight [9]. p-Hydroxybenzoic acid, in combination with other phenolic acids have been reported to act synergistically with vitamin C to enhance human and hamster low density lipoprotein resistance to oxidation [39]; and with gallic acid, gentistic and /or coumaric acid it act synergistically to modulate phenylsulfotransferase activity. Individually, p-hydroxybenzoic and other phenolic acids inhibit the activities of the two forms of phenylsulfotransferase enzymes involved in sulfate conjugation, in a manner that reflects their respective antioxidant activity [40]. Isoforms of this enzyme are important in the detoxication of chemical carcinogens.

The presence of catechol in *Canarium* oil is also of major interest in cancer chemoprevention. According to Manach and collegues [9], catechol and other polyphenols with catechol group may intervene in oxidation-precipitated diseases, such as cardiovascular diseases and cancer, because they competitively inhibit the catechol-O-methyltransferase-catalyzed O-methylation of endogenous catecholamines and catechol estrogens: Deregulation of the O-methylation metabolism of neurotransmitters and hormones in humans is an important risk factor for the development of some neurodegenrative diseases, cardiovascular disorders and hormone-dependent cancers [41, 42].

Several studies have suggested that other phenolic acids detected in *Canarium schweinfurthii* oil may also play some role in the chemoprevention of cancer and ROS induced diseases. p-hydroxybenzaldehyde, for instance, has been shown to significantly protect against lipid peroxidation [43], while phloretic acid, another component of *Canarium* oil, has been demonstrated to possess strong antioxidant activity with IC_{50} value of 3.0mM. [20].

Although some earlier investigators may have associated lignans with certain cancers, recent evidence however suggest that, pinoresinol, a common component of the lignan fraction of plants such as flaxseed, *Forsythia* specie and *Sesamum indicum* seeds [44-46], is believed to possess cancer chemopreventive properties [20, 30]. Similarly, detection of another lignan, secoisolariciresinol, whose major source is linseed, is also noteworthy. Different lignans have been shown to inhibit mammary carcinogenesis, lung cancer cell growth, skin cancer and colon cancer [41, 46], partly, because lignans, as phytoestrogens, exhibit structural similarity to the mammalian steroid hormone, 17 B-oestradiol [47]. Although, like matairesinol, secoisolariciresinol is not estrogenic by itself, it is readily converted by gut flora to the mammalian lignan, enterodiol and and entero-lactone which are estrogenic and have shown promise in reducing growth of cancerous tumors, especially hormone-sensitive ones such as those of the breast, endometrium and prostate [36, 48-50]. Lignans may also prevent cancer through their antioxidant and antiviral properties [36, 48-50].

Therefore, put together, these results indicate that fruit pulp oil of *Canariun schweinfurthii* is a rich source of cancer chemopreventive polyphenol compounds that possess strong *in vitro* antioxidant potential, as well as very potent radical scavenging capacity, suggesting that this oil might indeed play important roles in the chemoprevention of cancer and other diseases like diabetes, cardiovascular disorders, etc which are caused by oxidative damage. Studies are underway to identify the unknown phenolic components of the oil and demonstrate the antioxidant capacity of those components that have not been previously studied.

ACKNOWLEDGEMENT

I would like to thank Alexander von Humboldt Foundation (AvH), Bonn, Germany for grant of a research fellowship which made possible his stay as Guest Scientist at the Institute for Toxicology and Cancer Risk Factors, German Cancer Research Centre, Heidelberg in 2004/2005; and also for the subsequent award of material support for his work in Nigeria. He is grateful to Prof Dr H. Bartsch and his members of his group, especially, Drs R.W Owen and B. Pfundstein for hosting me in their laboratories. This article is dedicated to one of my mentors, Dr Bertold Spiegelhalder on the occasion of his retirement from active research at the German Cancer Research Centre in Heidelberg, Germany

REFERENCES

1. D'Archivio, M., C. Santangelo, B. Scazzocchio, R. Vari, C. Filesi, R. Masella and C. Giovannini, 2008. Modulatory effects of polyphenols on apoptosis induction: Relevance for cancer prevention. Intl. J. Molecular Sci., 9: 213-228.
2. Guo, W., E. Kong and M. Meydani, 2009. Dietary polyphenols, inflammation and cancer. Nutrition and Cancer, 61: 807-810.
3. Kim, K.W., A.R.M. Ruhul Amin and D.M. Shin, 2010. Chemoprevention of Head and Neck Cancer with Green Tea Polyphenols. Cancer Prevention Research, 3(8): 900-909.
4. Stan, S.D., S.V. Singh and R.E. Brand, 2010. Chemoprevention strategies for pancreatic cancer. Nature Reviews Gastroenterol. Hepatol., 7: 347-356.
5. Cosan, D.T., B. Bayram, A. Soyocak, A. Basaran, H.V. Gunes, I. Degirmenci and A. Musmul, 2010. Role of phenolic compounds in nitric oxide synthase activity in colon and breast adenocarcinoma. Cancer Biotherapy and and Radiopharmaceuticals, 25(5): 577-580.
6. Vauzour, D., A. Rodriguez-Mateos, G. Corona, M.J. Oruna-Concha and Jeremy P.E. Spencer, 2010. Polyphenols and human health: prevention of disease and mechanisms of action. Nutrients, 2: 1106-1131
7. Kim, E.S. and F.R. Khuri, 2002. Chemoprevention of lung cancer. Current Oncology Report, 4(4): 341-46.
8. Kampa, M., A.P. Nifli, G. Notas and E. Castanas, 2007. Polyphenols and cancer cell growth. Review of Physiololgy, Biochem. Pharmacolol., 159: 79-113.
9. Larsson, S.C., S.O. Andersson, J.E. Johansson and A. Wolk, 2008. Fruit and vegetable consumption and risk of bladder cancer: A prospective cohort study. Cancer Epidemiology and, Biomarkers and Prevention, 17: 2519-2522.
10. Boffetta, P., E. Couto, J. Wichmann, P. Ferrari, D. Trichopoulos, H.B. Bueno-de-Mesquita, F.J. van Duijnhoven, F.L. Buchner, T. Key and H. Boeing, 2010. Fruit and vegetable intake and overall cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC). Journal of National Cancer Institute, 102: 529-537.
11. Le Marchand, L., 2002. Cancer preventive effects of flavonoids--a review." Biomedicine and Pharmacotherapy, 56(6): 296-301.
12. Park, E.J. and J.M. Pezzuto, 2002. Botanicals in cancer chemoprevention. Cancer Metastasis Review, 21(3-4): 231-255
13. Cassileth, B.R. and A.J. Vickers, 2003. Soy: an anticancer agent in wide use despite some troubling data. Cancer Investigation, 21(5): 817-818.
14. Sheela, M.A., S. Sapna, K. Surabhi and B. Arunima, 2010. Dietary supplements as anti cancer agents. International Journal of Pharmaceutical Sciences. Rev. Res., 4(2): 159-163.
15. Adhami, V.M., N. Ahmad and H. Mukhtar, 2003. Molecular targets for green tea in prostate cancer prevention. Journal of Nutrition, 133(7)Suppl: 2417S-2424S.
16. Gupta, S., N. Ahmad and H. Mukhtar, 1999. Prostate cancer chemoprevention by green tea. Seminar in Urol. Oncol., 17(2): 70-76.
17. Katiyar, S.K. and H. Mukhtar, 1997. Tea antioxidants in cancer chemoprevention. Journal of Cellular Biochemistry, (Suppl): 59-67.
18. Simopoulos, A.P., 2004. The traditional diet of Greece and cancer. European Journal of Cancer Prevention, 13(3): 219-330.
19. Weisburger, J.H., 1998. Evaluation of the evidence on the role of tomato products in disease prevention. Proceedings of the society for experimental biology and medicine, 218(2): 140-143.
20. Owen, R.W., W. Mier, A. Giacosa, W.E. Hull, B. Spiegelhalder and H. Bartsch, 2000. Phenolic compounds and squalene in olive oils: the concentration and antioxidant potential of total phenols, simple phenols, secoiridoids, lignans and squalene. Food and Chemical Toxicolol., 38(8): 647-659.

21. Adebamowo, C.A. and O.O. Ajayi, 2000. Breast cancer in Nigeria. West African J. Med., 19(3): 179-191.
22. Oluwasola, A.O. and J.O. Ogunbiyi, 2003. Gastric cancer: aetiological, clinicopathological and management patterns in Nigeria. Nigerian J. Med., 12(4): 177-186.
23. Gyamfi, M.A. and Y. Aniya, 2001. Antioxidant properties of Thonningianin A, isolated from the African medicinal herb, *Thonningia sanguinea*. Biochemical Pharmacolol., 63(9): 1725-1737.
24. Wattanapenpaiboon, N. and M.W. Wahlqvist, 2003. Phytonutrient deficiency: the place of palm fruit. Asia Pacific J. Clin. Nutrition, 12(3): 363-368.
25. Abayeh, O.J., A.K. Abdulrazaq and R. Olaogun, 1999. Quality characteristics of *Canarium schweinfurthii* Engl. Oil. Plant Foods for Human Nutrition, 54(1): 43-48.
26. Agbonzi, G. and O.C. Kouame, 1996. Oil extraction improvement from *Canarium schweinfurthii* Engl fruit pulp using different enzymes. Sciences des Aliments, 16(1): 77-82.
27. Davidson, B.C. and B.N. Nkeh, 2003. The lipid and fatty acid profiles of the fruit of *Canarium schweinfurthii*. South African J. Sci., 99(7-8): 319-320.
28. Tchiegang, C., 2004. Comparative analysis of wet and dry softening of Schweinfurth's olives (*Canarium schweinfurthii* Engl.). Journal of Food Engineering, 62(4): 351-357.
29. Helene, T., Tamboue H.T. Helene, Fotso Serge, Bonaventure T. Ngadjui, Dongo Etienne and Berhanu M. Abegaz, 2000. Phenolic metabolites from the seeds of *Canarium schweinfurthii*. Bulletin of the Chemical Society of Ethiopia, 14(2): 155-159.
30. Owen, R.W., R. Haubner, W. Mier, A. Giacosa, W.E. Hull, B. Spiegelhalder and H. Bartsch, 2003. Isolation, structure elucidation and antioxidant potential of the major phenolic and flavonoid compounds in brined olive drupes. Food and Chem. Toxicolol., 41(5): 703-717.
31. Corona, G., M. Deiana, A. Incani, D. Vauzour, M.A. Dessi and J.P. Spencer, 2009. Hydroxytyrosol inhibits the proliferation of human colon adenocarcinoma cells through inhibition of ERK1/2 and cyclin D1. Molecular Nutrition and Food Research, 53: 897-903.
32. Morton, L.W., R.A. Caccetta, I.B. Puddey and K.D. Croft, 2000. Chemistry and biological effects of dietary phenolic compounds: relevance to cardiovascular disease. Clin. Experimental Pharmacol. Physiol., 27(3): 152-159.
33. Gosslau, A. and K.Y. Chen, 2004. Nutraceuticals, apoptosis and disease prevention. Nutrition, 20(1): 95-102.
34. Janne, P.A. and R.J. Mayer, 2000. Chemoprevention of colorectal cancer. New England J. Med., 342(26): 1960-1768.
35. Shureiqi, I., P. Reddy and D.E. Brenner, 2000. Chemoprevention: general perspective. Critical Rev. Oncol. Hematol., 33(3): 157-167.
36. Pusztai, R., M. Abrantes, J. Sherly, N. Duarte, J. Molnar and Ferreira M-J. U., 2010. Antitumor-Promoting Activity of Lignans: Inhibition of Human Cytomegalovirus IE Gene Expression. Anticancer Research, 30(2): 451-454.
37. Miro-Casas, E., E. Miró-Casas, M.I. Covas, M. Fitó, M. Farré-Albadalejo, J. Marrugat and R. de la Torre, 2003. Tyrosol and hydroxytyrosol are absorbed from moderate and sustained doses of virgin olive oil in humans. European. J. Clin. Nutrition, 57(1): 186-190.
38. Hashim, Y.Z., I.R. Rowland, H. McGlynn, M. Servili, R. Selvaggini, A. Taticchi, S. Esposto, G. Montedoro, L. Kaisalo, K. Wahala and C.I. Gill, 2008. Inhibitory effects of olive oil phenolics on invasion in human colon adenocarcinoma cells *in vitro*. Intl. J. Cancer, 122: 495-500.
39. Chen, C.Y., P.E. Milbury, H.K. Kwak, F.W. Collins, P. Samuel and J.B. Blumberg, 2004. Avenanthramides and phenolic acids from oats are bioavailable and act synergistically with vitamin C to enhance hamster and human LDL resistance to oxidation. J. Nutrition, 134(6): 1459-1466.
40. Yeh, C.T., P.H. Shih and G.C. Yen, 2004. Synergistic effect of antioxidant phenolic acids on human phenolsulfo-transferase activity. J. Agric. Food Chem., 52(13): 4139-4143.
41. Yang, C.S., J.M. Landau, M.T. Huang and H.L. Newmark, 2001. Inhibition of carcinogenesis by dietary polyphenolic compounds. Ann. Rev. Nutrition, 21: 381-406.
42. Zhu, Y.Z., S.H. Huang, B.K. Tan, J. Sun and M. Whiteman, 2004. Antioxidants in Chinese herbal medicines: a biochemical perspective. Natural Product Report, 21(4): 478-489.

43. Lee, K.H., K.C. Kim, Y.J. Jung, Y.H. Ham, J.J. Jang, H. Kwon, Y.C. Sung, S.H. Kim, S.K. Han and C.M. Kim, 2001. Induction of apoptosis in p53-deficient human hepatoma cell line by wild-type p53 gene transduction: inhibition by antioxidant. Molecules and Cells, 12(1): 17-24.
44. Sharp, H., D. Thomas, F. Curriea, C. Brighta, Z. Latifa, S.D. Sarkerb and R.J. Nash, 2001. Pinoresinol and syringaresinol: two lignans from *Avicennia germinans* (Avicenniaceae). Biochem. Systematic Ecol., 29(3): 325-327.
45. Meagher, L.P., G.R. Beecher, V.P. Flanagan and B.W. Li, 1999. Isolation and characterization of the lignans, isolariciresinol and pinoresinol, in flaxseed meal. J. Agric. Food Chem., 47(8): 3173-3180.
46. Thompson, I.M., D. Albanes, J.W. Basler, E.D. Crawford, L.J. Denis, B. Djavan, N. Fleshner, T.L. Johnson-Pais, E.A. Klein, A.R. Kristal, M.S. Lucia, H.L. Parnes, G.A. Piazza, E.A. Platz, B.H. Pollock, D.K. Price, J.K. Reichardt, C.M. Tangen, A.W. Tolcher and M.C. McMann, 2004. First International Conference on Chemoprevention of Prostate Cancer. Overview consensus statement. J. Urol., 171(2): S3-S4.
47. Limer, J.L. and V. Speirs, 2004. Phyto-oestrogens and breast cancer chemoprevention. Breast Cancer Research, 6(3): 119-127.
48. Tour´e, A. and X. Xueming, 2010. Flaxseed Lignans: Source, Biosynthesis, Metabolism, Antioxidant Activity, Bio-Active Components and Health Benefits. Comprehensive Rev. Food Sci. Food Safety, 9: 261-269.
49. Prasad, K., 2000. Antioxidant Activity of Secoisolariciresinol Diglucoside-derived Metabolites, Secoisolariciresinol, Enterodiol and Enterolactone. Intl. J. Angiol., 9(4): 220-225.
50. Touillaud, M.S., C. Anne, M. Thiébaut, A. Fournier, M. Niravong, M.C. Boutron-Ruault and F. Clavel-Chapelon, 2007. Dietary Lignan Intake and Postmenopausal Breast Cancer Risk by Estrogen and Progesterone Receptor Status. Journal of National Cancer Institute, 99: 475-486.