INAUGURAL LECTURE

Phenolics: A Class of Nature's Chemical Weapons of Self-Preservation

By

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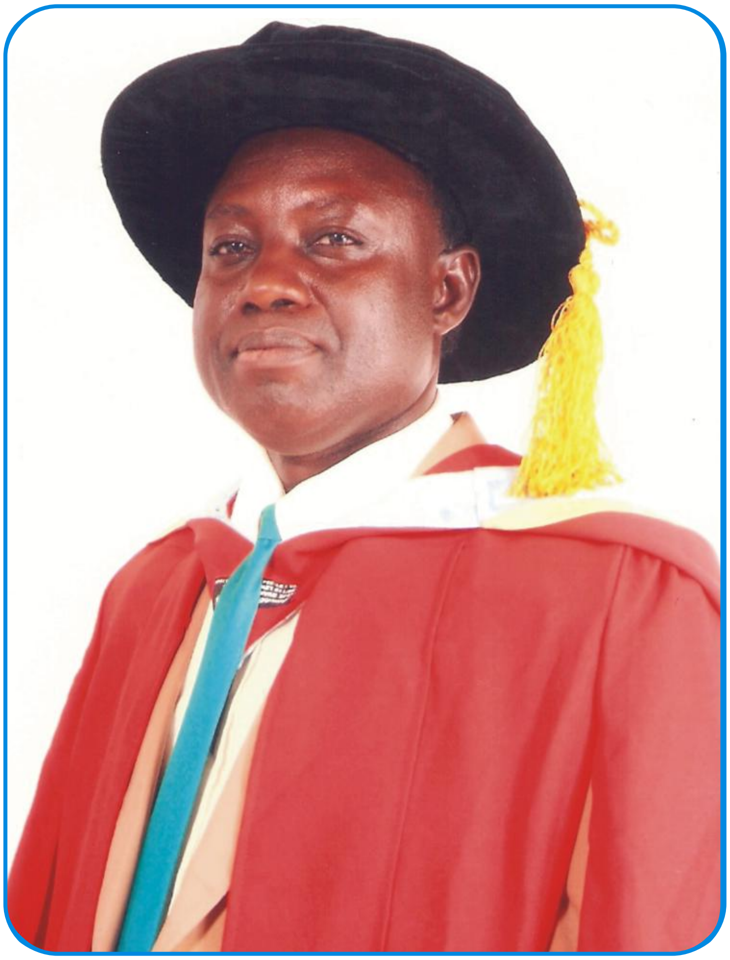

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Phenolics: A Class of Nature’s Chemical Weapons of Self-Preservation

The Vice Chancellor,
Deputy Vice Chancellors,
The Registrar and other Principal Officers,
My Great Heads of Departments in the Faculty of Natural Sciences,
Fellow Colleagues in Senate
Fellow Colleagues (Academic and Non-Academic) in KSU,
Friends of the University and Special Guests,
Gentlemen of the Press,
Distinguished Ladies and Gentlemen,
Great KSU Students.

PREAMBLE

G-O-D Almighty takes all the glory this day, Mr. Vice Chancellor as I mount this stage to present the paper titled “Phenolics: A Class of Nature’s Chemical Weapons of Self-Preservation”. One bags a first degree, second degree, and the third degree, and each time he convocates ceremonially. In a way therefore, the Inaugural lecture session is to me another Convocation ceremony in my academic life after being assessed externally and found worthy to join the Guild of Professors worldwide.

Secondly, I ‘clocked’ 50 years on 06th April 2008 and would therefore, consider the Inaugural lecture a mode to celebrate my golden Jubilee on this planet ! Thanks to the VC, and my co-members of Senate and the Governing Council.

From another perspective I consider the fact that Kogi State University was established in 1999/2000 session, barely eight years ago, and with Sunday Salifu Arogba as the first Professor on tenure appointment with effect from 1st June 2000 when I transferred my services to Anyigba. The Inaugural lecture therefore is the first of its kind, epoch-making, historical, and will remain memorable in the annals of Kogi State University. I rejoice with the beautiful audience gathered today for the lecture.

VC Sir, when the thought of my Inaugural lecture struck me, the topic on phenolics was loosely idealized or conceptualized. It kept resounding until crystallized thus. It is therefore, important to share the thought of a mind-set before beginning the lecture properly: When I first bagged the first degree in B.Sc. Biochemistry 1979 in Zaria, the project topic supervised by my mentor, Professor Godwin H. Ogbadu was titled "Aflatoxin and Tannin in different varieties of sorghum". In pursuit of the second degree obtained at the University of Reading, Reading, United Kingdom, in Food Quality Control 1982, the Masters thesis was titled "Quantitation of malic acid during primary fermentation of grape (red, and white), and apple juices". Finally in 1987, the Ph.D. thesis at Brunel (The University of West London), Uxbridge, Middlesex, U.K. was titled "Synthesis and analysis of antioxidants related to natural phenolic lipids and similar substances". From these topics, students of Biochemistry easily discern a common denominator: **Tannin – Wine – Phenolic lipids**. It is little surprising therefore to have crystallized 'Phenolics' into the topic of the day!

'Phenolics' is a wide topic that defies full discussion in few hours of a single day. The approach therefore is to:

i) introduce phenolics from general perspective,

ii) appraise their occurrence and positive contributions to the life of plant and animal cells. In other words, I shall describe the biological activity of phenolics with emphasis on their role as antioxidants in disease prevention and health promotion, and

iii) present a review of ***some*** of my research contributions to knowledge relevant to this area of specialization.

INTRODUCTION

Phenolics are organic compounds embracing a vast range of substances possessing, in the minimum, an aromatic ring bearing one or more hydroxy substituents as the basic structure. Aromatic compounds of different unique phenolic variations abound everywhere in the plant kingdom in their tens of thousands. Lignin, for instance, comprises over one-third of the dry weight of wood. Lignin is an extremely complex tangle of phenolic compounds that bind fibre bundles of cellulose in wood to give the main structural strength. In natural products chemistry, phenolics are considered as waste products of primary metabolic processes (biosynthesis, biodegradation, and other energy conversions of intermediary metabolism) in biological systems, and are therefore called ***secondary metabolites***. Other secondary metabolites include terpenes, and alkaloids. These metabolites were not initially thought to be of any particular use to plants in which they occurred, or to man but scientific evidence abound today identifying them with numerous biological activities of value.

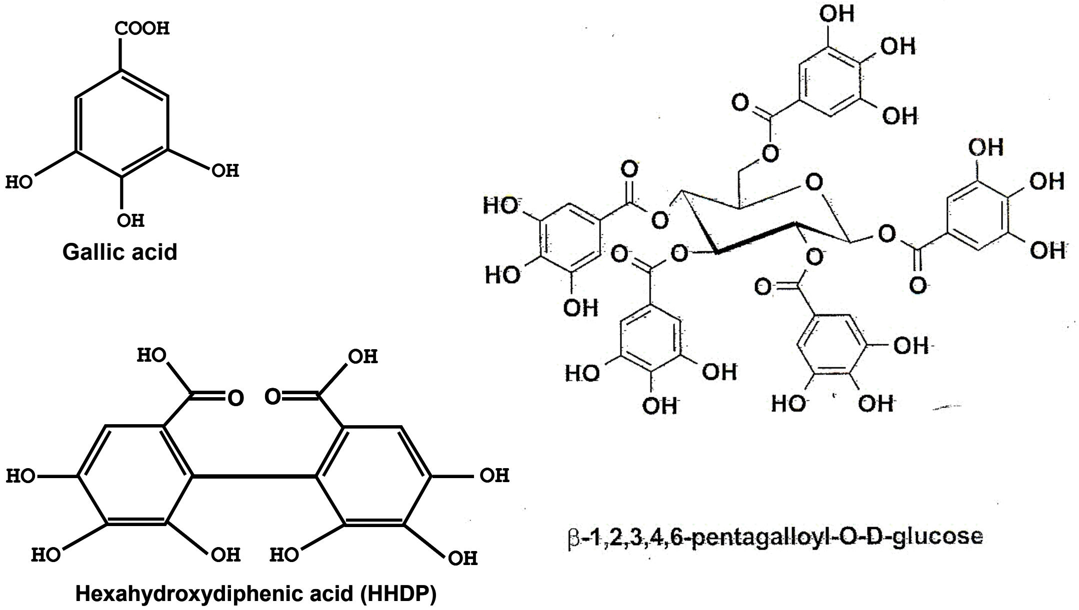
Phenolics cover a wide range of compounds from the familiar pigments of flower called *anthocyanidins* to the complex phenolics of plant cell wall earlier mentioned, as *lignin* which confer rigidity on the structure of plants. The simplest illustration of the classification, and examples of phenolic compounds among various fruits is shown in Table 1 (Antolovich *et al.*, 2000). The illustration exemplifies a common group of phenolics called *tannins*. The term 'tannin' comes from the ancient Celtic word for oak (*Quercus rubra)*, a typical source of tannins for leather-making. On the basis of structural chemistry, tannins are sub-classified as *Hydrolysable* tannins, and *condensed* tannins.

Table 1: Classification of phenolic compounds with characteristic examples in various fruits. (Antolovich *et al*, 2000)

Basic skeleton	Class	Common fruit source	Examples
C_6	*Simple phenols*	Widely distributed	*Catechol, hydroquinone, resorcinol*
C_6-C_1	*Benzoquinones* *Phenolic acids*	Widely distributed	p-Hydroxybenzoic acid, *salicylic acid*
C_6-C_2	Phenylacetic acids		p-Hydroxyphenylacetic acid
C_6-C_3	Cinnamic acids	Widely distributed	Caffeic acid, ferulic acid
	Phenylpropenes		Eugenol, myristicin
	Coumarins	Citrus	Umbelliferone, aesculetin, scopolin
	Chromones		Eugenin
C_6-C_4	Naphthoquinones	Walnut	Juglone
C_6-C_1-C_6	Xanthones	Mango	Mangostin, mangiferin
C_6-C_2-C_6	Stilbenes	Grape	Resveratrol
	Anthraquinones		Emodin
C_6-C_3-C_6	*Flavonoids*		
	Flavones	Citrus	Sinensetin, nobiletin, tangeretin, isosinensitin, various polymethoxylated flavones
	Flavonols	Apple	Quercetin, kaempferol
	Flavonol glycosides	Widely distributed	Rutin
	Flavanonols	Grape	Dihydroquercetin and dihydrokaempferol glycosides
	Flavanones	Usually found in citrus	Hesperitin, naringenin
		Tomato	Naringenin
	Flavanone glycosides	Citrus	Hesperidin, neohesperidin, narirutin, naringin, eriocitrin
	Anthocyanins	Apple	Cyanidin glycosides including acylated derivatives
		Sweet orange	Glycoside of pelargonidin, peonidin, delphinidin, Petunidin
		Grape	Glycosides of cyanidin, peonidin, delphinidin, petunidin, malvidin including acylated forms
	Flavanols (catechins)	Apple	(+)-Catechin, (-)-epicatechin
		Grape	(+)-Catechin, (-)-epicatechin, (+)-gallocatechin, (-)-epigallocatechin
	Chalcones	Tomato	Chalconaringenin
$(C_6$-$C_3)_2$	Lignins		Pinoresinol
$(C_6$-C_3-$C_6)_2$	Biflavonoids		Agathisflavone

Hydrolysable tannins are derivatives of *gallic acid*, 3,4,5-trihydroxybenzoic acid (Hagerman, 2002). Gallic acid is esterified to core polyol such as glucose, glucitol, quercitol, and the galloyl groups may be further esterified or oxidatively cross-linked to yield more complex hydrolysable tannins. Examples include (Fig 1):

Fig 1: Hydrolysable Tannins



Gallic acid

Hexahydroxydiphenic acid (HHDP)

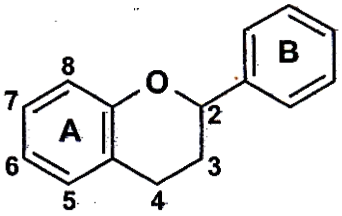
β-1,2,3,4,6-pentagalloyl-O-D-glucose

i) *gallotannins,* pentagalloyl glucose (PGG), *beta*-1,2,3,4,6-pentagalloyl-O-D-glucose and its isomers e.g. *beta*-1,2,2,3,6-pentagalloyl-O-D-glucose. Such isomers containing digalloyl ester (GOG) chains are formed by either *meta*- or *para*-depside bonds.

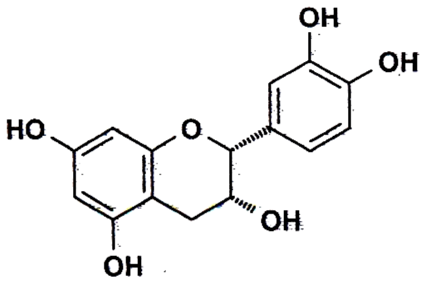
ii) *ellagitannins*, esters of hexahydroxydiphenic acid (HHDP); Galloyl groups on C-4 and C-6 or C-2 and C-3 of gallotannins commonly couple oxidatively and intramolecularly to form HHDP (e.g. eugenilin, casuarictin). In aqueous solution, HHDP spontaneously lactonizes to ellagic acid.

Condensed tannins are polymeric flavonoids. The flavonoid skeleton looks thus (Fig 2):

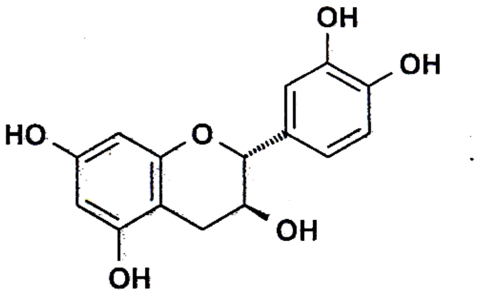
Fig 2: Condensed Tannins



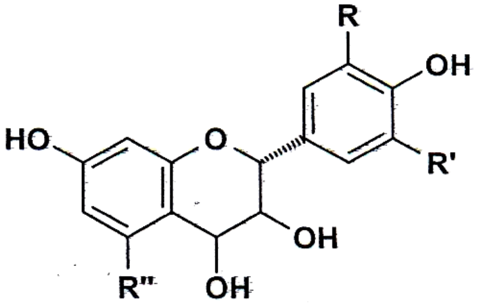
flavonoid skeleton



Flavan-3-ol (epicatechin)

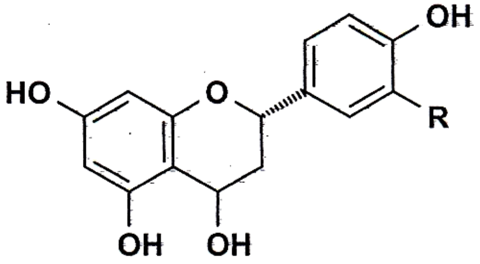


Flavan-3-ol (catechin)



Flavan-3,4-diols

R'' = H (stable)
  R = H, R' = OH, leucofisetinidin
R'' = OH (unstable)
  R = R' = H, leucopelargonidin
  R = H, R' = OH, leucocyanidin
  R = R' = OH, leucodelphinidin



Flavan-4-ols

R = H, apiferol (leucoapigeninidin)
R = OH, luteoferol (leucoluteolinidin)

Possible identification of hydroxy groups at positions 3,4,5,7, and the *meta* and *para* locations relative to C-2 on ring **B** accounts for the various structural monomeric forms called (leuco)cyanidins, upon oxidative cleavage of the condensed tannins (proanthocyanidins). Examples of these isomers, and which exhibit stereochemical isomers, include:

- i) Flavan-3-ols e.g. epiafzelechin/afzelechin, epicatechin/catechin, epigallocatechin/gallocatechin,
- ii) Flavan-4-ols e.g. apiferol (leucoapigeninidin), luteoferol (leucoluteolinidin),
- iii) Flavan-3,4-diols e.g. leucopelargonidin, leucocyanidin, leucodelphinidin.

Oxidative treatments of condensed tannins, by common use of acidified alcohol, yield products that have skeletons of cyanidin, that is, a flavylium cation (e.g. apigeninidin), a flavanone (e.g. naringenin), or a flavone (e.g. apigenin).

At a later point, I will present types of tannins associated with a fruit of interest during my post-doctoral research.

BIOLOGICAL ACTIVITY OF PHENOLICS

Phytochemicals constitute a wide family of natural substances that exist abundantly in legumes, whole grains, vegetables and fruits that are pigment-rich. Major phytochemicals are phenolic compounds such as phenolic acids, flavonoids, and coumarin derivatives as well as many other polyphenols which function as antioxidants (Zielinski and Kozlowska, 2000). Antioxidants reduce the rate of oxidation reactions that involve chemical transfer of electrons from a compound or substance to an oxidizing agent. Antioxidants play an important role in lipid and emulsion systems by preventing undesirable changes in flavour and nutritional quality of foods, and preventing oxidative damage to the living cells and tissues of plants, animals, and man.

Plant phenolics, as phytochemicals, are known to possess a considerable range of bioactive properties which are not only broadly classified as nutritional, but pharmacological, and anti-microbial. That is, phytochemicals have known and potential chemical applications as drugs against cancer, cardiovascular diseases, and microbial infections. We shall elaborate on these roles:

Generally, foods rich in potentially beneficial phytochemicals are acclaimed to promote overall health and prevent diseases (Table 2).

Table 2: A list of some phytochemicals:

TYPE	SOURCES	POSSIBLE ROLE
Allicin	Garlic, Onions	Lowering serum cholesterol against cardiovascular disease, anti-cancer enzyme production.
Capsaicin	Hot peppers	Reduces blood clotting
Carotenoids	Carrots, Pawpaw	Anti-cancer benefits
Tannins/flavonoids	Pigmented fruits (blue berries, black raspberries, apples) green tea, legumes, citrus fruits, wines	Against cardiovascular disease
Indoles	Cruciferous vegetables	Block carcinogens from damaging DNA
Isothiocyanates	Cruciferous vegetables, broccoli sprouts	Inhibit enzymes that promote cancer activity
Lignans	Whole grains, flax seed	Prevent estrogen +ve cancers from metastasizing
Limonene	Citrus fruits	Inhibit cancer growth
Lycopene	Tomatoes, pink grapefruit, watermelon, guava, pawpaw	Anti-prostate cancer
Phenolic acids	Fruits, coffee	Activate enzymes that promote excretion of carcinogen in water-soluble forms.
Phytic acid	Whole grains	Inhibit free-radical activity by binding minerals which are pro-oxidants
Phytosterol	Soyabeans	Slow growth rate of some cancers
Resveratrol	Grapes, peanuts, red wine	Lowers risk of blood clotting, inhibit cell growth.
Saponins	Legumes, some vegetables	Slows DNA replication in cancer cells
Vitamin C	Vegetables and fruits	Lowers risk of cancers of mouth, throat and eosophagus.

Quite a number of phytochemicals that function as antioxidants prevent cellular and tissue damage by

- limiting the formation of free radicals
- destroying free radicals
- restoring enzymes that have been damaged by free radicals
- stimulating the activity of these enzymes
- repairing the damage to tissues caused by free radicals.

Food industries have taken note of consumer interests by formulating ‘functional foods’ to which have been deliberately added phytochemicals or similar biologically active chemicals as supplements that promote health. Hence, supermarkets today are seen to shelf array of performance-enhancing beverages and snacks.

The aging process of man like other mammals is complex, and is beyond the known passive accumulation of wear and tear over the years. Free radicals (e.g. hydroxyl radical .0H), and other ROS (reactive oxygen species) (e.g. hydrogen peroxide H_2O_2, and superoxide anion O_2-) mediate the accumulation of these molecular injuries which can be reduced through consumption of antioxidant-rich foods. This dietary approach may slow down, prevent, or even reverse certain diseases by keeping in good shape or boosting the immune systems, and even slow down the natural aging process. This is the basis for the free-radical theory of aging (Wikipedia, 2006). Recently science has demonstrated that the total life span of mice can actually be extended to some measure by therapeutic manipulation of the ROS metabolism.

ROS mediated tissue injury is a common pathway for a number of disease processes. ROS can be generated within the cell not only by external sources of radiation (e.g sunlight and other forms of radiation), but also endogenously as byproduct of normal metabolic processes. The endogenous sources include drugs, pollutants, and other chemicals and toxins, collectively called xenobiotics.

Cancer and other malignancies all entail unconstrained cell growth and proliferation based on cell's genetic information. In most cases, for example, one or more genes that normally constrain replication is/are mutated, or otherwise inactivated. These genetic deficiencies correspond directly to sequence changes in the genetic code, resident in the cell's DNA. A frequently seen final common damage is free radical injury. Two known major cancers are the colon cancer, and breast cancer.

Atherosclerosis remains the major cause of death and premature disability in developed societies. It is predicted that by the year 2020 cardiovascular diseases, notably atherosclerosis, will be the most prevalent, globally. Atherosclerosis is a complex process that leads to heart attack, stroke and limb loss due to vasoconstriction of the arteries with atherosclerotic plaque. The plaque is a form of oxidized fat. A major factor is the ROS–mediated peroxidation of the low density lipoproteins (LDLs, or 'bad cholesterol'). The dietary approach in the prevention of heart disease and stroke is based partially on adding *dietary antioxidant* to limit LDL oxidation. A better remedy is to decrease the intake of fat itself.

Current trend in the therapy of aging, breast and colon cancers, and atherosclerosis appear to be potentially available in the administration of an oil palm trunk dietary fibre supplement (USA patent application 20060024390) because of its antioxidant capability. The African oil palm *(Elaeis guineensis)* is the test case.

From nutritional consideration, when bowel transit time with ingested food is perpetually prolonged, proteins putrefy, fats become rancid, carbohydrate ferment to produce toxins in feces. In most instances, blocked or slow-moving bowel can cause problem such a neck and shoulder pain, waist and skin problem, lower back pain, "brain fog", fatigue, sluggishness, common headache, and neurological problems. These are symptoms of ***nutritional disorders***.

Studies on structure–activity relationship have enabled researchers to understand the biological activities of some structurally related bioactive phenolic compounds such as ferulic, caffeic, gallic acids and curcumin (Croft, 1998). These hydroxylated acids acting as phenolic antioxidants show substantial in-vitro growth inhibitory activity against human breast cancer cells (Gomes *et al*, 2003, Indap *et al*, 2006).

The Mediteranean red and white wines, and virgin olive oil are known to contain simple phenolic antioxidants, tyrosol and hydroxytyrosol. In a wide range of up to 50 mg/kg total phenolics in olive oil, the complex related compound, oleuropein aglycone characterized by the catecholic structure, is also present (Galli *et al*, 2005, Pereira *et al*, 2006). These compounds are known to exert important biological activities *in-vitro*. The activities include anti-peroxidation, anti-atherogenic or atherosclerotic processes by binding to human cholesterol-rich low density lipoprotein (LDL) and functional inhibition of cells involved in inflammatory processes such as formation of leukotrienes via the lipoxygenase pathway. (Leukotrienes are part of the family of complex lipids called, the *eicosanoids* which I lecture in BCH 413 – Advanced Lipid Biochemistry to 400L undergraduate students, sessionally).

In vivo experiments recently conducted on rats, rabbits and man in Spain, Portugal, Netherlands, South Australia (Adelaide), Italy, Germany at various Institutes for pharmaceutical Sciences, Science of Nutrition - Dietetics and National Centre for Food Quality and Risk Assessment have demonstrated that after oral administration tyrosol, resveratrol, especially hydroxytyrosol, mono-acetylated tyrosol and diacetylated resveratrol are bioavailable in proportions sufficient to give biological effects such as in food or pharmaceutical formulations, and in the inhibition of platelet–activating-factor (PAF) in cardiovascular diseases. In olive oil solution, and aqueous solution of tyrosol and hydroxytyrosol have bioavailability estimates of 98-99% and 71-75% respectively. Unlike Vitamin E (an antioxidant) which is purely lipophilic, these compounds have amphiphilic characteristics. Hence, hydroxytyrosol will readily cross cell membranes and provide protection in both the cytosol and in membranes, including the water-lipid interface (Rietjens *et al*, 2006, Tuck *et al,* 2001, Gutierrez *et al,* 2001). The bioavailability of tyrosol from virgin olive oil has been demonstrated also, in human by Covas *et al* (2003). Bioavailability is determined quantitatively as fraction of compound and/or its transformed products excreted in urine relative to the total amount taken, or determination of changes in plasma concentrations at different times after administration, through radiolabeling techniques.

Since biological activities of phenolics include modulation of enzymes that regulate functions, *ellagitannins,* agrimonilin, pedunculagin, and genistein exhibit remarkable inhibitory activity against human neutrophil elastase (HNE) which is a serine protease in inflamed tissue as well as in psoriatic lesions (Hrenn *et al,* 2006). The presence of *ortho*-dihydroxy group and specific lipophilic shapes are prerequisites for an inhibitory activity.

Phenolic compounds have been isolated from several Asian traditional medicines as bioactive principles and assayed for their biological activities in respect of enzyme inhibition, anti-tumor promotion, and inhibitory activity of superoxide production (Nishibe, 1994). Similarly, the biological activity of hexylresorcinol, an alkyl dihydric phenols as an anthelmintic (Alexander and Trim, 1946), and synthesized derivatives of anacardic acid, an alkylphenolic acids from Cashew nut shell as anti-tumor agents have been reported (Logrado *et al,* 2005).

In summary, functions of several phenolic compounds are structure-dependent. We shall address the issue of chemical synthesis of similar alkylphenols later in my presentation of the doctoral research efforts.

THE UNDERGRADUATE PROJECT

Due to the ubiquitous distribution of phytochemicals in nature, tannins particularly are found in almost every plant organ and are assumed to be present in every cell especially in dead or drying cells, as secondary metabolites. Their production and accumulation is one of the major mechanisms by which plants defend themselves against herbivory, and attacks by invertebrate pests such as slugs, snails and insects, and microbial pathogens (Winks & Schimmer, 1999, Adams *et al*, 1991). Flavonoids constitute about 50% of all known phenolic compounds (Harbone, 1988). Flavonoid phytoalexins, for instance, offer resistance against fungal infection in sorghum (Wong, 1976, Shih *et al,* 2006).

The mould, *Aspergillus flavus,* is a well known environmental contaminant of agricultural foodstuffs, especially cereal grains. It induces food-poisoning in man and animals on ingestion of such infested foodstuff, through the production of a secondary metabolite, aflatoxin. The level of resistance of guinea corn (sorghum) to invasion by the mould was the subject of investigation in my undergraduate project (Arogba, 1979). The tannin content of 3 long and 4 short-season varieties of sorghum (B.E.S Ex-Bauchi, Farafara, Short Kaura; CK-60, Is-5790, Is-9289, and Serena) were to be correlated with the aflatoxin (B_1,B_2, G_1 and G_2) concentration. Results showed that the more colored or pigmented a variety, the higher the tannin content; tannin was of the magnitude 4 to 36 gm/kg sample and aflatoxin, 0.02 to 200 micrograms/kg sample. Aflatoxin B_2 was the predominant type but the total aflatoxin content and tannin content correlated positively and were higher in the short season varieties. It was deduced that fungal invasion of sorghum induced and enhanced production of secondary metabolites (aflatoxin, tannins and isoflavonoid phytoalexins inclusive). Indeed, the successful isolation of chloroflavonin and flavonin with known antimicrobial activity from another species of *Aspergillus* (*A. candidus*) had long been reported by Bird and Marshall (1969), and Marchelli and Vining (1973). I owe the knowledge and skill acquired at this level to my supervisor in Biochemistry and mentor of knowned academic standing, Professor GH Ogbadu.

THE POSTGRADUATE RESEARCH

Mr. Vice Chancellor sir, Ladies and Gentlemen, I have just highlighted how plants produce secondary metabolites of high astringency to enable them resist attacks from microorganisms, invertebrate pests and even deter forage digestibility in mammals. This self-preservative mechanism underlies the scientific knowledge in application today for the purpose of *food preservation* in order to extend shelf-life. For this same reason, man also needs to select and consume foodstuffs and processed foods safe enough not to cause cellular and tissue damage. Nutritional insults lead to disorders and diseases that reduce the longevity of man on earth.

a) **Exploratory study of nutshells**:

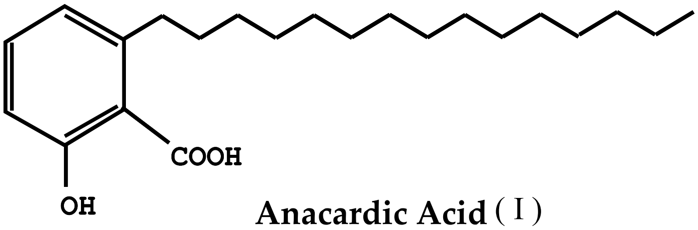
I should state at this juncture sir, that my interest for the second degree in the area of food Quality Control first arose during my primary posting, in the NYSC period of 1979-1980, to Nigerian Breweries, Aba. The Masters programme at the University of Reading, Reading, England exposed me more to statistical and chemical techniques in Food Analysis. The project on monitoring change in acidity of fermenting red and white grape juices was a re-assessment of wine quality. Grape (*Vitis vinifera*) extracts and wine are popular dietary supplements, and serve as sources of beneficial phytochemicals. In content, there are a large number of phenolic species including flavonoids (e.g quercetin, rutin), phenolic acids (gallic, caffeic, cinnamic acids), polyhydrophenols, and stilbenes (resveratrol). The red grape colour results from anthocyanidins, formed from proanthocyanidins.

The Doctoral programme at Brunel (The University of West London), Uxbridge, UK employed in addition, Instrumental techniques to explore for natural *phenolic constituent*s of the shells of various fruit-nuts, and thereafter got indulged in Chemical Synthesis and Analysis of several phenolic compounds with potential *antioxidant* characteristics.

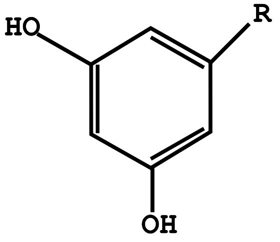
Providing more insight, my legendary supervisor in Chemistry and mentor at the doctoral level, Dr. JHP Tyman had explored and published several journal articles on Cashew nut-shell (*Anacardium occidentale*) and Natural Products Chemistry for over two decades before my studentship began with him.

On average, the raw cashew nut is composed of 74% shell, 24% kernel, and 2% testa. The liquid in the nut-shell of this tropical fruit (CNSL) contains *phenolic lipids,* namely, anacardic acid (6-alkylsalicylic acid, 65%), cardol (5-alkyl resorcinol, 12%), 2-methyl cardol (2-methyl-5-alkylresrcinol, 2%), cardanol (3-alkylphenol, 1%) and polymeric materials (20%) (Fig3).

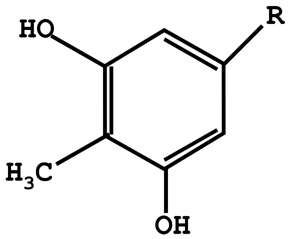
Fig 3: Components of Cashew Nut-Shell Liquid (CNSL)



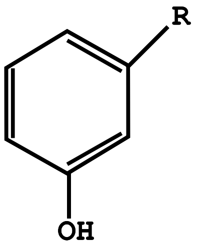
Anacardic Acid (I)



Cardol
5-alkyl resorcinol (II)



2- methyl Cardol (III)



Cardanol
3-alkyl phenol (IV)

In the above, when R = $C_{15}H_{31}$

the four corresponding compounds are (15:0) phenols,

I = 6-pentadecylsalicylic acid
II = 5-pentadecyl resorcinol
III = 2-methyl-5-pentadecyl resorcinol
IV = 3-pentadecyl phenol

when R = $C_{15}H_{29}$

the compounds are the (15:1) series.

I = 6-[8'(Z)-pentadecenyl] salicylic acid
II = 5-[8'(Z)-pentadecenyl] resorcinol
III = 2-methyl-[8'(Z)-pentadecenyl] resorcinol
IV = 3-[8'(Z)-pentadecenyl] phenol;

when R = $C_{15}H_{27}$

the compounds are dienoid (or 15:2) in type,

I = 6-[8'(Z), 11'(Z)-pentadecadienyl] salicylic acid
II = 5-[8'(Z),11'(Z)-pentadecadienyl] resorcinol
III = 2-methyl-[8'(Z),11'(Z)-pentadecadienyl] resorcinol
IV = 3-[8'(Z),11'(Z)-pentadecadienyl phenol;

when R = $C_{15}H_{25}$

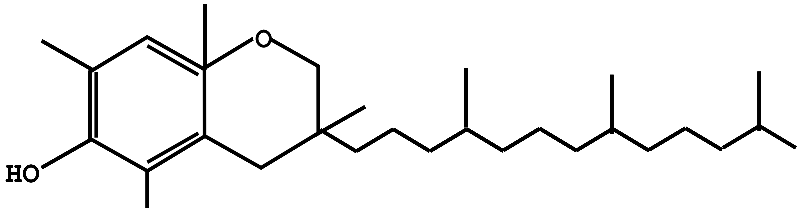
the series is (15:3) and comprises,

I = 6-[8'(Z),11'(Z),14'-pentadecatrienyl] salicylic acid
II = 5-[8'(Z),11'(Z),14-pentadecatrienyl]] resorcinol
III = 2-methyl-[8'(Z),11'(Z)14-pentadecatrienyl] resorcinol
IV = 3-[8'(Z),11'(Z),14'-pentadecatrienyl phenol.

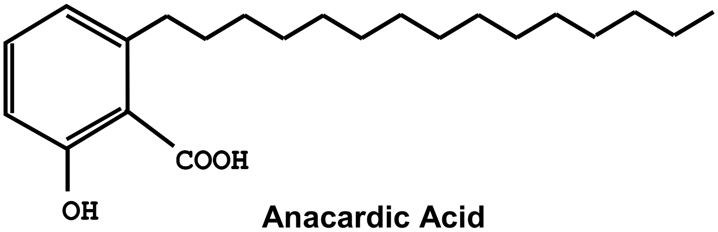
Each of the phenolic lipid types has a C-15 alkyl chain existing in the forms: saturated, monoene, diene, and triene constituents. The traditional names of cardanol, cardol etc are still reservably used in literature but the systematic names are preferred to reduce ambiguity in differentiating positions of unsaturation in the various constituents and sources. Structural analysis of the unsaturation were possible by means of oxidative degradation, fractional crystallization, argentation thin-layer chromatography, column chromatography and, NMR and IR spectroscopy. Thus, positions and geometric configurations were known (Tyman and Morris, 1967). More recently, both qualitative and quantitative analyses were conducted readily on hydrogenated and methylated products using techniques of gas-liquid chromatography (glc) and mass spectrometry.

Just as fatty acids commonly characterize lipids, *phenolic lipids* are fatty-like compounds having a phenolic ring instead of carboxyl as the polar group. Structural similarity in the methylenic chain exists between fatty acids and phenolic lipids. The latter bear one or two –OH groups with or without an additional carboxyl group in the ring. There are two types of phenolic lipids; those with 'isoprenoid' side-chain e.g vitamin E (the tocopherols), and the 'unbranched' e.g anacardic acid. Respectively, they are thought to be biogenetically derived through the 'malonate' and 'polyketide' pathways (Tyman, 1979) (Fig 4).

Fig 4: *alpha*-tocopherol and Anacardic acid



α-tocopherol (Vitamin E)



Anacardic Acid

As part of my contribution to knowledge, gas chromatography-mass fragmentation (GC-MS) study of the 20% polymeric residue from the CNSL revealed the presence of tocopherol and tocopherol-like substances with potential antioxidant activity (Arogba, 1987, Arogba, 1998). The two phenolic lipid types were thus seen to co-exist in the same natural source.

Phenolic lipids are widespread in the plant kingdom and bacterial sources e.g. urushiol in the *Rhus* species, microphyllinic acid in *lichens*, and 2,5-dialkylresorcinol in *Bacterium pseudomonas.* By classification, six plant families are known to contain phenolic lipids, namely, *Anacardaceae, Compositae, Gymnospermae, Lichens, Protaceae,* and *Triticum.* Cashew nut is a member of the *Anacardiaceae* family. I extended the search for phenolic lipids to nutshells of other members, namely pistachio, 'kaffir plum', *Spondias mombin* (Hog plum), and mango, and even beyond the *Anacardiaceae* family to shells of almond nut (*Rosaceae*), brazilnut (*Lecythidaceae*), hazelnut (filbert, *Betulaceae*), pecan, and walnut (*Juglandaceae*). Results showed that these shells contained not more than 3% phenolic lipids in contrast to 20% in cashew nut-shell, which was suggestive of the degree of porosity or ‘woodiness’ of the shells. Phenolic acids were absent in the non-*Anacardiaceae* family but present in cashew, pistachio and ‘kaffir plum’ shell liquids of the *Anacardiaceae* members. However, mass spectral studies ***also*** showed the striking presence of tocopherol and tocopherol-like substances in the crude shell extracts of all these nuts, on methylation (Table 3).

Table 3a: Accurate mass measurement of purified methyl anacardates (Arogba, 1998)

Shell-type	Side chain	Formula	m/e Required (Found)	Re-normalized % intensity*	% relative natural abundance**
			362.2821		
Cashew			(.2831)	1.23	3.69
Pistacio	$C_{15}H_{31}$	$C_{23}H_{38}O_3$	(.2799)	11.90	43.94
Kaffir plum			(.2804)	14.94	34.09
			360.2662		
Cashew			(.2665)	12.72	38.17
Pistacio	$C_{15}H_{29}$	$C_{23}H_{36}O_3$	(.2637)	5.47	20.20
Kaffir plum			(.2648)	28.88	65.92
Cashew	$C_{15}H_{27}$	$C_{23}H_{34}O_3$	358.2508 (.2549)	5.50	16.50
Cashew	$C_{15}H_{25}$	$C_{23}H_{32}O_3$	356.2451 (.2477)	13.88	41.64
Pistacio	$C_{13}H_{27}$	$C_{21}H_{34}O_3$	334.2508 (.2483)	9.71	35.86

*Re-normalized % intensity was calculated from relative heights of molecula ion and base peak (m/e 166).
**% relative natural abundance of homologues in a shell-type total to 100%.

Table 3b: Mass spectral examination of crude nut-shell extracts (Arogba, 1998)

Shell extract	Formula			m/e Found (Required)	Inference
	C	H	O		
Cashew, almond,	29	50	2	430.3806 (.3811)	Tocopherol (T)
pecan, hazelnut.	29	48	2	428.3649 (.3654)	
Cashew, kaffir	29	51	1	415.3933 (.3940)	T-1 (Oxygen atom)
plum, almond,	29	50	1	414.3857 (.3861)	
pecan, hazelnut.	29	48	1	412.3727 (.3705)	
Cashew. kaffir	29	50	0	398.3876 (.3912)	T-2 (Oxygen atoms)
plum, almond,	29	49	0	397.3810 (.3834)	
pistacio, walnut.	29	48	0	396.3751 (.3756)	

At that stage, it was inferred that the presence of phenolic lipids in nut-shells might be a natural protective device for the embryo, the kernel. This finding is of strong significance to this lecture as we address the issue of prolongation of life.

The exploratory study indicated that C-15 anacardic acid was predominant in pistachio nut-shell liquid, similar to the well-known case of CNSL, but only as (15:1)-monoene. My novel finding was in kaffir plum which contained saturated (15:0)- anacardic acid and its (15:1)-monoene in respect of composition, and in a ratio of 1:2.

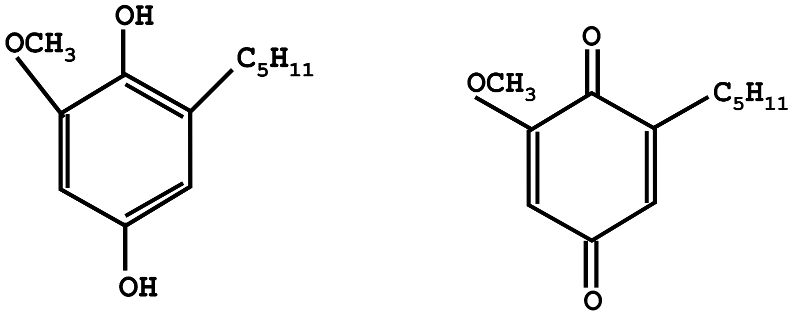
b) **Synthesis of alkylphenols and hydroquinones**:

The limited abundance of phenolic lipids in the nut-shells, which otherwise would have served as starting materials on purification, for the synthesis of antioxidants posed a research challenge. This was overcome through chemical synthesis of homologues of similar monohydric and dihydric phenolic lipids. Homologues of 3-alkylphenol and the intermediate secondary alcohols, and 6-n-alkylsalicylic acid synthesized were purified and characterized by elemental analysis, spectroscopy (IR, proton-NMR, and mass/charge ratio), tlc, and their glc behaviour. Details of the methodologies employed are available is the PhD Thesis (Arogba, 1987) and as published (Arogba, 1988*a,b*).

Again, isomeric forms of alkyl-quinones and hydroquinones, akin to some natural hydroquinones such as pirolagenin in *Pirola japonica* leaves flavoglaucin (*Pyrolaceae*), auroglaucin in *Aspergillus* mould species, and miconidin in *miconia* root-bark (*Melastomaceae*) were synthesized and characterized by the techniques mentioned earlier. Differences in melting points of the isomers were additional useful diagnostic indices. Synthetic procedures that ensured regiospecificity of functional groups in the endproducts were also adopted (Arogba, 1999*a,*Tyman and Arogba, 1999).

VC sir, Ladies and Gentlemen, the significance and impact of the results of these hydroquinone synthesis cannot be overemphasized. Hydroquinones have long-standing history in Science and Technology particularly in chemical, cosmetic, pharmaceutical and food industries. At varied levels of applications, hydroquinones act as bleach, skin toner or as antioxidants. *Primin* is a quinone extractable from primrose leaves (*Primula obconica)* and its reduced form, the hydroquinone, is called *miconidin*. (Fig. 5) with antimicrobial property (Bloch and Karrer, 1928, Schildknecht and Schmidt, 1967, Marini–Bettolo *et al,* 1971, Gonclaves *da* Lima *et al,* 1972.

Fig 5: Miconidin and primin



Miconidin

primin

These early researchers had controversially labelled primin and had not synthesized miconidin either, before my doctoral programme began. Primin was only extracted from the primrose plant. I state categorically that for the first time, our methodology in *chemical synthesis* and analysis identified an intermediate Quinhydrone (Arogba, 2000) from which we confirmed the structures of primin and micondin. The VC sir, Ladies and Gentlemen, this modest achievement was published in a highly–rated United States of America Journal (Organic Preparations & Procedures International) (Arogba, 1991*a*), and which had earned the Inaugural Lecturer a memorable landmark in the United States of America Patent No 6951856 acquired by Boss *et al* (2005). Their invention, thereafter, has been cited by several researchers. Primin was the active ingredient in the processes for the preparation of related pharmaceutical compounds, compositions, and applications as endothein receptor antagonists.

I have followed with interest in literature and on the Internet to note that no other synthetic work has been conducted to disprove the structural elucidation of primin and miconidin. Rather, researchers have been correctly labelling miconidin as 2-methoxy-6*n*-pentyl-1,4-hydroquinone, and primin as 2-methoxy-6*n*-pentyl-1,4-benzoquinone.

Furthermore, I note that since my publication in 1991, only Gupta (2005) acquired the USA patent to use isosteric forms of micondin *extracted* from the *Primula* plant in a formulation as skin-whitening toner.

Records of comparative antioxidant efficiency or activity of known phenolic compounds used in food systems abound in literature (Bickoff, 1951, Miller and Quackenbush, 1957) without recourse to the influence of structural configuration. Recent advances in the knowledge of biochemistry, particularly food chemistry has enabled plausible interpretations to be cautiously offered (Table 4).

Table 4: Antioxidant efficiency of some phenolic compounds for carotene in mineral oil solution at 75°C (Bickoff, 1951).

Phenolics	***Stability Value$_{20}$*** **(hours)**
Gallic acid (3,4,5-trihydroxybenzoic acid)	7
p-Hydroquinone	8
Catechol	84
3-BHA (3-*t*-butyl-4-hydroxyanisole)	130
Pyrogallol (1,2,3-trihydroxybenzene)	155
alpha-tocopherol (Vitamin E)	160
Ethylgallate	165
4-laurylcatechol	166
NDGA (nordihydroguaiaretic acid)	208
3-laurylcatechol	226

I did as a long essay in partial fulfilment for the award of the PhD and in a publication (Arogba, 1991*b*) while stating that formation of free radicals was the favoured mechanism of phenolic antioxidancy. Antioxidancy was interpreted in the light of the effects of position and nature of substituents on the phenolic ring and the reactivity of the resultant phenoxy radical with active autoxidation species such as alkyl and alkylperoxy radicals. Generally, steric requirements, electro-affinity, and the location of substituents on the aryl ring relative to the phenolic group were observed to strongly affect antioxidant activity.

c) **Synthesis of bis-phenols**:

The appreciation of this understanding of Nature on how reknowned phenolic antioxidants exhibited potency through structural configuration, led me on exploratory experiments to effect for the first time, regiospecific chemical synthesis of guaiaretic acid (GA) and nor-dihydroguaiaretic acid (NDGA). These are naturally-occurring bis-phenols that are comparatively little known as against the synthetic ones. NDGA and GA are obtainable from *Guaiacum officinalis and Larrea divaricata* plants respectively.

By the 'sequence rule' or the R,S convention, and using the Fisher projection for compounds with more than one asymmetric carbon atom, NDGA could exist in only four stereochemical forms namely, the inactive racemic mixture, the inactive diastereomeric mixture (meso), and the active (+ or -) forms. For an additional reason of geometric isomerism in GA, there would be six forms (Figs. 6 and 7). From synthon analysis, eight (8) reaction schemes were drawn to effect their synthesis (Arogba, 1987). Previous researchers (Schrecker and Hartwell, 1955, Schrecker, 1957, King and Wilson, 1964) attempted analysis of natural extracts of lignans from *Guaiacum officinalis* without clear elucidation of the stereochemical forms obtained. Here again, available commercial samples obtained similarly, and used as reference materials had limited value in the identification of some of my synthetic intermediates and products.

A conventional synthetic methodology particularly, the Wittig reaction was a failure until it was modified. Key intermediates of guaiaretic acid were successively and successfully obtained by a Reformatsky-based procedure. The organolithium procedure, and the McMurry-based reductive coupling gave analytical yields of NDGA, NDGA dimethyl ether, and NDGA tetramethyl ether. Charaterization by melting point determinations, and comparative molecular ions of the 'theoretical' with the observed value based on accurate mass spectroscopy analysis were very useful diagnostic tools. In closing the research, three (3) and five (5) suggestions were proposed for future work on GA and NDGA respectively (Arogba, 1987).

Fig 6: Absolute Configurations of NDGA (dimethyl ether)

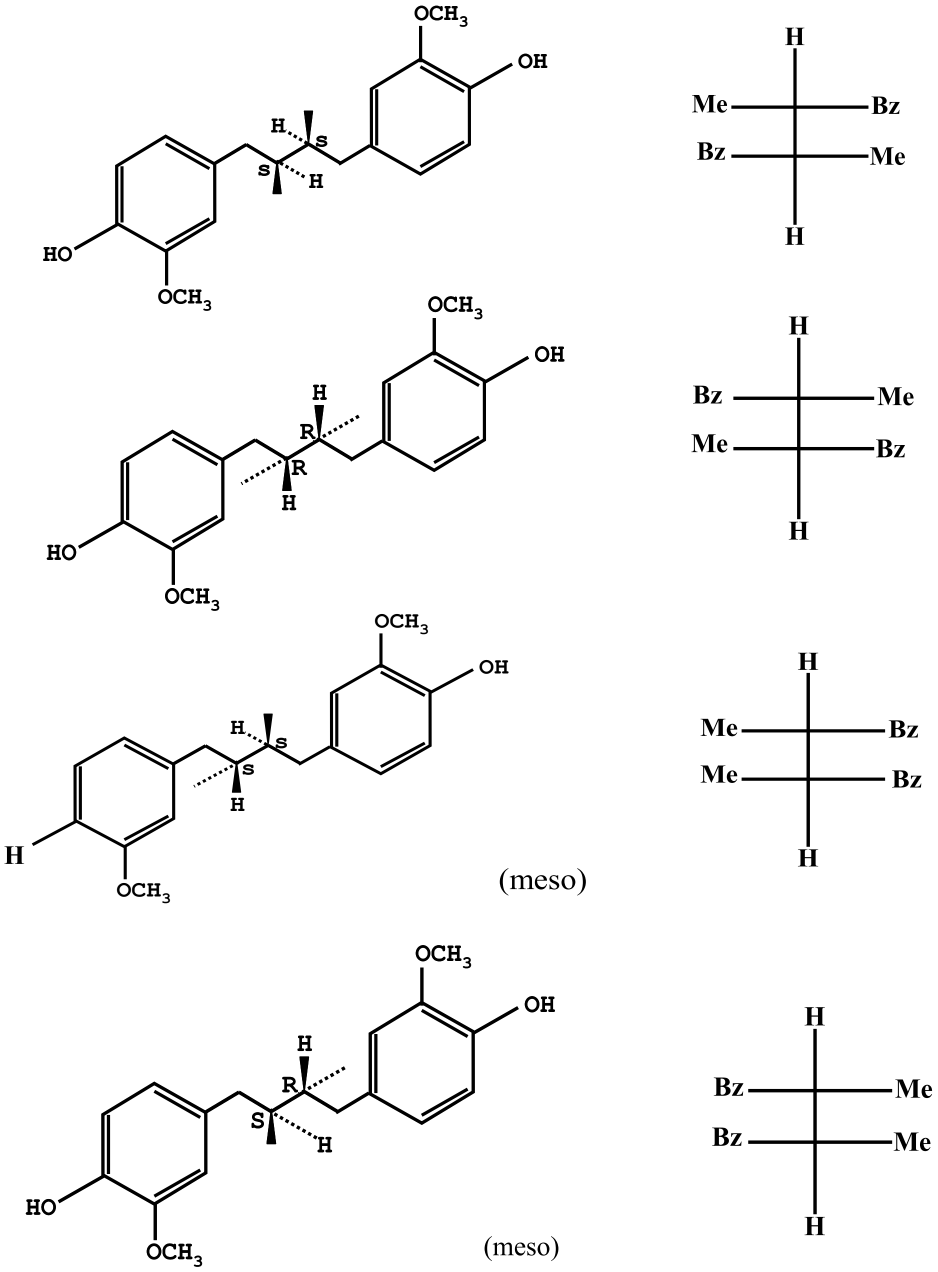
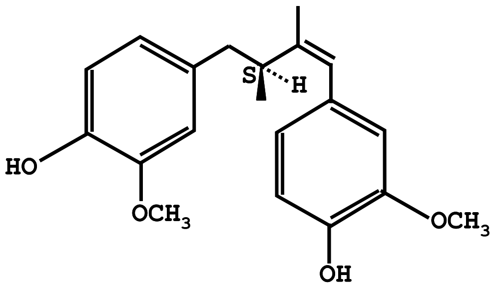


Fig 7: Geometric and Stereochemical Forms of Guaiaretic acid



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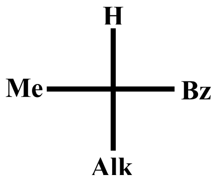

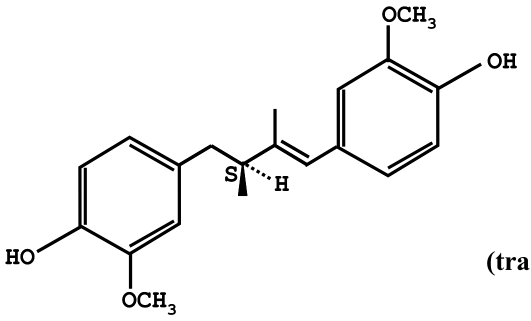

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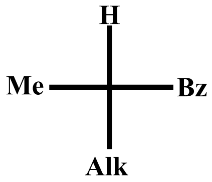

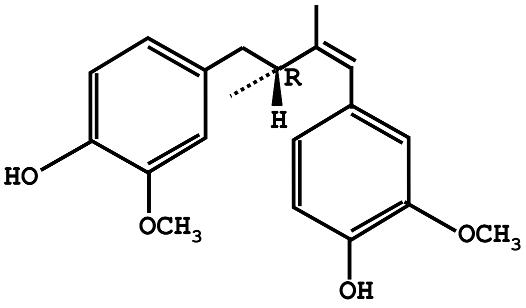

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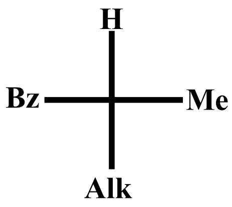

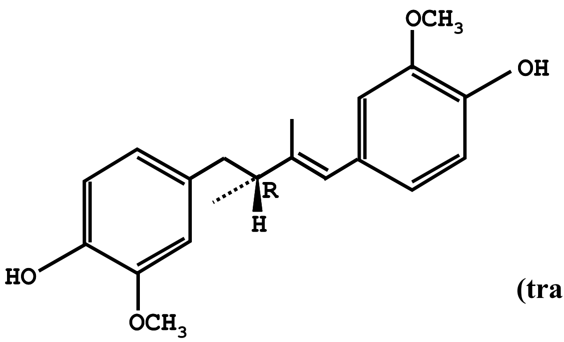

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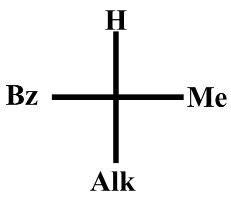

POST-DOCTORAL RESEARCHES

The VC, Sir, Ladies and Gentlemen, I have expressed academic interests through attendance of several professional conferences and journal article publications on food and potential food items since leaving the formal floor of the main Laboratory of Brunel (The University of West London) at Uxbridge, UK. These research interests ranged from handling:-

1. Nigerian traditional condiments for improved shell-life and level of acceptability by consumers (Arogba *et al,* 1995, Arogba, unpublished).
2. Velvet tamarind (*Dialium guineense*) fruit (Ayigele – Igala) for compositional analysis and a study on shelf-stability and improved tartness of the fruit drink through partial deacidification (Arogba *et al,* 1994, Arogba and Ajiboro, 1996). The pulp contains 0.42% (w/w) tannin as catechin equivalent.
3. Cowpea (*Vigna unguiculata*) seed to study effects of post-harvest handling on its properties (Abu *et al,* 1999) which on given treatments, improved nutrient retention (Arogba *et al,* 1996), storability (Arogba *et al,* 1999), and by contrast analysis technique understood better the "hard-to-cook" phenomenon (Arogba and Abu, 1999). Recently harvested beans of *Phaseolus vulgaris* have the highest amount of condensed tannins in the seed coat as 0.22g (+)catechin equivalents per gram and dropping to 0.04g per gram after 2 years of storage (Aparicio-Fernandex *et al*, 2005). The raw seeds with antimutagenic activity contain 0.14g tannins per gram, 70% of which is extractable by thermal treatment.

4. Basil (*Ocimum gratissimum*) leaf (Anyeba - Igala) for its composition and physicochemical properties (Arogba and Akisanya, 1999).

5. Kolanut (*Cola nitida*), bitter kola (*Garcinia kola*), and cashew (*Anacardium occidentale*) kernels for compositional analysis, moisture adsorption isotherm, physical and functional properties (Arogba, 1999*b,c*).

6. Grape and apple fruits: monitoring the acidity of the fermenting musts (Arogba, 2001*a*).

7. Black plum (*Vitex doniana*) (Ejiji – Igala) syrup and syrup/starch mixtures: high temperature drying characteristics and their moisture adsorption isotherms (Abu and Arogba, 2001).

8. Natural distribution of sodium and potassium ions in selected fruits, roots and tuber crops grown in Igalaland (Arogba *et al,* 2006) and the salt consumption pattern of foods prepared there-of by the Igala people (Arogba, 2006).

9. Wild mango (*Irvingia gabonensis var. gabonensis*) kernel (with or without testa) popularly known as "ogbono" seed: properties of polyphenol oxidase (Arogba and Ezeonye, 1999), browning index, physicochemical and functional properties and, effects of brining and pH (Arogba, 2001*b,c*). At this point, it is relevant to mention that the intact kernel with testa has 2% (w/w) tannin content, expressed as catechin equivalent. The testa alone contributes a significant 11% by proportion. Free polyphenols (as pyrogallol equivalent) constitute about 0.55 to 0.61% (w/w) of the kernel. The browning process, as the colour of the testa shows, is catalysed by enzymes (polyphenol oxidases) (Arogba, 1998*b*) and air. In the experiments conducted, results showed that the

presence of testa on the intact kernel retarded enzymic browning by a factor of (2.3). This knowledge allows for better handling and application of the seed in food systems.

10. Conventional mango (*Mangifera indica*) kernel: This formed the niche of my research interests since my Post-Doctoral years, spanning two decades. Research achievements here-in facilitated the identification of the topic of this Inaugural Lecture!

THE MANGO KERNEL

As I present the final segment of my short lecture, I recall that my earliest survey led to the report (Arogba, 1997) where-in the dimensions and kernel pH (or acidity) of several Nigerian mango seeds (shell + kernel) were measured and correlated. The kernels were acidic with pH between 4.8 and 5.0. The dimensions (that is, length and breadth at half-length) showed higher linear correlation coefficient of +0.95 in the unshelled seeds but a lower correlation (+0.65) with the fresh kernels. As against pH, either of the dimensions showed positive correlation in the unshelled seeds but negative correlation was recorded with the fresh kernels (Arogba, 1997). These characteristics are typical of Nigerian mango kernels.

The Ikanekpo variety of mango kernel has 45g tannin per kg sample. This is high but extractable by a combined soaking and thermal treatment. The calculated lethal dose (LD_{50}) per 70kg body weight was 0.78kg for the raw kernel and 1.5kg for the processed flour (Arogba, 1997). Processing the kernel therefore, has implicit beneficial effects in food systems for human consumption or for use as animal feed. The processed flour was rated above an average score by potential consumers in several sensory evaluation experiments when used in traditional Nigerian flour-based foods such a ‘Oje-akpa’ (Igala), ‘Agidi’ (Yoruba) or ’ijobu’ (Igala), ‘Akamu’ (pap – English) and ‘moin-moin’ (Yoruba) or ‘ile-le’ (Igala). Conventional snacks such as fish-pie, and biscuits were also experimented upon as model food systems (Arogba, 1999*d*, Arogba, 2002).

The initial concept was to convert the much discarded waste to human food and animal feed. Recently indeed, graduating project students in Biochemistry of 2005/2006 session whom I supervised, successfully conducted feeding experiments to albino rats in order to evaluate the protein quality of the processed mango flour against the reference use of a commercial formulation. The protein content of 7% (w/w) and the relative protein quality in respect of the available essential amino acids (Arogba, 1997) plausibly explained its higher biological value rating.

The mango kernel research project was promoted by the NBTE (National Board for Technical Education) Awards I won twice after successful defence of my proposals in 1993, and 1997 respectively. Highlights of the first work were reflected in their Newsletter (Arogba, 1994). Today, sufficient literature on mango kernel has been made available as my contributions to knowledge in respect of

- physical characteristics
- chemical composition
- protein constituents
- lipid constituents (Arogba, 1997)
- treatment techniques for improved utilization (Arogba, 1999*d*)
- functional properties and product formulation (Arogba, 1997, Arogba, 1999*c*)

- enzyme activity related to phenolic substances
- (polyphenol oxidase, catalase, peroxidase) (Arogba *et al*, 1998, Arogba, 1999*d*)
- water activity studies of kernel, processed flour, or products to prolong shelf-life, that is, moisture adsorption studies and effect of temperature (Arogba, 2001*d*, Arogba, 2002)
- compositional analysis of the phenolic substances (Table 5) (Arogba, 1999*e*). The study showed that mango kernel contained hydrolysable tannin which was about 75% of the total tannin content. The principal constituents were gallotannins (namely gallic acid, and tannic acid), and epicatechin. The condensed tannins were ellagic acid, gallocatechin, *n*-butyl-cyanidin. All the studies conducted indicated that a simple and effective process can be carried out at the cottage industry level to reduce toxicity by water–blanching at 90^{0}C for 5 min to leach the tannic substances and inactivate the polyphenol oxidase.

TABLE 5: Content of individual polyphenols in dry mango kernel meal (Arogba, 1999)

	Mango kernel meal	
Constituents	% of total tannins	% (w/w) of dry matter
Before hydrolysis		
Total tannin-related substances	100.0	6.37
Gallotannin	74.5	4.75
Tannic acid	46.6	2.97
Gallic acid	27.9	1.78
Condensed tannin-related polyphenols	25.5	1.62
Epicatechin	2.7	0.17
After acid hydrolysis		
Condensed tannin-related polyphenols	22.8	1.45
Ellagic acid	11.0	0.70
Gallocatechin	6.9	0.44
n-butyl-cyanidin	4.9	0.31

CONCLUSION

In this lecture I have illustrated what phenolic compounds are, both descriptively and by structural representations. They vary widely in composition as they are ubiquitous in nature. Several alkyl monohydric and dihydric phenols, as well as bis-phenols were synthesized during a postgraduate programme. Their functions and relative potency are to greater extent structure-dependent. In the barks, trunks, and leaves of plants are phytoalexins for instance, to fight-off attack from herbivores and the invertebrate pests. In pigmented fruits are tannins and the flavonoid compounds to prophylactically combat cardiovascular diseases and other nutritional disorders. In the shells and kernels of fruit-nuts, e.g cashew nutshell, intact bush mango kernel, and the conventional mango kernel are tannins, phenolic lipids and tocopherol-like substances that possess preservative properties, or better still, exhibit antioxidant activity in these natural habitats.

This class of nature's chemicals by deduction serves to extend the shelf-lives of these plant sources, propagate their species, or from pharmaceutical viewpoint, exist to serve as remedies for ailments in man and animals. When viewed from nutritional standpoint, the presence of these phenolics at certain levels of their natural abundance in already edible foodstuffs as mentioned serve as antioxidants or when harnessed from other sources, processed and utilized at controlled concentrations, these non-edibles become convertible to edibles for man and animals alike to enable life-span to be prolonged.

We are left without doubt to view nature as self-preserving through the contributions of phenolics as chemical weapons of life !

TRIBUTES AND ACKNOWLEDGEMENT

It is extremely difficult to mention all names that influenced my academic achievements positively except to highlight the following personalities:

1. God Almighty for His Will and Mercies shown in my academic successes!
2. My late parents Mr. Joseph Egene Arogba (father) and Mrs. Serah Asana Arogba (*nee* Haruna) (mother) for their wisdom to educate, sponsor, and encourage me. May your Souls remain rested in the Perfect Peace of Jesus Christ;
3. My teachers and lecturers for imparting relevant knowledge and skills all through 1963 to 1987, particularly my supervisors and mentors namely, Professor Godwin H. Ogbadu then at Ahmadu Bello University, Zaria, and Dr. John H.P. Tyman then at Brunel (The University of West London), Uxbridge, Middlesex, UK,
4. Scholarship sponsors namely:
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   b) Benue State Government for my Undergraduate programme at Ahmadu Bello University, Zaria, 1976 – 1979;
   c) *Ditto:* for my Masters programme at the University of Reading, Reading, Berkshire, UK 1981-1982, and the Federal Government of Nigeria: though not used simultaneously for the same programme;
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6. The Federal Polytechnic, Idah for the enabling environment to conduct and publish virtually all my Post-Doctoral Journal articles within Nigeria and Internationally;

7. Dr. Yusufu W. Awodi, substantive Rector, then at the Federal Polytechnic Idah, during whose period academic activity was made more fertile while I lectured and published;

8. Colleagues at the Department of Food Science & Technology, Federal Polytechnic Idah during my Doctoral and Post-Doctoral years spanning 19 years (1981 – 2000);

9. Dr. & Prof. (Mrs.) Abel S. Agbaji for the receptions at the National Research Institute for Chemical Technology (NARICT), Zaria, previously Leather Research Institute, that facilitated my Mango Kernel projects;

10. Heads and Staff of Biochemistry Department, A.B.U., Zaria for the laboratory space to conduct some experiments during my active Post-Doctoral research years;

11 Management staff and the Governing Council of Kogi State University, Anyigba at 2000 for elevating me into the Professorial Chair after due process of external assessment;

12. Colleagues and students of the Department of Biochemistry and the Faculty of Natural Sciences, Kogi State University, Anyigba since 2000 till date for the co-operation at work;
13. The Vice Chancellor, Professor Francis S. Idachaba for an overall exemplary leadership, and to particularly at the consent of Senate commence with me, the Inaugural Lecture Series of K.S.U, Anyigba; and
14. Finally to my home with the lovely and understanding wife, Mrs. Justina Aishatu Arogba (*nee* Onuh) and our happy children namely, Iye Serah Arogba, Esther Ufedo Arogba, Ojonugwa Juliet Arogba, and Victoria Aladi Arogba.

To God be the Glory, Amen !

Mr. Vice Chancellor, and distinguished audience, I thank you all for listening !

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