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ARTICLES

**Anthology of historical development and some research progress
glimpses on phytochemical antioxidants phenomenon**
Daramola, B.

Review

Anthology of historical development and some research progress glimpses on phytochemical antioxidants phenomenon

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The inevitability of applications of antioxidants in protection of human and preservation of dormant living systems such as food, drugs, cosmetics and other allied chemicals against internal and external stresses have made antioxidants an area of active research world over. Study impact of antioxidant research has advanced from topic level to subject level as revealed by the volume of antioxidants research publications. Each cardinal research interest is engaged in scientific exploration with view to gain mechanistic understanding and consequently raise the bar of knowledge in order to maximise exploitation of antioxidants benefits. The cardinal research interest is so diverse and the volume of publications is so enormous and profusely dispersed. Therefore, it is academically worthwhile to pool some of the research developments into a single piece which could offer accessibility to array of study briefs that appeared in many journals which could motivate readership to seek further knowledge on specific area of antioxidant research interest. In this review, glimpses of developments on antioxidant research that transverse research cardinal perspectives such as phytochemical antioxidant genesis, highlights mechanism of action. Modern methods of extraction and evaluation techniques, quantitative structure-activity relationship (QSAR) and rational design strategy for antioxidants were reviewed. The research future of antioxidants of phytochemical origin was projected. These academic collections should motivate readership to seek further knowledge for product research and development of antioxidants of phytochemical origin and natural product analogues.

Key words: Antioxidants, phytochemicals, cardinal research glimpse, lead references.

INTRODUCTION

All organisms or substance whether living or non-living are vulnerable to oxidative process with several consequences. For instance, in a living thing oxidative process result to a number of oxygenated radicals which are cytotoxic. Consequently, this leads to a spectrum of

disease condition such as cancer, cognitive dysfunctions, coronary heart disease and many more (Finkel and Holbrook, 2000; Steinmetz and Potter, 1991) in human beings. In food, oxidative radicals lead to loss of nutritional and sensory quality and consequently loss of

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economic value (Giese, 1996). This undesirable oxidation phenomenon could be prevented using antioxidants.

In term of origin, antioxidant can be natural or synthetic. Synthetic antioxidant are effective in inhibiting peroxidation especially in non-edible products, such as petroleum based chemicals, but are surrounded with league of limitations largely based on health risk concern. However, natural antioxidants are safe and renewable among other advantages hence preferred to synthetic antioxidants for application in foods, drugs and cosmetics. Recalling that oxidative stress occurs in all living systems, nature endowed the systems with a means of preventing lipid peroxidation. The activity of substances that prevent oxidation is antioxidation and the group of the substances is called antioxidants. In plants, this group of substances that prevent oxidative damaged have genealogical linkage to plant chemicals called phytochemicals or secondary metabolites.

Frontier studies have been conducted on the application of antioxidants with view to gain knowledge and exploit the preservation and protection endowment of antioxidants.

This review reports a survey on major classes of frontier researches of antioxidants scattered in many scholarly journals largely from the perspective of phytochemicals or analogues. This review on phytochemical antioxidant research status should update readership on present knowledge and motivate reader to seek further information for phytochemical antioxidant research and product development.

Phytochemical evolution and defence arguments

Phytochemicals also called nutraceuticals, phytochemicals, phytonutrients and sometimes referred to as functional foods possess antioxidants activity, some modify the immune system and others alter enzymes that metabolize drugs in our system. However, this review limits its purview to antioxidants. As implied by the name, phytochemicals are the chemicals produced by plants. These plant chemicals are biologically active but are not considered essential or priority nutrients such as proteins, carbohydrates, fat, minerals and vitamins. Therefore, it mainly contributes health maintenance and therapeutic properties of food to its consumers. That is, play a role of secondary and tertiary functions of food. In the beginning, phytochemicals were first known to be extradites and as such thought to be waste product. No sooner than later it became clear that phytochemical are arsenal of defence mechanism. The following relates the genesis of phytochemicals. When plants first evolved, there was little free oxygen in the atmosphere, as oxygen level increased as a result of plant metabolism (plants take in carbon dioxide and give off oxygen) their environment became polluted, and production of highly reactive oxygen which are cytotoxic follow afterwards. In order to combat the adverse

effect of the reactive species, plant cell mutates to synthesis chemicals referred to as plant chemicals (Figure 1) that can neutralize or absorb the toxic radicals. In addition, the plants are prone to environmental stress such as heat and radiation and biological attack notably bacteria and viruses and pests.

The following evidences signalled that phytochemicals are strictly for protection and defence purpose. First, phytochemicals are localized at the peripheral surfaces, such as bark of plant stem because it is vulnerable to attack because it exposes directly to attacking agents. However, the trunk cortex at the central of the stem, covered by the bark has low amount and concentration of phytochemicals because it is covered by the stem therefore protected from biological and environmental attacks.

Second, flower and immature leaves contain high amount of phytochemicals because they are structurally weak, therefore their presence will afford defence option for the vulnerable plant part. However, it is noted that as the plant leaves and flower grow and mature and structurally strong, the phytochemical content of the named plant parts proportionally decrease. Thirdly, similar explanation holds for root parts and seeds (plant young in embryo). Fourth, plants that survive in the desert and hot regions are known to be rich in phytochemicals. The basis for this is that such desert surviving plants have an efficient mechanism of mopping off radiation (sun) generated radicals that are cytotoxic to the plant.

Fifth, when any part of plant is injured, it quickly respond by secreting extrudates to heal the cut as well as offering protection against probably invading micro and macro organisms.

Ultraviolet (UV) screen

The energy of UV light reaching the earth is sufficient to induce photochemical degradation of many plant components; however, nature equipped terrestrial plants with capability of evolution of barriers to absorb this UV radiation by increasing accumulation of plant chemicals to avert damage. It is apparent that flavonoids such as flavones and flavonols are responsible for UV screen because they are prominent in plant parts such as flower and leaves (Harborne, 1980). Also, phytochemicals such as flavonoids are often concentrated in or around epidermal tissues, where their screening potential will be greatest (Ibrahim et al., 1987; Schmelzer et al., 1988).

Pharmacology of phytochemicals

The aim of the review in this section is not designed to treat pharmacological principles of phytochemicals, but to allude to the relationship between disease condition and

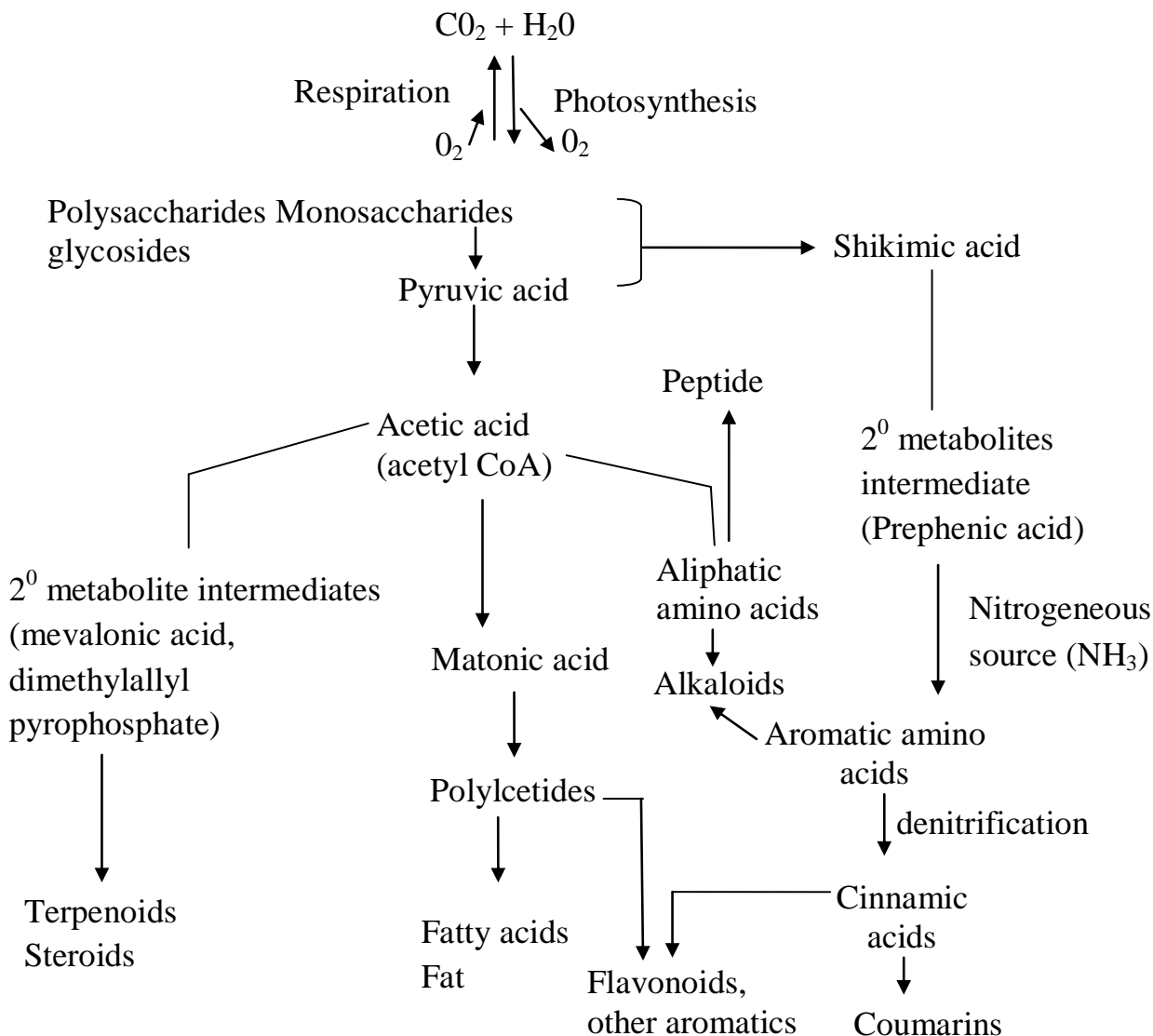


Figure 1. Notable streams of secondary metabolism adapted for academic use from: Essential Inorganic and Organic Pharmaceutical Chemistry. Olaniyi et al., 1998 Shaneson C.I Limited.

antioxidant- pharmacology activities of phytochemicals. For thorough understanding of the mechanism process readers are urge to seek detail information in professional texts.

Gastric ulcer

Simply, the aetiology of gastric ulcer involves increase effect of stress hormones which lead to increased glandular secretion which denatures proteins in plasma membranes and catalyses the hydrolysis of polysaccharide moieties of proteoglycans in the protective mucous coat covering the luminal surface of the stomach and the upper intestine to a perilous extent during prolong stress. Consequently, making the lumen surfaces

to be weak and vulnerable to injury described as gastric ulcer. Phytochemicals such as flavonoids and glycosides have been reported to protect against gastric disorders such as cancer. This activity is accomplished by ability to transfer acyl group to hydroxyl group of cyclooxygenase (Kumar et al., 2011).

Inflammation

Inflammation is the integrated response of many defence systems of the body to the invasion of a foreign body. Inflammation involves action of the complement systems, blood coagulation, humoral and cellular immunity, cytokines, tissue hormones, angiogenesis and repair processes. It is produces free radicals (Miller, 1996).

Phytochemicals such as flavonoids have been found to be prominent inhibitors of cyclooxygenase (COX) and Lipooxygenase (LOX) that affects inflammation. The activity of flavonoids such as quercetin, apigenin, tea catechin in the inhibition of COX and inducible nitric oxide synthase was related to antioxidant activity (Kumar et al., 2011).

Cancerous conditions

Cancerous condition is abnormal growth condition resulting from disturbance in growth metabolism. Cancer cells manifest in uncontrolled proliferation, differentiation, loss of functions, invasiveness and metastasis that differentiate it from normal cells. Cancer undergoes the stage of initial development, and progression by modulation of cellular proliferation, differentiation, apoptosis, angiogenesis and metastasis (Rang et al., 2007). Phytochemicals such as flavonoids have been shown to be highly effective scavengers of most types of oxidizing molecules, notably singlet oxygen and other free radicals that are probably involved in DNA damage and tumour promotion. This mechanism of therapeutic action expresses antioxidative action.

Antimicrobial activity

Phytochemicals of various classes have been used extensively since pre-historic times for treatment of various diseases. Propolis has been used, referred in old testament for its healing properties. Crude and fractionated extracts of tropical seed of bitter kola (*Garcinia kola*) had been reported to exhibit antimicrobial activities against some micro-organisms notably *Candida albicans*, *Escherichia coli*, *Salmonella enteric* that are important in food spoilage and intoxication (Daramola and Adegoke, 2007). Flavonoids and alkaloids are the dominant bio-active components identified in the fractional flavonoids isolated from *Termanalia ballerica* possess antifungal activity against *C. albicans*. And some glycosylated flavonones are effective against *Aspergillus flavus*. A summary of antibacterial, antimicrobial and antiviral activities of flavonoids a class of phytochemical is tabulated and presented (Kumar et al., 2011; Cushnie and Lamb, 2005).

Treatment of thrombosis

Platelet aggregation progressively contributes to atherosclerosis and acute platelet thrombus formation. Activated platelets adhering to vascular endothelium generate lipid peroxides and oxygen free radicals which inhibit endothelial function of prostacyclin and nitric oxide (Middleton et al., 2000).

Etiology and treatment of cardiovascular diseases

Cardiovascular diseases (CVS) are today the principal cause of death in both developing and developed countries. CVS diseases include atherosclerosis, coronary heart disease, arterial hypertension and heart failure. The major reason behind CVS Diseases is oxidative stress. Oxidative stress is a condition of imbalance endogenous oxidants and reactive oxygen/nitrogen species (RONS) with predominance of reactive species. Products of oxidative stress modify low density lipoprotein particles and subsequent induction of inflammation (Kumar et al., 2011).

Endothelial dysfunction associated with increased AMI platelet aggregation. Common consequences of AMI are heart failure and arrhythmias because patients with heart failure and arterial hypertension have been diagnosed with increased production of reactive oxygen species (ROS) (Mladenka et al., 2010). Studies revealed that long-term administration of flavonoids can decrease the incidence of cardiovascular disease and their consequences, since all the disorders can be mitigated by flavonoids. Flavonoids are the most studied phytochemicals in relation to antioxidant activity. All these revealed the capability of antioxidant in management of various diseases conditions.

Antioxidants phenomenon

Although, metabolic process channel the provision of energy for vital cell activities in a living system, however, this process leads to the unavoidable production of oxygen derived free radicals (Mccord, 1994) and harmful toxins such as tobacco smoke and indiscriminate burning of materials such as tyre and ageing related diseases that have been confirmed to be causative agents for myriad of diseases notably cancer, rheumatoid arthritis, heart attack, stroke and Alzheimers disease and others. In addition, free radicals and other oxidants are known to cause oxidative damage to lipids, proteins and nucleic acids. These oxidants are important factors in the development of a number of diseases as itemised above (Ames et al., 1993). Ordinarily, there is a balance between antioxidants and oxidants. When the equilibrium between oxidants and antioxidants defence systems is impaired in favour of the oxidants, the condition is known as oxidative stress (Halliwell and Gutteridge, 1999). Abundant evidences exist that oxidative stress triggers many undesirable processes at cellular, tissue and organism levels, consequently playing a major role in pathogenesis of many human diseases named above and others such as ischemia, atherosclerosis, chronic renal failure and many more. These undesirable consequences of oxidative stress accounts for the justification for application of exogenous antioxidants with view to abort or prevent undesired chemical damage caused by

oxidative stress (Augustin et al., 1997)

Some aspect of biological organelle as targets of endogeneous radicals

The basis of the spectrum of diseases is stemmed from susceptibility of biological molecules to radical attack. For example susceptible lipid molecules present in the biological membrane are most prone to free radical attack (Roy et al., 2013). In addition, DNA and protein-like macro molecules also constitute vulnerable targets for free radical attack. Free radical mutates the DNA and RNA by pairing with electrons in the DNA chains leading to cellular electronic imbalance (Evans and Cooke, 2004). Ageing is often characterized by the accumulation of mitochondrial DNA mutations as well as improper clearance of reactive oxygen species produced by the respiratory chain resulting in early aging. More importantly, when transport proteins (protein critical for rapid homeostatic mechanism) are affected by free radicals, or when inherent antioxidant defences are overwhelmed, toxics occurrence ensues (Salvi et al., 2001). Such occurrences hand-in spectrum of chronic pathological conditions as well as other known fatal degenerative diseases. In terms of electronic transport chain, free radicals are produced inevitably during cellular metabolism such as by electron leakage from the electron transport chain and redox enzymes as well as by some lymphocytes while defending the human system against foreign organism (St Pierre, 2002).

Antioxidants

Antioxidants simply means against oxidation. Their physiological effects have been harnessed for management of public health. Going by the definition of oxidation and reduction for antioxidants then it can be claimed that the mechanism of therapeutic and pharmacological actions of phytochemicals illustrated here-above is in accord with at least one of the oxidoreduction definition of antioxidants. Hence all the pharmacological exhibition of phytochemicals expresses one form of antioxidants or another. The broad spectrum of biological activities within the group and the multiplicity of actions displayed by certain individual members make the flavonoids group one of the most intriguing classes of biologically active compounds and thus these are often termed 'bioflavonoids' (Singla et al., 2001). Flavonoids occur in practically all parts of plants including fruits, vegetables, nuts, seeds, leaves flowers and bark (Middleton, 1984). Phytochemicals are very important because some symptoms originally thought to be due to vitamin C deficiency such as bruising due to capillary fragility were found in early studies to be relieved by crude vitamin C extract but not by purified vitamin C.

Bioflavonoids were found to be essential components in correcting this bruising tendency and improving the permeability and integrity of the capillary lining. These bioflavonoids include hesperidin, citrin, rutin, flavones, flavonols, catechin and quercetin (Singla et al., 2001).

Also, the antioxidative properties have been employed in preservation of lipid food systems thereby circumventing undesirable changes such as objectionable odour and flavour, rancidity and bleaching of fatty food colours, consequently prolong the shelf-life of the food (Giese, 1996).

Antioxidants in food

One of the major causes of loss of quality in foods is liquid peroxidation which leads to quality deterioration, rancidity, discoloration and loss of nutrients such as vitamins (Nawar, 1996; Hidalgo et al., 1998). The deteriorative process of oxidation occurs naturally in all foods, not just those with high fat content (Giese, 1996). This makes the addition of antioxidants useful in most fat-containing foods.

Although, oxidative damage to foods can be prevented or delayed using improvements in food processing and preparation, refrigeration and packaging but they are much more expensive in comparison to the cost of adding antioxidants. In terms of origin, antioxidants can be synthetic or natural; though synthetic antioxidants are effective in preventing lipid peroxidation but their activity is surrounded by league of limitations that are centrally linked to health risk and poor process carry-through. However, exploitation of natural antioxidants especially from plant sources has greatly increased in recent years. The development on application of antioxidants from natural sources is favoured by a number of factors notably: (1) safety, since they are part of food man has been eating for thousands of year. (2) effectiveness since they survive processing operations as found in institutional food preparations (3) Their use is not guided by regulatory rules (4) Their source is renewable (Daramola et al., 2009).

Han-Seung et al. (2004) listed in their report several factors such as light, relative humidity, temperature, availability of oxygen and some metals that affect the production of lipid oxidation products. For example, lipid oxidation is likely the most common mechanism of oxygen uptake in fried foods such as potato chips. Also, lipid oxidation in meat and meat products is one of the major causes of spoilage and deterioration of organoleptic properties leading to off-flavour development, colour degradation and nutritive loss (Genot et al., 1997). This undesirable occurrence can be inhibited by the use of antioxidants.

Nature of antioxidant action

As the name implies antioxidants could function by any of

Table 1. Definition of oxidation and reduction.

Oxidation	Reduction
Addition of oxygen	Removal of oxygen
Addition of electro negative element (s) or ion	Addition of electronegative elements or ions
Removal of hydrogen	Addition of hydrogen
Removal of electro positive elements	Addition of electronegative elements or ions
Loss of electron(s)	Gain of electron
Increase in oxidation	Decrease in oxidation number

reductive action process presented in Table 1. However, the principal natures of antioxidant action are nomenclaturally designated as:

Reducing agents: Reducing agents function by transfers of hydrogen atom. Such compounds include ascorbic acid, erythorbic acid, ascorbyl palmitate and sulphites.

Chelating agents: Although some scientific opinion holds that chelating agents are not true antioxidant but are often used with antioxidative compounds. This is because chelating agents grab and hold or complex with pro-oxidative metal ions such as iron and copper. Examples of chelating agents are citric acid and its salt, phosphates and the salts of ethylene diamine tetraacetate (EDTA). However, considering the universal definition of antioxidant in Table 1, reducing agents are functionally antioxidants. Generally, the mode of antioxidant action can be oxygen scavenger, radical scavenger and capability to repair damaged molecules. This is customarily expressed by enzymic antioxidants

Natural antioxidant - plant phenolics functional groups

Virtually all phytochemicals would exhibit antioxidative properties. A treaty on comprehensive list that include: simple phenolics, tannins, coumarins, anthraquinones, xanthenes, chromones, flavonoids, anthocyanins saponins, alkaloids, and steroids and respective glycosides can be found in pharmacognosy text such as Trease and Evans (2002) and natural products texts. However, this review shall center on phenolics and derivatives with reference to flavonoids. Phenolics are virtually ubiquitous to all plant bio-constituents therefore, it is logical to think that they play a critical role with respect to the survival of plants. As already stated phenolics are important in playing defence role in plants, more importantly as an antioxidant. This section reviews fundamental chemical properties of phenolics that accounts for their antioxidative properties.

The common feature of phenolics is the presence of a hydroxyl-substituted benzene ring within their structure. However, some non-hydroxylated precursors or derivatives eg cinnamic acid (3-phenyl propenic acid), are sometimes tagged 'honourary phenolics' although these do not exhibit all the characteristics of true phenol-

lics (Parr and Bolwell, 2000). The interaction of the hydroxyl groups of phenolics with the λ -electrons of the benzene ring gives the molecules special properties, most notably the ability to generate free radicals, where the radical is stabilized by delocalization. By this formation, the relatively long lived radicals are able to modify radical-mediated oxidation processes. Phenolics which possess two ortho-positioned hydroxyl groups are very good antioxidants. Many phenolics chelate metal ions, but tight binding requires vicinal hydroxyl groups such as those present in the B-ring of quercetin and on caffeic acid (Morei et al., 1993). This metal-binding capacity is relevant to one aspect of phenolic antioxidant activity since free transition metals ions are pro-oxidant because they are profoundly present in biological systems. Also, they produce free radicals easily in the presence of hydrogen peroxide. Besides, phenolic hydroxyl groups, increase the potential of electronic delocalization. More importantly phenolic groups are easily ionized, thus serve as weak acids, which ultimately influence the chemical reactivity of total phenolics (Parr and Bolwell, 2000). Others such as phenolics are good hydrogen donor in the formation of hydrogen bonds. Some polymeric phenolics carry large numbers of such donor groups, with the result that complexes formed with other molecules are very stable and tend to precipitate out. This is the basis for tanning abilities of tannins (hydrolysable tannins; for example, esters of gallic acid and the condensed tannins; for example, flavan-3-ol polymers).

Mechanism of oxidation: Autoxidation

As reported by Giese (1996), oxidation occurs in three steps as explained below:

First initiation step: Fat free radicals are formed when hydrogen atoms are lost from the fatty acid group. The resultant fat free radicals react with oxygen to form peroxy free radicals. The peroxy free radicals act as strong initiators or catalysts for further oxidation by extracting hydrogen from another molecule triggering propagation.

Second propagation step: The earlier formed peroxy radicals remove a hydrogen atom from a lipid to form a relatively stable hydroperoxide and a new unstable fatty

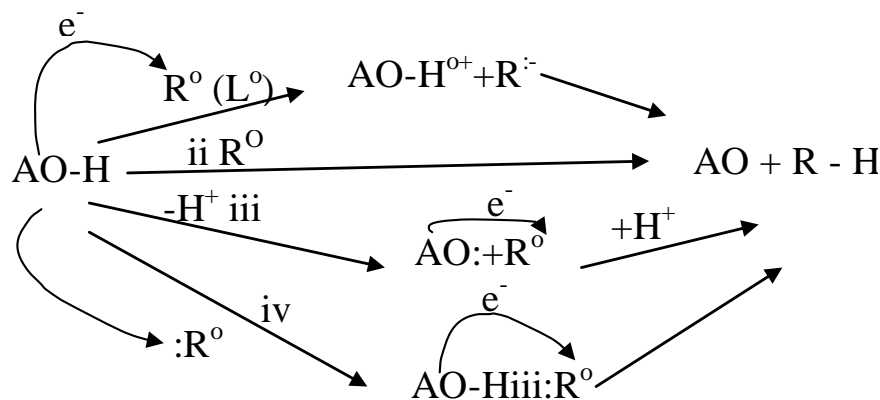


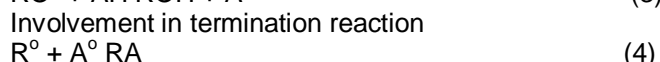
Figure 2. Mechanism of radical scavenging by an antioxidant.

radical. The unstable fatty radical will then react with oxygen to form another new reactive peroxy radical.

Third, termination step: The final step in autoxidation involves the split of hydro peroxides to form smaller short chain organic compounds such as aldehydes, ketones, alcohols and acids which are responsible for the off-odours, off-flavour characteristics of rancid fats, oils and their products. It is important to state that autoxidative process is ended when two unstable radicals react. Besides, the termination step can also be accomplished when a fatty radical reacts with a stable antioxidant radical. The mechanism of autoxidation and antioxidant action has been structurally represented in the work of Dorko and Edwards (1993).

Mechanism of antioxidant action

According to the mode of action, food antioxidants can be classified as free radical terminators, chelators of metal ions or as oxygen scavengers that react with oxygen in a closed system (Dziezak, 1986). Free radical terminators also known as primary antioxidants react with higher energy lipid radicals to convert them to thermodynamically more stable product. They are believed to contribute hydrogen or electron from the phenolic hydroxyl groups. These effects may either inhibit the initiation or propagation step (Giese, 1996) as illustrated in the equations below. An antioxidant, AH apparently reacts with radical produced during autoxidation according to the scheme (Dugan, 1985):



The antioxidant free radicals formed in (1), (2) and (3) are stable and low energy free radicals that do not start a chain propagation process but rather enter some termination reaction with lipid radical to form lipid - antioxidant complex.

Generalised pathways of radical scavenging DA-2 by antioxidant

The generalized pathways depicted in Figure 2 according to Evans et al. (1992), Mukai et al. (1992) and Van Acker et al. (1996) explains mechanism of antioxidant action in four different mode designated as follows: (i) electron transfer from antioxidant to active radical produces a cation radical and an anion. The electron transfer is succeeded by proton transfer from the cation radical to the anion. (ii) Direct hydrogen atom transfer between the antioxidant and the active radical (iii) Deprotonation of the antioxidant followed by electron transfer from the resulting anion to the active radical. This is immediately followed by protonation of the anion derived from the active radical and (iv) proton-coupled electron-transfer for phenolic antioxidants as a fourth pathway distinguished by some DA-3 author notably Mayer et al. (2002), DiLabio and Johnson (2007) and Tishchenko et al. (2008).

Extraction and analysis of plant phenolics

All plants are endowed with phenolic compounds and plant foods and medicinal plants are rich sources of phenolics that can act as antioxidants that can prevent heart diseases (Jin et al., 2010; Wijngaam et al., 2009), reduce inflammation (Mohammad et al., 2012), lowers the incidence of cancers and diabetes. The protection arrogated to the consumption of food plant products such as fruits, vegetables and legumes is essentially associated with the presence of phenolic compounds.

Therefore, there is need for their isolation prior to characterisation. The objective of this section is to review current methods of extracting natural product of phenolic origin that are known to exhibit antioxidant activity from plant materials.

Sample preparation

The fundamental factors for consideration during preparation of plant samples are: nature of sample matrix, chemical properties of the phenolics, with respect to polarity, concentration, number of aromatic rings and hydroxyl groups (Roberts et al., 2013). Consequently, no single extraction procedure is suitable for the extraction of phenolics from all plant materials. Other factors for consideration include particles since, temperature and all forms of pretreatment to enhance the extraction of phenolic compounds. Such pre-treatment include removal of fat especially when necessary to remove lipid from fat - containing samples as accomplish by Weidner et al. (2012) prior to extraction of phenolics from ground seeds of grapes.

Solvent extraction is the most common technique employed for the extraction of phenolics. However, notable parameters influencing the yield of phenolics include nature or chemical property of the solvent, extraction time, temperature, solvent to sample ratio, number of repeat extraction of sample. As well as nature of bioactive or phenolic compound to be isolated. The commonest solvents used are: water, acetone, ethyl acetate diethyl ether, chloroform, alcohols (methanol, ethanol, propanol). A comprehensive review on accomplishments with respect to extraction process and factors affecting phenolic extraction can be found in Roberts et al. (2013). However, it is imperative to state that solvent modification do greatly influence extraction of phenolic compounds such that solvent modification could be by acid, alkaline or sulphated water.

Modern extraction techniques for phenolics

Although, there are merits such as simplicity, cheap apparatus and high extraction rate, the convectional procedures of Soxhlet, tested reflux extraction procedure is still used in many research and institutional centres especially in developing countries. It has some draw backs, notably (1) long extraction time, (2) the need to use large volumes of hazardous organic solvents, which constitute environmental pollutants, and health hazards to workers (3) interference with and degradation of targeted components due to both internal and external factors such as light, air, high temperature (Santana et al., 2009; Altuner et al., 2012). The flaws associated with Soxhlet solvent extraction method necessitate the evolution of new extraction techniques outlined herein: Ultra-

sound-assisted extraction (UAE), microwave assisted extraction (MAE), ultrasound-microwave assisted extraction, supercritical fluid extraction (SFE), sub-critical water extraction (SCWE) and high hydrostatic pressure processing. These methods are characterized by short extraction times, decrease release of toxic pollutants as a result of reduction in the amount of organic solvent used. Summary of the novel extraction techniques for phenolic constituents and lead references are presented in Table 2.

ROS and methods of assessment of antioxidant activity

Since it is ROS that are the most important substrates for target by antioxidants to scavenged, summary of ROS that are relevant to the food matrix and ROS that could be found *in vivo* is shown in Table 3. There many methods for the assessment of antioxidative potentials of samples. Notable methods (Table 4) outlined herein are limited to physical and chemical methods.

Improvement of antioxidant activity

There are two broad types of strategies to be employed for the designing of novel antioxidant. They are: (1) Modification of the existing antioxidant to meet specific requirement or demand. (ii) Molecular construction/synthesis of antioxidants based on theoretical/data. This is often referred to as *de novo* design (Zhang, 2005). The efficacy of both strategies depends on antioxidant structural requirements obtained from qualitative SARS and QSARS of antioxidants.

Modification of existing antioxidants

Activities of antioxidants are governed by many factors and antioxidative properties requirement are diverse. Therefore there are needs to improve existing ones and design new ones with superior activity to existing ones. In this section, highlights of modification of antioxidant with view to improve activity are presented. The molecular structure of antioxidant could be altered with view to enhance its activity.

Qualitative SARS for phenolic antioxidants and analogues

Of all the natural antioxidants, flavonoids and its analogues have the highest frequency of study hence used for illustration herein. The essence of structure activity relationship study is to define dominant structural factors that influence activity of a molecule. Bors et al.

Table 2. Some novel extraction techniques for the extraction of phenolic compounds.

Name	Operation Principles	Examples of plant extraction	Lead Ref Samples
Ultrasound- assisted extractors	Application of ultra sonic radiation (waves) that create cavitation bubbles around sample tissue to disrupt cell wall thereby releasing content.	- <i>Potentilla altrosanguinea</i> - Pinus radiata	Toma et al., 2001 Vinatoru, 2001 Kalpana et al., 2008
Microwave assisted extracted	Microwaves are non-ionizing radiation, inducing molecular motion in materials resulting in sample heating, the steam generated swells and ruptures the cells thereby releasing their active components	- <i>Andropogon paniculata</i> - Green tea polyphenols	Wang et al., 2010 Vasu et al., 2010
Ultrasound/microwave assisted extraction	The coupling of ultrasonic and microwave to extract bioactive constituents. The principle is the simultaneous exertion of the two methods explained above. The synergism of the technique leads to reduction of extraction time, consumption of lower volume of solvents and comparative high yield.	- <i>Spatholobus suberectus</i> - Burdock leaves	Lou et al., 2010 Xian et al., 2011
Super critical fluid Extraction	The transformation of a solvent to a fluid at particular temperature and pressure marks its critical points. Such fluid is termed super critical fluid. It has penetration power thus efficient for extraction of bioactive components	- Sweet basil - <i>Momordica charantia</i>	Leal et al., 2008 Shan et al., 2012
Subcritical water extraction	Also called superheated water, pressurized water or hot liquid water extraction. It is simple, high extract quality, low extraction time. Water becomes subcritical when its liquid form is preserved at 100-347°C and 10-60 bar. It extracts essentially polar compounds.	- <i>Morinda citrifolia</i> - Green tea	Pongnaravane et al., 2006 Etoh et al., 2010
High Hydrostatic pressure extraction (HHPE)	This method is non-thermal but super-high hydraulic pressure (1000-8000 bar). HHPE involves creation of huge pressure difference between the cell membrane interior and exterior consequently allows solvent to penetrate into the cells leakage of cell components (bioactive substances)	- <i>Maclura pomifera</i> - Cashew apples	Altuner et al., 2012 Queiroz et al., 2010
Pulse electric field	Also a non-thermal technique with low energy, that is effective in cell membrane breakdown and efficient mass transfer.	- Strawberry juice - Apple juice	Odriozola et al., 2008 Turk et al., 2010
Accelerated solvent extraction	An automated solvent technique that operates under nitrogen, high pressure and temperature. These conditions lead to high solvent penetration and dislodgement of bioactive constituent		Wibisono et al., 2009 Richier et al., 1996

Table 3. Relevant ROS for examining potential antioxidants.

<i>In vivo</i>	Food matrix
O ₂ (Superoxide radical)	O ₂
Singlet O ₂	Singlet O ₂
H ₂ O ₂	H ₂ O ₂
Lipid peroxides	Lipid peroxides
HOCl*	-
RO (alkoxy radicals) ^a	RO·
RO ₂ (Peroxyl radical) ^a	R O ₂ ·
NO (Nitric oxide) NO ₂ (nitrogen dioxide)	NO, NO ₂
ONOO (Peroxynitrite)	(Nitrite preservation)
OH (hydroxyl radical)	OH

*HOCl is produced by activated phagocytes in the human body. Many bleaches and disinfectants contain its sodium salt (NaOCl), so it is possible that food constituents might sometimes come into contact with this molecule. ^aRadicals formed by the breakdown of lipid hydroperoxides, either thermally (as in heated oils/fats) or catalysed by transitional metal ions (both in foods and *in vivo*). Source: Aruoma et al., 1997.

Table 4. Notable methods of assessment of antioxidant activity

Method/ instrument	Principal oxidant/ Reagent	Antioxidant activity unit	References
Electron spin resonance spectroscopy	Methyl linoleate	Measure rate of oxygen depletion	Skibsted et al., 2001
Free-radical scavenging activity UV-Spectro photometer 515 nm	DPPH 2, 2 di-phenyl -1-picrylhydrazyl	2 DPPH radical scavenging activity	Brand-Williams et al., 1995
Free-radical scavenging activity UV-Spectro photometer 532 nm	Deoxyribose model	% Hydroxyl radical scavenging activity	Halliwell et al., 1987
Ferric Reducing antioxidant power 500 nm (FRAP)	Ferric thiocyanate method	% oil-acid peroxidation	Osawa and Namiki, 1981
Total phenolic content/spatrophatium	Folin Ciocalteau reagent	Total phenolic content	Taga et al., 1984
Total phenolic content/spectrophotometer 470 nm	β -carotene linoleic acid bleaching method	Bleaching/double bond oxidation	Taga et al., 1984
fluorescence	ORAC method	Peroxyl radical scavenging activity	Cao and prior, 1995; Ou et al., 2001
Copper chelating capacity 200-600 nm	CuCl ₂		Afanasen et al., 1989
ABTS ⁺ radical cation 658nm, 734 nm	MnO ₂ ABTS spectro photometer	ABTS radical cation scavenging activity	Re et al., 1999
Superoxide anion radical scavenging activity 550nm	Nitroblue tetrazolium salt (NBT)	Anion scavenging activity	Kuda et al., 2005
Spectrophotomer 515 nm	1, 3 Diethyl-2-thiobarbituric acid method	% Lipid peroxidation	Furuta et al., 1997, Suda et al., 1994
Conjugated diene mtd spectrophotometer 234 nm	Linoleic acid-emulsion		Lingnert et al., 1979
Total antioxidant capacity method	Oxygen radical absorbance capacity		Cao et al., 1996

Ferric reducing antioxidant power = FRAP; oxygen radical absorbance capacity = ORAC.

(1990) indicated three structural factors being the most crucial for flavonoids antioxidant activity namely; i) an ortho-dihydroxy (catechol) structure in the B ring; ii) a 2, 3-double bond in conjugation with a 4-oxo function (1, 4, pyrone moiety) in the C ring; iii) the additional presence of both 3 and 5 hydroxyl group. These structural factors could be manipulated to improve the antioxidant activity of flavonoids. For example it has been proposed and confirmed that a five - membered analog of α - tocopherol is more active than the six - member

analogue because the formal is more planer than the latter and consequently, enables λ -type lone pairs to exert its full stabilizing effect on the radical. This lay the foundation for the designing and improvement of a novel antioxidant 2,3-dihydro -5- hydroxy-2,2- dipentyl 4,6 ditertbutyl benzofuran. The major improvements as reviewed by Zhang (2005) are: i) reduction of OH BDE and prohibition of the free radical of BO - 653 (Figure 3) from attacking other biological targets due to substitution of two O - tert butyl moiety, ii)

Improved cellular mobility of BO - 653 within and between membranes and lipoproteins, and iii) the opened- 7 - position, which facilitates the addition of peroxy radical to the phenoxy radical of BO - 653.

Rational design strategy for antioxidants

The most important features of an excellent radical-scavenging antioxidants are: (a) the

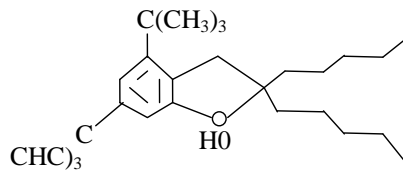


Figure 3. Structures of BO - 653.

X(O,S,N,C)- H BDE or IP should be appropriate. Low BDE and IP are beneficial to enhance the direct radical-scavenging activity in non-polar or polar solvents. However, in polar solvents, possible proton dissociation should be considered because anions are more active than neutral counterparts to donate an electron and some electron-withdrawing groups become electron donating in anion forms; for example, - COOH, CHCOOH (b) the solubility should be suitable in the applied environments. For example, hydrophobic antioxidants tend to exhibit better activity in an emulsion system, while hydrophilic antioxidants are more effective in bulk lipids, often observed as 'polar paradox'. (c) The toxicity of antioxidant and their metabolites, including antioxidant derived radicals should be as low as possible.

De novo antioxidant design

This implies either the assembling of the good features of two or more antioxidants into one molecule or to find new structures by computer aided methodologies. Some examples as illustrated by Zhang (2005) are explained herein: By combining the essential moiety of α -tocopherol and uric acid into one molecule a new antioxidant lead structure; hydroxyphenyl-urea was proposed. Most of which their derivatives exhibit 10 times higher antioxidant activity than α -tocopherol or uric acid. The enhanced radical scavenging activity of hydroxyphenyl-urea derivatives were attributed to the fact that the odd electron can be better stabilized by the P-Ome group and the urea moiety.

Similarly, combining the aromatic moiety of structure of α -tocopherol and diene moiety of lycopene, Palloza et al. as reported by Zhang (2005) designed a new antioxidant tagged FeAOX-6 which was a better antioxidant than α -tocopherol and lycopene, alone or in combination. In addition, a planar catechin analogue was designed and synthesized with view to enhance radical scavenging activity and reduce the pro-oxidant toxicity of catechin. More examples and instructive details can be found in the report of Zhang (2005).

FABRICATION OF ANTIOXIDANTS USING QSAR

Since un-denied facts exist that there is need for exogenous antioxidants, research has gathered a library of information with respect to activity and other properties of

antioxidant. The available information has revealed that no single antioxidant is adequate to address all oxidative stress conditions. Secondly, existing antioxidants application is associated with one form of limitation or the other.

Consequently, there is need for designing an antioxidant with an improved potency and functional specificity (St Piere, 2002). This feat would be accomplished by the advancement accomplished in quantum mechanical treatment of atoms and progress made in application of computer information technologies in chemistry. Therefore, antioxidants are now being fabricated with view to carry all the potentials needed to prevent, cure or at least manage disease conditions associated with all radicals.

The first step in accomplishing this ambition is by design of antioxidant using quantitative structure-activity relationship (QSAR). The QSAR technique mathematically correlates the biological activity of the molecules with their various structural features that impart a distinct variation in the different physicochemical properties of the molecules. Therefore, the QSAR technique provides an easy, non-destructive and economical route for identification and designing of novel antioxidants prior to synthesis and assessment. Descriptors could be used for either homogenous or heterogeneous groups. All the descriptors used have correlation with antioxidant activity. The fundamental descriptors used for QSAR study is presented in Table 5.

FUTURE RESEARCH DIRECTION ON PHYTOCHEMICAL ANTIOXIDANT

Since the application of antioxidants is inevitable for maintenance of human health and preservation of foods and non-food products, its demand shall continue to increase and research would need to address the following among others: while extensive research work had been accomplished on primary antioxidant functionality, there are sparse studies on secondary and tertiary functionalities of antioxidants. Consequently, secondary and tertiary functionalities antioxidants should be designed and fabricated for treatment of spectrum of disease conditions implicated with oxygenated radicals. In addition, there would be adoption of green extraction protocols in obtaining antioxidants of natural origin by application of methods such as plant milking technology (Chemat et al., 2012).

Similarly, enhancement of anti-oxidant metabolites accumulation by manipulation of factors such as environmental and cultural as demonstrated by Karaaslan et al. (2013) should be prioritised in phytochemical antioxidant research.

CONCLUSION

Since the functionality of antioxidants in the prevention or

Table 5. Some Types of QSAR Descriptors

Model, Number	Descriptor	Type	Unit
Semi-empirical descriptors			
1.	Homo energy (HOMO)	Electronic	eV
2.	LUMO energy (LUMO)	Electronic	eV
3.	Energy gap (HOMO-LUMO)	Electronic	eV
4.	Electronic energy (EE)	Electronic	Kcal/mol
5.	Polarizability (P)	Electronic	Å ⁰³
6.	Energy of heat of formation (HP)	Thermodynamic	Kcal/mol
7.	Total energy (TE)	Thermodynamic	Kcal/mol
8.	Binding energy (BE)	Thermodynamic	
9.	Hydration energy (HE)	Thermodynamic	
10.	Log P	Thermodynamic	
11.	Refractivity	Thermodynamic	A ⁰³
Dragon 3D descriptor			
1.	Randic molecular profiles		
2.	Geometrical descriptors		
3.	RDF descriptors		
4.	3D-MORSE descriptors		
5.	WHIM descriptors		
6.	GETAWAY descriptors		Bo-2

Sources: OM and KIM (2008).

mitigation against degenerative diseases and shelf life extension of oxidizable foods and drugs have been established, efforts have been concerted to engage in frontier research for maximum exploitation of protection and preservation potentials inherent in antioxidants of phytochemical origin. This review pooled up-to-date research briefs on antioxidants of phytochemical origin that are scattered in many journals and articles into one piece. The future of research on phytochemical antioxidants was projected. This paper should offer antioxidant status information to readership with view to stimulate readership to seek further information for research and development of improved antioxidant

Conflict of Interests

The author(s) have not declared any conflict of interests.

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