THOMSON REUTERS ENDNOTE

[Devi*, 5(3): March, 2016] ISSN: 2277-9655
(I2OR), Publication Impact Factor: 3.785

IJESRT

INTERNATIONAL JOURNAL OF ENGINEERING SCIENCES & RESEARCH TECHNOLOGY

HERBAL ANTIOXIDANTS- A REVIEW

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DOI: 10.5281/zenodo.47002

ABSTRACT

Reactive oxygen species, circulating in the human body tend to react with the electron of other molecules which may initiate the chain reaction and contribute to adverse health effects in the body. Antioxidants possess anti-inflammatory property, antitumor property, anticarcinogenic property, antimutagenic property and metal chelating potential which inturn terminates the chain reaction by arresting free radical intermediates. Natural antioxidants derived from plant sources are effective and nontoxic to meet the increasing consumer demands. The present review aims at reviewing (1) the mechanism of oxidation and anti-oxidation; (2) methods available for the measurement of antioxidant capacity (3) the potential source of herbal antioxidants.

KEYWORDS: Natural antioxidant, antioxidant analysis, anticarcinogenic property, anti-inflammatory property.

INTRODUCTION

Oxidation reaction produces free radicals in human body which start the chain reaction and has the capability to damage the active human cells. Many health effects are caused, by the reaction of free radicals with the electron of other molecules in the body which may contribute to condition such as cancer, ischemia, aging, adult respiratory distress and rheumatoid arthritis (Kokate *et al.* 2008). Antioxidant agents terminate those chain reactions by arresting free radical intermediates (Sies 1996). Generally antioxidant capacity is due to its effectiveness either in absorbing and neutralizing the free radicals, or quenching singlet and triplet oxygen (Zhang *et al*. 2010). Similar property of antioxidant permits them to act as reducing agents and hydrogen donors. Some of the properties of antioxidant include anti-inflammatory property, antitumor property, anticarcinogenic property, antimutagenic property and metal chelating potential.

Antioxidant plays a vital role in effective functioning of the human body due to the invasion of life threatening diseases like coronary heart disease and cancer. There is an increasing demand for the antioxidants among the eminent food manufacturers. The total antioxidant capacity and the antioxidant property of a plant material depend on the nature of the phenols, composition of the phenols, climatic conditions and the growth factors. Antioxidants can be natural, nature identical or synthetic components. Synthetic antioxidants like butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), tetrabutylated hydroxy quinone (TBHQ) are currently used in food industries which have the ability to suppress the rate of oxidation. Continuous consumption of synthetic antioxidants results in liver damage and kidney failure. Such drawback of synthetic antioxidants is replaced by natural antioxidants. Natural antioxidants derived from plant sources are effective and nontoxic to meet the increasing consumer demands. The research on the extraction of synthesis antioxidants from plant and other sources are in intensive care among the food scientists. Our paper aims in reviewing the mechanism of oxidation and anti-oxidation, methods of antioxidant analysis and the potential source of herbal antioxidants.

MECHANISM OF OXIDATION

Oxygen is important for living organisms and acts as a source of endogenous oxidants. Normal metabolic processes produce various reactive species that are either radicals or non-radicals which has a potential of producing radical species. Oxidative stress leads to cancer, cardiovascular, neurodegenerative and oncological disease. Auto-oxidation may activate polycyclic aromatic hydrocarbons and induce the production of hydrogen peroxide (Lorentzen and Ts'o 1977). Oxidation reaction takes place as a chain reaction consisting of initiation, propagation and termination steps (Wayner *et al*. 1986). The initiation step comprises of the production of a radical.

$$RH \rightarrow R* \quad (1)$$

Propagation is a multistep process. Radical reacts with non-radical compounds to produce new reactive species.

$$R^* + O_2 \rightarrow ROO^* \quad (2)$$
$$ROO^* + RH \rightarrow ROOH + R^* \quad (3)$$

These chain reactions will continue until either two radicals react with each other to produce a non-reactive molecular product or an antioxidant breaks the chain through reaction with a radical. This step is called termination.

$$ROO^* + ROO^* \rightarrow ROO^* \quad (4)$$

Sources of exogenous oxidants include various air pollutants, cigarette smoke, ionizing radiation and exposure to heavy metals. The living organism consumes these oxidative compounds through food which initiates redox-type reactions.

MECHANISM OF ANTIOXIDATION

Antioxidants are compounds that can fight oxidative damage to a given molecule in different ways. Antioxidants possess free radical scavenging, quenching and transition metal chelation properties which inturn convert free radicals into non-radical products. They are further characterized by lowering the localized oxygen concentrations and chain breaking reaction through propagating radicals (Halliwell and Gutteridge 1990). The action of an antioxidant can be based on hydrogen or electron donation to the radical and sequential proton loss electron transfer (Musialik and Litwinienko 2005). Antioxidant sometimes becomes a radical itself when reacting with free radicals (Burton and Ingold 1986).

Mechanism of antioxidant activity of natural and synthetic compounds (AH) is encompassed by a redox transition which involves the donation of a single electron to a free radical ion (R). The radical ion is transferred to the antioxidant, yielding the antioxidant-derived radical (A). The radical adduct displays limited reactivity and, in addition, it prevents the further reactions of the radical R.

$$AH + R \rightarrow RH \quad (5)$$

This reaction mechanism transfers the radical character to the antioxidant which results in the formation of antioxidant derived radicals. The antioxidants technically termed as antioxidant derived radicals may be highly reactive, stable or inert based on the reactants. Three molecular mechanisms for antioxidant activities can be described as process of (1) transferring radical ion with the formation of a reactive antioxidant-derived radical; (2) transferring the radical ion accomplished with formation of a stable, inert antioxidant-derived radical; and (3) molecules which mimic enzyme activities (Cadenas 1997).

ANTIOXIDANT ANALYSIS

Antioxidants can deactivate radicals by two major mechanisms namely hydrogen atom transfer reaction (HAT) and single electron transfer reaction (SET). Both mechanisms give the same end result but with different kinetics and potential for side reactions. HAT based methods measure the classical ability of an antioxidant to quench free radicals by hydrogen donation. HAT methods include oxygen radical absorbance capacity (ORAC) assay, total radical-trapping antioxidant parameter (TRAP) assay and total oxidant scavenging capacity (TOSC). SET based methods are ferric reducing antioxidant power (FRAP) method and copper reduction assay (CUPRAC) method which will detect the ability of a potential antioxidant to transfer one electron to reduce any compound including metals, carbonyls and radicals. Combination of both the mechanisms include 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity assay method and trolox equivalent antioxidant capacity (TEAC) assay method. Less used methods include crocin bleaching assay, in which the discoloration of the reaction mixture is monitored by an UV spectrometer (Huang *et al*. 2005).

HYDROGEN ATOM TRANSFER REACTION-BASED ASSAYS

ORAC ASSAY

This method is suitable for measuring the hydrophilic and lipophilic compounds of antioxidant against peroxide radicals (Huang *et al*. 2005). Samples are incubated with a fluorescent probe at neutral pH and the radical initiator is added to start the reaction. The reaction is monitored spectrophotometrically as fluorescence attenuation, which is inhibited by an antioxidant, with regarding to its chain-breaking capacity. The peroxyl radical reacts with a fluorescent probe to form a non-fluorescent product, which can be quantified by fluorescence. Capacity of antioxidant is determined by the rate decreased and the product formed during the quantitative estimation. This method provides the controllable source of peroxyl radical which is the main advantage.

TRAP ASSAY

TRAP assay method, developed by Wayner (1985) is used to evaluate the antioxidant status of biological fluids. TRAP values are usually expressed as, a lag time or reaction time of the sample compared to corresponding times for Trolox. An azo compound used for initiation of peroxidation and induction period is measured. Induction period is the time before the antioxidant becomes ineffective in inhibiting the initiation of peroxidation.

TOSC METHOD

This method quantifies the absorbance capacity of antioxidants toward radicals. The substrate R-keto-*ç*-methiolbutyric acid (KMBA) is oxidized in this assay, which forms ethylene. The ethylene formation is followed by headspace analysis of the reaction cell by gas chromatography and the antioxidant capacity is quantified by the ability of the antioxidant to inhibit ethylene formation relative to a control reaction. This method predicts an area under the curve that best defines the experimental points during the reaction time (upto 300 min). Linear dose-response curves for antioxidants can be generated from kinetics of the reaction (Prior *et al.* 2005).

ELECTRON TRANSFER REACTION BASED ASSAYS

FRAP ASSAY

The reaction measures reduction of ferric 2,4,6-tripyridyl-*s*-triazine (TPTZ) as a colored product. FRAP is a reasonable screen because of its ability to maintain redox status in cells or tissues at the redox potential of less than 7. Reducing power appears to be related to the degree of hydroxylation and the extent of conjugation in polyphenols. However, FRAP cannot detect compounds that act by radical quenching (H transfer), particularly thiols and proteins (Ou *et al.* 2002). This causes a serious underestimation in serum.

CUPRAC ASSAY

These assays are based on the reduction of Cu(II) to Cu(I) by the combined action of all antioxidants using neocuproine (2,9-dimethyl-1,10-phenanthroline) at 450 nm. A dilution curve generated by uric acid standards is used to convert sample absorbance to uric acid equivalents. Copper has advantages over iron, for antioxidant assays in all classes of antioxidants including thiols, which are detected with little interference from reactive radicals and the copper reaction kinetics are faster than iron.

COMBINATION OF HAT AND SET

DPPH ASSAY

DPPH free radical scavenging assay is based on the use of 1,1-dipenyl-2-2picrylhydrazyl. This assay is based on the measurement of the reducing ability of antioxidants towards DPPH. The assay donates hydrogen to DPPH and a consequent reduction of free radical results in change of color from purple to yellow. The ability can be evaluated either by electron spin resonance (ESR) or by measuring the decrease in absorbance at 515nm (Foti *et al*. 2004). Reaction kinetics can be found by comparing the free radical scavenging activities of samples.

TEAC ASSAY

The TEAC assay is a method for finding the antioxidant capacity of both hydrophilic and lipophilic samples (Huang *et al*. 2005). The oxidant solution is mixed with a sample and the absorbance is measured at several points. The discolouration of an oxidant in the presence of antioxidant causes a change in absorbance which is compared with the standard to assess the antioxidant capacity.

OTHER STANDARDISED METHODS

TBRAS ASSAY

ISSN: 2277-9655
(I2OR), Publication Impact Factor: 3.785

The most important method in detecting lipid oxidation is thiobarbituric acid reactive substances (TBARS) assay measuring the malondialdehyde (MDA) formation (Antolovich *et al*. 2002). A transition metal ion or a free radical source is used to oxidize the substrate resulting in the formation of MDA (for example, LDL or tissue sample). The produced MDA reacts with thiobarbituric acid to form a coloured complex, which is measured spectrophotometrically. The presence of antioxidant reduces the absorption power by complex formation.

IRON (II) CHELATION ASSAY

Iron chelation assay method can be used to assess the preventive antioxidant capacity of a sample. It is based on the reaction of ferrozine with ferrous ions (Stookey 1970). This reaction leads to the formation of a coloured complex and is disrupted by the chelation of ferrous ions. The concentration of chelating sample reduces the ferrous ion concentration, which can be detected by the reduction in absorbance using a spectrophotometer.

HERBS AND ITS ANTIOXIDANT PROPERTY

Ganodema lucidum

*Ganodema lucidum, "*King of herbs" belonging to the family *Ganodermataceae* is commonly called as lingzi mushroom. It has been widely used through the centuries for numerous pharmacological benefits, including immuno-modulating, anti-inflammatory, anti-cancer, anti-diabetic, antioxidative, and radical-scavenging activities. *G. lucidum* contains a wide variety of bioactive components such as terpenoids, steroids, phenols, glycoproteins, and polysaccharides (Boh *et al*. 2007). Literature has shown that triterpenes and polysaccharides are the major physiologically active components of *G. Lucidum*.

Ocinum sanctum Linn.

Ocinum sanctum Linn., "Queen of herbs" belongs to the family *lamiacea* and is commonly called as thulsi. The sacred thulsi is renowned for its religious and spiritual sanctity, as well as for its important role in the traditional Ayurvedic and Umami system of holistic health and herbal medicine of the East. Volatile oil, terpenoids, eugenol, thymol, estragole are the compounds present in thulsi which posses significant health benefits in curing diseases like bronchitis, cutaneous diseases, stomachic, gastric disorders of children and earache (Archana and Namasivayam, 2000).

Curcuma domestica valeton

Curcuma domestica valeton, belongs to the family *Zingiberaceae* and is commonly referred to as turmeric. It is a widely cultivated tropical plant of India having yellow flowers and a large, aromatic, deep yellow rhizome, which is the source of yellow dye and is also used as a condiment. The leaf of this herb contains chemical constituents such as curcumin, β-pinene, camphene, euglenol, β-sitosterol which are responsible for purification of blood, cough relief and curing of dyspnoea management. They act as an effective anti-fertility agent, curing agent for malarial fever, precursor for gastro-intestinal complaints, antifungal agent and as an effective insecticide (Reddy *et al*. 2005).

Magnifera indica Linn.

Mangifera indica, a species of mango in the Anacardiaceae family, is found to be a pharmacologically active flavonoid and a natural xanthone. C-glycoside is extracted from mango at high concentrations from the young leaves. These leaves possesses anti-diuretic, anti-diarrheal and anti-emetic properties. Cyanogenetic glycosides, polyphenols, vitamin A, vitamin C, mangiferin, β-sitosterol, qucertin, ellagic acid and gallic acid are the chemical constituents present in *magnifera indica*. It helps in curing leucorrhea, dysentery, bronchitis, biliousness and haemorrhage in the uterus, lungs or intestine (Martinez *et al*. 2000).

Momordica charantia Linn.

Momordica charantia Linn, commonly called as bitter melon belongs to the family of *cucurbitaceae*. Bitter melon leaves has been used as a folk remedy for a variety of ailments, particularly stomach complaints. Different parts of bitter melon is used as a traditional medicine in India for the treatment of cough, respiratory diseases, skin diseases, wounds, ulcer, gout and rheumatism. According to the Memorial Sloan Kettering Cancer Center, *Momordica charantia* has a number of uses that are thought to be beneficial which includes cancer prevention, treatment of diabetes, fever, HIV and infections. The compounds responsible for its therapeutic properties are stearic acid and tri-terpene glycosides (Leelaprakash *et al*. 2011).

Solanum nigrum Linn.

Solanum nigrum Linn is a medicinal plant, belonging to the family of *Solanaceae. S. nigrum* possesses various components that are responsible for medicinal properties. The major active components are glycoalkaloids, glycoproteins, and polysaccharides. It also contains polyphenolic compounds such as gallic acid, catechin, protocatechuic acid (PCA), caffeic acid, epicatechin, rutin, and naringenin (Jain *et al*. 2011). It plays a vital role in curing diseases such as diuretic, laxative, dropsical actions, virulent gonorrhoea, malaria, dysentery and hepatoprotective conditions.

Withania somnifera Dunel

Withania somnifera commonly called as Ashwagndha or Indian Ginseng belongs to *Solanaceae* family and has been used for centuries in Indian systems as an alternative medicine to treat various ailments. It is acclaimed for its adaptogenic activity in alleviating stress induced illness (James Daniel *et al*. 2011). It has bioactive components such as steroidal lactone, withanolides, glycine and withanone which promotes analgesic effect, increases immunity and hepatoprotective activity (Gupta & Sharma, 2006).

Eclipta prostrata

Eclipta prostrata L. belonging to the family *Asteraceae,* is a herb used in traditional Chinese medicine. It have been extensively used for tonifying the liver and kidney, promoting hair growth, resisting hyperlipidemia, increasing the antioxidant capacity in the human body and treating snake venom poisoning (Kumari *et al*. 2006; Kim *et al*. 2008). *E. prostrata* is rich in flavonoids accounting for the beneficial functions on the human health (Zhang & Guo, 2001). Luteolin is the main flavone possessing a wide spectrum of pharmacological properties including anti-amnesic, anti-tumorigenic, anti-hepatotoxic, anti-osteoporotic, anti-proliferative, anti-inflammatory and antioxidant activities. Eclipta alba saponins, wedelolactone, demethyl wedelolactone in the leaves are responsible for its antioxidant properties.

Coleus aromaticus

Coleus aromaticus belonging to the family, *Laminaceae* is well known for its medicinal properties. It is used for its therapeutical efficacy against common cold, cough, fever, headache and indigestion. The leaves are said to have specific action on the bladder and to be very useful in urinary disease. The uses of medicinal plants in traditional medicine are widespread and still serve as leads for the development of novel pharmacological agents. Some of the compounds like rosmarinic acid, chlorogenic acid and caffeic acid are responsible for many medicinal properties such as hepatoprotective, neuroprotective, anti-inflammatory and also antioxidant or radical-scavenging properties (Kumaran 2006). Therefore, clinical medicine emphasis is placed on the use of antioxidants mainly for intervening and for treating several human ailments in the recent years.

Catharanthus roseus

Catharanthus roseus is an important medicinal plant of the *Apocynacae* family. The species has long been cultivated for herbal medicine and as an ornamental plant. The extracts of various parts of the plant is used against several diseases like cancer, diabetes, malaria, and Hodgkin's lymphoma. Many of the vinca alkaloids were first isolated from *Catharanthus roseus*. The substances vinblastine and vincristine extracted from the plant are used in the treatment of leukemia. Kaempferol and isorhamnetin are the compounds responsible for its flavour and acts as an effective antioxidant. (Kabesh *et al*. 2015).

Andrographis paniculata

Andrographis paniculata, known as Kalmeghin in the Indian system of Ayurveda, belongs to the family *Acanthaceae*. It is a widely used home remedy for common cold. The plant is native to India and Sri Lanka. The effectiveness of this herb has been widely recognized and its demand is on the rise. The major active compound in the plant is andrographolide, apigenin and onysilin (Mishra *et al*. 2009). This is often called, a wondrous compound which possesses antiinflammatory, anti-cancer, anti-viral and even anti-malarial properties.

Cissus quadrangularis

Cissus quadrangularis, belonging to the family *vitaceae* is a perennial climber widely used in traditional medicinal systems of India where it has been reported to posses bone fracture healing, anti-bacterial, anti-fungal, antioxidant, anthelmintic, anti-hemorrhoidal and analgesic activities. *Cissus quadrangularis Linn*. has been recognized as a rich source of carotenoids, triterpenoids and ascorbic acid and is proved to have potential for medicinal effects, including gastroprotective activity and oxidative stress. The isolated chemical constituents from *Cissus quadrangularis* extract includes gallic acid derivatives, steroids, iridoids, flavonoids, stilbenes and triterpenes. (Subhashri *et al*. 2013).

Solanum trilobatum

Solanum trilobatum, belonging to the family *solanaceae,* commonly available in Southern India has been used in the treatment of various diseases. It was reported that *S.trilobatum* possesses antioxidant and hepatoprotective activity. *S.trilobatum* was reported effective in treating respiratory disorder, cancer, tumour reduction and protect peneaus modon past larvae from bacterial attack. New concepts such as nutraceutical nutritional therapy, phytonutrients and phytotherapy using *Solanum trilobatum* as a curing agent is emerging. This functional or medicinal foods and phytonutrients or phytomedicines play positive roles in maintaining well-being, enhancing health and modulating immune function to prevent specific disease. Phytochemical screening of leaf extract of the plant revealed the presence of tannins, saponins, flavonoids, carbohydrates and alkaloids (Jagadeesan *et al*. 2011).

Cardiospermum halicacabum

Cardiospermum halicacabum belonging to the family *Sapindacea* was used in the oldest traditional medicines. This cures chronic hyperglycaemia of diabetes which is associated with long-term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels (ADA 2007). Preliminary phytochemical analysis of the *Cardiospermum halicacabum L*. revealed the presence of alkaloids, coumarine, flavones, saponins, steroids, sugar, tannins and terpenoids (Aishwarya *et al*. 2014).

***Piper betel L*.**

Piper betel L. commonly known as Paan belongs to the family *Piperaceae*. The leaves are pungent, bitter, sweetish and acidic in nature. *Piper betel* is used as an anthelmintic agent, astringent, blood purifier and stimulant for preventing excessive thirst, fever, loss of appetite and nasal inhalation. It acts as an antibacterial agent and is used for treating boils, dandruff, discoloration of the skin, eczema, leprosy, tetanus, urticaria, and wounds (Chakraborty & Shah 2011). Chemical components present in betel leaves are alcohols (α-cadinol, τ-muurolol), esters (methyl salicylate, chavibetol acetate, allylpyrocatechol diacetate), aldehydes (n-decanal), phenols (chavicol, eugenol, chavibetol, methyl eugenol), monoterpenes (trans-sabinene hydrate) and sesquiterpenes (caryophyllene, δ-cadinene, α-humulene, muurolene).

Alternanthera sessilis

Alternanthera sessilis, belongs to the family of *Amarantheceae*. This is a perennial herb with prostrate stems, rarely ascending and often rooting at the nodes. Leaves posses a shape of obovate to broadly elliptic and occasionally linear-lanceolate. It is known for its therapeutic property among traditional medicines. The plant has been scientifically proven to posses chemical constituents like α-and β- spinasterols, lupeal, β- sitosterol and stigmasterol which acts as antioxidants (Subhashini *et al*. 2010).

HERBAL ANTIOXIDANT- SIGNIFICANCE

Food products naturally contain varying amounts of antioxidants. Plant foods are rich in vitamins and phenolic substances. Plant-derived foods are often thought to be the effective sources of antioxidants, but generally animal-derived foods also contain antioxidative compounds in much lower concentrations (Carlsen *et al*. 2010). Antioxidant-derived preservatives can help to prolong the shelf life of commercial products such as various juices, sauces and fat-containing foods. Synthetic antioxidants used in food industries are efficient and inexpensive preservatives are used to prevent oxidative rancidity of fat-containing foods for decades (IARC 1986). Herbs have a great potential as food preservatives (Hinneburg *et al.* 2006; Szabo *et al*. 2010). The extract obtained from a mixture of herbs, has proven to be a potential preservative which may replace notorious synthetic antioxidants such as BHT, at least in some food products. Unfortunately, strong aroma of natural herb-derived antioxidants may restrict their use to some extent (Szabo *et al*. 2010).

Epidemiological studies shows that, the consumption of plant foods can have a protective effect against cardiovascular diseases and cancer (Bazzano *et al*. 2002; Riboli and Norat, 2003). Plant foods rich in antioxidant property have become more important in the prevention and treatment of various diseases. Herbs, a remarkable antioxidant source, are used as traditional medicines in effectively protecting the living cells against oxidative and nitrosative stress (Drangland, 2003). Antioxidant property of herbs is influenced by the processing conditions like extraction temperature, extraction time, drying temperature, drying time and storage condition. Plant habitat can also influence the concentrations of active compounds. Fresh herbs seem to lose phenolic compounds and their antioxidant-related activities due to enzymatic degradation and atmospheric oxygen promoted oxidation. Hence it is advisable to store them in a dry condition.

Herbal antioxidants are widely used as dietary supplements for promoting longevity in alternative medicine systems of Ayurveda and Siddha, which originated from India. Everyday metabolic activities, including breathing, may lead to the exposure of cells to biochemical substances such as free radicals and antioxidants which can be employed to prevent the gradual accumulation of free radical induced cellular damage during aging. Literature studies have shown that the life span of an organism can be extended by diminishing oxidative stress (Finkel and Holbrook, 2000). Medicinal herbs are considered to possess neurohormetic property because of their ability to protect neurons against injury and disease by stimulating the production of antioxidant enzymes, neurotrophic factors, protein chaperones and other proteins that help cells to withstand stress (Mattson and Cheng 2006).

CONCLUSION

The immense effort of the potential use of antioxidants from herbal plants and the antioxidant analysis techniques are summarised in this paper. Based on the review of literature, it is clear that the utilization of natural antioxidants rather than synthetic antioxidants can improve the consumer acceptance and quality of the processed foods. The stability of natural antioxidants can be improved by encapsulation technologies. Though some of the encapsulation techniques are adopted for other food grade products, technologies like air suspension coating, molecular inclusion, and freeze drying are not being commercially employed for herbal antioxidants. However this does not necessarily mean that the above mentioned technologies are not suitable for the production of herbal antioxidants. Fairly compressed overview of the commercially available herbs and their potential use as antioxidants are discussed in this paper. Bioavailability of raw materials and cost estimation plays a vital role in industrialization and commercialization of herbal antioxidants. It is clearly foreseen that a deep understanding of the natural antioxidants and improvements in the manufacturing technologies will play a significant role in the formulation of herbal antioxidants incorporated processed food over the next decade.

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