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Extraction and analysis of bioactive compounds as antioxidants from plants: Review

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ABSTRACT

Free radicals, defined as species contains one or more electrons that are not arranged in pairs, have the ability to exist independent. These species are the major cause of various diseases such as coronary heart diseases, carcinogenesis, mutagenesis etc. Research being conducted to stabilize the unpaired electrons containing groups, to avoid diseases by using effective bioactive compounds as antioxidant. Plants are the major source of bioactive compounds. Several techniques been conducted to extract and estimate the bioactive compounds from plants. This review paper briefly describes about the extraction and screening techniques to estimate the antioxidant activity that are derived from plants.

KEY WORDS: Free Radicals, Diseases, Bioactive compounds, extraction and screening techniques.

1. INTRODUCTION

An atom or molecule that contains one or more electron not arranged in pairs, in the outer orbit called free radicals. The chemical reactivity of unpaired electron is higher than the non-radicals. Free radicals brings about free radicals, that is they can be generated from a compound on a continuous basis as result of chain reaction. Free radicals are the major cause of various diseases such as cardiovascular diseases (CHD), Cancer, Respiratory diseases, Cataract, Parkinson's diseases, Alzheimer's diseases, etc. The major sources of free radicals are cellular metabolism and environmental effects.

Free radicals responsible for diseases are mainly because of oxidative stress. If there is any shift in balance between the producing and degrading process of Reactive oxygen species(ROS), result in oxidative stress and it can lead to harmful effects on living cells. As like coin, ROS has two faces, one is harmful and other one is beneficial in physiological condition of living system. ROS play a major role in defense against infectious agents and participate in cellular signaling events, and its undesirable effects are mainly controlled by antioxidant mechanism, (as a series of reactions are carried out to stabilize the free radicals) present in cells. In the absence of antioxidant, ROS can be a causative agent of diseases such as cancer, arteriosclerosis, etc. ROS can be divided into two parts i.e. radical and non-radical. Superoxide, Hydroxyl, Peroxyl, etc. belongs to the radicals group. Non-radicals include Hydrogen peroxide, Hypochlorous acid, Ozone, Singlet oxygen. Non radicals generally don't fall under the category of free radical because they don't have any unpaired electrons in their outer orbit. Apart from ROS, Reactive nitrogen species derived from the reaction of nitric oxide NO* and superoxide anion $*O^{2-}$ which is an ROS, result in the formation of peroxynitrite $ONOO^-$, which deactivates Nitrogen oxides, that plays major role in regulating blood pressure, platelet activation etc. Peroxynitrite is a highly reactive species and itself can directly react with biological components such as amino acid residues, lipids, DNA etc. and it can generate various RNS as free radicals by reaction with other molecules and these include Nitric oxide NO* and Nitrogen dioxide NO_2*.

Endogenous source of ROS includes Mitochondria, Peroxisomes and inflammatory cell activation, Xanthine oxidase, neutrophils, eosinophils, and macrophages, and source of RNS especially for NO* include Nitric oxide syntheses (NOSs) which plays a major role in the formation of NO* by metabolizing arginine to citrulline. Exogenous source of ROS and RNS include ionizing radiations (eg. X-rays and gamma rays), electromagnetic radiation can out-turn in the breakdown of O_2 molecule and lipid peroxidation occurs as of interaction between the alcohol and lipid membrane, etc.

In their definition of the term antioxidant, given by Satyanarayana and Chakrapani (2013), states that, A compound that are capable of delaying or inhibiting the action of oxidation by a substrate called antioxidant and it can also be considered as a scavenger of free radical. Antioxidant can be broadly classified in two types, they are water soluble and fat soluble compound. Water soluble or hydrophilic antioxidants reacts with oxidants present in the cell cytosol, while fat soluble or lipophilic antioxidants scavenge the unpaired electron, that can induce lipid peroxidation on cell membrane. To stabilize the free radical we need the antioxidant, some compound can be synthesized by body or the requirement has to be fulfilled by means of diet. Apoptosis, is the programmed cell death as a result of increase in level of ROS, this can be prevented by means of antioxidants such a Vitamin E (alpha-tocopherol), if it get partitioned into lipid compartment of cell and N-acetylcysteine and glutathione free radical scavengers get partition into liquid compartment of cell, and thus programmed cell death can be inhibited.

Extraordinary source of antioxidant is plants and so these antioxidants are to be extracted from plants by different techniques and analyzed, to examine their activity of stabilizing the free radicals. These things are elaborated and have been segregated into two parts as extraction and analysis part as follows.

2. EXTRACTION PART

Different extraction modes are done to meet out an bioactive compounds from plants as antioxidant, they are Hexane extraction, Ethanol and Methanol Extraction for polar one and chloroform extraction for non-polar ones, alternatively supercritical water extraction pressurized liquid extraction microwave assisted extraction supercritical fluid extraction apart from this, soxhlet extraction has been proposed is also used to extract or isolate antioxidants from plant.

Let us start with detailed information about the phenolic compounds extraction from plants. Phenolics are pervasive substance found in plants and they are synthesized by plants during stress conditions such as infection, radiation etc. Phenolics as phytochemicals are derived from phenylalanine and tyrosine and there been several forms such as simple phenols, flavonoids, tannins, phenolic acids (gallic acid, tannic acid etc.), Hydroxycinnamic acid (cinnamic acid, caffeic acid, ferulic acid etc.), curcuminoids, etc.

The chemical nature of the phenolics plays a major part in the extraction system and their complexity may vary from simple to higher form, such as protein, or can be found in association with high molecular weight phenol compounds etc. Because of this complexity in nature there is no perfect procedure to extract the phenolics from plant. There were some techniques being handled routinely such as Methanol, ethanol, acetone, dimethyl formide, propanol etc. acts as solvent for the extraction of phenolic compounds from plants.

Phenolic compounds present in plants that have antioxidant property, were illustrated as follows.

Anti-oxidant Plants:

Green tea: Experiments been conducted to segregate the particular phenolic compounds from green tea leaves, particularly catechins were investigated because of their antioxidant activity. The prominent catechins in green tea include (-) epicatechin, (-) epigallocatechin, (-) epicatechin-3 gallate, (-) epigallocatechin-3 gallate. Epigallocatechin-3 gallate and Epicatechin are known to inhibit the carcinogenic bacterium Streptococcus mutans. These catechin compounds were successfully isolated by using column chromatography using ethanol as solvent and separated compounds were identified by silica gel thin layer chromatography techniques.

Diospyros abyssinica: It was found that root of this plant contains highest phenolic compounds with antioxidant activity. Compounds includes triterpenoids butulin, lupeol etc, these compounds possess anti-inflammatory properties. Compounds are mainly extracted by using solvents such as methanol, ethanol, ether etc. Sometimes soxhlet apparatus also used to extract the compounds.

Pistacia lentiscus: The leaves of *pistacia lentiscus* contains enormous amount of phenolic compounds of which most of them are monoterpenes, and substances that found to have antioxidant properties were extracted by using solvents such as water, ethanol, hexane, chloroform etc.

Geranium sanguineum: Its root extract contains the phenolic compounds such as tannins, flavonoids, catechins, proanthcyanidines. It's been used to treat skin diseases, infection etc. It has significiant antioxidant and anti-inflamatory properties.

***Ficus microcarpa*:** Its phenolic compounds are obtained by methanol extraction and compounds include protocatechuic acid, catechol, vanillin etc.

***Leea indica*:** The methanol extract of this plant is found to have various chemical compounds such as phthalic acid, farneso, solaneso, hydrocarbon, gallic acid, etc.

***Salvia officinalis*:** This plant contains antioxidant properties, due to its phenolic composition. The plant extracts were prepared by using solvents such as methanol and water and phenolic constituents are rosmarinic acid, caffeic acid, ferulic acid, flavonoid etc.

***Rheum ribes*:** It's been used as a laxative and antipsoriatic drug. The extracts are prepared by using solvents such as chloroform and methanol, its antioxidant property is mainly contributed by quercetin (flavonoid part).

***Cynara scolymus*:** It was found that leaves of this plant contain the polyphenolic compounds. The extracts of this plant leaves have shown antoxidative, antibacterial, have the ability to inhibit the cholesterol biosynthesis, etc. The compounds were extracted by using chromatography with the combination of different solvents such as methanol, chloroform, distilled water etc.

Hibiscus sabdariffa: It was found to have antioxidant and anticarcinogenic property, these characteristics are mainly due to presence of phenolic compound is anthocyanin.

***Pistacia vera*:** It has antioxidative property, is mainly contributed by the phenolic content present in this plant are anthocyanins, flavonoids include luteolin, genistin and naringenin, quercetin etc. The phenolic compounds are extracted by using solvents such as water and methanol.

***Ocimum gratissimum*:** It has medicinal values, used in the treatment of rheumatism, paralysis, epilepsy, mental illness, gonorrhea, high fever etc. The phenolic content responsible for the antioxidant activity are tannins, steroids,

terpenoids, and flavonoids. These phenolic compounds are mainly extracted by using solvents such as methanol and water.

***Morinda citrifolia*:** The parts of the tree is mainly used for rheumatic diseases, infections etc. The root of this plant contain anthraquinones and other phenolic compounds. The compounds are mainly extracted by using methanol as solvent.

***Acacia nilotica*:** The bioactive compounds were extracted by using different solvents such as methanol, ethanol etc. The leaf extract of this tree contains flavonoids, tannins, triterpenoids, saponines. The bioactive compounds of the plant contains some medicinal properties such as anti-hypertensive, antispasmodic, anti-platelet agregatory properties etc.

3. ANALYSIS PART

Antioxidant activity of various plant extract can be determined by different assays such as DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS [2,2'-azinobis (3-ethyl benzothiazoline 6-sulfonate)], FRAP (ferric reducing antioxidant potential), ORAC (oxygen radical absorption capacity), beta-carotene linoleic acid assay, Ferric thiocyanate assay (FTC), and HPLC with UV detection by using extracted compounds can be detected and moreover bioactive extracted part can be analysed by using chromatography techniques such as Stranded HPLC, thin-layer chromatography, gas-liquid chromatography, and can also be determined by means of UV spectrophotometry and capillary electrophoresis.

DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay (decolorisation method): This method is first introduced by bolis in 1958 and after there been several modification according to the experiment conducted by the researchers. Free radical scavenging method, in which DPPH (dark colored crystalline powder) is reduced in methanol solution in the presence of antioxidant. Absorbance is measured at a wavelength of 515nm, if results in large decrease in absorbance indicate the free radical stabilizing capacity of the compound and ascorbic acid is normally used as reference while measuring the DPPH assay value of antioxidants.

ABTS [2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonate)] assay (Decolourisation method): This method is developed by Rice evans and Miller in1994 and modified by Re in 1999. 7.4mM ABTS is mixed with 2.6mM Potassium persulfate solution is prepared as stock and kept in dark for a period of 12h at room temperature and then 1ml of stock solution is diluted with 60ml of methanol, again kept in dark for a particular period. Then the sample is mixed with the working solution and absorbance is measured at 734nm and then trolox is used as a control. Antioxidant activity is measured in terms of (TEAC/mg). ABTS assay can be applied to both lipophilic and hydrophlic antioxidants.

FRAP assay: Stock solution is prepared as follows ,300mM acetate buffer (3.1g of sodium acetate trihydrate and 16 ml of acetic acid) and pH has to be maintained at 3.6 and 10mM of TPTZ (2,4,6 tripyridyl-s-triazine) with 40mM of HCL and Fecl3.6H2O (iron chloride hexahydrate) of 20 mM is mixed. Then respective ml of acetate buffer, TPTZ solution and iron (iii) chloride hexahydrate has to be taken as working solution and mixed with sample, finally absorbance has to be measured at 593 as a result of which colored complex (ferrous tripyridyltriazine) is formed.

ORAC assay: Peroxy radicals are produced as an outcome of thermal decomposition of AAPH [2, 2' azobis (2-amidino propane) dihydrochloride] in aqueous buffer and fluorescein is used as a substrate commonly Beta-phycoerithin is used as fluorescent target. Then antioxidant activity are quite commonly measured at 520nm. It is the only type of assay to use area under curve technique to measure both the inhibition percentage and time for the quantification assessment.

Ferric thiocyanate assay: First of all, stock solution 50mM phosphate buffer has to be prepared, deionised water (0.4ml) +methanol (0.5ml) is added along with 1ml of phosphate buffer. 2.5g of linoleate is mixed with water of 100ml (2.5%) prepared is added to above solution (0.5ml). Sample is mixed with working solution and incubated at 50^{o}C and analysed for every 24 hr interval. 50 microlitre of incubated sample is added with 70% of ethanol of desired ml (should be between 7-10 ml), 30% of thiocyanate (0.1 ml) is added to above mixture. For a period of 3mins mixture has to be kept at room temperature. Solution of 20mM ferric chloride solution of 0.1ml is being added to the above solution and methanol is used as a reference, absorbance is measured at 500nm.

Trolox equivalent antioxidant capacity assay: The analysis is mainly conducted in a microwell plate. ABTS of 7mM is taken as a stock solution using Potassium persulfate as an oxidant. Stock solution is diluted with ethanol to give an absorption 0.70 at 734 nm. Sample or trolox is diluted with ethanol and pour down into the microwell with the addition of 290µl ABTS and the reaction begins.

Let us move on to the further step by discussing about the extraction and analysis of various plants, with evidence about the phenolic compound, which were described in the following tabular column.

Scientific name	Extraction method	Total phenolic content	Antioxidant activity assay	Polyphenolic compounds
Cinnamomum cassia	Solvent extraction (acetone, methanol, hot water)	9.6 mML^{-1}	DPPH 84-90% FRAP 17-33$\mu moll^{-1}$ FTC 53-82%	Cinnamaldehyde
Curcuma longa	Solvent extraction (acetone, methanol, hot water)	2.6mML^{-1}	DPPH 22-40% FRAP 7-11 $\mu moll^{-1}$ FTC 53 -81%	Curcumin
Coptidis rhizome	Solvent extraction (acetone, methanol, hot water)	4.3mML^{-1}	DPPH 53-64% FRAP 18-26 µmol/l FTC 59 82%	Berberin
Polygonum multiforum Thunb	Solvent extraction (80% methanol, hot water ext)	Water extract-33.9±.62 mgGAE/g	FRAP 343±1.7µmol/g [88]	Hydroxybenzoic acid, hydroxyl cinnamic acid, flavonoids etc
Byrsonima crassifolia	Solvent, Methanol, ethanol, water	45.5±1.9mgGAE/g	TEAC 347.1±.7 µmol/g ORAC 778.8±54.4	Epicatechin-3 gallate catechin, epicatechin, 3-o-galloylquinic acid, etc.
Inga edulis	Solvent, Methanol, ethanol, water	9.8±6.7mgGAE/g	TEAC 58.1±44.9 µmol/g ORAC 239.5±47.4	Gallic acid, catechin, epicatechin, myricetin, etc.
Pennisetum glaucum	80% methanol extract	1387±13.3 µg/g	ABTS 21.4±.43 µmol/g DPPH 23.83±.067µmol/g	Flavonoids, tricin, acacetin, phenolic acid, vanillic acid, syringic acid etc.
Sorghum bicolour	80% methanol extract	4128±9.3 µg/g	ABTS 51.7±.57 µmol/g DPPH 195.8±8.82µmol/g	Hydrobenzoic acid, coumaric acid, ferulic acid etc.
Murraya koenigii	Methanol extraction	38.60 mg/g	FTC 70.60%	Alkaloid, phenolic compound, flavonoids
Ocimum grattissimum	Methanol extract	5.68±.06 mg GAE/g	DPPH 84.6%	Alkaloids, tannins, flavonoids etc.
Lausonia intermis	Methanol extract	38.67±4.51m g GAE/g	DPPH67.67±5.87%	Alkaloids, phenols, etc.
Magnefera india	Methanol extract	135±9.54 mg GAE/g	DPPH 78.14±3.63	Flavonoids, glycosides
Terminalia chebula Retz.	Methanol extract	166.33±18.01 mg GAE/g	DPPH 85.36±2.44	Phenols, glycosides etc.
Punica granatum L.	Methanol extract	122±6.24 mg GAE/g	DPPH 75.50±3.90%	Alakaloids, phenols, Flavonoids
Rubus caucasius	Water	4.527±.008 g GAE/kg	FRAP 65669± 997µol/g	Catechins, flavonols

Abbrevations:

DPPH- (2, 2-diphenyl-1-picrylhydrazyl) assay
FRAP- Ferric reducing antioxidant potential assay
FTC- Ferric thiocyanate assay
ABTS- (2, 2'-azinobis (3-ethyl benzothiazoline 6-sulfonate)) assay

ORAC- Oxygen radical absorption capacity assay
TEAC- Trolox equivalent antioxidant capacity assay

4. CONCLUSION

As we know that plants are the rich source of bioactive compounds, they are synthesized by plants during adverse conditions, inorder to overcome the effects of free radicals. This review paper briefly describes about the extraction and analysis of bioactive compounds as antioxidant from certain plants with their phenolic content. Solvent extraction procedure is commonly preferred than any other procedure because of their high antioxidant characteristics exhibited after segregating the phenolic compounds from plant by the usage of various solvents such as methanol, ethanol, water etc., and their antioxidant characteristics are analysed by using various assays such as DPPH, ABTS, FRAP etc. Though synthetic antioxidants are being synthesized they possess certain side effects so it is important to add the naturally occurring antioxidants (fruits and vegetable) in diet, to prevent the effects caused by unpaired electrons.

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