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Review Article

An Overview of Screeing Methods of Antioxidant Activity

Dr. D. Rama Brahma Reddy, T. Jaganmohanrao, M. Premkumar, S. K. Sathaj, T. Dinesh Kumar, N. Hemanth Kumar, H. G. Diwakar, D. Khadar Basha

Nalanda institution of pharmaceutical sciences.

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ABSTRACT

Antioxidants are substances found in the medicinal plants which may have a protective role to play in certain conditions such as heart disease, stroke and some cancers. Polyphenols, which are antioxidant components, can be found in tea extracts. Natural antioxidants have gotten a lot of attention and research since they are effective due to free radical scavenger activity and are thought to be less hazardous than synthetic antioxidants. Tea, along with water, is one of the world's most popular beverages. Tea (Camellia sinensis) have classified into green tea, black tea, oolong tea. Spectroscopy is a common method for evaluating antioxidants. It is a hydrogen atom transfer (HAT) based approach that uses the following methods for antioxidant assay: DPPH, ABTS, FRAP, PFRAP and it shows that the tea plant possesses the antioxidant property.

INTRODUCTION

Antioxidant means "against oxidation".

Antioxidants are compounds found in medicinal plants that may have a preventive impact in illnesses such as heart disease, stroke, and cancer. Mechanisms of antioxidants like a chain-breaking process and quenching chainintiating catalyst to remove reactive oxygen species. Polyphenols, which are antioxidant components, can be found in tea extracts. Natural antioxidants have gotten a lot of attention and research since they are effective due to free radical scavenger activity and are thought to be less hazardous than synthetic antioxidants. Antioxidant activity and total antioxidant content in foods, beverages, dietary supplements, and herbal extracts have become increasingly popular. Regular consumption of antioxidant-rich foods and beverages can help to minimise the harmful effects of free radicals and oxidative stress. Antioxidants are chemical compounds which bind to free oxygen radicals and prevents these radicals from damaging healthy cells.

1.1Mechanism Of Antioxidant Activity

The mechanism of action is involved in two methods:

***Corresponding Author:** Dr. D. Rama Brahma Reddy
Address: Nalanda institution of pharmaceutical sciences.
Email ✉: dk4393924@gmail.com

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1)The first is a chain-breaking process in which the principal antioxidant donates an electron to the system's free radical.

2) The second method includes quenching chainintiating catalyst to remove reactive oxygen species ROS/reactive nitrogen species initiators (secondary antioxidants).

1.2. Pharmacology Role of Antioxidant Activity

An antioxidant is a molecule capable of inhibiting the oxidation of another molecule. It breaks the free radical chain of reactions by sacrificing their own electrons to feed free radicals, without becoming free radicals themselves.

Figure 1: Role Of Antioxidant

1.3. Classification of Antioxidant Activity

There are different attributes to classify the antioxidants. The first attribute is based on the function (primary and secondary antioxidants). The second attribute is based on enzymatic and non-enzymatic antioxidants

1. Primary antioxidants:

They are the chain breaking antioxidants which react with lipid radicals and convert them into more stable products. Antioxidants of this group are mainly phenolics, in structure and include the following: Antioxidant minerals, antioxidant vitamins and phytochemicals which include flavonoids, catechins, carotenoids, β-carotene, lycopene, diterpene of, black pepper, thyme, garlic, cumin and their derivatives (Hurrell, 2003).

2. Secondary antioxidants:

These are phenolic compounds that perform the function of capturing free radicals and stopping the chain reactions. The compounds include Butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT) and propyl gallate (PG).

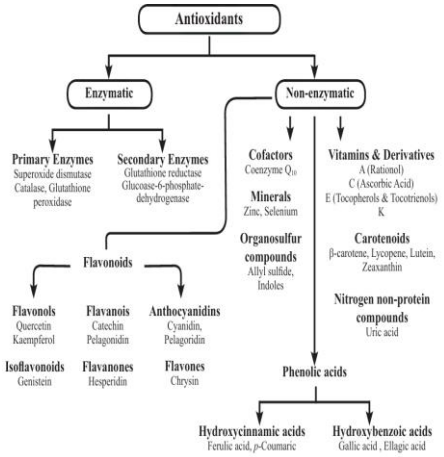

Figure 2: Classification Of Antioxidant Activity

1.4. Advantages Of Antioxidants:

Disease prevention: Antioxidants may help reduce the risk of many diseases, including heart disease, certain cancers, and type 2 diabetes.

Skin health: Antioxidants can reduce DNA damage from UV light, improve hydration, and reduce the appearance of wrinkles.

Eye health: Lutein, an antioxidant found in spinach and corn, may help prevent eye lens degeneration and vision loss in the elderly.

Brain function: Antioxidants can help aid brain function and contribute to mental health improvements.

Liver health: Antioxidants can help maintain the oxidative balance in the liver, which can help prevent liver diseases.

Urinary tract infections: Antioxidants can help bind urine during a UTI and prevent the growth of additional bacteria.

1.5. Disadvantages Of Antioxidant Activity

Increased risk of cancer: High doses of beta-carotene can increase the risk of lung cancer, especially in smokers. Antioxidants can also promote tumour growth by reducing oxidative stress in cancer cells.

Bleeding issues: High doses of vitamin E can increase the risk of bleeding by reducing the blood's ability to clot.

Stomach issues: High doses of vitamin C can cause diarrhea, nausea, and stomach cramps.

Mineral deficiencies: Taking too many antioxidants can prevent the body from absorbing minerals like iron and zinc.

Interactions with other drugs: Antioxidants can interact with other drugs, such as anticoagulants, antiplatelets, and cancer treatments.

Reduced exercise benefits: Taking antioxidant supplements can decrease the benefits of exercise.

Table 1: Antioxidant Activity from Natural Sources.

S.NO	Name	Biological name	Family	Part of the source	Antioxidant contents
1.	Tulasi	Ocimumsanctum linn	Lamiaceae	Leaves	Eugenol, ursolic acid volatile oils
2.	Turmeric	Curcumalonga	Zingiberaceae	Blub	Rhizome, curcumin, beta-pipene
3.	Ginger	Zingiber officinale	Zingiberaceae	Blub	Zingiberene, terpenoid, gingerol
4.	Lemon	Citrus lemon	Rutaceae	Fruit	Monoterpenes, sesquiterpens, Sesquiterpenoids.
5.	Grapes	Vitis vinifera	Vitaceae	Skin &seed	Zingiberene, terpenoid, gingerol

2.Screening methods for antioxidant activity

Screening: it is the systematic testing of compounds for their biological activity and therapeutic potential.

Screening methods

1.Test For Alkaloids:

Meyer's test: To the 5ml of extract 5ml of 2N HCL is added and boiled and then the mixture is filtered. To the filtrate a few drops of Mayer's reagent is added. A cream colour precipitate was produced immediately indicating the presence of alkaloids.

Wagner's test: About 10 mg of tea leaf and tea dust extract was taken and few drops of Wagner's reagent was added, and the formation of a reddish-

brown precipitate indicates the presence of alkaloids.

2. Test For Saponins:

Frothing test: Saponins are tested by boiling 5ml of extract in 10ml of distilled water in a test tube and are shaken vigorously for about 30 seconds. The test tube is allowed to settle for half an hour. Formation of froth indicates the presence of saponins.

3.Test For Phenols:

Ferric chloride test: Phenols are tested by adding 2ml of ferric chloride solution to 2ml of plant extract. Appearance of bluish green colour solution indicates the presence of phenols.

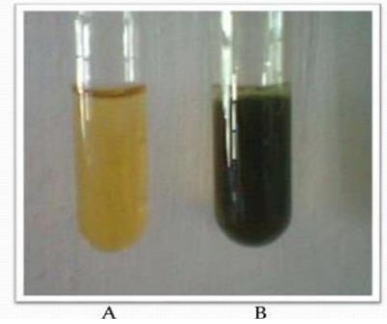

Figure 3. Detection Of Phenols

4.Test For Flavonoids:

Alkaline test:2ml of 2%sodium hydroxide mixture was mixed with tea leaf and tea dust extract, concentrated yellow colour was produced, which became colourless when we added 2 drops of diluted acid to mixture. This result showed the presence of flavonoids.

Lead acetate test: 10 mg of tea leaf and tea dust extract was taken and few drops of 10% lead acetate solution was added. Appearance of yellow colour precipitate indicates the presence of flavonoids.

5.Test For Carbohydrates:

Molisch's test: A Few drops of molischs solution were added to 2 ml of extract, thereafter a small volume of concentrated sulphuric acid was allowed to run down the side of the test tube to form a layer without shaking. The interface was observed for upper phase brown colour ring was indicative for carbohydrate.

Benedict's test :3 ml of extract and add a few drops of boiled benedict reagent and then heated. The interface was observed for blue colour was indicative for carbohydrate.

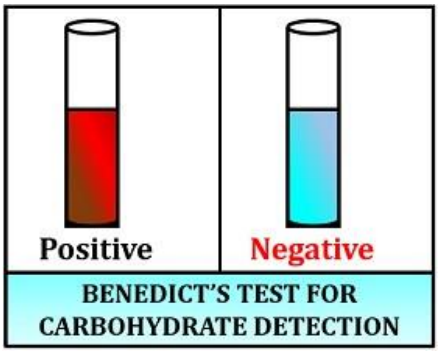

Figure 4. Benedict's Test

6.Test For Proteins:

Ninhydrin test: About 5 mL of extract was taken and 2 drops of freshly prepared 0.2% ninhydrin reagent were added and heated. The appearance of pink or purple colour indicates the presence of proteins, peptides or amino acids.

7.Test For Amino Acids:

Million tests: 2ml of the extract, added the million's reagent (mercuric sulphate and concentrated sulphuric acid) was added and heated for 10 minutes. After cooling few drops of 1% sodium nitrate was added. It gives the color of red brick indicates the presence of amino acids.

3. Bioactive Compounds:

1. **Determination of flavonoid content** (TFC): Flavonoid content of isolated crude (tea leaves and coffee seeds) was determined with this method (Gia et al., 1999). Take a clean test tube and add 0.5 ml of the specimen (extract) to 1.25 ml of distilled water. Then 0.075 ml of 5% sodium nitrite solution was added and allowed to stand for 5 minutes. 0.15 ml of 10% aluminium chloride was added, after 6 minutes, 0.5 ml of 1.0 ml sodium hydroxide was added, and the mixture was diluted with 0.275 ml of distilled water.

2.Determination of total phenolic content (tpc):

Total phenolic contents of each extract were

determined using a Folin-Ciocalteu colorimetric method (Singleton et al., 1999). 1 mL properly diluted of each extract solution were mixed with 0.5 mL of Folin-Ciocalteu reagent. The reagent was pre diluted, 10 times, with distilled water. After standing for 8 min at room temperature, 2 mL of (7.5% w/v) sodium carbonate solution was added. The solutions were mixed and allowed to stand for 30 min at room temperature. Then, the absorbance was measured with a spectrophotometer UV–visible Genesys 10 BIO at 765 nm.

4. Antioxidant Capacity:

Measured by two techniques electrochemical technique and chromatographic technique

1) Electrochemical technique:

A) Voltammetry: At the appropriate applied voltage, a chemical is reduced or oxidised at the surface of a working electrode, causing mass transport of fresh material to the electrode surface and the creation of a current.13 The current of the cathodic/anodic peak is measured.

B) Amperometry: In comparison to a reference electrode, the working electrode's potential is set at a predetermined value.14 The current generated by the oxidation/reduction of an electroactive analyte is measured.

C)Biamperometry: The analyte (antioxidant) reacts with the oxidised form of a reversible redox couple, suggesting a redox couple.15 Measurement of current flowing between two identical working electrodes immersed in a solution containing the examined sample and a reversible redox couple at a minor potential difference.

2)Chromatographic technique

A) Gas chromatography: The partition between a liquid stationary phase and a gas mobile phase is used to separate the components in a mixture. Thermal conductivity detection or flame ionization

B) High performance liquid chromatography: The separation of compounds in a mixture is based on the partitioning of a solid stationary phase and a liquid mobile phase with different polarities, at high flow rates and pressures of the mobile phase UV-Vis (e.g., diode array), fluorimetry detection, mass spectrometry, or electrochemical detection.

5. Applications of antioxidant activity:

Anti-diabetic Effect: In Type II diabetes, which is a heterogeneous disorder, there is resistance of glucose and lipid metabolism in peripheral tissues to the biological activity of insulin and insulin secretion by pancreatic β cells is inadequate.

Antiparkinsonian Effect: Parkinson's disease is a progressive, degenerative disorder of the central nervous system, resulting from the loss of dopamine-producing brain cells, and there is presently no cure.

Anti Alzheimer Effect: In an in-vitro study, it was found that green tea inhibited human acetyl cholinesterase, with an IC value of 0.03 mg/ml and, at an assay concentration of 0.03 mg/ml, inhibited β-secretase by 38%.

In Cardiovascular Disease: Cardiovascular disease (CVD) is a complex disorder involving multiple factors. Among those factors are inflammation, oxidative stress, platelet aggregation, and lipid metabolism. Some of these factors are also involved in other disease processes but will be discussed in this paper under CVD.

6. CONCLUSION

Camellia assamica has antioxidant effect that is more safe than synthesized antioxidants. The green tea having high antioxidant effect than other black tea and oolong tea. The polyphenols in tea plant shows antioxidant property. The content of antioxidant in various formulations of tea can be determine by amperomertymethod. The antioxidant content is different in different formulation of tea due to different processing is for make different tea formulation. Antioxidants

arebeneficials for health and tea is the good source of antioxidant.

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