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EXTRACTION AND EVALUATION OF ANTIOXIDANTS FROM NATURAL SOURCES

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ABSTRACT

Antioxidants from natural sources play an important role in preventing oxidative damage and promoting human health. This study focuses on the extraction and evaluation of antioxidants from plant-based materials using simple and effective methods such as solvent and ultrasound-assisted extraction. The obtained extracts were analyzed for their antioxidant activity using standard assays. Results showed that natural sources contain significant amounts of bioactive compounds, including polyphenols and flavonoids, which contribute to strong antioxidant properties. These findings highlight the potential use of natural antioxidants in food, pharmaceutical, and cosmetic industries.

KEYWORDS: Antioxidants, Natural sources, Extraction, Evaluation, Polyphenols, Bioactive compounds.

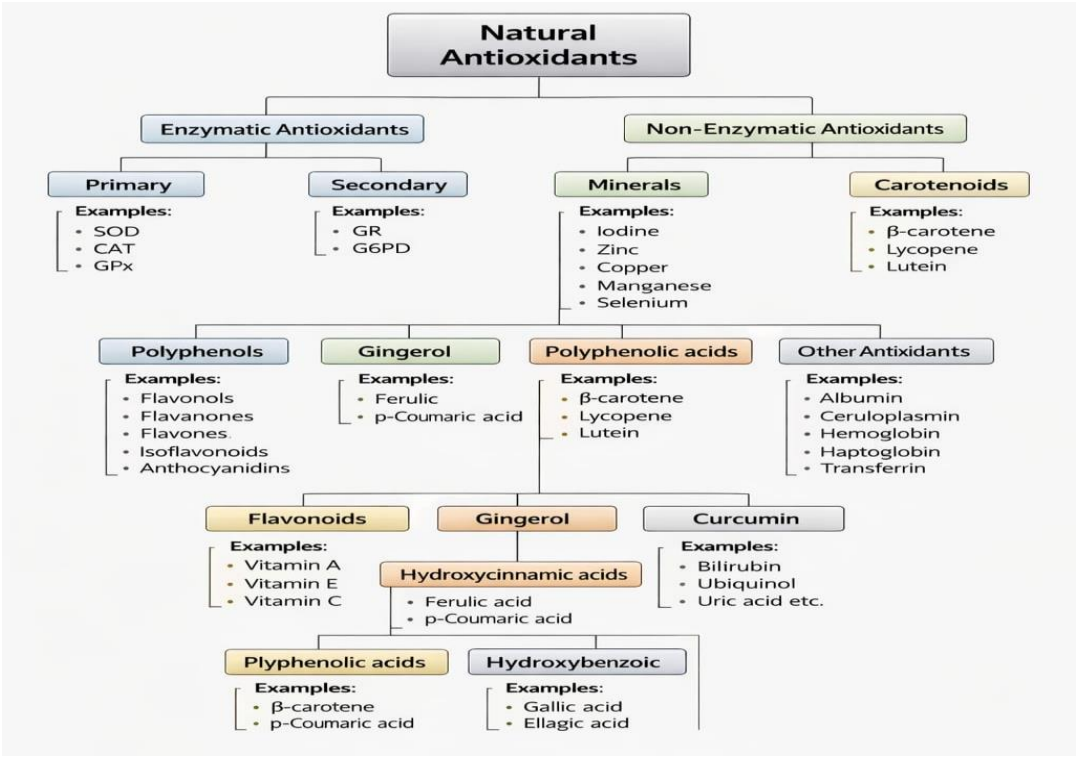
INTRODUCTION

Oxygen is very important for the life of aerobic organisms. It helps in normal metabolism, but it also can cause harmful reactions. Because of this, scientists are interested in studying reactive oxygen species (ROS). ROS include substances such as superoxide anion, singlet oxygen, lipid peroxides, and hydroxyl radicals. These reactive molecules are naturally formed during normal energy production in cells. They play useful roles in cell signaling, gene expression, apoptosis, and ion transport. However, when ROS levels become too high, they can be dangerous. Excess ROS can damage proteins, lipids, RNA, and DNA because of their high reactivity. Free radicals are produced not only inside the body during normal metabolism. They can also come from external sources like stress, radiation, pollution, pesticides, and industrial chemicals(1,6). Oxidative stress occurs when the production of reactive oxygen species (ROS) becomes too high. At the same time, the body's antioxidant defense system is unable to remove them effectively. This imbalance between ROS formation and their elimination leads to oxidative stress.[7]

Extraction is the process of isolating desired chemical constituents from natural or synthetic materials using appropriate solvents and techniques based on the solubility and chemical nature of the compounds.[8]

Evaluation is the systematic examination and analysis of extracted substances to determine their identity, purity, quality, quantity, and biological or pharmacological activity using suitable analytical methods.[9]

Antioxidant is a substance that helps slow down or prevent damage caused by unstable molecules in the body. These unstable molecules can harm cells and macromolecules, leading to oxidative stress, which plays a major role in many diseases. By reducing oxidative stress, antioxidants support overall health. Antioxidants exist in many forms ranging from common compounds like vitamin C to newly identified peptides obtained from plant and animal sources.[10]



Natural Antioxidants

Intense oxidative process in the human body produce reactive oxygen species, these reactive oxygen forms can cause serious damage to cells and tissues. Antioxidants act as protectors against this oxidative damage, they neutralize free radicals by getting oxidized themselves.[11] Even at very low concentrations, antioxidants are highly effective, they perform various important physiological functions in the body. Antioxidants help stop oxidation reactions, this action protects the body form harmful chain reactions.[12] Many researchers consider antioxidants as nature's defense system, they help the body fight physiological and environmental stress. Antioxidants play a role in preventing atherosclerosis, they also help slow aging and reduce the risk of cancer.[13] The body has its own defense system to fight free radicals, this defense system can be strengthened by eating antioxidant rich foods. Antioxidants are mainly classified into synthetic and natural types, free radicals mainly damage the body at the cellular level. Antioxidants protect cells from this damage, based on their action, antioxidants are divided into enzymatic and non-enzymatic types. Enzymatic antioxidants include glutathione peroxidase, catalase, and superoxide dismutase, other enzymes in the body also support overall antioxidant protection.[14] Non-enzymatic antioxidants include several important substances, these mainly include vitamins such as A, E, and C, and to a lesser extent vitamin D, they also include enzyme cofactors like coenzyme Q10. Peptides and minerals such as zinc and selenium are also part of this group. These compounds are known for their strong antioxidant activity.[15]

Polyphenols are natural substances found in fruits and vegetables. They can be small or large molecules. These compounds act as antioxidants and protect fats from damage caused by oxidation. Most polyphenols are linked with sugars and contain one or more phenolic rings. Some polyphenols also exist as ester or methyl ester forms. Polyphenols are commonly obtained from tea, especially green and red tea, and from fruits like grapes. Polyphenols from tea are more useful than those from fruits because they are better absorbed into the bloodstream. Nearly 15-20% of consumed polyphenols enter the blood. Absorption increases when polyphenols are free from sugar molecules. Since fruits contain high contain high amounts of sugar, polyphenols from tea are absorbed more easily than those from fruits. Flavonoids are another important group of antioxidants. They belong to the polyphenol family and are widely present in many foods. Common sources include potatoes, wheat, tomatoes, soft fruits, peaches, and almonds. Anthocyanins are a special type of flavonoid found in berries and red wine. They are strong antioxidants but are absorbed less efficiently than other flavonoids. Polyphenols protect health by preventing the oxidation of low-density lipoprotein (LDL). This helps reduce plaque formation in blood vessels. Some polyphenols also prevent oxidation of important enzymes and help maintain their normal function. Carotenoids from another major group of plant antioxidants after polyphenols. They are mainly present in fruits and vegetables. Rich sources include root vegetables and fruits such as potatoes, carrots, papaya, apples, berries, and apricots.[16]

Carotenoids are fat-soluble compounds made of 40 carbon atoms. They belong to the isoprenoid family. Carotenoids are the second most common natural pigments on earth. More than 700 different carotenoids have been identified. Their colors vary from colorless to yellow, orange, and red. These pigments give bright

colors to many fruits and vegetables.[17-18] It can be extracted using organic solvents like acetone, chloroform, hexane, isopropanol, methanol, methylene chloride, and diethyl ether. Using a combination of solvents often improves extraction because different solvents dissolve carotenoids more effectively due to their varying polarities. Therefore, selecting the right solvent or solvent mixture is crucial for achieving efficient carotenoid extraction.[19]

Vitamin E refers to a group of eight fat-soluble compounds found in nature. These include four tocopherols and four tocotrienols. All forms of vitamin E share a chromanol ring and a side chain with 16 carbon atoms. The two groups differ in their side chains. Tocopherols have a fully saturated phytyl chain. Tocotrienols contain an unsaturated geranylgeranyl chain with three double bonds.[20,22] Animals and humans cannot produce vitamin E on their own, so it must be obtained from plant- based foods, especially vegetables rich in tocopherols. Among these, Y-tocopherol is the most commonly found form in seeds and seed-based products.[23,24]

EXTRACTION METHODS OF ANTIOXIDANTS FROM FOODS AND MEDICINAL PLANTS

Extraction is the first and most important step in studying natural antioxidants from plants. The efficiency of extraction depends on several factors such as solvent type, solvent concentration, temperature, time, and pH. Among these factors, the choice of solvent plays a major role. Many different solvents are used to extract antioxidants from food and medicinal plants. Solvents are selected based on the chemical nature and polarity of the antioxidant compounds. Most phenolics, flavonoids, and anthocyanins are water-soluble antioxidants. Therefore, polar and moderately polar solvents like water, ethanol, methanol, propanol, acetone, and their mixtures are commonly used for antioxidant extraction.[25,28]

Different methods can be used to extract antioxidants from food and medicinal plants. Traditional methods include hot water bath, maceration, and Soxhlet extraction. These methods take a long time and use large amounts of organic solvents. They often give low yields and may damage heat-sensitive compounds due to long heating. To overcome these problems, modern non-conventional methods are used. These include ultrasound, microwave, pressurized liquids, enzyme hydrolysis, and supercritical fluids. Other advanced techniques are high hydrostatic pressure, pulsed electric fields, and high-voltage electrical discharge. These methods are more energy-efficient, economical, and environmentally friendly.[29,31]

Conventional extraction techniques

Solvent extraction can be done using solid-liquid or liquid-liquid methods. It requires selecting suitable solvents. Heat and stirring are often used to improve the extraction process.[30] Solid-liquid extraction is usually carried out using a Soxhlet apparatus. In this method, plant material is placed in contact with condensed solvent. Fresh solvent repeatedly passes through the solid sample, which improves extraction. Another advantage is that filtration is not needed at the end. However, this method also has some drawbacks. It requires a large amount of solvent, which later needs to be removed by evaporation. There is no stirring during the process. The extraction is done at the boiling point for a long time, which may damage heat-sensitive compounds.[33,35]

Non-Conventional extraction techniques

Supercritical Fluid Extraction (SFE)

SFE is a modern, non-conventional technique used for extracting used for extracting antioxidants from natural sources such as plants, fruits, seeds, and herbs. This method mainly uses carbon dioxide (Co_2) as the extraction solvent in its supercritical state. When CO_2 is maintained above its critical temperature (31.1□C) and critical pressure (73.8 bar), it exhibits properties of both gas and liquid, which enables it to penetrate plant tissues easily and dissolve bioactive compounds efficiently(36). In the SFE process, the plant material is first cleaned, dried, and ground into fine powder and then packed into a high-pressure extraction vessel. Liquid CO_2 is pumped into the system and heated to reach supercritical conditions. The supercritical CO_2 flows through the plant matrix and dissolves antioxidant compounds such as carotenoids, tocopherols, and essential oils. For extracting polar antioxidants like polyphenols and flavonoids, a small amount of ethanol is often added as a co-solvent to improve solubility and extraction efficiency.[37] The mixture of CO_2 and dissolved compounds is then transferred to a separator, where pressure is reduced and the antioxidants are released and collected in pure form. The CO_2 is converted back into gas and recycled for further use. Important operating parameters such as pressure, temperature, extraction time, and particle size strongly influence the yield and quality of the extract.[38] SFE offers several advantages, including high purity of extracts, absence of toxic solvent residues, low operating temperature, environmental safety, and short extraction time. However, it has limitations such as high equipment cost and technical complexity. Due to these benefits. SFE is widely applied in food, pharmaceutical, nutraceutical, and cosmetic industries and is considered a sustainable and green technology for antioxidant extraction.[34]

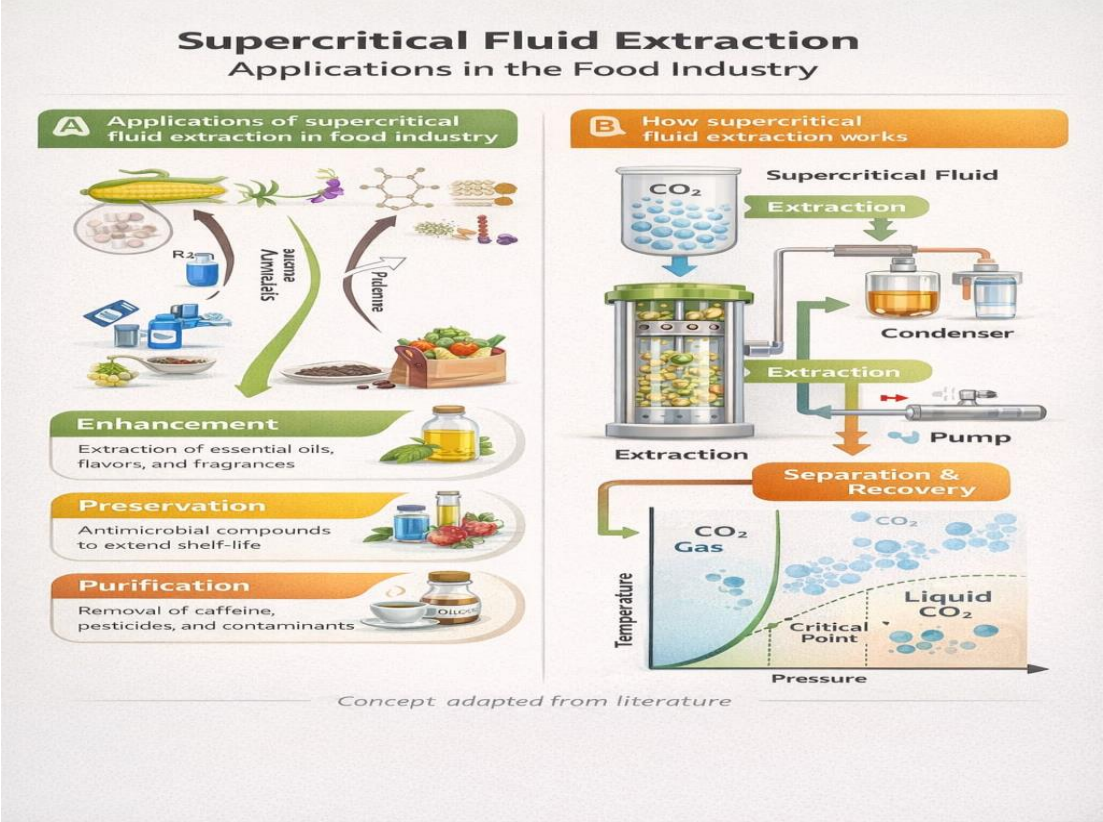


Figure:1.

High Hydrostatic Pressure (HHP) and Pressurized Liquid Extraction (PLE)

HHP and PLE are advanced technologies increasingly used for extracting antioxidants from natural sources. HHP involves applying very pressures (100-1000 MPa) to a solvent-plant matrix, which disrupts cellular structures and enhances mass transfer of bioactive compounds such as phenolics, flavonoids, and carotenoids, while preserving heat-sensitive antioxidants due to minimal thermal exposure.[38,39] Although HHP improves extraction yield and antioxidant activity compared with conventional methods, it requires specialized and high-cost equipment. In contrast, PLE (also called accelerated solvent extraction) employs moderate pressure (3-20 MPa) combined with elevated temperature (usually 40-200□C) to keep the solvent in a liquid state above its boiling point, increasing solubility and extraction efficiency of antioxidant compounds in shorter times and with reduced solvent use.[40,41] However, excessive temperature in PLE can lead to degradation of certain thermolabile antioxidants. Both techniques are considered "green extraction" alternatives to traditional solvent extraction due to their higher efficiency, lower solvent demand, and better preservation of antioxidant activity, making them promising tools in food, nutraceutical, and pharmaceutical applications.[39,41]

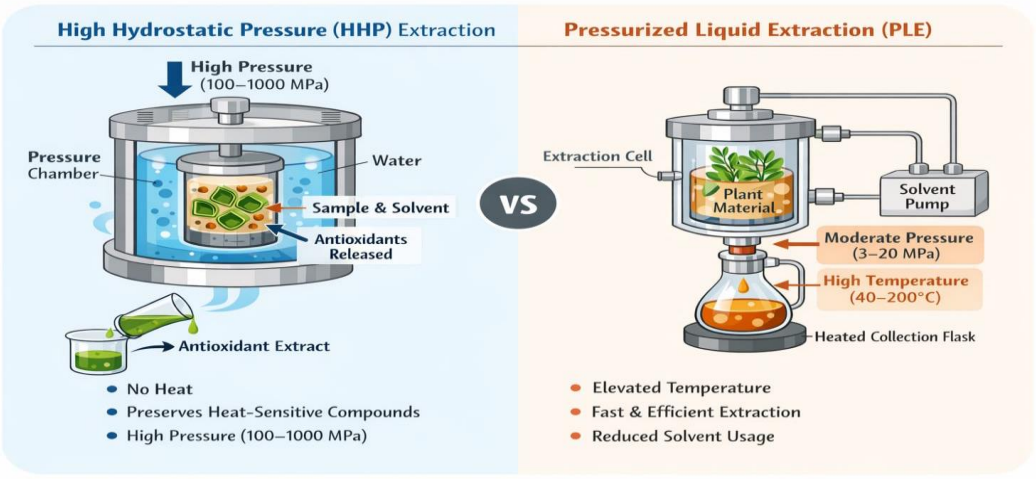


Figure:2.

Ultrasound-Assisted Extraction (UAE)

UAE is an advanced non-conventional technique used for extracting antioxidants such as polyphenols, flavonoids, and carotenoids from natural sources. It works by applying ultrasonic waves (20-100 kHz) to the extraction medium, which produces cavitation bubbles that collapse and generate strong micro-jets and shockwaves. These forces disrupt plant cell walls, enhance solvent penetration, and increase the release of intercellular bioactive compounds.[42] Compared with conventional extraction methods, UAE offers several advantages, including reduced extraction time, lower solvent consumption, higher extraction efficiency, and minimal thermal degradation of heat-sensitive antioxidants.[43] The effectiveness of UAE depends on parameters such as ultrasonic power, temperature, solvent type, extraction time, and solid-liquid ratio.[44] Due to its simplicity, energy efficiency, and scalability, UAE is widely applied in food, pharmaceutical, and nutraceutical industries for antioxidant recovery.[45]

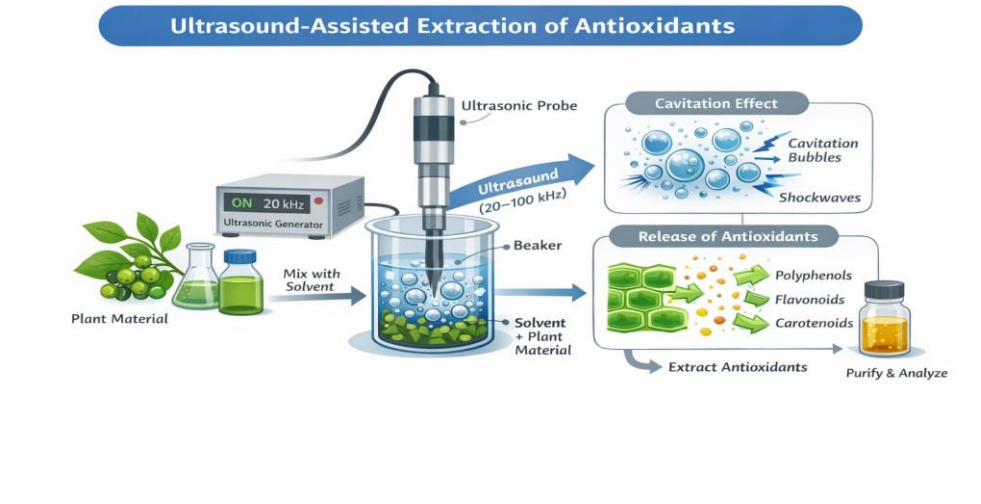


Figure:3.

Microwave-Assisted Extraction (MAE)

MAE is a modern non-conventional technique used for extracting antioxidants such as polyphenols, flavonoids, and carotenoids from natural sources. This method uses microwave energy (usually 300 MHz-300 GHz) to heat the solvent and plant matrix rapidly through diploe rotation and ionic conduction. As a result, internal pressure builds up inside plant cells, causing cell wall rupture and enhancing the release of antioxidant compounds into the solvent.[46]

Compared to conventional extraction methods, MAE offers shorter extraction time, reduced solvent consumption, higher extraction yield, and improved recovery of heat-sensitive bioactive compounds.[47] The efficiency of MAE depends on factors such as microwave power, extraction time, solvent polarity, temperature, and solid- liquid ratio.[48]

Due to its high efficiency, reproducibility, and environmental friendliness, MAE is widely applied in food, pharmaceutical, and nutraceutical industries for antioxidant extraction.[49]

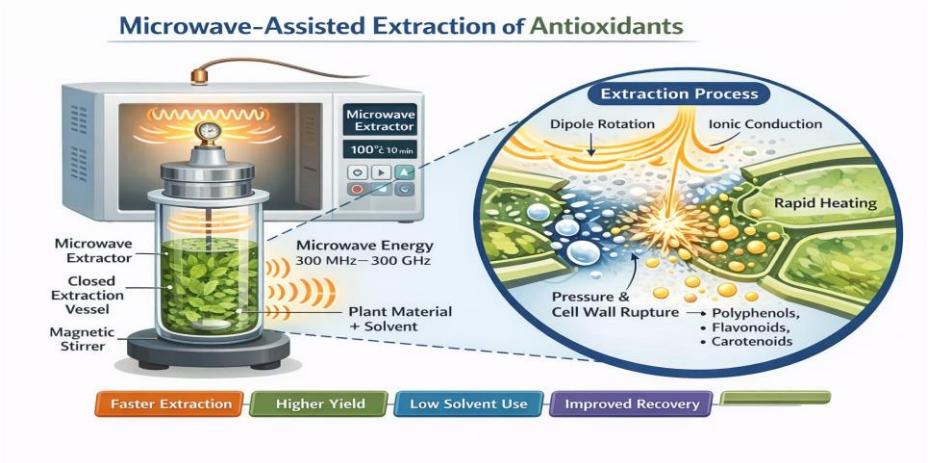


Figure:4.

Evaluation Methods

DPPH (2,2-Diphenyl-1-picrylhydrazyl) Radical scavenging Assay

The DPPH radical scavenging assay is one of the most widely used in vitro methods for evaluating the antioxidant activity of natural compounds and plant extracts. DPPH is a stable free radical with a deep violet color and shows maximum absorbance at 517nm. When an antioxidant donates a hydrogen atom or an electron to DPPH, the radical is reduced to its non-radical form (DPPH-H), resulting in a color change from violet to pale yellow.[50] In this method, a DPPH solution prepared in methanol or ethanol is mixed with different concentrations of the test sample and incubated in the dark for about 20-30 minutes at room temperature. After incubation, the absorbance is measured using a UV-Visible spectrophotometer, and the percentage inhibition is calculated using the formula $[(A_0-A_s)/A_0]\times100$(51). The antioxidant activity is commonly expressed as IC_{50}, which represents the concentration required to scavenge 50% of DPPH radicals, with lower IC_{50} values indicating higher antioxidant potential.[52] The DPPH assay is simple, rapid, economical, and requires only a small amount of sample, making it suitable for routine screening of natural antioxidants(53). However, it has certain limitations, such as sensitivity to light, interference from colored compounds, and limited applicability in aqueous systems; therefore, it is mainly used as a preliminary screening method and is often combined with other antioxidant assays for comprehensive evaluation.[54]

ABTS Radical Cation Scavenging Assay

The ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation scavenging assay is a widely used in vitro method for evaluating the antioxidant activity of natural compounds and plant extracts. In this method, ABTS is converted into a stable blue-green radical cation ($ABTS^{\cdot+}$) by reaction with an oxidizing agent such as potassium persulfate, and this radical shows maximum absorbance at 734nm. When antioxidants present in the sample donate electrons or hydrogen atoms to $ABTS^{\cdot+}$, the radical is reduced to its colorless neutral form, resulting in a decrease in absorbance.[55] For the assay, the $ABTS^{\cdot+}$ solution is usually prepared by mixing ABTS with potassium persulfate and allowing the mixture to stand in the dark for 12-16 hours before use. The radical solution is then diluted with ethanol or phosphate buffer to obtain an initial absorbance of about 0.70 ±0.02 at 734nm. Different concentrations of the test sample are mixed with the $ABTS^{\cdot+}$ solution and incubated for 6-10 minutes at room temperature, after which the absorbance is measured using a UV-Visible spectrophotometer.[56] The percentage inhibition is calculated using the formula $[(A_0-A_s)/A_0]\times100$, where A_0 represents the absorbance of the control and A_s represents the absorbance of the sample. The antioxidant activity is often expressed as Trolox equivalent antioxidant capacity (TEAC) or IC_{50} values, which allow comparison between different samples.[57] The ABTS assay has several advantages, including high sensitivity, rapid analysis, and applicability to both hydrophilic and lipophilic antioxidants, making it more versatile than some other methods such as DPPH.[58] However, the method has certain limitations, including the need for prior radical generation, possible interference from colored compounds, and lack of direct correlation with in vivo antioxidant effects. Therefore, the ABTS assay is commonly used as a complementary method along with other antioxidant assays for comprehensive evaluation of natural products.[59]

Nitric Oxide (NO) Scavenging Assay

The nitric oxide (NO) scavenging assay is an important in vitro method used to evaluate the ability of natural compounds and plant extracts to neutralize nitric oxide radicals, which are involved in inflammation, oxidative stress, and various pathological conditions. In this method, nitric oxide is generated in aqueous solution from sodium nitroprusside under physiological conditions (pH 7.2), and it reacts with molecular oxygen to form stable nitrite ions(NO_2^-). These nitrite ions are then detected using Griess reagent, which converts them into a pick-colored azo dye that shows maximum absorbance at 546 nm.[60] When antioxidants are present in the reaction mixture, they compete with oxygen for nitric oxide and inhibit the formation of nitrite ions, resulting in reduced color intensity.[61] For the assay, sodium nitroprusside solution is mixed with different concentrations of the test sample and incubated at room temperature for 2-3 hours. After incubation, Griess reagent is added, and the absorbance is measured using a UV-Visible spectrophotometer.[62] The percentage inhibition of nitric oxide is calculated using the formula $[(A_0-A_s)/A_0]\times100$, where A_0 represents the absorbance of the control and A_s represents the absorbance of the sample. The antioxidant activity may also be expressed in terms of IC_{50} values, which indicate the concentration required to inhibit 50% of nitric oxide radicals. The nitric oxide scavenging assay is particularly useful for evaluating the anti-inflammatory and protective effects of natural products, as excessive nitric oxide production is associated with tissue damage and chronic diseases.[63] However, the method has certain limitations, such as long incubation time, sensitivity to environmental conditions, and possible interference from colored compounds. Therefore, it is commonly used in combination assays to obtain a comprehensive evaluation of antioxidant potential.[64]

Superoxide Radical Scavenging Assay

The superoxide radical scavenging assay is widely used to evaluate the antioxidant activity of natural compounds based on their ability to neutralize superoxide anion radicals (O_2^-), which are primary reactive oxygen species involved in oxidative stress and cellular damage. In this method, superoxide radicals are usually generated in vitro by the phenazine methosulfate (PMS)-nicotinamide adenine dinucleotide (NADH) system.

These radicals reduce nitroblue tetrazolium (NBT) to form a blue-colored formazan compound, which can be quantitatively measured at approximately 560 nm using a UV-visible spectrophotometer. When plant extracts or natural antioxidants are added to the reaction mixture, they compete with NBT for superoxide radicals and inhibit formazan formation, resulting in decreased absorbance. The scavenging activity is expressed as percentage inhibition, and the IC_{50} value represents the concentration of extract required to scavenge 50% of the superoxide radicals. This assay is considered biologically relevant because superoxide radicals contribute to lipid peroxidation, DNA damage, inflammation, and the development of chronic disease. Therefore, the superoxide radical scavenging assay is as important tool for assessing the protective and therapeutic potential of antioxidants derived from natural sources.[65,69]

CONCLUSION

Natural antioxidants from plant biological sources are important for protecting the body against oxidative stress and related diseases. Effective extraction and proper evaluation are essential to obtain high-quality antioxidant compounds. Conventional extraction methods are simple and economical but often require more time and solvents and may reduce antioxidant activity. In contrast, non-conventional techniques such as SFE, HHP, PLE, UAE, and MAE provide higher efficiency, shorter extraction time, lower solvent consumption, and better preservation of bioactive compounds.

The antioxidant potential of extracts is commonly evaluated using assays such as DPPH, ABTS, nitric oxide, and superoxide radical scavenging methods, which help in determining their free radical-scavenging ability. The combined use of advanced extraction methods and reliable evaluation techniques ensures the effective utilization of natural antioxidants in food, pharmaceutical, and nutraceutical applications. Further research should focus on developing sustainable and scalable processes for maximizing antioxidant recovery and biological effectiveness.

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