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Methodology for In-Vitro Evaluation of Antioxidant Properties

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ABSTRACT:
This article provides details concerning various methods that are useful for carrying out the In-vitro antioxidant activity. After going through a large number of research articles and abstracts published, thus far, for calculating the antioxidant action of several samples of scientific interest and came across the number of in-vitro methods used to perform an antioxidant activity, from which 20 methods were described in this review article. It provides basic information about Antioxidants and their classification under Enzymatic and Non-enzymatic antioxidants classes. It emphasizes the method simplicity, the time necessary, reagent requirement and instrumentation, methodology, that makes to determine and which method to be followed to carry out for checking antioxidant properties depend on the feasibility and afforded to determine it. It also provides a comparison of the most frequently used methods like DPPH a radical scavenging method, among all those methods which act as a good reference for further research. This article will be an encyclopedic prepared reference for them that are excited about the study of Antioxidants.

Keywords: Antioxidants, in-vitro methods, scavenging activity, DPPH, Reducing power.

I. INTRODUCTION:

Antioxidants can be described as "The molecules that, when present at low concentrations as compared to that of an oxidizable molecule, can significantly reduce or inhibit oxidation of that molecule"[1]. A physiological function by antioxidants, it explained by definition that suppresses, destruction of cellular part occurred due to result by chemical reactions including of free radicals. Antioxidants are chemicals that inhibit the oxidation of other chemicals[2-3]. The free radicals are less stable as compared to non-radicals and they are the ability to react randomly to the molecules[4]. Free radicals are mainly involved in causes of a wide range of diseases like cancer, cardiovascular disease, neurological disorders, pulmonary diseases[5-7]. It also includesrheumatoid arthritis, ocular disease, and aging[8-10]. The protection in opposition to free radicals may be increased by sufficient consumption of the dietary antioxidants. The Antioxidant nutrients can play a crucial role in the prevention of disease[11-12].

Antioxidants play a vital role that neutralizes overmuch of free radicals and protects the cells in opposition to toxic effects and is also involved in the prevention of disease[13]. The supplement with large-dose nutritional antioxidants can finally obtain a major function to the prevention including treatment of cancer, inflammatory disorders, cardiovascular disease as well as several diabetic problems[14]. The various antioxidants like beta carotene, quercetin, lycopene, resveratrol, vitamin C, and vitamin E show a preventive as well as a therapeutic advantage in various kinds of cardiovascular disorders[15]. Antioxidants like carotenoids and flavonoids can help to reduce the inflammatory response of our body[16]. The greatest way to reduce inflammation naturally is by incorporating vegetables, fruits, and spices that contain antioxidants into our daily diet[17].

There are several In-vitro as well as In-vivo techniques that are useful for determining the antioxidant activity of the Samples, which include plant extracts and viable antioxidants[18]. The article aims to acquire each possible In-Vitro method that is useful for determining the antioxidant properties of several samples. The compiled explanation of all possible In-Vitro antioxidant methods that give a prolific benefit to researchers in this field to lessen the time for review of literature as well as method formation. Some review articles are published before on methods of assessment of antioxidant action[19-20]. In this review article, attempts are taken to present more specific In-vitro methods of antioxidant detection.

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II. OVERVIEW OF ANTIOXIDANTS

Antioxidants have been described as any substances that prevent the oxidation of another substance[21]. These substances break the reaction of a free radical chain. It feeds the free radicals by sacrificing their electrons, without becoming free radicals themselves[22]. Antioxidants are substances that against oxidation and prevent reactions assist by free radicals[22]. With the general use of oxygen, free radicals are synthesized constantly in the body[23]. Oxygen is essential for life[24]. Free radicals are described as any molecular species possessing unpaired electrons, the unpaired electron only into the atomic and molecular orbital[25]. The free radicals are produced through the molecule by a way to break the chemical bond in such manner every segment holds one electron, to cleave the radical that provide other radical further ultimately through redox reactions[26]. While cells used oxygen to form the energy, free radicals are formed in the mitochondria[27]. Free radicals are chemical species, that are the ability of independent presence they consist of one or more unpaired electrons[28].

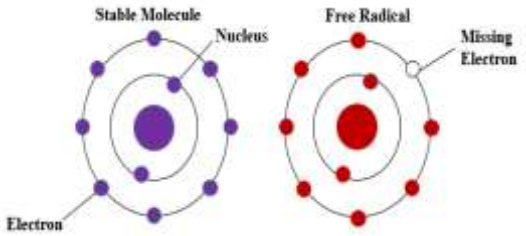

Figure 1. Stable Molecule and Free Radical.

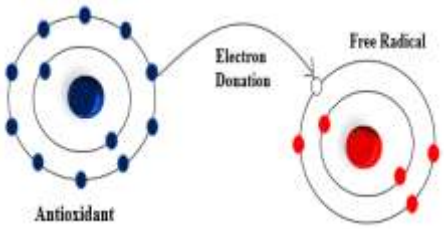

Figure2. Antioxidant and Free Radical.

Based on the activity antioxidants are mainly divided into two categories, Enzymatic along non-enzymatic antioxidants[29].

Enzymatic Antioxidants

Enzymatic antioxidants are specially synthesized into the human body. They are subclassified in primary as well as secondary antioxidants[30]. Primary antioxidants consist of glutathione peroxidase (GPx), catalase (CAT), including superoxide dismutase (SOD)[31]. The Glutathione peroxidase enzyme (GPx) is found in the cytoplasm of nearly all mammalian tissues, which are helpful for the removal of hydrogen peroxide[32]. The Catalase enzyme (CAT) is present in blood as well as majorly in the living cells and that are break H_2 O_2 into the water as well as oxygen. The Superoxide dismutase (SOD) is present in the epidermis as well as the dermis[31]. That eliminates superoxide radical (O.−) further it repairing body cells injured through free radicals[33]. Secondary antioxidants include glutathione reductase (GR) and glucose-6-phosphate dehydrogenase (G_6 PDH)[34-35]. Glutathione reductase is also referred to as the GSH reductase, which is present in human cells and it changed oxidized glutathione (GSSG) into the two molecules of the reduced glutathione (GSH)[36].

Non-enzymatic Antioxidants

Non-enzymatic Antioxidants is further a part of antioxidants that do not naturally present in the body although it is essential for supplementing the appropriate metabolism[37]. The other certain nonenzymatic antioxidants like vitamins, minerals, polyphenols, carotenoids, as well as other antioxidants[38].

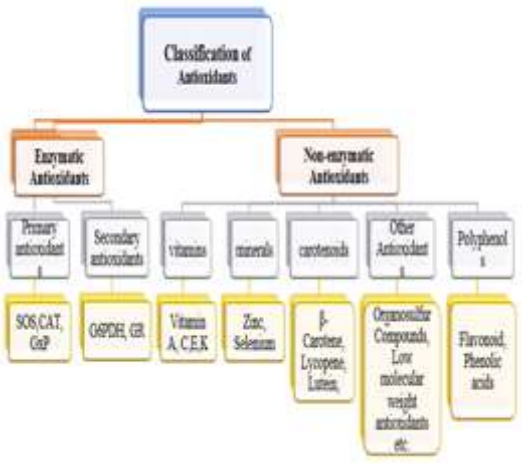

Figure 3. Classification of Antioxidants

In-Vitro Evaluation of Antioxidants

Researchers are critically checking methods of investigation before acquiring that for the research intended. Researchers should not rely on a single antioxidant test method to conclude the antioxidant activity. Mainly, in-vitro antioxidant methods used free radical traps that are comparably simple for carrying out[39]. DPPH method is

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moreover fast, simple, as well as low-cost as compared to other test models[40]. That is crucial to note that one can optimize logically but no one method is complete rather than an example.

III. METHODS OF IN-VITRO EVALUATION OF ANTIOXIDANTS

1. DPPH Free Radical Scavenging Activity

The molecule DPPH (1,1-Diphenyl-2-picrylhydrazyl) is the free radical, and that is constant at room temperature, that is formed a violet-colored solution inside the ethanol[41]. That is decreased in the existence of antioxidant molecules, the result gives the uncolored solution.

The molecule DPPH (1,1-Diphenyl-2-picrylhydrazyl) is generally constant, it is nitrogen centered radical and readily receives the electron or hydrogen radicals that develop in the stabilized diamagnetic particle[42]. The DPPH assay in which violet DPPH solution is converted into the yellow-colored product, i.e Substances can donate the electrons or hydrogen atoms may change DPPH (that is purple) to non-radical form is 1,1-diphenyl-2-picrylhydrazine (that is yellow)[43]. This method is useful considerably to evaluate antioxidant actions due to generally less time is used for the examination. The total free radical scavenging capacity to extracts from various plant samples is to be analyzed following the previously described method[44]. 2.4 mg DPPH that is soluble into the 100 ml methanol through make a solution of the free radicals. A test solution of plant extract 5µl that added to 4 ml methanolic DPPH. A combination of samples including DPPH solution that was shaken actively that placed for 30 min at room temperature in dark. After 30 min absorbance, a combination of reactions is determined at 515 nm on UV-visible Spectrophotometry. Blank is determined to the absorbance of DPPH radical lacking of antioxidant or test sample. All the calculations were carried out in triplicate. A potential to test samples that scavenge DPPH radical to be determined with the help of expression[45].

DPPH Scavenged (%) = [(A control – A sample) / A control] × 100

In which, A control is the absorbance of the control, A sample is the absorbance of the sample. A graph plotted to % DPPH scavenged vs concentration of standard antioxidant[46]. The Ascorbic acid to use as a standard antioxidant[47].

2. Nitric Oxide Radical Scavenging Activity

In accord with referred experiment, the usual examination in which the reaction mixture consists of 2ml of sodium nitroprusside(10mM), as well as 0.5ml of phosphate buffer (pH 7.4), is combined to the 0.5ml sample solution that is incubated for 150min at 25℃. Then later of incubation 0.5ml incubated solution is removed then combined with 1ml of the sulfanilic acids reagent (0.33% of sulfanilic acid in 2% glacial acetic acid) then kept for about of 5min. After 1ml 0f 1%t naphthyl ethylene diamine dihydrochloride (NEDD) that are included then let for the 30min at 25℃[48]. Then absorbance of the pink color of the solution was taken at 540nm. The presence of nitric oxide inhibition is determined by the formula:

% Inhibition of NO radicals = $[(A_0 - A_1)/ A_0] \times 100$

In which, A_0 is the absorbance of the control, A_1 is the absorbance of the treated sample.

3. Hydrogen Peroxide Scavenging Activity

Following the referred experiment, in a typical experiment, 0.4ml of the sample solution that added into 0.6ml of 40mM H_2O_2 solution then formed a 2ml with the help of 50mM sodium phosphate buffer (pH 7.4) then incubation for the 40min at 30℃. Then measured absorbance at 230nm[48-49]. Percentage of inhibition of H_2O_2 that was evaluated with help of expression:

% H_2O_2 Scavenging = $[(A_0 - A_1)/ A_0] \times 100$

In which, A_0 is the absorbance of the control, the A_1 is the absorbance of the treated sample.

4.Total Antioxidant Activity (Phosphomolybdenum Method)

The total antioxidant activity is a spectrophotometric technique for quantitative estimation of antioxidant ability to make phosphomolybdenum complex[50].

The complete antioxidant capability may be determined according to the 3referred method, 0.1ml of the sample solution is mixed to the 1ml reagent solution (0.6M sulfuric acid, 28mM sodium phosphate, as well as 4mM ammonium molybdate). Test tubes are closed then incubated at 95℃into the boiling water bath for 90min. Then later cooling sample absorbance to determine at the 695nm opposed blank in the UV spectrophotometry. The normal blank solution consists of 1ml of sample reagent and a proper volume of sample solvent it is useful for samples that are incubated during similar conditions[51]. In a sample of unknown constituents, antioxidant capacities were shown as an equivalent of α- tocopherol and ascorbic acid respectively[52].

5. Ferrous Ions Chelating Activity (FIC)

The Ferrozine are make the complex along with red color to form chelates to the Fe^{2+} [53]. That reaction is prevented in the existence of certain chelating agents that consequence to reduced red

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color of ferrozine- Fe^{2+} composite[54]. The ferrous ion chelation is measured with the help of the preferred method in which 0.1ml of the extract is put in the solution of the 0.5ml ferrous chloride (0.2mM). A reaction begins with the inclusion of 0.2ml of ferrozine (5mM) that are incubated for 10 min at room temperature then absorbance is measured the 562nm[55]. Percentage inhibition of ferrozine- Fe^{2+} composite production to determine as:

FIC % = $[(A_0 - A_1)/ A_0] \times 100$

In which A_0 is the absorbance of the control, A_1 is the absorbance of the treated sample. EDTA or citric acid are used for positive control[56].

6. Cupric Ion Reducing Antioxidant Capacity (CUPRAC Assay)

A CUPRAC method can be efficiently useful for antioxidant capacity assay[57]. According to the slightly modified method, 0.05ml $CuCl_2$ solution (0.01M), 0.05ml of MeOH neocuproine solution (7.5mM), as well as 0.05ml of ammonium acetate buffer solution (1M) are included into the test tube along to the 0.05ml sample solution (0.5mM). This combination is to be placed for 30min at room temperature. An absorbance is determined at 450nm as opposed to the reagent blank. An excess absorbance of reaction combination in differentiation to control shows to reduced ability of the test composite[58]. The results are given by TEAC (Trolox Equivalent Antioxidants Capacity) acquired with the help of absorbance information than linear calibration curve to plot with absorbance versus Trolox concentration[59].

7. Reactive Nitrogen Species (RNS) Inhibition Capacity

Nitric oxide (NO) produced via the sodium nitroprusside (SNP) into the solution of physiological pH is determined to help of Griess reaction[60]. According to the preferred method, 3ml reaction combination consists of 2ml of 10mM sodium nitroprusside, 0.5ml phosphate buffer saline (pH 7.4) as well as 0.5ml sample extract to distinct concentrations (10- 100µg/ml) are incubated at the 25℃ for 150min. The later of incubation, 0.5ml of an incubated solution consisting of nitrite are pipette out then combined to the 1ml sulfanilic acid reagent (0.33% in 20% glacial acetic acid) Then permit at 25℃ for the 30min. The absorbance of pink-colored chromophores produced in diazotization is determined at 540nm[61]. Butyl Hydroxy Toluene (BHT), as well as catechin, are useful for the comparison[62]. Scavenging actions of the extract are shown to IC_{50} (Inhibition Concentration) this is a concentration of extract in which no radicals are quenched to 50%[63].

Nitric oxide scavenging capacity (%) = $[(A_0 - A_1)/ A_0] \times 100$

In which A_0 is the absorbance of the control, A_1 is the absorbance of the treated sample.

8. ABTS Radical Scavenging Activity

ABTS radical scavenging actions of plant extract are estimated according to the preferred methods[64-66]. A reaction combination is prepared to the inclusion of the same volume of 7mM ABTS as well as 2.45mM potassium persulfate. A combination is placed at room temperature for 12h in the dark that eliminates ABTS radicals. To produce a green-colored solution is diluted to the inclusion of 3ml of ABTS solution to 150ml of the methanol by obtaining the absorbance of 0.700 ± 0.005 at 734nm. The necessary absorbance is acquired, 1ml of obtained solution is mixed to 1ml sample extract in different concentrations (0.005-0.08 mg/ml). Afterward nearly 7min later, reduced absorbance is determined at 734nm. The percentage inhibition of the ABTS radical to extracts or standard are measured with the use of the formula:

ABTS (%) = $[(A_0 - A_1)/ A_0] \times 100$

In which A_0 is the absorbance of the control, A_1 is the absorbance of the treated sample.

9. Ferric Reducing Antioxidant Activity

A reducing capability of sample extracts with positive standard controls is estimated with the help of the potassium ferricyanide reduction method, 0.5ml sample extract at varying concentrations (10- 100 µg/ml) are made into a particular solvent combined to the 2.5 ml of (0.2M) sodium phosphate buffer (pH 6.6) as well as 2.5 ml of potassium ferricyanide (1%) solution to vortexed incubation at the 50℃ for 20min and later incubation 2.5 ml trichloroacetic acid (TCA)(10% w/v) are included each tube and centrifuged at 3,000rpm for 10min. Afterward uppermost layer from the solution (Supernatant) 5ml is taken and combined with 5ml deionized water. In that solution, 1ml of $FeCl_3$ (1%) is included in every tube then incubated at 35℃ for 10min. The production of Perl's Prussian color is determined at 700nm in UV- Vis Spectrophotometry[67-68]. The rising absorbance of the reaction combination increased the reducing ability. Ascorbic acid, as well as Butyl Hydroxy Toluene (BHT), are used as standard antioxidants[69].

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10. Superoxide Radical Scavenging Activity

The effects of extraction of solvents to different plant materials of superoxide radicals are estimated using the referred methods[70-71]. The reaction mixture contains 1ml nitro blue tetrazolium (NBT) solution (312 µM make into the phosphate buffer, pH 7.4), 1ml Nicotinamide adenine dinucleotide (NADH) solution (936 µM make into the phosphate buffer, pH 7.4) as well as 100 µl of distinct solvents extraction. Ultimately, the reaction is fast to the inclusion of 100 µl of Phenazine Metho Sulphate (PMS) solution (120 µM make into the phosphate buffer, pH 7.4) of combination. A reaction combination is incubated at 25℃ for about 5 min then absorbance is determined at 560 nm as opposed to ascorbic acid like control. The capability of scavenging superoxide radicals are determined with help of a formula:

Activity (%) = $[(A_0 - A_1)/ A_0] \times 100$

In which A_0 is the absorbance of the control, A_1 is the absorbance of the treated sample.

11. Ferric Reducing Antioxidant Power Assay

The test is evaluated to check the capability of the antioxidants in reducing ferric ions. A reducing capability of sample extract is estimated to use of the test in which, 10 µl sample extract added in 300 µl freshly prepared FRAP reagent (Consist of acetate buffer pH 3.6, 10 mM 2,4,6- tripyridyl-5-triazine (TPTZ) in 40 mM HCl and 20 mM $FeCl_3 .6H_2 O$ in ratio 10:1:1). The absorbance to taken at 593 nm later 30 min of incubation in the 37℃[72-73]. FRAP value may be occurred to compare the absorption alter in the test combination to formed from the rising concentration of Fe3+ and shown to mM of Fe2+ equivalents per Kg (Solid food) or per liter (Beverages) of a sample[20].

12. Reducing Power Method (RP)

The method mainly depends based on the rising absorbance of the reaction mixture. A rising absorbance that shows elevated antioxidant actions[74]. This method in which antioxidant compounds made of colored composite to the potassium ferricyanide trichloroacetic acid as well as ferric chloride, that is determined at 700 nm[20]. The reduction of various extracts is analyzed under the referred methods[75-76]. Various concentration of the extracts (20, 40, 60, 80 and 100 µg/ml) dissolved into 1.0 ml distilled water are combined to 2.5 ml phosphate buffer (0.2 M, pH 6.6) as well as 2.5 ml potassium ferricyanide (1% w/v) (K_3 Fe (CN)6). The combination is incubated at 50℃ for 20 min come after the inclusion of 2.5 ml trichloroacetic acid (10% w/v) with a centrifuge for 10 min at 3000rpm. An uppermost layer of 2.5 ml is assembled and combined with 2.5 ml distilled water and added to 0.5 ml fresh solution (0.1% w/v). The absorbance of the sample is determined at 700 nm.

13. Ferric- Bipyridine Reducing Capacity of Total Antioxidants (FBRC)

Bipyridine is a partly selective as well as very sensitive compound for Fe_2 arising to the consequences of reduction[77]. The essay is mainly based on the reduction of the Fe_3 - Bipyridine compound into constant pink-colored Fe_2 - Bipyridine chelated to antioxidants into buffer medium[78]. The 0.04 ml of 10% aqueous extract are reacted to 1.0 ml of $FeCl_3$ solution (0.01 M) 1.0 ml bipyridine as well as 2.0 ml acetate buffer (0.3 M, pH 4). That combination is diluted with 10 ml of distilled water. To incubation for 10 min at the room temperature then determined colorimetrically at 535 nm opposed to blank containing 1.0 ml $FeCl_3$ solution, 2.0 ml acetate buffer (0.3 M, pH 4) formed until 10 ml by distilled water.

14. Ferric Thiocyanate Method (FTC)

To determine the antioxidant activity this method can be exploited as illustrated by the reference[79]. To the sample extract of different concentrations (2,4,6,8 and 10 mg/ml) 120 µl of 95% ethanol, 100µl of 2.5% linolic acid as well as 9 ml of 40 mM phosphate buffer (pH 7) was included. Additionally, a solution is kept at 40℃ in the dark for 40 min. From the 100 µl was taken out to this solution, 9.7 ml of 75% ethanol,100 µl of 30% ammonium thiocyanate, as well as 20 mM $FeCl_3$ were added in 100 µl of 3.5% HCl. An absorbance of subsequent combination (Red color) to determine of 500 nm for each 24 h up to absorbance of control achieved to greatest standard antioxidant (ultimate concentration of 0.02% w/v) it useful like a positive control another is the combination in the absence of sample is useful as a negative control[20].

15. Thiobarbituric Acid (TBA)

The test explained is as followed: 1 ml of 2.50% linolic acid, 200 µl of 20% trichloroacetic acid, along with 200 µl of 0.67% thiobarbituric acid was included in the sample extract of varying concentrations (2,4,6,8 and 10 mg/ml) then keep for the 10 min into the boiling water bath. Afterward, centrifugation at 3000 rpm for 20 min, absorbance for supernatant is determined at 532 nm[79].

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16. β- Carotene Bleaching Assay

The preferred method is explained as, an emulsion is formed by combining 11 µl of β-Carotene (8.2 µM), 4.4 µl of linolic acid (628 µM), as well as 22 µl of tween 40 (0.2 gm/ml) after the solvent is removed to the emulsion, 2.4 ml of phosphate buffer (0.02 M, pH 7) the sample extract to distinct concentration (2,4,6,8 and 10 mg/ml) was included. A solution was placed at 50℃ for 10 min then absorbance is determined at 460 nm[79-80]. A presence of inhibition shown by distinct concentrations of extracts to measured as:

Percent inhibition (%) = $[(A_0 - A_1)/ A_0] \times 100$

In which control is designed to solution consist of all the compounds are useful for every assay and have the sample extract. A concentration in that 50% of radicals scavenged is determined to the percent inhibition values[80].

17. Oxygen Radical Absorbance Capacity (ORAC)

ORAC is determined by the radical chain baking capability of antioxidants[81]. The test may be carried out with the use of either β- phycoerythrin (β PE) or fluorescein of the particular atoms. The test is carried out with the help of Trolox (the Water-soluble relative of the vitamin E) to a standard of estimated Trolox equivalent (TE). ORAC value is determined to Trolox equivalent and show ORAC value[82]. The "Antioxidant Power" is always greater when the ORAC value is higher[20]. That essay is mainly based on the formation of the free radical use in AAPH (2,2 azo bis -2- amido propane dihydrochloride) the evaluation to reduce the fluorescence to the existence of the free radical scavengers. After the inclusion of AAPH into the test solution, A fluorescence to noted then antioxidant actions appeared like Trolox equivalent[83]. An ultimate reaction combination of the assay consists of 1.67× 10-8 M β- PE as well as 3×10-3 M AAPH in 7.5 × 10-2 M phosphate buffer, pH 7.0. The ultimate volume of 2 ml is useful for 10 mm broad cuvettes. every sample tube in which 20 µl, diluted sample, or another antioxidant solution are included. The Sample of α-tocopherol acid succinate, β- carotene, or bilirubin are initially dissolved into the acetone afterward included in the reaction combination. For blank 20 µl, acetone rather than phosphate buffer is used. The AAPH is useful for peroxyl radical formation that begins the reaction[84]. The AAPH is included, the reaction combination is incubated at 37℃. The Fluorescence is determined each 5 min of radiation at 565 nm to the excitation of 540 nm with the help of a fluorescence spectrophotometer up to the zero fluorescence take place. For the standard 20 µl of 100 µM (1µM in ultimate concentration) Trolox stock, the solution is evaluated in every run.

ORAC value (U/ml) = 50 k (S Sample – S Blank) / (S Trolox – S Blank)

In which K is a dilution factor, S is an area under a quenching curve of β-PE, Blank (20 µl Phosphate buffer or acetone instead of sample).

18. Xanthine Oxidase Inhibitory Activity Method

The xanthin oxidase action is measured spectrophotometrically by the referred modified method[85-86]. By calculating uric acid generation spectrophotometrically at 295nm using: The reaction combination involved 300µl of phosphate buffer (pH 7.5), 100µl of plant extract of the varying concentrations (50,100,150 and 200µg/ml), 100µl of XO enzyme solution (0.2 units/ml into the buffer, pH 7.5 at 25℃) and 100µl of the distilled water, whole the solutions are formed newly. Later of pre-incubation at 37℃ for 15min, the reaction is inhibited by the inclusion of 200µl of xanthine substrate solution (0.15mM) in combination. The combination is incubated for 30min at 37℃. Ultimately, the reaction is stopped to the inclusion of 200µl of 0.5M hydrochloric acid. Absorbance is measured at 295nm. Allopurinol is useful like a positive control. XOI action of the analyzed sample appeared as percentage inhibition of the XO[87]. The blank is formed in a similar way however the XO is not included in the blank solution. The inhibition percentage is measured with the help of the formula:

(%) XO Inhibition = $[1- (As/ Ac)] \times 100$

In which, As is XO action with extract and Ac is XO action without extract.

19. DMPD (N,N-dimethyl-p-phenylenediamine dihydrochloride) Method

DMPD radical cation decolorization assay is established to the estimation of antioxidant activity in a portion of food as well as biological samples[88]. The process consists of the analysis of reduced the absorbance of DMPD to the existence of the scavengers in which absorbance greatest of 505nm[89]. An action is a show as a percentage lessen of DMPD estimate the action[90]. The standard process is formed is the combination of 1ml DMPD solution (200mM), 0.4ml ferric chloride (0.05M), as well as 100ml of sodium acetate buffer solution at 0.1M, adjust pH to 5.2. A reaction combination is placed in darkness, under refrigeration, that is at small temperatures (4-5℃). A reaction has occurred once 50µl of sample is included in the 950µl of DMPD solution (Dilution of 1:10 in water)[91]. Then

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Absorbance is determined afterward of 10min of constant stirring. That is the time needed to achieve constant decolorization value. Then results are quantified in mM Trolox of the applicable curve[20,92].

20. Total Radical- Trapping Antioxidant Parameter (TRAP) Method

The test depends on protection provided to the antioxidants of fluorescence degrade R-phycoerythrin (R-PE) in regulated peroxidation reaction[93-94]. The fluorescence of the R-phycoerythrin is quenching to ABAP (2,2-azo-bis(2-amido-propane) hydrochloride) like radical producer[95-96]. In the presence of antioxidants, quenching reactions were measured. The antioxidant capacity is estimated to determine the degradation in decolorization[20,97]. In accordance to the reference, 120 µl diluted sample included in 2.4 ml phosphate buffer (pH 7.4), 375 µl double distilled water, 30 µl diluted R-PE, as well as 75 µl of ABAP. Reaction kinetics in the 38℃ is noted for the 45 min with luminescence spectrometer[98]. The TRAP value was measured to the length of the lag-phase because the sample is differentiated to the standard[99-100].

IV. CONCLUSION

To date, a wide range of analytical methods for the evaluation of antioxidant ability is accessible as noted here. All the given assays are different from one another in case of reaction mechanism, target species, reaction state, and form the results have appeared. That is considering in broad range of region there, these methods are recently used, it consists of Nutrition, Pharmacology, Physiology, as well as Agrochemistry, it can be hard to choose the most accurate method to prevent inaccurate application and distortion of the results. This context provides knowledge regarding the main component are considered to choose a method that is the mechanism of the reaction. That is also important to choose methods that are generally approved, validated, as well as standardized to the great body of comparable details accessible in literature. This article will be an encyclopedic prepared reference for them that are excited about the study of antioxidants.

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