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A REVIEW ON DIFFERENT METHODS OF DETERMINATION OF ANTIOXIDANT ACTIVITY ASSAY OF HERBAL PLANTS

Kundan Ojha, Satish Dubey, Jagriti Chandrakar, Robin Anigo Minj,

Rashmi Dehariya, Ashwini Kumar Dixit*

Laboratory of Molecular Taxonomy & Medicinal Plant Biology

Department of Botany, Guru GhasidasVishwavidyalaya, Bilaspur, Chhattisgarh, India

ABSTRACT: In this review paper represented of the mechanism of action and various methods for measurement of oxidative scavenging capacity and also tries to presenting of different type of molecules delectation technique and most of important advantages and shortcoming of each method. Antioxidant is reacting with oxidative agent; this antioxidant reaction is able to inhibit the oxidation processes. In this present study provides diverse rationales for developing standardized method for antioxidant scavenging capacity for the food, pharmaceuticals and nutritional industries. In organism defense mechanism for the oxidative agent is antioxidant and this is also played against the harmful agent, those mechanisms are associated with the free radicals, various plants oxidative scavenging capacity was are responsible of plant-derived antioxidant.

KEYWORDS: Oxygen quenching, oxidation processes, free radicals, standardized antioxidant capacity, oxidative stress.

Corresponding Author: Dr. Ashwini Kumar Dixit* Ph.D.
Department of Botany, Guru Ghasidas Vishwavidyalaya, Bilaspur, Chhattisgarh, India
Email Address: dixitak@live.com

1. INTRODUCTION

In present Era Natural antioxidants have received great attention and have been studied extensively, since they are effective due to free radical scavenger's activity and are assumed to be less toxic than synthetic antioxidants. A natural antioxidant was introducing as a substance, in the small amount for the capable of preventing in a major way, the oxidation process of easily done by oxidizable material, such as fats. In the natural system two types of radicals, first are oxygen based radicals and second one is nitrogen based radicals. Oxygen based free radicals, such as superoxide,

hydroxyl radicals and peroxyl radicals, with the adding up known-radicals, such as hydrogen peroxide and hypochlorous acid are known as reactive oxygen species (ROS), these are generate during the metabolism oxygen process and reactive nitrogen species (RNS), including radicals which is based on nitrogen radicals and non- radicals, such as nitrogen dioxide [54]. In biological systems, DNA and protein are also susceptible to the oxidation processes. Consequently, antioxidants are also defined as molecule, these are low in quantities when compare to those are oxidizable substrate is able to prevent the substrate become oxidization the oxidizing action depends on velocity constants [36].That sense, the hydroxyl radical is enormously burly oxidant, it's not just because of its highly strong reduction potential. Order of magnitude the velocity content characteristics of reaction limited only by molecule movement. In the medical importance of the plant has assumed a more important scope in the past few year owing largely to the discovery that extracts from plant contains, not only primary metabolites are important but also various secondary metabolites with antioxidant potential [70]. A large number of medicinal plants which is have show the beneficial potential by their purified constituents like carotenoids, cinnamic acid, tocopherols, folic acid, flavonoids, ascorbic acid, tocoterienols, banzonoic acid etc are some most important antioxidant produced by plant substance [84, 41]. In a current time, widely used secondary metabolites in antioxidant are ascorbic acid and alpha-tocopherol [14]. At a time not only it is complicated to describe the term of antioxidant because of there no common definition of antioxidant and no accepted international definition for that term, as there also universal 'best' antioxidant. Such as ascorbate protecting for the plasma lipid peroxidation, these are caused by tobacco smoke, but this is not against protein damage [61]. In the present review, various antioxidant such as plant and their use of different disease and efficiency of the different plant through various screening procedures and attempts are made by researchers.

1.2 Secondary Metabolites as Antioxidants

Plant secondary metabolites are the most valuable phytochemicals of plant secondary metabolism and possess sufficient chemical or structural complexity so that artificial synthesis is difficult or not currently possible. For the production of specific secondary metabolite, activation of a specific path way is necessary. This may occur when transcription triggers the expression of a specific gene to participate in production of those proteins that are further taking part in biosynthetic pathways as activator molecules for triggering specific secondary metabolites. This whole process requires a combined knowledge of transcriptonomics, genomics, proteomics, and metabolomics. Metabolic engineering is a branch to engineer/optimize parameters involved in the high production of secondary metabolites by using various tools such as proteomics, genomics, transcriptonomics, and metabolomics. The primary purpose involved here is to produce high-quality secondary metabolites with improved yield. Metabolic engineering also creates a good understanding of

biosynthetic pathways and their respective end products. In Recent time numerous studies related to evaluation of antioxidant capacity of different plant materials.

Table No. 1 Secondary metabolites compound of different Plants

S. N.	COMPOUND NAME	PLANT USED	ASSAY	REFRENCES
1.	Quercetin	*Carthamus tingtorius*	DPPH and ABTS	[23]
2.	Quercetin, kaempferol	*Cordia macleodii*	DPPH	[57], [67]
3.	Apigenin	*Lippia alba*	DPPH	[39]
4.	Chlorogenic acid	*Anona squamosa*	ORAC, DPPH	[55]
5.	Quercetin	*Pongamia pinnata*	FRAP, DPPH	[102]
6.	Kaempferol-3-o-glucoside	*Commiphera wightii*	DPPH, ABTS	[94]
7.	Phenyl dimethylsiloxane	*Hyptis Suaveolens*	DPPH, TEAC	[68]
8.	Chlorogenic acid	*Heydichiun coronerium*	DPPH,	[95]
9.	Linoleic acid	*Achillea santolina*	DPPH	[5]
10.	Glutathione	*Adiantum capillus-veneris*	DPPH, ABTS,TPTZ	[81], [80]
11.	Flaven-3- catechin , procyanidins	*Agrimonia eupatoria*	DPPH, ABTS	[5]
12.	Quercetin, kaempferol,Isoquercetin,	*Ailanthus altissima*	DPPH, FRAP, ABTS	[3]
13.	Isorhamnetin-3-O-[αl-rhamnopyranosyl-(1 --> 3)]-β D-glu.	*Alhagi maurorum*	FRAAP, DPPH	[1], [69]
14.	3,4′-O-β-D-diglucoside,	*Allium cepa*	HPLC Method, DPPH	[50], [34]
15.	DipropylTrisulfide	*Allium porrum*	DPPH	[63], [40]
16.	Diallyl disulfide	*Allium sativum*	DPPH	[17]
17.	Quercetin	*Allium schoenoprasum*	DPPH	[49]
18.	1'-acetoxychavicol acetate	*Alpinia galangal*	DPPH	[13]
19.	7-hydroxy-6-methoxy coumarin	*Althaea officinalis*	ABTS, DPPH	[14],[104]
20.	Carbon trichloride	*Ammania baccifera*	SOD	[69],[25],[99]
21.	3- methoxylated, rhamnetin	*Ammi visnaga*	DPPH	[13]
22.	A-linolenic acid	*Anchusa italic*	DPPH	[5]
23.	4′-O-β-D-glucopyranoside	*Anchusa strigosa*	DPPH, ABTS	[5]
24.	N-hexane fraction	*Antirrhinum majus*	HPLC, GCMS	[87],[5]
25.	Diallyl disulfide, Anthocyanins	*Arachis hypogaea*	DPPH	[105],[24]
26.	Quercetin	*Arctium Lappa*	DPPH	[76],[91],[5]
27.	Aesculetin, Quercetin	*Artemisia campestris*	TBARS, DPPH, ABTS	[60],[5]
28.	Rutin	*Asparagus officinalis*	DPPH, ABTS	[26],[16]
29.	Trifolin	*Astragalus hamosus*	RGP,DPPH	[18]
30.	Catechin	*Avena sativa*	DPPH	[45]
31.	Quercetin	*Aronia melanocarpa*	DPPH	[33]

1.3 Health Benefit of Antioxidant

Antioxidants have concerned in relation to radical and stress, and different therapy and also it's effective the long life [43,74,70]. Phenolic compound and polyphenols is target analyte. The final HORAC in such many cases; they are detected a target by several enzyme such as tyrosine's or

another phenol oxidizes, or even by plants tissue containing these enzyme [53]. To make certain the best defense against for the different disease caused by different oxidative stress agents, such as cancer, heart disease, prevented from the fewer processed staple food like fruits and vegetable for the epidemiological study. [37, 74, 10]. The valuable health effect of different antioxidant is present in different fruits and vegetable [104]. According to the dietitian consensus that all plant-based diet rich in fruit and vegetable pulses and minimally processed starch staple food are reduced the risk development of these diseases significantly. All the recordation, basically in based on epidemiological studies are that fruits, vegetables and less processed staple foods are provided the protection of the expansion of sickness [37,35]. Various type of antioxidant are present in the plant this are containing the compounds, like benzoic acid, phenol, carotenoids, flavonoids proanthocyanidins, coumarins, stilbenes, lignin's. Various plant extract play a very important function in protective of the oxidative agent [78, 45]. Various type of fruit juice are contains the high amount of antioxidant properties such as polyphenols, vitamin C, vitamin E, carotene, and many other [37,74]. The potential of antioxidant reflection of food stuffs is depended on the cooperative and redox communication surrounded by the different molecule present in food [83, 74]. When the conducted of regional surveys for antioxidant, so environmental differences in food constituent is also considered. If the investigation of connection between dietary antioxidant and pathogenesis induced by the oxidative stress in the compare with total antioxidant potential, it is very relevant tool [74, 10]. Various degenerative diseases caused by oxidative stress such as cancer, Parkinson, Alzheimer etc is also prevented by plant derived antioxidant [39, 29, 5]. In this overview we are presenting two type of mechanism, first is hydrogen atom transfer (HAT) reaction mechanism, and single electron transfer (SET) reaction mechanism, which is most relevant to human biology [74]. Spectroscopy is another major method for used the determining the antioxidant, in this technique is relies on the reaction of radical, it is (HAT) based technique, for the assay of antioxidant, this technique has following method, DPPH, ABTS, FRAP, PFRAP etc are used. Apart from these in recent time electrochemical techniques is used for determination of antioxidant through the measurement of intensity of the due to the current generated by the oxidants of electro active analyte [74].

1.4 Mechanism of Antioxidant Activity

The antioxidant activity mechanism has been based on hydrogen atom transfer mechanism; it is two type of antioxidant mechanism there are the differentiation between hydrogen atom transfer (HAT) and single electron transfer (SET) [89]. In the mechanism of (HAT) and (SET) reaction, The end of result is the same, apart from of mechanism, but these mechanism are different for the kinetics and probable for surface reaction, proton- incorporated electron transfer and HAT reaction

occurred in corresponding, and the mechanism dominant in a giving will be decisive by antioxidant structure properties, and partition coefficient and system solvent are determining factor for the efficiency of antioxidant is determined by bond dissociation energy and ionization potential (IP) [65]. Certainly, procedure is desired that involves measurement of two to three properties because phenolic compounds like polyphenols have been shown the multiple activities, and medium of the substrate for the testing are decided by the dominant activity of compound [78, 52].

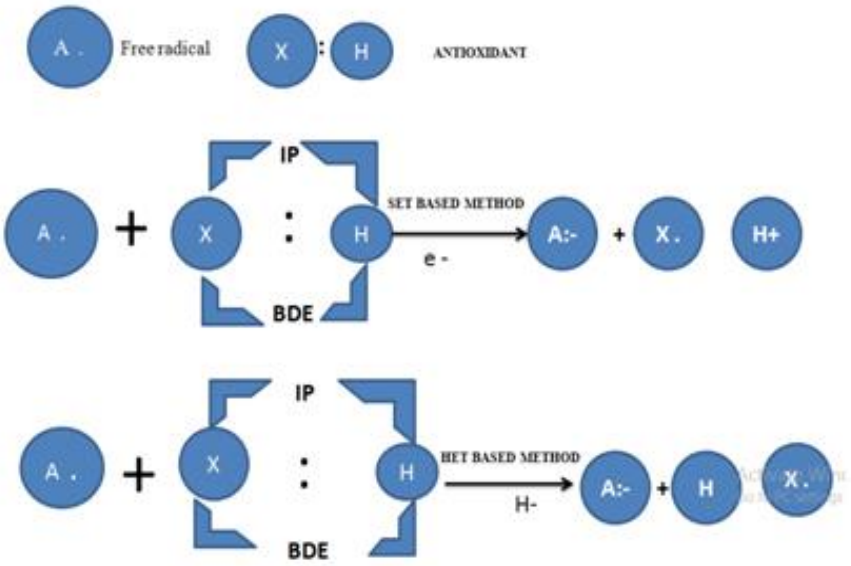

Fig 1: Mechanisms of antioxidant react with free radical: single electron transfer (SET) and hydrogen atom abstraction (HAT). For SET mechanism, the IP of the antioxidant is the most important energetic factor in evaluating the antioxidant action. The BDE (bond dissociation enthalpy) of the antioxidant is the important parameter in evaluating the antioxidant action in a HAT mechanism.

1.4.1 The Mechanism of Single Electron Transfer (HAT) Analysis

The HAT method measurement used for the antioxidant is classical ability, this method is depend in the reduce free radicals by hydrogen donation and any other donor [78, 52]. Many scientists feel these are most relevant to reactions where act very typically. In HAT method relativity for the reaction is determined by the Bond dissociation enthalpy for the grouping of H-donating group in potential antioxidant [52]. Competition kinetics is used for antioxidant reactivity or capability measurements. Basically pH is independent and HAT reaction are solubilizing agent, this reaction is quite rapid, and completed by minutes. HAT analysis can be lead high apparent reactivity for the presence of scavenging agents, including metals [78].

1.4.2 Single Electron Transfer Mechanism (SET) Based Method Mechanism

The determination of ability of the potent antioxidant, in this method antioxidant was transfer one electron for decries the oxidative rate on compound in including carbon, radical. [78, 89].

$$Y^* + AH \longrightarrow Y^- + AH^{*+} \quad (1)$$

$$AH^{*+} \xrightarrow{H_2O} A^* + H3O^+ \quad (2)$$

$$Y^- + H3O^+ \longrightarrow YH + H2O \quad (3)$$

$$M(III) + AH \longrightarrow A\,H^+ + M(II) \quad (4)$$

These two (SET and HET) are same mechanism together with the all sample, for the help of structure of sample and pH of the sample balance are determined. SET method is based on the primary exclusion of the hydrogen atom for reactive functional group. SET reaction based on pH, increasing of the pH so, IP value is decried in the SET method, this method pH and IP value is inversely proportional [78,90]. pH is increasing for the process of de-protonation because of the electron donating capacity. The reaction of the SET based method is very slow and can required long time for the completion, therefore antioxidant capacity is calculated by decreasing value for comparison of kinetics [52]. SET method is very susceptible to same acid like ascorbic acid and uric acid, which is important in maintaining plasma redox tone, and reducing polyphenols are also detected. This reaction is very sensitive, trace component and same contamination particularly metals interfere with SET methods [78].

1.5 Bioassay of Different Antioxidants Activity Methods

Plants are containing the various type of essential oil, this oil is most effective antioxidant in all the essential oil and pure compounds can be analyzed using various in vitro assays. Like Spectrometry technique, electrochemical techniques and Chromatography techniques.

1.5.1 Spectroscopic Technique for the Evaluation of Antioxidant

In an assessment of antioxidant through the spectrometric method technique relies for the interaction of radical's, free radical contain or compound with an antioxidant a molecule capable to denote a hydrogen atom.

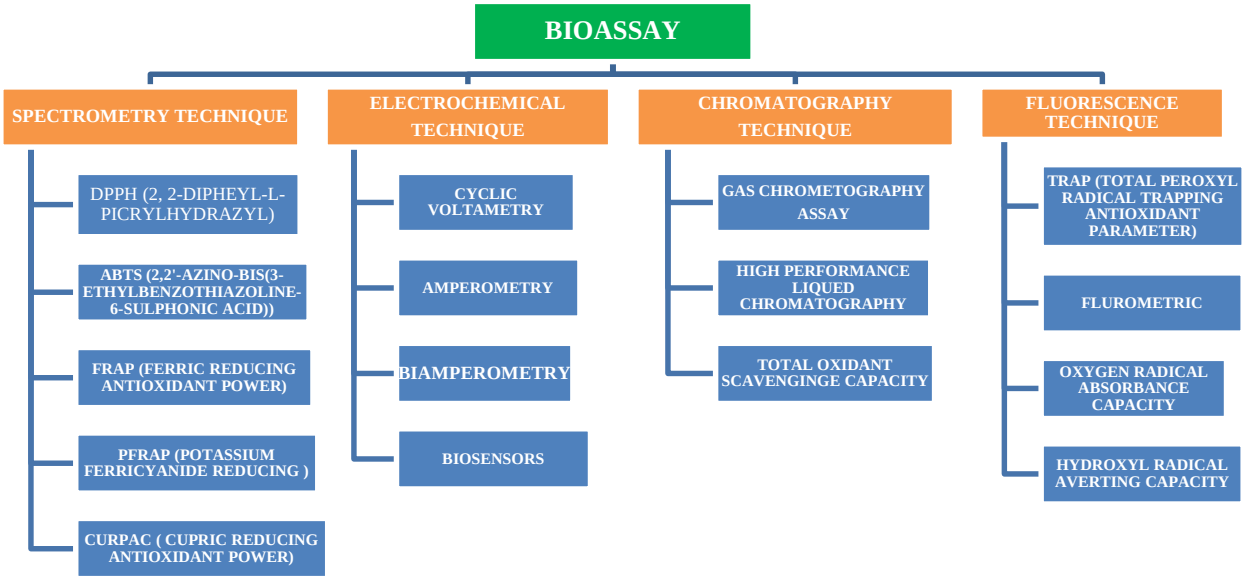

Fig No 2: Graphical representation of different methods of Antioxidants bioassay

Table no. 2 Different Spectrometric techniques of Antioxidant activity assay

S. No.	Antioxidant capacity assay	Principle	Dissolving agent	Techniques	Limitations	Reference
1.	DPPH	Antioxidant was react with an organic cation radical	DMSO (dimethyl sulphoxide)	Colorimetry	DPPH also is decolorized by reducing agent as well as H transfer	[4],[56] [74],[96]
2.	ABTS	organic cation radicals are react with the antioxidant solvent	ACS water	Colorimetry	many phenolic compound have low redox potentials can be thus react with ABTS, its also the TEAC reaction may not be the same for slow reaction, and it may take a long time to reach	[4] [56],[6], [71],[7]
3	FRAP	Antioxidant reaction with a Fe(III) complex	Acetate buffer	Colorimetry	The FRAP assay dose not measure thiol antioxidant, such as glutathione	[72],[27], [72]
4.	PFRAP	This method was reacting in antioxidant determination for the Reduction of, Potassium ferricyanide reduction for the help of potassium ferrocyanide Fe3+.	Potassium ferricyanide	Colorimetry		[19],[74]
5.	CUPRAC	the antioxidants Cu (II) was reduction to Cu (I)	Cupric Sulphate (CuSO4)	Colorimetry	The CUPRAPC assay is complete in minutes for ascorbic acid and so all, but requires 30- 60 min for more complex molecule	[8],[9],[62]

1.5.1.1 Assessment of Antioxidant through the DPPH Method

DPPH (2, 2-dipheyl-l- picrylhydrazyl) is stable free radical, because of decolorized after reduction with an antioxidant (AH) or a radical (R) thus not dimerize as happens with most free radicals [98]. If the DPPH molecule determination, the molecule will be the occurrence of a purple color. If DPPH react with electron donor, the reduced form is generated accompanied, disappearance of the violet color [78, 98]. Absorption band was set in a maximum around 520 nm to determination in fruit juices and extract [78, 96, 71]. In this method radical is reduced by antioxidant therefore absorbance was decreases. In other investigations contrast of the absorbance was measured at a wavelength of 550 and 517 nm. The 550 wavelength is facilitated by the measurement of the stable free DPPH radical, this DPPH radical was stable with interference pigment [95]. This method was stabiles for the estimation of the methanolic compound extract for two different DPPH concentrations [98, 79]. After the estimation of methanolic extract change in absorbance is calculated with help of IC 50 value this formula was determined the decrease the absorbance of DPPH by 50 %.It calculated by the graphical representation of different solution of the essential oil [98].

1.5.1.2 ABTS Method

ABTS radical method is used in assays for the determined for the concentration of free radicals [93]. ABTH method contained the positive radicals (ABTS•+) which absorbs Abs 743 nm and its view as bluish –green color due to the loss an electron by the nitrogen atom ABTS (2,2- azino-bis (3-ethylbenzthiazoline-6-sulphonic acid)[78,89]. In the presence of another hydrogen-donating antioxidant ascorbic acid, the nitrogen atom quench by the hydrogen atom, and yielding the solution decolonization. Various oxidizing agent of ABTS, such as Manganese dioxide and potassium– persulphate, these are giving rise the ABTS contain radical (ABTS•+). In the presence of ascorbic acid the absorbance of ABTS was diminution at 743 nm method; this absorbance was monitored by spectroscopy [74, 93]. ABTS method are based on spectroscopy method this ABTS contain radical are decrease in nm, this method is applied for the antioxidant determined for food extract [74].This reaction is monitored by spectrophotometrically method by the change of the absorption spectrum [96].

1.5.1.3 FRAP Method

This is another method in determination of antioxidant, in the FRAP (ferric reducing antioxidant power) method is based on the decreasing of ferric –tripyridyltriazing by antioxidant present in the sample, blue color product is forming in the presence of ferrous tripyridyltriazing, this method legend bind with Fe2+ ion and forming navy blue color [96]. The antioxidant assay was reduced the ferric to ferrous ion, theses assay is the only method that directly measures in the sample and caperedto the other assay measuring inhibition of free radicals [72,27]. On the bases of Absorbance

and test the amount in iron reducing can be correlated with the amount of antioxidants [96].

1.5.1.4 The Potassium Ferricyanide Reducing (PFRAP) Method

In the PFRAP method antioxidant extract measurement by calorimetric technique. Increased absorbance indicated that reducing ability of antioxidant extract. In this method firstly any compound contain with antioxidant capacity reacted with potassium ferricyanide, an altered with ferrocyanide and furthermore ferric trichloride reacted and yielding ferric ferrocyanide, and formed blue the color complex, this color is showing the maximum absorbance at 700 nm [78, 19].

1.5.1.5 The CUPAC (Cupric reducing antioxidant power) Assay

The CUPAC assay method is the wildly accepted method, it these method standard antioxidant extracts is mixed with Copper (II) sulfate and neocuproine. After this mixture absorbance was measured through the Colorimetric technique [78]. This technique was used by utilizing the copper (II)-neouproin [Cu (II)-NC] chemical reagent, this reagent act as the chromogenic oxidizing agent for indexing for nutritional polyphenols and vitamin C and E. This method is done for the copper (II) reducing capacity of polyphenols is measure [8].This method based on mixing of antioxidant solution like chloride and cupper (II) solution, and alcoholic solution a neocuproine, ammonium acetate buffer solution was maintained at pH 7, absorbance will be measured at 450nm after 30 min [8].

Table No. 4: Electrochemical Techniques of Antioxidant activity assay

S. No.	Antioxidant capacity assay	Principle	Dissolving agent	Techniques	Limitations	Reference
1.	Cyclic voltammetry	The potential of a working electrode is linearly varied from an initial value to a final value and back, and the respective current intensity is recorded	DMSO(dimethy l sulphoxide)	Measurement of the intensity of the cathodic/ anodic peak	Determined compounds with low oxidation potential.	[9], [46], [96], [74]
2.	Amperometry	The potential of the working electrode is set a fixed value with respect to a reference electrode	KCl, Phosphate buffer	Basedon oxidation/reduction of an electroactive analyte	Low biological material stability	[18],[75],[100]
3.	Biamperometry	The oxidized form of reversible indicator redox couple is reacting with antioxidant with the oxidized form.	ACS water, DMSO(di-methyl sulphoxide)	Based on reversible redox couple	Its selectivity depending on the specificity of the reaction involving the oxidized or reduced form of the redox pair and the analyst.	[100],[75]
4.	Biosensors method	Intensity the result is depends on the current being proportional to residual concentration of control solution.	Phosphate buffer solution.	the analysis of the antioxidant was measured by spectrophotometer	Low biological material stability	[100],[20], [32]

1.6. Electrochemical Techniques

1.6.1 The Cyclic Voltammetry Method

The potential dynamic electrochemical are used for this method. This method was analysis using bio analytical system at 100 electrochemical analyzer [46, 82]. Cyclic voltammetry is working electrode potential. It's a glassy carbon electrode which is cleaned by aluminum powder and Ag/ Ag No^3 was used as a reference electrode for the analysis [46]. In this method is scanned for the initial to final value of the working potential for the electrode while recording the respective current intensity. This method is useful for the quantification of the molecular weight of antioxidant capacity of plant extract [96].

1.6.2 Amperometric Method

The amperometric method current flow intensity is measured by two different electrodes, these two electrodes one is the working electrode and another is a reference electrode, for the applied value of potential. Current is generated by process of oxidation and reduction [96, 18]. This reaction is done for the reduction of 2, 2-diphenyl-1-picrylhydrazyl for analysis of DPPH method [18].At the glassy carbon; electrode is determined by the antioxidant activity. Ethanolic solution a KCL, phosphate buffer and electrode electrochemical cell will be used for all the experiment for method, evaluation of antioxidant activity of beverages and tea industry, will be applied for the method. [101, 61].

1.6.3 The Biomperometric Method

This method is based on the measurement of the current flowing between two identical working electrodes polarized at a small potential difference and immersed in a solution containing a reversible electron couple. A common electron pair chosen in biamperometric studies was DPPH [100]. Antioxidant are react with the free radical such as DPPH and generated the reduced form, in this method the intensity the result is depend on the current being proportional to the residual concentration of DPPH. In the biometric method the potential deference between two electrodes is depending upon the controlled parameter. The potential value of the two different electrodes is not controlled by the reference electrode [61]. The biamperometric detector is reacting with component of the electron couple; this electron couple is present in lower concentration. Operational condition was chosen for oxidized form DPPH• is similar the electron form DPPH•. After the addition of the antioxidant contained redox couple DPPH then concentration of the oxidized form is reduced and concentration of the reduced form is increased which generate a current in proportion of the concentration of the antioxidant. This cathodic current is the limited by the lower concentration of the DPPH in the mixture of this method [61, 78].

1.6.4 Biosensors Method

The biosensor applications often used oxidoreductases, Because of their electron transferring

properties during catalysis. Oxidoreductase enzyme offer the advantages of being stable and this enzyme are not required coenzyme for some situations. Other electron–proton couple used in biamperometric method for antioxidant is peroxidase enzyme, for produced tubular flow-through reactor. This bioreactor performance was tested by different concentration of immobilized enzyme, and hydrogen peroxide [31, 77, 23]. Evaluation of antioxidant is Important applications of biosensors, it is also monitoring of superoxide (O2•) radical, nitric oxide, and monitoring of various phenolic compounds like glutathione, uric acid ascorbic acid [82, LD *et al.,* 2007). An electrocatalytical evaluation of total scavenging capacity was constructed by carbon paste DNA-based biosensor [78,12]. This method is based on the damage of the DNA layer, it is damage for the absorbed in electrode surface by OH radicals; this method is generated for Fenton reaction, and subsequent electrochemical oxidation of the whole adenine bases, to generate an oxidation product that was able to catalyze the oxidation of NADH [69]. The presence of antioxidant compound scavenging activity is depend by un- oxidized adenine molecules and hydroxyl radical, after the scavenging the un-oxidized adenine molecule and hydroxyl radical electrolytic current of NADH was increased, this increases was measured by differential pulse voltammeter. The finding of amount of ascorbic acid in aqueous solution is possible for biometric method [78]. Several plants are contained polyphenols these polyphenols are the main content of antioxidant. Several amperometric biosensors have been developed, for the detection these biosensors are detected for phenolic compound on the basis of enzymes, such as lactase, peroxides, and tyrosinase (16,32,78]. Enzyme is based on biosensors; it is allow the evolution of the “total phenol content”. Since the tyrosinase acts on the hydroxyl group of phenolic compounds, the determination by enzyme electrodes activity obtaining through the total amount of –oh group of red wines. In the biosensor method spectrometer is used for the detection of antioxidant activity in wine and all type of fomenter product [20,47,78]

1.7 Chromatography Technique

1.7.1 GAS Chromatography Method

This method is new and advance technique of chromatography; it is also useful for the determined by antioxidant activity [67]. For use the separating and analyzed the compound that can be vaporized without decomposition. In this method compound mixture are separated is two phase first is stationary phase this phase is liquid and the second phase is gas mobile phase. With the help of chromatographic method determined oxidative scavenging capacity of turmeric oil [48]. Gas chromatography with flam isolation detector and Gas chromatography attached with mass Spectrometry are analyzed by turmeric oil and fraction [96, 35].

1.7.2 High Performance Liquid Chromatography Method

HPLC is one of the methods to determination of different type of antioxidant profiling. In this

method is typically utilized by various type of stationary phase, a pump are moving the mobile phase, it is transfer to analyze for the help of column, after that detector was provided a retention time for characteristic to the analyte [96,106]. HPLC is providing information related to different phytochemical such as phenol and phenolic component of the plants and detector are providing retention time that show the different characteristic during analyst. For the free radical identification is done by the comparing with standards of identified compound for the help of retention time of running the sample after the addition of pure standard and absorbance spectrum profile [101]. The Pump is providing higher pressure that enquired to move the mobile phase. After the separation detector provides characteristic information of analyte. The separation of the plant samples on the HPLC column the elate was heading for to a PDA (photodiode array) detector and then mixed with a stabilized of the ABTS solution it is cation radical and the solution was heading for to a detector monitored of absorbance. The ABTS cation solution is appearing deep blue color and any invigorating of the results of solution is defeat of color indicated by a negative peak on the HPLC trace [78,101].

1.7.3 Total Oxidant Scavenging Capacity (TOSC) Method

The total oxidant scavenging Capacity was developed to quantification of the absorbance capacity of antioxidant, this method was specifically quantified by three Significance oxidants such as peroxyl radicals, hydroxyl radical and peroxynitrite [30]. This method is very important in the term of being biologically relevant radical sources. For this method R-keto-c- methiobutric acid (KMBA) is used oxidization, and this is forms ethylene. The formation time of ethylene is followed by headspace analysis, this analysis is done separation of gas chromatography, this method for quantification of antioxidant capacity is determined by gas Chromatography and the quantification of the ability of antioxidant for scavenge of ethylene formation is determined by relative to a control reaction [28]. This method is permits the quantification of the antioxidant capacity for using gas chromatography to the measurement of high- throughput analysis for antioxidant [30].

Table No. 4: Chromatographic techniques of Antioxidant activity assay

S. No.	Antioxidant Capacity assay	Principle of the used method	Dissolving agent	Techniques	Limitations	Reference
1.	Gas Chromatography Assay	Liquid phase are play the important role in separation of the compound.	Hydroxyaniso le (BHA)	Flame ionisation or thermal conductivity detection	It is very sensitive method on the any type of contamination.	[67],[74]
2.	High performance liquid chromatography Assay	repartition of mixture are depend on the separation of compound between stationary phase and a liquid mobile phase with different polarities, at high flow rate and the pressure of mobile phase	ACS water,	UV-VIS (e.g. diode array) detection, fluorescence, mass spectrometry or electrochemical detection	The analytical signal is sometimes difficult to measure and does not account for all antioxidants.	[106],[101],[96]
3.	Total oxidant Scavenging Capacity (TOSC) Method	Determination of the absorbance capacity of the antioxidant with oxidized for R- keto-c- methiolbutyric acid, and forms ethylene.	ACS water,	Chromatography	The main limitation of this technique is the long reaction time and the necessity of multiple chromatographic analyses for each experiment.	[30],[7],[74]

1.8. Fluorescence Method

1.8.1 TRAP Method

The TRAP method the ability of the luminol-enhanced chemiluminescence (CL) antioxidant compound to interfere with the reaction between peroxyl radicals generated by AAPH method .The basic reaction of this method was used in measure the antioxidant scavenging activity against the peroxyl radical, and determination of antioxidant as a time of consuming, by the extension of the lag time for appearance of the oxidized probe when antioxidants are present [74,78]. The TRAP method has been used in chemistry for uptake the oxygen the basic reactions assay is almost identical to those of ORAC. This assay is basic required for the probe are reacted with ROO at low concentration, oxidized probe and radical chain in the dramatic change in spectroscopic reaction away from probe oxidation should occur [9].The help of fluorescence oxidation probe is followed optically have been a determination of antioxidant activity by the time consumed, percent reduction of reaction by extension of the initial time is appearance of the oxidized probe when the antioxidants are present [78]. Trap value which is used for reaction time of the sample that can be compared to the corresponding time of trolox. TRAP method problem is may be comparisons between laboratories are difficult is various method in this method last stage have been used for comparison [78, 90].

1.8.2 Fluorimetry Method

Fluorescence is used for the determination of fluorimetry method. In this method the emission of light is determined by a substance that absorbed light or other electromagnetic radiation of

different wavelength. Many cases light wavelength in longer and therefore energy is lower, than the absorbance radiation. Florescence is release, when an orbital electron of a unwind state to emitted of photon of exited state by some type of energy [79, 82]. With the help of florescence spectroscopy is determined antioxidant through the fluorescence assay. It is also applied for determined by the same phenolic compound. Quantify the butihylhydroxyanisole (BHA and tert-butylhydroquinone antioxidant concentration in biodiesel produced from sunflower and soybean oil are proposed for fluorescence spectroscopy. The evaluation range is 320-800 nm in fluorescence and spectra is obtained under excitation at about 310 nm. To the strength of antioxidant affect for organization of membrane of sterol Assess of possible adverse effect lipid-soluble antioxidant is use to this information, which was reported in recent studies. In this method presence of antioxidant, is reflects the strength the antioxidant. The time is producing free radical-induced through the sterol oxidation in lipid vesicles reflects [74].

Table no. 5: Fluorescence techniques of Antioxidant activity assay

S. No.	Antioxidant Capacity assay	Principle of the used method	Dissolving agent	Techniques	Limitations	Reference
1.	TRAP	Antioxidant capacity to scavenge luminol-derived radicals, generated from AAPH decomposition	2,2'-azobis-(2-amidino-propane) dihydrochloride (AAPH	Chemiluminescence Quenching	Many different end points have been used, so comparisons between laboratories are difficult. It is relatively complex and time consuming. It also requires a high degree of expertise and experience	[74],[75],[78]
2.	Fluorimetry	Emission of light by a substance that has absorbed light or other electromagnetic radiation of a different wavelength	Butylhydroxyanisole (BHA)	Recording of fluorescence excitation/ emission spectra	Nil	[78],[74]
3.	ORAC	Antioxidant reaction with peroxyl radicals, induced by AAPH,2'-azobis-2-amidino-propane)	2,2'-azobis-(2-amidino-propane) dihydrochloride (AAPH)	Loss of fluorescence of fluorescein	Limitation of ORAC is the area under the curve, its move toward to measure the protective effect of antioxidant, and the relativity of fluorescent is low on the way to ROO• Radicals that proceeding is out the result for antioxidant quality.	[22],[7],[92],[74]
4.	HORAC Assay	Antioxidant capacity to quench OH radicals generated by a Co(II) based Fenton-like system	Gallic acid solutions	Loss of fluorescence of fluorescein	Nil	[2],[74]

1.8.3 ORAC Method

The OROC (oxygen radical absorbance capacity) assay this is another method for determined antioxidant. Inhibition percentage are used for antioxidant in this method is combined for both

inhibition percentage in this methods that combines both inhibition percentage are used [22].The method is based upon the early work were further developed by this method [74, 30]. ORAC was used in human plasma pure antioxidant compounds and antioxidant food compound has been largely applied to the assessment of free radical [22]. The ORAC method is measured by the induced oxidation and peroxyl radical of oxidative scavenging capacity for Hydrogen atom transfer by the breaking classical radical chain. Antioxidant was measured by this method for the help of antioxidant scavenging activity agents the peroxyl radical [96]. In this method is used fruit extract of guava, and grape fruit for methanol extract. Fluorescing is using as the fluorescent probe. If the fluorescence is loss, this is indicator of the extent of the decomposition from the peroxyl radical reaction [96].This method provides for peroxyl radicals as controllable source, reactions of antioxidants with lipids simultaneously food and physiological systems, and it can be adapted to detect as well as hydrophilic and hydrophobic antioxidants by altering the radical source and solvent the reaction has been determined [78].

1.8.4 The Hydroxyl Radical Averting Capacity (HORAC Method)

This method was depended for the measurement of the metal- chelating activity of antioxidants. This method uses as Evaluation of protecting ability the hydroxyl radical co (II) complex and therefore against the formation of hydroxyl radical [96]. For the blank sample, phosphate buffer solution is used. 100, 200, 600, 800 and 1000 μM standard antioxidant solutions (in phosphate buffer 75 MM, pH 7.4) are used for building the standard curve. In this method hydrogen peroxide is prepared in distilled water by dissolving agent, of picolinic acid. The initial fluorescence is measured after which the readings are taken every min. calculating the value of HORAC are used for regression equation for standard scavenging concentration and their total area of curve [2].

2. CONCLUSION

For the health benefit understanding the free radical and provide interest gained by plant based Antioxidant activity. In the recent time various industries used plant based antioxidant compound, these compound are present in plant in low molecular weight. For the determined by Antioxidant activity various methods are available. These techniques are determining the various types of Antioxidants with the help of various instruments. These all method is only determining qualitative and quantitative estimation of antioxidant, but these methods is not determining the gravening factors of antioxidant capacity. Antioxidant material will be preventing the occurrence of oxidative-stress related diseases, these oxidative –stress related diseases are started the attack of free radicals on key bio-components. Were antioxidant was play very significant role in our body but recently there are various problem in the practical application of herbal antioxidant therapy, it dose and duration of therapy to be employed, such as nature of antioxidants is play the different role.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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