REVIEW ON PRINCIPLES OF ANTIOXIDANT METHODS

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ABSTRACT

The antioxidant is defined as any substance that could put off or help oxidative harm by way of loose revolutionaries. The antioxidant inhibits the oxidative harm with the aid of unfastened revolutionaries through several mechanisms. The assets of the antioxidant are abundantly available in nature and may be set up in the diurnal food plan similar as fruit, veggies, seeds, nuts, leaves, roots, and barks. several principal composites related as antioxidants comparable as polyphenols, vitamins, and carotenoids. This composition provides a trendy summary of the most common in vitro patterns for determining antioxidant exertion. It emphasizes the operating principle, method, advantages, and drawbacks of various styles. The dedication of antioxidant exertion could not simplest comply with one single approach due to the diversity of antioxidant mode of conduct. accordingly, the selections of the applicable system of analysis need to be considered to achieve the reason of the exploration.

Key words: antioxidant activity, spectrometry, free radical , reactive oxygen species.

I. INTRODUCTION

The document of world health corporation (WHO) noted the burden of non-communicable diseases (NCDs) (e.g.cancer, cardiovascular sicknesses, continual respiration diseases, neurodegenerative sicknesses and diabetes) in many nations, as their numbers are regularly growing (Grosso G 2018). It money owed for almost -thirds of annual mortality, mainly in low- and center-income international locations (Engelgau M.M et al. 2018). The NCDs have been related with unhealthy existence such as imbalanced weight-reduction plan (excessive calories and fat intake) and mild physical pastime. Besides, the prevalence of NCDs has been related to oxidative stress, wherein the manufacturing of free radicals [reactive oxygen species (ROS) and reactive nitrogen species (RNS)] and the biological antioxidant defense gadget is not proportional (Sies H et al. 2005). ROS include superoxide anion radical ($O_2^{\bullet}$), singlet oxygen ($^1O_2$), hydroxyl radical ($^{\bullet}OH$) and perhydroxyl radical ($HO_2^{\bullet}$) (Ahsan et al . 2003). RNS consist of radical nitrogen oxide ($^{\bullet}NO$), nitrogen dioxide ($^{\bullet}NO_2$), and non-radicals S-nitrosothiols, peroxynitrite ($ONOO^-$), nitroxyl anion (NO^-), nitrate (NO_3^-), nitrosonium cation (NO^+), dinitrogen trioxide (N_2O_3), dinitrogen tetroxide (N_2O_4), nitryl chloride (NO_2Cl), and nitrous acid (HNO_2) (Saddhe A.A et al.2019). The loose radicals are volatile, reactive, and have a tendency to capture other stable molecules. Consequently, if the number of loose radicals is in high concentration, the oxidative tissue harm can occur within the body (Huang Y.J and Nan G.X 2019).

Antioxidants are compounds which, found in meals or the human body in very Low concentrations, delay, control or save you oxidative processes leading to meals quality. Deterioration of food or the occurrence and propagation of degenerative illnesses within the organism. Some of techniques are concerned in the technique of inhibiting the oxidation
By means of those antioxidant compounds (Shahidi F and Zhong Y 2015). Fats-soluble antioxidants are critical in preventing the peroxidation of polyunsaturated Fatty acids (PUFA) in biological membranes. Water-soluble antioxidants, like nutrition C, Play a key position in neutralising ROS within the hydrophilic segment. This evaluation provides a fashionable and updated evaluation of methods available for measuring Antioxidant activity and the chemistry precept behind them. Similarly, the maximum Critical advantages and shortcomings of each technique had been analysed and highlighted.Understanding the principle mechanisms, benefits and barriers of the dimension Assays is important for correct choice of technique(s) for legitimate evaluation of antioxidant Potential in practical applications.(Irina Georgiana Munteanu and Constantin Apetrei 2021).
The part of exogenous antioxidants comes into play when endogenous species are unfit to give full protection against the reactive oxygen.Vitamin C, vitamin E, vitamin D, vitamin K3, flavonoids, minerals and β- carotene are among the utmost effective and important exogenous antioxidants used as active constituents in utmost medicinals and food supplements. composites like butylhydroxytoluene, butylhydroxyanisole, gallates etc are among the synthetic exogenous antioxidants, while they can be attained from natural sources as well like flavonoids, vitamins, anthocyanins and some minerals (Litescu SC et al. 2011).In order to help dangerous goods of free revolutionaries in the body, also for the regression of food ingredients and fats, interest in the use of antioxidants is fairly adding (Molyneux P.2004).

II Benefits of antioxidants

The relation of antioxidants to the cancer prophylaxis, remedy, life and oxidative stress has gained notable attention in recent days (Kalcher K et al.2009). Recent studies suggest that the conditions related to oxidative stress like cancer, coronary heart complaint, hypertension, rotundity, diabetes and cataract are stylish defended by the use of vegetables, fruits and lower reused foods (Halvorsen.B.L et al.2002).This is made possible due to the positive health goods of vegetables and fruits containing salutary antioxidants. Walnuts, cranberries, blackberries, strawberries, blueberries, snorts, brewed coffee, cloves, grape juice, unsweetened chocolate are at the top of the food list containing antioxidants. On assaying 50 food particulars containing high quantum of antioxidants, 13 of them were spices, 5 were berries, 8 of them were vegetables and fruits, 5 were cereals and 4 of them were nuts (Halvorsen.B.L et al. 2006). Antioxidants regulate colorful oxidative responses naturally being in apkins. likewise, terminates or retards the oxidation process by scavenging free revolutionaries, chelating free catalytic essence and also by acting as electron benefactors.
A diet high in foods of animal origin and impregnated fats increases the threat of cardiovascular conditions and some cancers (Pierart Z.C et al.2006). which has generated interest in promoting the consumption of factory- deduced proteins (Espin J.C et al.2007). Legumes similar as cereals, fruits, and vegetables have health- promoting composites and nutritive value (Shetti A et al.2009). The nutritive quality and nutraceutical content associated with the antioxidant exertion of legumes similar as common bean are important sources of

nutritive factors (proteins, carbohydrates, fiber, vitamins, and some minerals). (Duarte-Martino HC et al.2012).

In vitro antioxidant methods through several mechanisms of actions makes the comparison of each antioxidant method impossible. However, the *in vitro* antioxidant approach can provide a measurement of the effectiveness of compounds. Generally, the assessment of *in vitro* antioxidant activity can be divided into two main mechanisms. The first category is assessment in relation to free radicals transfer (hydrogen atom transfer, single electron transfer, or the combination of both). The second category is related to the evaluation of the damaging effect on biological markers and substrates, which is based on lipid peroxidation (Litescu S C et al.2010). In vitro testing of antioxidants using free radical scavengers is relatively easy to perform. Among the free radical scavenging methods, the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method is faster, simpler (i.e., does not require many steps and reagents) and less expensive than other test models. On the other hand, the decolorization assay of 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diamonium salt (ABTS) is applicable to both hydrophilic and lipophilic antioxidants.

III Discussion

1. *DPPH (2,2-diphenyl-1-picrylhydrazyl) Scavenging Activity*

The DPPH assay is very easy and rapid method for manual analysis of antioxidant content. This method can be used for solid or liquid samples and is applicable not only to specific antioxidants, but also to the total antioxidant capacity of the sample. The DPPH test is based on the ability of the stable free radical 2,2-diphenyl-1-picrylhydrazyl to react with a hydrogen donor (Inoue K et al 2005 and Fahey JW et al. 2002). The DPPH assay method is based on the depletion of DPPH, a stable free radical. The free radical DPPH with an odd number of electrons gives an absorption maximum at 517 nm (purple). When antioxidants react with DPPH, stable free radicals are reduced to DPPHH in the presence of hydrogen donors (e.g., free radical scavenging antioxidants), resulting in reduced uptake compared to DPPH. The radical form of DPPH-H causes a color change (yellowing) depending on the number of electrons captured. The stronger the discoloration, the higher the elasticity. This test is the most widely used model for evaluating the free radical scavenging activity of any new drug. The DPPH radical exhibits a strong absorption spectrum in the UV visible region. When a solution of DPPH is mixed with a solution of a substance capable of donating hydrogen atoms, it gives a reduced form (diphenylpicrylhydrazine; non-radical) that loses the purple color (pale yellow when picrylic acid groups are present). (Bondet V et al.1997). Approximately 4.3 mg of DPPH was dissolved in 3.3 ml of methanol and the tube was covered with aluminum foil to block light. 150 ml of DPPH solution was added to 3 ml of methanol and the absorbance at 517 nm was immediately measured as a control. Take 50 ml of compounds of varying concentrations and a standard compound (e.g. ascorbic acid) and bring to a volume of 150 ml with methanol. Each sample was then further diluted to 3 ml with methanol and DPPH was added to each 150 ml. The absorbance was taken after 15 minutes at 517 nm using methanol as blank on UV-vis spectrometer. The IC50 values for each drug compounds as well as standard preparation were calculated. The free radical scavenging activity was calculated using the following formula:

% scavenging=[Absorbance of control−Absorbance of test sample/Absorbance of control]×100 .The effective concentration of sample required to scavenge DPPH radical by 50% (IC50 value) was obtained by linear regression analysis of dose-response curve plotting between % inhibition and concentrations. The best way to compare antioxidant activity between samples is by using IC50 values. The inhibitory concentration value (IC50) is defined as the sample concentration required for 50% free radical inhibition. IC50 is determined in triplicate in the graph between residual free radical uptake and concentration in each assay. This test uses quercetin, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), tocopherol and ascorbic acid as positive controls (Ferreira IC et al.2009 and Piljac-Zegarac J et al.2009). The DPPH Assay is a quick and simple test that provides reliable results. It is also widely used to screen for antioxidant properties because it only requires a UV-Vis spectrophotometer to operate. However, this method is sometimes complicated when a test compound has a spectrum overlapping the DPPH spectrum at 515 nm.

2. ***FRAP (Ferric Reducing ability plasma)***

Assay Based on PubMed, the FRAP method has been described and used to measure the antioxidant efficacy in 3400 papers. In this method, the evaluation of antioxidant activity is characterized by the calculation of the low pH mixture of iron (III) and 2,3,5-triphenyl-1,3,4-triaza-2-azoniacyclopenta-1,4-dienechloride (TPTZ) reduction to its ferrous form (II), change the solution into violet-blue color. The changes was evaluated using spectrophotometer at the wavelength of 593 nm (Benzie I F.F and Strain J.J 1996). It has been applied to detect the antioxidant activity of honey (Moniruzzaman M et al.2016). FRAP method has some limitations. The antioxidant activity of slow-reacting compounds such as polyphenols cannot be accurately measured, nor can samples below the physiological pH value (pH 3.6) be measured and could give false-positive results if the samples have lower redox potential value than the redox pair Fe3+/Fe2+(Moniruzzaman .M et al.2016).

3. ***TEAC (Trolox Equivalent Antioxidant Capacity)***

The data searching from PubMed shows that the TEAC assay has been utilized for measuring the antioxidant capacity, approximately 1100 papers.The principle of this method is based on the scavenging of stable ABTS+ (2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) radical by antioxidants in comparison with Trolox. The reduction causes the color loss of ABTS. The color loss is measured at λmax 750 nm. Generally, the polar analog of vitamin E, namely Trolox, is used to be the standard of the antioxidant calculation, and the result is interpreted as Trolox equivalents. The advantages of this method are simple, reproducible, a wide range of samples (e.g. a wide range of samples polarity (hydrophobic and hydrophilic compounds) (Awika J.M et al.2003). However, it should be noted that the different reaction rates between the antioxidants and ABTS radical, cause the different reaction times for the complete measurement of antioxidant capacity (Zheng.L et al.2016)

4. ***ORAC (Oxygen Radical Absorbance Capacity)***

ORAC is one of the popular methods for determining the antioxidant activity according to the data from PubMed, where there are 1610 papers conducting this method. The measurement of antioxidant activity is based on the scavenging activity of oxyradical-induced oxidation of 2,2′-azo-bis-(2-methylpropionamidine) dihydrochloride (AAPH) by samples with antioxidants. In this method, phycoerythrin or fluorescein is used as a target molecule. Over time, the fluorescent signal of these compounds will be lost when the antioxidant compounds

appear. When the solution tested containing antioxidant properties, the fluorescent reduction is inhibited and measured at the excitation wavelength of 485 nm and emission wavelength of 520 nm (Alam Md.N et al.2013 and Awika J.M et al.2003). The test uses Trolox as a reference standard, and the values usually expressed as Trolox Equivalent (TE).The antioxidant capacity results have a correlation with the total fluorescence area under reaction curves where the values of antioxidant are measured by subtracting the area with the blank, which contain no antioxidant (Apak R et al.2007). ORAC method is suitable to be conducted in a microtiter plate with constant temperature for automation of experiments in screening of biological samples matrices, relevant to *in vivo* conditions, considers both inhibition time and degree of inhibition of free radical action, and compliant for various sample matrices, including food (e.g., sorghum, orange juice, blueberries, nuts),pharmaceutical, and blood plasma (Alam Md.N et al.2013 and Prior R.L 2015). However, some difficulties occur when applying this method such as the control of ORAC reaction temperature, reagent concentration, and oxygen (Schaich K.M et al. 2015).

5. ***HORAC (Hydroxyl Radical Averting Capacity)***

The working principle of this assay is the measurement of the antioxidant activity by the calculation of the inhibitory activity of antioxidants against the oxidation of the fluorescent probe (fluorescein) by hydroxyl radicals. This radical is produced through the radical initiation process by the catalyzer hydrogen peroxide (H2O2) and Fenton reagent. Over time, the radicals bind the fluorescein, until the appearance of antioxidant, gradually preventing the oxidation of the probe (Borges R.S et al.2013). The standard of an antioxidant such as gallic acid is used to make the calibration curve. The value of antioxidants is then compared and correlated to the total area fluorescence decay curve (AUC) of the samples. The strong point of this assay is that the value of antioxidant capacity is directly calculated against the hydroxyl radicals produced from the breaking of the hydrophilic chain (Moharram H.A and Youssef M.M 2014). determining the antioxidant activity of vegetables (tomato, parsley, celery, chili pepper, radish, capsicum, eggplant, broccoli) (Číž M et al.2010).

6. ***CUPRAC (Cupric Ion Reducing Antioxidant Capacity)***

CUPRAC is one of electron-transfer based assay for measuring the antioxidant capacity. The method is based on the utilization of the pigment-oxidizing agent (chromogenic), namely bis-copper (II) neocuproine. When the antioxidants mix with the reagent, the reduction of chromogenic probe occurs and changes the color of the solution. The degree of color change is correlated with the concentration of antioxidants in the sample. The change is measured at a wavelength of 450 nm (Ozyürek M et al.2011). The CUPRAC method is simple and adjustable. It is beneficial for high-throughput screening of the antioxidants in matrices of food (egg white, whey protein, gelatin) and blood serum. It was also applicable to measure the hydrophilic and lipophilic antioxidant, due to the high solubility of the reagent in a polar and non-polar solvent (Cekiç S.D et al.2009). In addition, CUPRAC assay works at physiological pH (pH 7) compared to another the electron-transfer assay such as ABTS and FRAP (Apak R et al.2007).

7. ***Total Radical Trapping Antioxidant Parameter (TRAP)***

The TRAP method was firstly described by Wayner *et al*. to measure the total antioxidant capacity of blood plasma or serum (Wayner D.D et al et al.1985). This method is based on the generation of peroxyl-radicals from 2,2′-azo-bis(2-amidinopropane) hydrochloride

(AAPH). The surface of the oxygen electrode can be used to monitor the lag time of oxidation. The induction period of APPH was measured to determine the ability of antioxidants to inhibit the oxidation. Then, the total antioxidant capacity of samples was measured by a comparison of the time interval of the reaction induction and the interval time generated by the reference compound, Trolox.It has been applied to measure the antioxidant activity of vegetables, berries, apple, banana, pepper, and green bean (Pellegrini N et al 2003). This method, however, has a significant drawback, such as lack of oxygen electrode stability. Therefore, some modification has been done by the addition of chemiluminescence such as R-phycoerythrin. This pigment is able to give a brief point where the reaction reaches the completion. The addition of the antioxidants could quench the chemiluminescencein the system (Huang D et al 2005).

8. ***Folin-Ciocalteau method***

At present, the Folin–Ciocalteu test is commercially available from several important commercial societies, so this method is widely used to quantify polyphenols in plantderived extracts (Blainski A et al. 2013). as well as in foods and beverages (Apak R et al. 2018). The pharmacopoeia includes the Folin–Ciocalteu test (Apak R et al.2018). and Europe adopted it as an official procedure of measuring the total phenol contents in wines (European Community 1990). The Folin–Ciocalteu test is based on reducing the Folin–Ciocalteu reagent with phenolic compounds in an alkaline state. The exact chemical nature of the Folin–Ciocalteu reagent is not clearly defined, but it is believed that it may contain a complex of the phosphomolybdic/ phosphotungstic acid which are reduced to obtain a blue chromophore with the maximum absorption at 765 nm (Magalhaes L.M et al.2008). The central molybdenum ion in the complex is accepted as a reducing site, where the Mo6+ ion is reduced to Mo5+ by accepting an electron donated by the phenolic antioxidant.

9. ***TBARS (Thiobarbituric Acid Reactive Substances)***

TBARS is widely known as an assay for measuring the inhibition of lipid peroxidation product by antioxidant*in vitro*. This assay measures the susceptibility of the samples to the peroxyl radical-induced by Cu2+/H2O2.When peroxidation occurs, the lipid peroxides and peroxyl radicals are formed subsequently and decompose the lipid to create aldehydes and derivatives, such as 4-hydroxy-2- nonenal, malonic aldehyde (MDA), and hexanaldehyde (Singh S and Singh R P). This metabolite will react with TBA (thiobarbituric acid) and form red or pink chromophore. The changes of color are then measured at the wavelength of 530-540 nm. TBARS assay is comparable with other assays because it needs simple instrumentation, good reproducibility, adaptable for measuring lipophilic and hydrophilic antioxidant and suitable for running „high-throughput" analysis (Ghani M.A et.al 2017). It has been used for determining the antioxidant activity in fried fast food, essential oils, and medicinal plants (Galego L et al.2008 and Karagöz A et al. 2015).However, some drawbacks of this method have been described such as absorbance interference by samples containing aldehydes and sugars, unsuitable for measuring lipid peroxidation *in vivo*, takes longer time, and more complex preparation (Singh S and Singh R P and Ghani M.A et al.2017).

IV Conclusion

This review is focused on the most common method of *in vitro* antioxidant activity. Each method has its advantages and disadvantages, depends on the characteristic of sample materials and mode of action of antioxidants. There is no single approach could describe all antioxidant activity. The evaluation of antioxidant activity should be carried out using several methods to measure the diverse inhibition mechanism of free radicals. Researchers have to critically consider the method of the antioxidant analysis before carried out that for the research purpose.

V References

1. Ahsan H, Ali A and Ali R 2003 Oxygen free radicals and systemic autoimmunity Clin. Exp. Immunol.131 398–404.
2. Alam Md N, Bristi N J and Rafiquzzaman Md ,2013. "Review on in vivo and in vitro methods evaluation of antioxidant activity", Saudi Pharm. J21 143–52.
3. Apak R, Güçlü K, Demirata B, Özyürek M, Çelik S E, Bektaşoğlu B, Berker K I and Özyurt D 2007. Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay Molecules **12** 1496–547.
4. Apak, R.; Çapano ˘glu, E.; Shahidi, F. Measurement of Antioxidant Activity and Capacity—Recent Trends and Applications; John Wiley & Sons Ltd.: Hoboken, NJ, USA, 2018; pp. 1–283.
5. Awika J M, Rooney L W, Wu X, Prior R L and Cisneros-Zevallos L 2003. Screening methods tomeasure antioxidant activity of sorghum (Sorghum bicolor) and sorghum products J. Agric. Food Chem. 51 6657–62.
6. Benzie I F F and Strain J J 1996 The ferric reducing ability ofplasma (FRAP) as a measure of "antioxidant power": The FRAP assay Anal. Biochem.239 70–6
7. Blainski, A.; Lopes, G.C.; De Mello, J.C.P. Application and Analysis of the Folin Ciocalteu Method for the Determination of the Total Phenolic Content from Limonium Brasiliense L. Molcules 2013, 18, 6852–6865.
8. Bondet V, Brand-Williams W, Berset C. Kinetics and mechanisms of antioxidant activity using the DPPH free radical method. Food Sci Technol 1997;30:609-15.
9. Borges R S, Pereira G A N, Vale J K L, França L C S, Monteiro M C, Alves C N and Silva A B F da 2013. Design and evaluation of 4-Aminophenol and salicylate derivatives as free-radical scavenger Chem. Biol. Drug Des. 81 414–9.
10. Cekiç S D, Başkan K S, Tütem E and Apak R 2009. Modified cupric reducing antioxidant capacity (CUPRAC) assay for measuring the antioxidant capacities of thiol-containing proteins in admixture with polyphenols Talanta79 344–51.
11. Číž M, Čížová H, Denev P, Kratchanova M, Slavov A and Lojek A 2010 Different methods for control and comparison of the antioxidant properties of vegetables Food Control 21 518–23.
12. Duarte-Martino HC, Bigonha SM, De Morais CL, De Oliveira BR, Brunoro CN, Ramirez CL, et al. Nutritional and bioactive compounds of bean: Benefits to human health. In: Tunick MH, González DE, Mejía E. editors. Hispanic Foods: Chemistry and Bioactive Compounds (ACSSymposium). USA: American Chemical Society; 2012. p. 233-58.

13. Engelgau M M, Rosenthal J P, Newsome B J, Price L, Belis D and Mensah G A 2018. Non communicable diseases in low- and middle-income countries: a strategic approach to develop a global implementation research workforce Glob. Heart 13 131–7.
14. Espín JC, García-Conesa MT, Tomás-Barberán FA. Nutraceuticals: Facts and fiction. Phytochemistry 2007;68(22-24):2986-3008.
15. Fahey JW, Stephenson KK. Pinostrobin from honey and Thai ginger (Boesenbergia pandurata): A potent flavonoid inducer of mammalian phase 2 chemoprotective and antioxidant enzymes. J Agric Food Chem 2002;50(25):7472-6.
16. Ferreira IC, Aires E, Barreira JC, Estevinho LM. Antioxidant activity of Portuguese honey samples: Different contributions of the entire honey and phenolic extract. Food Chem 2009;114:1438-43.
17. Galego L, Almeida V, Gonçalves V, Costa M, Monteiro I and Matos F 2008; Antioxidant activity of the essential oils of Thymbra capitata, Origanum vulgare, Thymus mastichina, and Calamintha baetica Acta Hortic.765 325–34.
18. Ghani M A, Barril C, Bedgood D R and Prenzler P D 2017;Measurement of antioxidant activity with the thiobarbituric acid reactive substances assay Food Chem.230 195–207.
19. Grosso G 2018; Dietary antioxidants and prevention of non-communicable diseases Antioxidants (Basel)71-3.
20. Halvorsen BL et al. 2002; A systematic screening of total antioxidants in dietary plants. The Journal of Nutrition 132(3):461-471.
21. Halvorsen BL et al. 2006; Content of redox-active compounds (ie, antioxidants) in foods consumed in the United States. The American journal of clinical nutrition 84(1):95-135.
22. Huang D, Ou B and Prior R L 2005; The Chemistry behind Antioxidant Capacity Assays J. Agric. Food Chem.53 1841–56.
23. Huang Y J and Nan GX 2019; Oxidative stress-induced angiogenesis J. Clin. Neurosci.63 13–6
24. Inoue K, Murayarna S, Seshimo F, Takeba K, Yoshimura Y, Nakazawa H. 2005; Identification of phenolic compound in manuka honey as specific superoxide anion radical scavenger using Electron Spin Resonance (ESR) and liquid chromatography with coulometric array detection. J Sci Food Agric 85:872-8.
25. Irina Georgiana Munteanu and Constantin Apetrei 2021, Analytical Methods Used in Determining Antioxidant Activity: A Review y. Int. J. Mol. Sci. 22, 3380.
26. Kalcher K et al.2009; Electrochemical sensors and biosensors based on heterogeneous carbon materials Monatshefte für Chemie-Chemical Monthly 140(8):861-889.
27. Karagöz A, Artun F T, Özcan G, Melikoğlu G, Anıl S, Kültür Ş and Sütlüpınar N 2015 In vitro evaluation of antioxidant activity of some plant methanol extracts Biotechnol. Biotec. Eq.29 1184–9
28. Litescu S C, Eremia S and Radu G L 2010; Methods for the Determination of Antioxidant Capacity in Food and Raw Materials Bio-Farms for Nutraceuticals vol 698, ed M T Giardi, G Rea and B Berra (Boston, MA: Springer US) pp 241–9

29. Litescu SC et al.2011; Biosensors Applications on Assessment of Reactive Oxygen Species and Antioxidants. Environmental Biosensors .
30. Magalhaes, L.M.; Segundo, M.A.; Reis, S.; Lima, J.L. 2008, Methodological aspects about in vitro evaluation of antioxidant properties. Anal. Chim. Acta 613, 1–19.
31. Moharram H A and Youssef M M 2014;Methods for determining the antioxidant activity: A review Alex. J. Fd. Sci. and Technol.11 31–42.
32. Molyneux P. 2004; The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J Sci Technol 26(2):211-219.
33. Moniruzzaman, M, M I Khalil, S A Sulaiman, and S H Gan 2016. "Advances in the analytical methods for determining the antioxidant properties of honey: a review." Afr. J. Tradit. Complement. Altern. Med. 9 36–42.
34. Ozyürek M, Güçlü K, Tütem E, Başkan K S, Erçağ E, Çelik S E, Baki S, Yıldız L, Karaman Ş and Apak R 2011; A comprehensive review of CUPRAC methodology Anal. Methods3 2439.
35. Pellegrini N, Serafini M, Colombi B, Del Rio D, Salvatore S, Bianchi M and Brighenti F 2003; Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different invitroassays J. Nutr.133 2812–9.
36. Pierart ZC, Rozowsky NJ. 2006; Papel de la nutricion en la prevencion del cancer gastrointestinal. Rev Chil Nutr 33(1):8-13.
37. Piljac-Zegarac J, Stipcevic T, Belscak A. 2009; Antioxidant properties and phenolic content of different floral origin honeys. J Api Prod Api Med Sci .1:43-50.
38. Prior R L 2015 Oxygen radical absorbance capacity (ORAC): New horizons in relating dietary antioxidants/bioactives and health benefits J. Funct. Foods18 797–810
39. Saddhe A A, Malvankar M R, Karle S B and Kumar K 2019; Reactive nitrogen species: Paradigms of cellular signaling and regulation of salt stress in plants Environ. Exp. Bot.161 86–97.
40. Schaich K M, Tian X and Xie J 2015; Hurdles and pitfalls in measuring antioxidant efficacy: A critical evaluation of ABTS, DPPH, and ORAC assays J. Funct. Foods14 111–25.
41. Shahidi, F.; Zhong, Y. Measurement of antioxidant activity. J. Funct. Foods 2015; 18, 757–781.
42. Shetti A, Keluskar V, Aggarwal A. Antioxidants enhancing oral andgeneral health. J Indian Acad Oral Med Radiol 2009;21(1):1-6.
43. Sies H, Stahl W and Sevanian A 2005; Nutritional, dietary and postprandial oxidative stress J. Nutr.135 969–72.
44. Singh S and Singh R P, "In vitro methods of assay of antioxidants: an overview," Food Rev. Int.24 392-414.
45. Wayner D D M, Burton G W, Ingold K U and Locke S 1985;Quantitative measurement of the total, peroxyl radical-trapping antioxidant capability of human blood plasma by controlled peroxidation FEBS Letters187 33–7.
46. Zheng L, Zhao M, Xiao C, Zhao Q and Su G 2016; Practical problems when using ABTS assayto assess the radical-scavenging activity of peptides: importance of controlling reaction pHand time Food Chem.192 288–94.