International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Review Article **CODEN: IJPNL6**

METHODS TO STUDY ANTIOXIDANT PROPERTIES WITH SPECIAL REFERENCE TO MEDICINAL PLANTS

Susmita Mahapatra[1], Yugal Kishore Mohanta[2] and Sujogya Kumar Panda[1]*

[1]Department of Zoology, North Orissa University, Baripada-757003, Odisha, India-757003
[2]Department of Life Science, National Institute of Technology, Rourkela, India -769008

***Corresponding author e-mail:** sujogyapanda@gmail.com

ABSTRACT

Antioxidant properties have been credited to medicinal plants because plants have to counteract themselves from stress caused by oxygen. Plants have no side effects and provide protection against free radicals, thus prevent our body from oxidative damage. As a result, it is very much necessary to screen medicinal plants for their antioxidant properties. There are several *in vitro* assays are reported for measurement of antioxidant properties. Results comparability is largely dependent on the techniques employed in the investigations and conclusive results can only be obtained if methods are standardized and worldwide. In the light of the fact, the present review is designed for the simplicity and feasibility of the authors to use the appropriate methods based on the availability of the instruments, so as to reduce the time consumption. This review offers an idea behind the mechanism involved in each test assay. At the same time it also provide information on the most common methods used in the present day to study antioxidant activity with their advantages and comparison of different standards used by several authors.

Keywords: Antioxidants; *In vitro* methods; Oxidative stress; Reactive oxygen species; Plant extract

INTRODUCTION

Recent developments in biomedical science emphasize the involvement of free radicals in many diseases. In the body, the oxidation of free radicals may contribute to a number of chronic and degenerative diseases such as cancer, cardiovascular diseases, atherosclerosis, diabetes and cataracts as well as the process of ageing [1]. There is increasing evidence to suggest that many degenerative diseases such as brain dysfunction, cancer, heart disease and immune system decline could be the result of cellular damage caused by free radicals and that anti-oxidants may play an important role in disease prevention [2]. Since those chronic diseases are associated with increased oxidative stress, these have been suggested that the protective effects of polyphenolic components are related to their antioxidative properties [3]. Anti-oxidants are compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. The anti-oxidant activities of natural substances are based on their ability to donate hydrogen atoms to free radicals. Plants are known as potential source of natural anti-oxidants. The antioxidant activities of plants mainly depend on their free radical scavenging abilities which is determined by their reducing properties as hydrogen-or electron-donating agents. Plants contain flavonoids and phenolic compounds are very effective scavengers of hydroxyl and peroxyl radicals [4]. In addition, they have a metal chelation potential and inhibit the Fenton and Haber-Weiss reactions which produce active oxygen radicals [5]. An important characteristic of flavonoids is that they can maintain their free radical scavenging capacity after forming complexes with metal ions. The following scheme illustrates the interference of an oxidation reaction by donation of a hydrogen atom from the phenolic compound to radicals [4]:

ROO• + PPH ⟶ ROOH + PP•

RO• + PPH ⟶ ROH + PP•

Several *in vitro* studies have pointed out the strong antioxidant activity of polyphenols due to their low redox potential and their capacity to donate several electrons or hydrogen atoms. The antioxidant potential of the polyphenol depends on the extent of absorption and metabolism of these compounds. A lot of phenolic compounds exhibit a wide range of biological effects including antibacterial and anti-inflammatory activity [6]. Studies have also shown that phenolic compounds are potent scavengers of free radicals and are potentially useful in the prevention of number of diseases [7]. There are two primary conditions can be able to define a polyphenol as an antioxidant: (i) it should delay or prevent the oxidation of the substrate when they are present in low concentration compared to the oxidizable substrate; (ii) it should form stable phenoxy radical intermediates that act as terminators of the propagation step by reacting with other free radicals [5].

ROO• + PP• ⟶ ROOPP

RO• + PP• ⟶ ROPP

Plants have played a significant role in the development of new drugs and in many developing countries attention has been paid to exploring natural substances as substitutes for synthetic compounds. The commonly used anti-oxidants such as butylated hydroxyanisol and butylated hydroxytolune are synthetic chemicals and the possible toxicity of these anti-oxidant has been resulted in their reduced usage [8]. Due to health concern, natural anti-oxidants have been extensively employed in recent years [9]. Plants and other natural products generally contain many hundreds compounds of natural antioxidant. Therefore, several methods have been described to quantify the phytocompounds. These standard assays differ from each other in terms of reagents, difference in the physical and chemical properties of oxidizable substrates, experimental condition, reaction medium, effectiveness and sensitivity [10, 11, 12]. Methods that are widely used to measure the antioxidant activity level in herbal sample, fruits and vegetables and their products are thiobarbituric acid reactive species (TBARS) [13], oxygen radical absorbance capacity (ORAC) [14,15,16], β-carotene bleaching test (BCBT) [17], Trolox equivalent antioxidant capacity (TEAC)/ ABTS radical-cation [18,19], DPPH titration [20], Folin Ciocalteu [21] and Ferric ion reducing antioxidant power **(**FRAP).

ORAC assay: It uses a peroxyl radical induced oxidation reaction to measure the antioxidants chain breaking ability. It uses beta-phycoerythrin (PE) as an oxidizable protein substrate and 2, 2'-azobis (2-amidinopropane) dihydrochloride (AAPH) as a peroxyl radical generator or $Cu2^{+}$. H_2O_2 as a hydroxyl radical generator is the only method that takes free radical action to completion and uses an area under curve (AUC) technique for quantitation. It combines both inhibition percentage and the length of inhibition time of the free radical action by antioxidants into a single quantity. The capacity of a compound to scavenge peroxyl radicals generated by spontaneous decomposition of 2, 2'-azo-bis, 2-amidinopropane dihydrochloride (AAPH) is calculated in terms of standard equivalents using the ORAC assay [22]. The reaction mixture (4.0 ml) consist of 0.5 ml extract in phosphate buffer (75 mM, pH 7.2) and 3.0 ml of fluorescein solution both are mixed and pre-incubated for 10 min at 37°C. Then, 0.5 ml of 2, 2'-azo-bis, 2-amidinopropane (AAPH) dihydrochloride solution is added and immediately the loss of fluorescence (FL) is observed at 1 min intervals, continuously for 35 min. The final results are calculated using the differences of areas under the FL decay curves between the blank and a sample and are expressed as micromole Trolox equivalents (TE) per gram (μmol TE/g).

TRAP assay: TRAP is the most widely used *in vivo* method for measuring total antioxidant capacity of plasma or serum during the last decades. It uses peroxyl radicals generated from 2, 2'-azobis (2-amidinopropane) dihydrochloride (AAPH) and peroxidizable materials contained in plasma or other biological fluids. After adding AAPH to the plasma, the oxidation of the oxidizable materials is monitored by measuring the oxygen consumed during the reaction. During the induction period, this oxidation is inhibited by the antioxidants in the plasma. The length of the induction period (lag phase) is compared to that of an internal standard Trolox (6-hydroxyl-2, 5, 7, 8, - tetramethylchroman-2-carboxylic acid), and then quantitatively related to the antioxidant capacity of the plasma. Although TRAP is a useful assay for antioxidant measurement activity, the precision and reliability of the method is problematic due to the fact that that antioxidant activity can be continued after the lag phase.

Dichlorofluorescin-diacetate (DCFH-DA) based assay: TRAP can also be measured

spectrophotometrically by using dichlorofluorescin-diacetate (DCFH-DA) [23]. This assay uses AAPH to generate peroxyl radicals and DCFH-DA as the oxidizable substrate for the peroxyl radicals. The instant oxidation of DCFH-DA by peroxyl radicals converts DCFH-DA to dichlorofluorescein (DCF). DCF is highly fluorescent having an absorbance value at 504 nm. Therefore, the produced DCF can be monitored in either spectrofluorometer or spectrophotometer.

Total phenolic content: The amount of total phenolic content can be determined by Folin-Ciocalteu reagent (FCR) method [24, 25, 26, 27, 28, 29]. Commonly 0.5 ml of extract and 0.1 ml of Folin-Ciocalteu reagent (0.5 N) are mixed suitably and incubated for 15 min at room temperature. Then 2.5 ml of saturated sodium carbonate is added and further incubated for 30 min at room temperature and absorbance is measured at 760 nm. Gallic acid [27], tannic acid [30], quercetin [24] or guaiacol [31] can be used as positive controls. The total phenolic content is expressed in terms of standard equivalent (mg/g of extracted compound).

Total flavonoid content: The antioxidative properties of flavonoids are due to dissimilar mechanisms including scavenging of free radicals, chelation of metal ions and inhibition of enzymes responsible for free radical generation [32]. Depending on their structure, flavonoids are able to scavenge practically all existing as well as known ROS. The amount of total flavonoid content can be determined by Aluminum chloride method [33]. The reaction mixture (3.0 ml) comprised of 1.0 ml of extract, 0.5 ml of aluminum chloride (1.2%) and 0.5 ml of potassium acetate (120 mM) is incubated at room temperature for 30 min and absorbance is measured at 415 nm. Quercetin [34] or catechin [35] can be used as positive control. The flavonoid content can be expressed in terms of standard equivalent (mg/g of extracted compound).

Reducing power: Reducing power showcase the major antioxidant activity of different plant samples [36]. Compounds having reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation process. The reducing power can be determined by the method of Athukorala [37]. 1.0 ml extract is mixed with 2.5 ml of phosphate buffer (200 mM, pH 6.6) and 2.5 ml of potassium ferricyanide (30 mM) and incubated at 50°C for 20 min. Thereafter 2.5 ml of trichloroacetic acid (TCA) (600 mM) is added to the reaction mixture and centrifuged for 10 min at 3000 rpm. The supernatant (2.5 ml) is mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (6 mM) and absorbance is measured at 700 nm. Ascorbic acid, a-tocopherol, butylated hydroxyanisole (BHA) and Trolox can be used as positive control.

FRAP: The FRAP assay measures the reduction of a ferric salt to a blue colored ferrous complex by antioxidants under acidic condition (pH 3.6). The FRAP unit is defined as the reduction of one mole of Fe (III) to Fe (II). Ferric ion reducing ability of plasma (FRAP) determines the total antioxidant power as the reducing capability. The increase in absorbance (ΔA) at 593 nm is measured and compared with ΔA of a Fe (II) standard solution. The results were expressed as micromole Trolox equivalents (TE) per gram on dried basis. 0.2 ml of the extract is added to 3.8 ml of FRAP reagent (10 parts of 300 mM sodium acetate buffer, pH 3.6 + 1 part of 20 mM $FeCl_3.6H_2O$ solution + 1 part of 10 mM TPTZ solution), incubated at 37°C for 30 min and the increase in absorbance at 593 nm is measured. $FeSO_4$ solution is used for calibration. The antioxidant capacity is based on the ability to reduce the ferric ions of sample is calculated from the linear calibration curve and expressed as mM $FeSO_4$ equivalents per gram of sample. BHT, BHA, ascorbic acid, quercetin, catechin or Trolox [38] can be used as positive controls. The FRAP assay is simple, economic and reproducible method which can be applied to both plasma and food extracts. This method has the advantages of determining the antioxidant activity directly in whole plasma; it is not dependent on enzymatic as well as non-enzymatic methods to generate free radicals prior to the valuation of antiradical efficiency of the plasma.

DPPH method: This method uses a stable chrogen radical, DPPH (2, 2-diphenyl, 1-picrylhydrazyl) in methanol, which gives deep purple color. By addition of DPPH in the test solution, the color of the solution fades and the reduction is monitored by the decrease in the absorbance at 515 nm. When a solution of DPPH is mixed with a substance that can donate a hydrogen atom, the reduced form of the radical is generated accompanied by loss of colour. Thus delocalization is also responsible for the deep violet colour and characterized by an absorption band at about 515 nm. The reaction mixture (3.0 ml) consists of 1.0 ml of the extract, 1.0 ml of DPPH in methanol (0.3 mM) and 1.0 ml of methanol. It is incubated for 10 min in dark and then the absorbance is measured at 520 nm. In this assay, the positive controls can be ascorbic acid, gallic acid [39] and BHT [40]. The percentage of inhibition can be calculated using the

formula, Inhibition (%) = (A0 - A1 / A0) × 100 (Where, A0 is the absorbance of control and A1 is the absorbance of test).

This assay is simple and widely used. However, it has a disadvantage i.e. reactive peroxyl radicals react slowly with DPPH. The reaction kinetics between the DPPH and antioxidants are not linear consequently EC_{50} measurement is problematic for DPPH assay.

ABTS or TEAC assay: TEAC assay is a decolonization assay applicable to both lipophillic and hydrophillic antioxidants. The TEAC assay is based on the inhibition by antioxidants of the radical cation of 2, 2'-azinobis (3-ethylbenzothiazoline 6-sulfonate) (ABTS), which has a characteristic of long-wavelength, shows absorption maxima at 660, 734 and 820 nm. Generation of the ABTS radical cation forms the basis of one of the spectrophotometric methods that have been applied to the measurement of the total antioxidant activity. The experiments are carried out using a decolorisation assay, which involves the generation of the ABTS chromophore by the oxidation of ABTS with potassium persulphate. The ABTS free radical-scavenging activity of plants samples is determined by the method of Re et al. [41]. The radical cation, is generated by persulphate oxidation of ABTS. A mixture (1:1 v/v) of ABTS (7.0 mM) and potassium persulphate (4.95 mM) is allowed to stand overnight at room temperature in dark to form radical cation $ABTS^+$. A working solution is diluted with phosphate buffer solution to absorbance value in between 1.0 and 1.5 at 734 nm. An aliquot (0.1 ml) of each sample is mixed with the working solution (3.9 ml) and the decrease of absorbance is measured at 734 nm after 10 min incubation at 37°C in the dark. Aqueous phosphate buffer solution (3.9 ml, without $ABTS^+$ solution) is used as a control. The $ABTS^+$ scavenging rate is calculated. The reaction is pH - independent. A decrease of the $ABTS^+$ concentration is linearly dependent on the antioxidant concentration. Trolox, BHT, rutin [42], ascorbic acid [43] or Gallic acid [44] can be used as a positive control. The only problem with ABTS does not resemble the radical found in the biological system. However, this assay is widely used because of its simplicity and automation.

Assay of Superoxide radical (O_2^-) scavenging activity: Superoxide anion generates powerful and dangerous hydroxyl radicals as well as single oxygen, both of which contribute to oxidative stress [45]. In PMS/NADH-NBT system, the superoxide anion derived from dissolved oxygen from PMS/NADH coupling reaction, reduces NBT. The decrease of absorbance at 560 nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture. The scavenging activity of superoxide anion is measured as described by Dasgupta and De [46]. The superoxide anion radicals are generated in 3.0 ml of Tris-HCl buffer (16 mM, pH 8.0) which is containing 0.5 ml of NBT (0.3 mM), 0.5 ml NADH (0.936 mM) solution and 1.0 ml extract. The reaction is started by adding 0.5 ml PMS solution (0.12 mM) to the mixture incubated at 25°C for 5 min and then the absorbance is measured at 560 nm. Later, the method [47, 48] was modified using riboflavin-light-NBT system. Each 3 ml mixture contains 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 2 µM riboflavin, 100 µM EDTA, 75µM NBT and 1 ml sample solution. Gallic acid [46], BHA, ascorbic acid, a-tocopherol and curcumin [49] can be used as positive controls.

Assay of Hydroxyl radical (-OH) scavenging activity: Plant extracts have ability to inhibit non-specific hydroxyl radical (hydroxyl radical reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and causes damage to cell [50, 51]. The model uses ascorbic acid-iron-EDTA model of OH generating system in which ascorbic acid, iron and EDTA work together with each other to generate hydroxyl radicals. The reaction mixture (1.0 ml) consist of 100 µl of 2-deoxy-D-ribose (28 mM in 20 mM KH_2PO_4-KOH buffer, pH 7.4), 200 µl EDTA (1.04 mM) and 200 µM $FeCl_3$ (1:1 v/v), 100 µl of H_2O_2 (1.0 mM), 500 µl of the extract and 100 µl ascorbic acid (1.0 mM) which is incubated at 37°C for 1 hour. 1.0 ml of thiobarbituric acid (1%) and 1.0 ml of trichloroacetic acid (2.8%) are mixed and incubated at 100°C for 20 min. After cooling, absorbance is measured at 532 nm against a blank. Gallic acid, catechin [52] and vitamin E [53] can be used as positive controls. Later, this method was modified by Dasgupta [48] based on benzoic acid hydroxylation using spectroflurometer. The reaction mixtures (2 ml) consist of 200 µl each of sodium benzoate (10mM), $FeSO_4.7H_2O$ (10mM) and EDTA (10mM). The volume of the solution is made up to 1.8 ml by adding phosphate buffer (pH 7.4, 0.1 M) and finally 0.2 ml of H_2O_2 (10mM) is added and incubates at 37 ^oC for 2 hours. The fluorescents are measured at 407 nm emission (Em) and excitation (Ex) at 305 nm.

Hydrogen peroxide radical scavenging assay: Hydrogen peroxide naturally occurs at low concentration in air, water, human body, plants, microorganisms and food. Hydrogen peroxide enters the human body through inhalation of vapor or mist

and through eye or skin contact. Inside body, H_2O_2 is rapidly decomposed into oxygen and water, ensuing hydroxyl radicals (OH^-) that can initiate lipid peroxidation and cause DNA damage. The methods comprise solution of hydrogen peroxide (40 mM), prepared in phosphate buffer (50 mM, pH 7.4) [54]. Extract concentration (20-50 g/ml) aqueous is added to hydrogen peroxide and absorbance at 230 nm after 10 min. incubation against a blank solution (phosphate buffer without hydrogen peroxide). The percentage of hydrogen peroxide scavenging is calculated as follows:

% scavenged (H_2O_2) = (A0 - A1 / A0) × 100 (where, A0 is the absorbance of control and A1 is the absorbance of test). Ascorbic acid, rutin, BHA [55] can be used as a positive control.

Nitric oxide radical scavenging assay: Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions. This can be measured using the Griess reaction reagent (1% sulphanilamide, 0.1% naphthyethylene diamine dihydrochloride in 2% H_3PO_3) [56]. 3.0 ml of 10 mM sodium nitroprusside in phosphate buffer is added to 2.0 ml of extract. Similarly standard compound in different concentrations (20-100 µg/ml), methanol as a blank will prepared and incubated at 25°C for 60 min. After incubation, samples (5.0 ml) are added with 5.0 ml of Griess reagent and absorbance is measured at 540 nm. Percentage of inhibition of the nitrite oxide generation is measured by comparing the absorbance values of control and test. Curcumin, caffeic acid, sodium nitrite [57], BHA, ascorbic acid and rutin [55] can be used as positive controls.

Xanthine oxidase assay: To determine the activity of superoxide anion-scavenging, two different assays can be used: (i) the enzymatic method with cytochrome C [58] and (ii) nonenzymatic method with nitroblue tetrazolium (NBT) [59]. With cytochrome C method, superoxide anions can be generated by xanthine and xanthine oxidase system. The extract (500 µl of 0.1 mg/ml) and allopurinol (100µg/ml) (in methanol) is mixed with 1.3 ml phosphate buffer (0.05M, pH 7.5) and 0.2 ml of (0.2 units/ml) xanthine oxidase solution. After 10 min of incubation at 25°C, 1.5 ml of 0.15 M xanthine substrate solution is added to this mixture. The mixture is reincubated for 30 min at 25°C and then the absorbance is taken at 293 nm using a spectrophotometer against blank (0.5 ml methanol, 1.3 ml phosphate buffer and 0.2 ml xanthine oxidase). BHT [60] can be used as a positive control. Percentage of inhibition was calculated using the formula, Inhibition (%) = [1 - (As / Ac)] × 100 (where, As and Ac are the absorbance values of the test sample and control respectively).

Metal chelating activity: Ferrozine can be chelated with Fe^{2++} and form a complex with a red color. This reaction is limited in the presence of other chelating agents and results in a decrease of the red color of the ferrozine-Fe^{2+} complexes. Measurement of the color reduction estimates the chelating activity to compete with ferrozine for the ferrous ions [61]. The ferrous ions chelating activity can be measured by the decrease in absorbance at 562nm of iron (II)-ferrozine complex [62]. 1 ml of the extract is added to a solution of 1 ml of ferrous sulphate (0.125 mM). The reaction is initiated by the addition of 1 ml of ferrozine (0.3125mM) and incubated at room temperature for 10 min and then the absorbance is measured at 562 nm. EDTA or citric acid [62] can be used as a positive control. The ability of sample to chelate ferrous was calculated relative to the control using formula, Chelating effect (%) = (Ac-As/Ac) ×100 (where, Ac-Absorbance of control and As-Absorbance of sample).

Lipid peroxidation: The oxidation of linoleic acid generates peroxyl free radicals due to the abstraction of hydrogen atoms from diallylic methylene groups of linoleic acid. These free radicals later oxidize the highly unsaturated beta carotene (orange colour disappear) and the results can be monitored by spectrophotometer follow the conjugated diene method [63]. Different concentration of extracts (0.1-20 mg/ml) in water or ethanol (100 µl) is mixed properly with 2.0 ml of 10 mM linoleic acid, emulsion in 0.2 M sodium phosphate buffer (pH 6.6) and kept in dark at 37 °C. After 15 hours of incubation, 0.1 ml from each tube is mixed with 7.0 ml of 80% methanol in deionized water and the absorbance of the mixture is measured at 234 nm against a blank in a spectrophotometer. Later this method is modified by several authors using thiocyanate. 0.5 ml of each extract sample with different concentration is mix up with linoleic acid emulision (2.5 ml 40 mM, pH 7.0). The final volume was adjusted to 5 ml by adding with 40 mM phosphate buffer, pH 7.0. After incubation for 72 hours at 37 °C in dark, 0.1 ml aliquot are mixed with 4.7 ml of ethanol (75%), 0.1 ml $FeCl_2$ (20mM) and 0.1 ml Ammonium thiocyanate (30%). The absorbance of this mixture can be measured at 500 nm in spectrophotometer. Ascorbic acid, BHT, gallic acid, α-tocopherol [63] can be used as positive control. The % of inhibition or antioxidant activity is calculated using formula, Antioxidant activity (%) =

(Ac-As/Ac) ×100, (where Ac-Absorbance of control; As-Absorbance of sample)

Cyclic voltammetry method: The cyclic voltammetry procedure evaluates the overall reducing power of low molecular weight antioxidant. The sample is introduced into a well in which three electrodes are placed: the working electrode (e.g., glassy carbon), the reference electrode (Ag/AgCl) and the auxiliary electrode (platinum wire). The potential should be applied to the working electrode at a constant rate (100 mV/s) either toward the positive potential (evaluation of reducing equivalent) or toward the negative potential (evaluation of oxidizing species). During operation of the cyclic voltmeter, a potential current curve is recorded (cyclic voltammogram). Raymundo et al. [64] described quantitative determination of the phenolic antioxidants using voltammetric techniques. Chatterjee et al. [65] determine the antioxidant property from plant sample of *Myristica fragrans* and *Piper nigrum*.

Photochemiluminescence (PCL) assay: PCL assay was initially used to determine water-soluble and lipid-soluble antioxidants [66, 67]. The photochemoiluminescence measures the antioxidant potential in lipidic and water phase towards the superoxide radical. This method allows the quantification of both the antioxidant capacity of hydrophilic as well as lipophilic substances either as pure compounds or complex matrix from different origin. The PCL method is based on the acceleration of the oxidative reactions *in vitro.* The PCL is a very quick and sensitive measurement method (1000 times faster than the normal conditions) [68] determined antioxidant property in marigold flowers.

CONCLUSION

Considerate the properties of antioxidants in plants are demanding. The use of single method to evaluate versatile antioxidants is extremely complicated. Due to the complexity of the phytochemicals in plant products, purifying the individual responsible compounds are costly, uses sophisticated instruments, inefficient without possible synergistic interactions. The major problem is the lack of a validated assay that can consistently measure the antioxidant capacity with plant extracts. Several reviews have been published and the opinions differ greatly for which method is best. The present review is a compilation of different *in vitro* assay methods used in determining the antioxidant activity of different plant extracts. *In vitro* assays can only rank antioxidant activity for their particular reaction system and their significance in *in vivo* test system is doubtful. Although *in vitro* antioxidant assays have been carried out in a number of medicinal plants, yet *in vivo* tests are yet to be carried out. Active compounds of many plants extract possessing antioxidant activity are yet to be identified. This will ultimately develop many lead molecules which could help in disease prevention and cure.

ACKNOWLEDGEMENT

We wish to express our profound gratitude to Dr. S. K. Dutta, Dr. A. K. Bastia and Dr. G. Sahoo (North Orissa University) for their cooperation and critical suggestion on the preparation of the manuscript.

REFERENCES

1. Shahidi F, Naczk M. CRC Press LLC. Boca Raton, FL 2003.
2. Aruoma OI. J Am Oil Chem Soc, 1998; 75: 199-212.
3. Lotito SB, Frei B. Free Radical Biol Med, 2006; 41: 1727-46.
4. Bravo L. Nutr Reviews, 1998; 56: 317-33.
5. Rice-Evans CA, Miller NJ, Paganga G. Trends Plant Sci, 1997; 2: 152-9.
6. Cook NC, Samman S. Nutr Biochem, 1996; 7: 66-76.
7. Zainol MK, Abd-Hamid A, Yusof S, Muse R. Food Chem, 2003; 81: 575-81.
8. Ito N, Fukushima S, Tsuda H. CRC Critical Rev Toxicol, 1985; 15: 109-50.
9. Yen GC, Chang YC, Su SW. Food Chem, 2003; 83: 49-54.
10. Khal R, dan Hilderbrant AG. Food Chem Toxicol, 1986; 24: 1007-14.
11. Frankel EN. Trends Food Sci Technol, 1993; 4: 220-25.
12. Koleva II, Van Beek TA, Linssen JPH, de Groot A. dan Evstatieva LN. Phytochem Anal, 2002; 13: 8-17.
13. Roberto G, Baratta MT, Deans SG, dan Dorman HJD. Planta Medica, 2000; 66: 687-93.
14. Tsai P, McIntosh J, Pearce P, Camden B, Jordon BR. (2002). Food Res Int, 2002; 35(4): 351-56.
15. Wang H, Cao G, dan Prior RL. J Agric Food Chem, 1996; 44: 701-05.
16. Zheng W, dan Wang SY. J Agric Food Chem, 2001; 49(11): 5165-70.

17. Gazzani G, Papetti A, Massolini G, Daglia M. J Agric Food Chem, 1998; 46: 4118-22.
18. Arena E, Fallico B, dan Maccarone E. Food Chem, 2001; 74(4): 423-27.
19. Miller NJ, Diplock AT, dan Rice-Evans CA. J Agric Food Chem, 1995; 43: 1794-1801.
20. Imark C, Kneubuhl M, dan Bodmer S. Innovative Food Sci Emerging Technol, 2000; 1(4): 239-43.
21. Donovan JL, Meyer AS, Waterhouse AL. J Agric Food Chem, 1998; 46: 1247-52.
22. Prior RL, Wu X, Schaich K. J Agric Food Chem, 2005; 53: 4290-4302.
23. Amado LL, Jaramillo MD, Rocha AM, Ferreira JLR, Garcia LM, Ramos PB et al. Comp Biochem Physiol-Part A, 2007; 148: S75-S76.
24. Singleton VL, Rossi JA. Am J Enol Viticult, 1965; 16: 144-58.
25. Velioglu YS, Mazza G, Gao L, Oomah BD. Agric Food Chem, 1998; 46: 4113-17.
26. Kahkonen MP, Hopia AI, Vuorela HJ, Rauha J. Agric Food Chem, 1999; 47: 3954-62.
27. McDonald S, Prenzler PD, Antolovich M, Robards K. Food Chem, 2001; 73: 73-84.
28. Huang D, Ou B, Prior RL. Agric Food Chem, 2005; 53: 1841-56.
29. MacDonald-Wicks LK, Wood LG, Garg ML. J Sci Food Agric, 2006; 86: 2046-56.
30. Wolfe K, Wu X, Liu RH. J Agric Food Chem, 2003; 51: 609-14.
31. Yildirim A, Oktay M, Bilaoglu V. Turkey J Med Sci, 2001; 31: 23-7.
32. Benavente-Garcia. J Agri Food Chem, 1997; 45: 4505-15.
33. Chang C, Yang M, Wen H, Chern J. Estimation J Food Drug Analysis, 2002; 10: 178-82.
34. Ordonez AAL, Gomez JD, Vattuone MA, Isla MI. Food Chem, 2006; 97: 452-58.
35. Kim DO, Jeong SW, Lee CY. Food Chem, 2003; 81: 321-26.
36. Oktay M, Gulcin I, Kufrevioglu OI. Leb-Wissen Technol, 2003; 36: 263-71.
37. Athukorala Y, Kim KN, Jeon YJ. Food Chem Toxicol, 2006; 44: 1065-74.
38. Benzie IFF, Strain JJ.. Methods Enzymol, 1999; 299: 15-27.
39. Blois MS. Nature, 1958; 181: 1149-50.
40. Liyana-Pathirana CM Shahidi F. J Agric Food Chem, 2005; 53: 2433-40.
41. Stratil P, Klejdus B, Kuban V. J Agric Food Chem, 2006; 54: 607-16.
42. Re R, Pellegrini N, Proteggente A, Pannala A,Yang M, Rice-Evans C. Free Radiation Biol Medicine, 1999; 26: 1231-37.
43. Alzoreky N, Nakahara K. Food Sci Technol Res, 2001; 7: 141-44.
44. Auddy B, Ferreira M, Blasina F, Lafon L, Arredondo F, Dajas F, Tripathi PC, Seal T, Mukherjee B. J. Ethnopharmcol, 2003; 84: 131-38.
45. Meyer-Isaksen A. Trends Food Sci Technol, 1995; 6: 300-04.
46. Robak J, Gryglewski RJ. Biochem Pharmacol, 1988; 37: 837-41.
47. Dasgupta N, De B. Food Chem, 2004; 88: 219-24.
48. Dasgupta N, De B. Food Chem, 2007; 101: 471-74.
49. Nishikimi M, Rao NA,Yagi K. Biochem Biophy Res Communication, 1972; 46: 849-54.
50. Halliwell B, Gutteridge JMC. *FEBS* Letter, 1981; 128: 347-52.
51. Hinneburg I, Dorman HJD, Hiltunen R. Food Chem, 2006; 97: 122-29.
52. Kunchandy E, Rao MNA. Int J Pharmacol, 1990; 58: 237-40.
53. Halliwell B, Gutteridge JMC, Aruoma OI. Ann Biochem, 1987; 165: 215-19.
54. Ruch RJ, Cheng SJ, Klaunig JE. Carcinogenenesis, 1989; 10: 1003-8.
55. Jayaprakasha GK, Jaganmohan, Rao L, Sakariah KK. Bioorg Med Chem, 2004; 12: 5141-6.
56. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Ann Biochem, 1982; 126: 131-8.
57. Sreejayan Rao MN. J Pharm Pharmacol, 1997; 49: 105-7.
58. McCord JM, Fridovich I. J Biol Chem, 1969; 244: 6049-55.
59. Zhang HY, Lu CS. Acta Biochem Biophys Sin (in Chinese), 1990; 22: 593-4.
60. Chang WS, Lin CC, Chuang SC, Chiang HC. Am J Chin Med, 1996; 24: 11-17.
61. Soler-Rivas C, Espin JC, Wichers HJ. Phytochem Analysis, 2000; 11: 330-8.
62. Dinis TCP, Madeira VMC, Almeida LM. Arch Biochem Biophys, 1994; 315: 161-9.
63. Lingnert H, Vallentin K, Eriksson CE. J Food Processing and Preservation, 1979; 3: 87-103.
64. Raymundo MS, Paula MMS, Franco C, Fett R. Food Sci Technol, 2007; 40: 1133-9.
65. Chatterjee S, Niaz Z, Gautam S, Adhikari S, Variyar PS, Sharma A. Food Chem, 2007; 101: 515-23.
66. Popov IN, Lewin G. Free Radical Biol Med, 1994; 17: 267-71.
67. Popov IN, Lewin G. J Biochem Biophys Methods, 1996; 31: 1-8.
68. Wang M, Tsao R, Zhang S, Dong Z, Yang R, Gong J et al. Food Chem. Toxicol, 2006; 44: 1522-9.