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Review

In vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview

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Reactive oxygen species (ROS) are a class of highly reactive molecules derived from the metabolism of oxygen. ROS, including superoxide radicals, hydroxyl radical and hydrogen peroxide molecules are often generated as by products of biological reactions or from exogenous factors. There is extensive evidence to involve ROS in the development of degenerative diseases. Evidence suggests that compounds especially from natural sources are capable of providing protection against free radicals. This has attracted a great deal of research interest in natural antioxidants. It is necessary to Screen out medicinal plants for their antioxidant potential. Therefore an attempt has been made to review different *in vitro* models for estimating antioxidant properties of natural products from medicinal plants. All the models are described along with the different standards that can be used for estimation. In the end, a large number of plants showing *in vitro* antioxidant activity are listed but *in vivo* studies are lacking.

Key words: Antioxidant assay, *in vitro* models, antioxidant medicinal plants.

INTRODUCTION

Oxidative stress depicts the existence of products called free radicals and reactive oxygen species (ROS), which are formed under normal physiological conditions but become deleterious when not being eliminated by the endogenous systems. In fact, oxidative stress results from an imbalance between the generation of reactive oxygen species and endogenous antioxidant systems. ROS are major sources of primary catalysts that initiate oxidation *in vivo* and *in vitro* and create oxidative stress which results in numerous diseases and disorders (Halliwell, 1994; Rackova et al., 2007) such as cancer (Kinnula and Crapo, 2004), cardiovascular disease (Singh and Jialal, 2006), neural disorders (Sas et al., 2007), Alzheimer's disease (Smith et al., 2000) mild congnitive impairment (Guidi et al., 2006), Parkinsons disease (Bolton et al., 2000), alcohol induced liver disease (Arteel, 2003), ulcerative colitis (Ramakrishna et al.,1997), ageing (Hyun et al., 2006), atherosclerosis (Upston et al., 2003). Oxygen derived free radicals such as superoxide anions, hydroxyl radicals and hydrogen peroxide are cytotoxic and give rise to tissue injuries (Jainu and Shyamala Devi, 2005). Excessive amount of ROS is harmful because they initiate bimolecular oxidation which leads to cell death and creates oxidative stress. In addition, oxidative stress causes inadvertent enzyme activation and oxidative damage to cellular system (Wiseman and Halliwell, 1996).

Free radical is a chemical compound which contains an unpaired electron spinning on the peripheral layer around the nucleus. The family of free radicals generated from the oxygen is called ROS which cause damage to other molecules by extracting electrons from them in order to attain stability. ROS are ions, atoms or molecules that have the ability to oxidize reduced molecules. ROS are various forms of activated oxygen, which include free radicals such as superoxide anion radicals ($^{\cdot}O_2^-$) and hydroxyl radicals (OH·), as well as non-free radicals (H_2O_2) and singlet oxygen (Halliwell, 1995). In the body, free radicals are derived from two sources: endogenous sources, e.g. nutrient metabolism, ageing process etc and exogenous sources e.g. tobacco smoke, ionizing radiation, air pollution, organic solvents, pesticides, etc

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(Buyukokuroglu et al., 2001).

When oxygen traps single electron, it becomes unstable and thus very reactive, since it generates harmful chain reactions against many biological molecules. The extreme toxicity of oxygen is related to its high capability of generating free radicals and in turn destroying many major biological molecules. They can attack on lipids and proteins and destroy membranes. ROS can damage DNA and lead to mutation and chromosomal damage. Oxidized cellular thiols abstract hydrogen atoms from unsaturated fatty acids to initiate the peroxidation of membrane lipids (Valko et al., 2006). ROS can attack various substrates in body and contribute to development of chronic diseases. For example, oxidatively modified LDL has been hypothesized to be a causative agent in the development of cardiovascular diseases (Touyz, 2004).

Exogenous chemicals and endogenous metabolic processes in human body produce free radicals, especially oxygen derived radicals, which are capable of oxidizing biomolecules, resulting in cell death. Superoxide anion radicals increase under stress conditions such as heavy exercise, certain drugs, infection and various disease states. During normal metabolic processes, human body generates more than 2 Kg of $^{\cdot}O_2^-$ per year (Evans and Halliwell, 1999).

Cells are equipped with different kinds of mechanisms to fight against ROS and to maintain the redox homeostasis of cell. For example, antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) play important roles in scavenging the free radicals and preventing cell injury (Bergendi et al., 1999). Molecules such as vitamin C and E inhibit lipid peroxidation in cell. When the mechanism of antioxidant protection becomes unbalanced in human body, antioxidant supplement may be used to help reduce oxidative damage.

Natural sources of antioxidants

Medicinal plants are an important source of antioxidants (Rice-Evans, 2004). Natural antioxidants increase the antioxidant capacity of the plasma and reduce the risk of certain diseases such as cancer, heart diseases and stroke (Prior and Cao, 2000). The secondary metabolites like phenolics and flavonoids from plants have been reported to be potent free radical scavengers. They are found in all parts of plants such as leaves, fruits, seeds, roots and bark (Mathew and Abraham, 2006). There are many synthetic antioxidants in use. It is reported, however, they have several side effects (Ito et al., 1983), such as risk of liver damage and carcinogenesis in laboratory animals (Gao et al., 1999; Williams et al., 1999; Osawa and Namiki, 1981). There is therefore a need for more effective, less toxic and cost effective antioxidants. Medicinal plants appear to have these desired comparative advantages, hence the growing interest in natural antioxidants from plants.

Evaluation of antioxidant activity

A great number of *in vitro* methods have been developed to measure the efficiency of natural antioxidants either as pure compounds or as plant extracts. *In vitro* methods can be divided into two major groups: 1) Hydrogen atom transfer reactions like Oxygen Radical Absorbance Capacity (ORAC), Total radical trapping antioxidant potential (TRAP) and β carotene bleaching; 2) Electron transfer reactions like trolox equivalent antioxidant capacity (TEAC), Ferric reducing antioxidant power (FRAP), α, α-diphenyl-β-picryl-hydrazyl radical scavenging assay (DPPH), Superoxide anion radical scavenging assay, Hydroxyl radical scavenging assay, Nitric oxide radical scavenging assay and Total phenol assay (Huang et al., 2005a). These methods are popular due to their high speed and sensitivity. However, it is essential to use more than one method to evaluate antioxidant capacity of plant materials because of the complex nature of phytochemicals (Salazar et al., 2008). The most commonly and uncommonly used antioxidant assays along with various standards that can be used as positive control are described below.

SCREENING METHODS OF ANTIOXIDANT ACTIVITY: AN OVERVIEW

Total phenolic content (TPC)

Plant polyphenols, a diverse group of phenolic compounds (flavanols, flavonols, anthocyanins, phenolic acids, etc.) possess an ideal structural chemistry for free radical scavenging activity. Antioxidative properties of polyphenols arise from their high reactivity as hydrogen or electron donors from the ability of the polyphenol derived radical to stabilize and delocalize the unpaired electron (chain-breaking function) and from their potential to chelate metal ions (termination of the Fenton reaction) (Rice-Evans et al., 1997).

The amount of total phenol content can be determined by Folin-Ciocateu reagent method (McDonald et al., 2001). 0.5 ml of extract and 0.1 ml of Folin-Ciocalteu reagent (0.5 N) are mixed and incubated at room temperature for 15 min. Then 2.5 ml saturated sodium carbonate is added and further incubated for 30 min at room temperature and absorbance measured at 760 nm. Gallic acid (McDonald et al., 2001), tannic acid (Wolfe et al., 2003), quercetin (Singleton and Rossi, 1965), chlorogenic acid (Singleton et al., 1999), pyrocatechol (Slinkard and Singleton, 1977) or guaiacol (Yildirim et al., 2001) can be used as positive controls. The total phenolic content is expressed in terms of standard equivalent (mgg^{-1} of extracted compound).

Total flavonoid (TF)

The antioxidative properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper, and inhibition of enzymes responsible for free-radical generation (Benavente-Garcia, 1997). Depending on their structure, flavonoids are able to scavenge practically all known ROS.

The amount of total flavonoid content can be determined by Aluminum chloride method (Chang et al., 2002). 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 measured at 415 nm. Quercetin (Ordonez et al., 2006) or catechin (Kim et al., 2003) can be used as a positive control. The flavonoid content is expressed in terms of standard equivalent (mgg^{-1} of extracted compound).

Reducing power (RP)

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity (Oktay et al., 2003). Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants (Yen and Chen, 1995).

The reducing power can be determined by the method of Athukorala et al. (2006). 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 (600 mM) is added to the reaction mixture, centrifuged for 10 min at 3000 rpm. The upper layer of solution (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, butylated hydroxyanisole (BHA), α-tocopherol, trolox (Oyaizu, 1986) or butylated hydroxytoluene (BHT) (Jayaprakasha et al., 2001) can be used as positive control.

FREE RADICAL SCAVENGING ASSAYS

α, α-Diphenyl- β-picryl-hydrazyl radical scavenging (DPPH) Assay. The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant compounds. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen–donating antioxidant due to the formation of the nonradical form DPPH-H (Blois, 1958). This transformation results in a color change from purple to yellow, which is measured spectrophotometrically. The disappearance of the purple color is monitored at 517 nm. The free radical scavenging activity can be measured by using 2, 2-diphenyl-1-picryl-hydrazyl or 1, 1-diphenyl-2-picryl-hydrazyl by the method of McCune and Johns (2002). The reaction mixture (3.0 ml) consist of 1.0 ml of DPPH in methanol (0.3 mM), 1.0 ml of the extract and 1.0 ml of methanol. It is incubated for 10 min in dark, then the absorbance is measured at 517 nm. In this assay, the positive controls can be ascorbic acid, gallic acid (Blois, 1958), BHA, α-tocopherol (Shimada et al., 1992), quercetin (Shon et al., 2003), BHT (Liyana-Pathirana and Shahidi, 2005), rutin (Yamasaki et al., 1994), catechin (Astudillo et al., 2000) or glutathione (Kato et al., 1988). The percentage of inhibition can be calculated using the formula:

$$\text{Inhibition (\%)} = (A_0 - A_1 / A_0) \times 100$$

Where; A_0 is the absorbance of control and A_1 is the absorbance of test.

Superoxide anion radical scavenging (SO) assay

Although superoxide anion is a weak oxidant, it gives rise to generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress (Meyer and Isaken, 1995). Numerous biological reactions generate superoxide anions which are highly toxic species. In the 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 thus indicates the consumption of superoxide anion in the reaction mixture.

The superoxide anion scavenging activity is measured as described by Robak and Gryglewski (1988). The superoxide anion radicals are generated in 3.0 ml of Tris-HCl buffer (16 mM, pH 8.0), containing 0.5 ml of NBT (0.3 mM), 0.5 ml NADH (0.936 mM) solution, 1.0 ml extract and 0.5 ml Tris-HCl buffer (16 mM, pH 8.0). 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 against a blank sample. Gallic acid (Robak and Gryglewski, 1988), BHA, ascorbic acid, α-tocopherol, curcumin (Nishikimi et al., 1972), quercetin (Beauchamp and Fridovich, 1971) or trolox (Fernandes et al., 2003) can be used as a positive control.

Xanthine oxidase method

To determine superoxide anion-scavenging activity, two different assays can be used: the enzymatic method with cytochrome C (McCord and Fridovich, 1969) and non-enzymatic method with nitroblue tetrazolium (NBT) (Zhang and Lu, 1990). With cytochrome C method, superoxide anions can be generated by xanthine and xanthine oxidase system.

The xanthine oxidase activity with xanthine as the sub-

substrate is measured spectrophotometrically, by the method of Noro et al. (1983). The extract (500 µl of 0.1 mg/ml) and allopurinol (100 µg/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 room temperature (25°C), 1.5 ml of 0.15 M xanthine substrate solution is added to this mixture. The mixture is again incubated for 30 min at room temperature (25°C) and then the absorbance is measured at 293 nm using a spectrophotometer against blank (0.5 ml methanol, 1.3 ml phosphate buffer, 0.2 ml xanthine oxidase). The solution of 0.5 ml methanol, 1.3 ml phosphate buffer, 0.2 ml xanthine oxidase and 1.5 ml xanthine substrate is used as a control. Percentage of inhibition was calculated using the formula:

Percentage of inhibition = $[1 – (A_s / A_c)] \times 100$

Where; A_s and A_c are the absorbance values of the test sample and control, respectively. BHT (Chang et al., 1996) or catechin (Schmeda-Hirschmann et al., 1996) can be used as a positive control.

Hydrogen peroxide radical scavenging (H_2O_2) assay

Hydrogen peroxide occurs naturally at low concentration levels in the air, water, human body, plants, microorganisms, food and beverages. It is widely used as a bleaching agent in the textile, paper and pulp industries. Human beings exposed to H_2O_2 indirectly via the environment are estimated as 0.28 mg/kg/day with intake from leaf crops contributing most to this exposure. Hydrogen peroxide enters the human body through inhalation of vapor or mist and through eye or skin contact. In the body, H_2O_2 is rapidly decomposed into oxygen and water and this may produce hydroxyl radicals ($OH^{\cdot}$) that can initiate lipid peroxidation and cause DNA damage.

The ability of plant extracts to scavenge hydrogen peroxide is determined according to the method of Ruch et al. (1989). A solution of hydrogen peroxide (40 mM) is prepared in phosphate buffer (50mMpH 7.4). The concentration of hydrogen peroxide is determined by absorption at 230 nm using a spectrophotometer. Extract (20 - 60 µg/ml) in distilled water isadded to hydrogen peroxide and absorbance at 230 nm is determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging is calculated as follows:

% Scavenged (H_2O_2) = $(A_0 – A_1 / A_0) \times 100$

Where; A_0 is the absorbance of control and A_1 is the absorbance of test. Ascorbic acid, rutin BHA (Jayaprakasha et al., 2004), α-tocopherol (Gulcin et al., 2003) or quercetin (Ruch et al., 1989) can be used as a positive control.

Nitric oxide radical scavenging (NO) assay

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interact with oxygen to produce nitrite ions, which were measured using the Griess reaction reagent (Green et al., 1982). 3.0 ml of 10 mM sodium nitroprusside in phosphate buffer is added to 2.0 ml of extract and reference compound in different concentrations (20 - 100 µg/ml). The resulting solutions are then incubated at 25°C for 60 min. A similar procedure is repeated with methanol as blank, which serves as control. To 5.0 ml of the incubated sample, 5.0 ml of Griess reagent (1% sulphanilamide, 0.1% naphthyethylene diamine dihydrochloride in 2% H_3PO_3) is added and absorbance of the chromophore formed is measured at 540 nm. Percent inhibition of the nitrite oxide generated is measured by comparing the absorbance values of control and test preparations. Curcumin, caffeic acid, sodium nitrite (Sreejayan, 1997), BHA, ascorbic acid, rutin (Jayaprakasha et al., 2004), BHT or α-tocopherol (Garrat, 1964) can be used as a positive control.

Hydroxyl radical scavenging (HO) assay

Hydroxyl radical is one of the potent reactive oxygen species in the biological system. It reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and causes damage to cell (Halliwell and Gutteridge, 1981). The model used is ascorbic acid-iron-EDTA model of $HO^{\cdot}$ generating system. This is a totally aqueous system in which ascorbic acid, iron and EDTA conspire with each other to generate hydroxyl radicals. The scavenging ability for hydroxyl radicals is measured by the method of Kunchandy and Rao (1990). 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), 500 µl of the extract, 200 µl EDTA (1.04 mM) and 200 µM $FeCl_3$ (1:1 v/v), 100 µl of H_2O_2 (1.0 mM) and 100 µl ascorbic acid (1.0 mM) which is incubated at 37ºC for 1 h. 1.0 ml of thiobarbituric acid (1%) and 1.0 ml of trichloroacetic acid (2.8%) are added and incubated at 100ºC for 20 min. After cooling, absorbance is measured at 532 nm, against a blank sample. Gallic acid, mannitol, catechin (Kunchandy and Rao, 1990), vitamin E (Halliwell et al., 1987), quercetin, BHA (Halliwell and Gutteridge, 1981), α-tocopherol (Klein et al., 1981), rutin or ascorbic acid (Jayaprakasha et al. 2004) can be used as a positive control.

Metal chelating activity

Ferrozine can quantitatively chelate 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 (Soler-Rivas et al., 2000).

The chelation of ferrous ions is estimated using the method of Dinis et al. (1994). 0.1 ml of the extract is added to a solution of 0.5 ml ferrous chloride (0.2 mM). The reaction is initiated by the addition of 0.2 ml of ferrozine (5 mM) and incubated at room temperature for 10 min and then the absorbance is measured at 562 nm. EDTA or citric acid (Dinis et al., 1994) can be used as a positive control.

Total antioxidant activity

The oxidation of linoleic acid generates peroxyl free radicals due to the abstraction of hydrogen atoms from diallylic methylene groups of linoleic acid (Kumaran and Karunakaran, 2006). The free radicals then will oxidize the highly unsaturated beta carotene. Consequently, the orange coloured chromophore of beta carotene would be degraded and the results can be monitored spectrophotometrically.

The antioxidant activity is determined by the conjugated diene method (Lingnert et al., 1979). Each extract (0.1 - 20 mg/ml) in water or ethanol (100 µl) is mixed with 2.0 ml of 10 mM linoleic acid emulsion in 0.2 M sodium phosphate buffer (pH 6.6) in a test tube and kept in dark at 37°C to accelerate oxidation. After incubation for 15 h, 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. The antioxidant activity is calculated as follows:

Antioxidant activity (%) = $(A_0 – A_1 / A_0)$ X 100

Where; A_0 is the absorbance of control and A_1 is the absorbance of test. Ascorbic acid, BHA, α-tocopherol (Lingnert et al., 1979) or trolox (Mitsuda et al., 1996) can be used as a positive control.

Oxygen radical absorbance capacity (ORAC) assay

The capacity of a compound to scavenge peroxyl radicals, generated by spontaneous decomposition of 2, 2'-azo-bis, 2- amidinopropane dihydrochloride (AAPH), was estimated in terms of standard equivalents, using the ORAC assay (Prior et al., 2005).

The method of Ou et al. (2002a, 2002b) is used for the estimation. 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 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^{-1}).

Ferric reducing antioxidant power (FRAP) assay

FRAP assay is based on the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} in the presence of 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), forming an intense blue Fe^{2+}-TPTZ complex with an absorption maximum at 593 nm. This reaction is pH-dependent (optimum pH 3.6). The absorbance decrease is proportional to the antioxidant content (Benzie and Strain, 1996). 0.2 ml of the extract is added to 3.8 ml of FRAP reagent (10 parts of 300 mM sodium acetate buffer at pH 3.6, 1 part of 10.0 mM TPTZ solution and 1 part of 20.0 mM $FeCl_3$. $6H_2O$ solution) and the reaction mixture is incubated at 37°C for 30 min and the increase in absorbance at 593 nm is measured. $FeSO_4$ is used for calibration. The antioxidant capacity based on the ability to reduce ferric ions of sample is calculated from the linear calibration curve and expressed as mmol $FeSO_4$ equivalents per gram of sample. BHT, BHA, ascorbic acid, quercetin, catechin or trolox (Benzie and Strain, 1996) can be used as a positive control.

Trolox equivalent antioxidant capacity (TEAC) assay

The $ABTS^{\cdot+}$ formed from the reaction ABTS-e^- → $ABTS^{\cdot+}$ reacts quickly with ethanol/hydrogen donors to form colourless 2, 2'-azinobis (3-ethyl-benzothiazoline 6-sulfonate (ABTS). The reaction is pH - independent. A decrease of the $ABTS^{\cdot+}$ concentration is linearly dependent on the antioxidant concentration.

The ABTS free radical-scavenging activity of plants samples is determined by the method of Stratil et al. (2006). The radical cation $ABTS.^+$ is generated by persulfate oxidantion of ABTS. A mixture (1:1, v/v) of ABTS (7.0 mM) and potassium persulfate (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 values 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 at 37°C in the dark. Aqueous phosphate buffer solution (3.9ml, without $ABTS.^+$ solution) is used as a control. The $ABTS^+$ scavenging rate is calculated. Trolox, BHT, rutin (Re et al., 1999), ascorbic acid (Alzoreky and Nakahara, 2001) or gallic acid (Auddy et al., 2003) can be used as a positive control.

Conclusion

Many solvents are used for extraction of bioactive compounds from the plants. The solvents used vary in their polarity. The most commonly used solvent is metha-

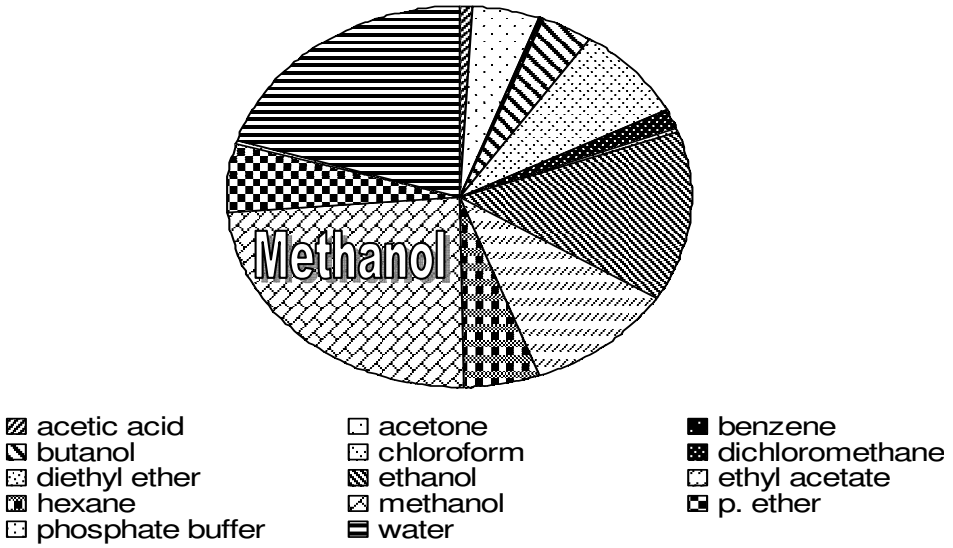

Figure 1. Various solvents used for plants extraction.

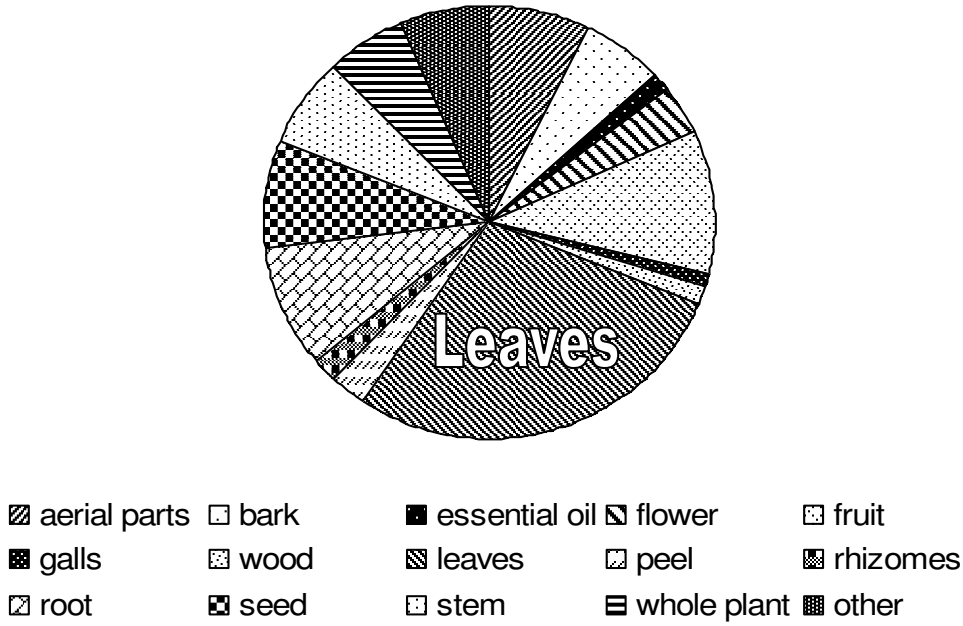

Figure 2. Different plant parts used for the study of antioxidant activity.

nol as shown in Figure 1. Traditionally, water is used for extraction but it is next to methanol. The use of non polar solvents is comparatively less indicating that the active constituents are soluble in polar solvents only. Generally, any part of the plant can be used for antioxidant studies but most commonly used part is leaf followed by fruit (Figure 2).

Table 1 lists some of the plants which show antioxidant activity. It also shows which part of the plant is used for antioxidant studies, which solvent is used and which assays are employed. It is generally believed that plants which are having more phenolic content show good antioxidant activity that is there is a direct correlation between total phenol content and antioxidant activity (Brighente et al., 2007; Biglari et al., 2008; Salazar et al., 2008; Saravana et al., 2008). However there are reports which do not show this correlation (Agbor et al., 2005; Maisuthisakul et al., 2007). It can be stated that phenolic content of the plant may be a good indicator of its antioxidant capacity.

Table 1. List of medicinal plants, their family, parts used solvents used for extraction and assay employed, for antioxidant studies.

Plants (family)	Parts used	Solvent	Assay	References
Acacia auriculiformis A. Cunn. ex Benth. (Mimosaceae)	bark	HE, C, A, ET, ME	TPC, DPPH, HO, RP, TBARS	Singh et al. (2007)
Achillea millefolium subsp. Millefolium Afan. (Asteraceae)	essential oil	C, ME, Water	DPPH, SO, HO, TBARS	Candan et al. (2003)
Aegle marmelos Correa(Rutaceae)	fruit	ME	DPPH, RP, NO,SO	Dhalwal et al. (2008)
Allanblackia floribunda Oliv (Guttiferae)	leaves, fruit	ME	DPPH, TPC, TF	Ayoola et al. (2008)
Amaranthus lividus L. (Amaranthaceae)	stem, leaves, flower	EA, ME Water	TEAC, DPPH , RP, Metal chelating, HO	Ozsoy et al. (2009)
Aporosa lindleyana Baill. (Euphorbiaceae)	root	PE, C, EA, ME	DPPH, NO	Badami et al. (2005a)
Argyreia cymosa R. Sweet (Convolvulaceae)	bark	PE, C, EA, ME	DPPH, HO, ABTS, NO, H_2O_2	Badami et al. (2008)
Aristotelia chilensis Maqui (Elaeocarpaceae)	fruit	EA, ME, Water	ORAC, FRAP , TPC	Céspedes et al. (2008)
Azadirachta indica A. Juss var. siamensis Valeton (Meliaceae)	leaves, raw fruit, ripe fruit, flower stem bark	HE, ME, Water	DPPH, total antioxidant activity, TBARS	Sithisarn et al. (2005)
Azadirachta indica A. Juss var. siamensis Valeton (Meliaceae)	leaves	ET	DPPH	Sithisarn et al.(2006)
Byrsonima crassifolia H. B. and K.(Malpighiaceae)	leaves, bark, fruit	ME, Water	TPC, TF	Souza et al. (2008)
Bergia suffruticosa (Delile) (Elatinaceae)	whole plant	ME	TPC, DPPH, SO, RP	Anandjiwala et al. (2007)
Burkea africana Hook (Leguminocaea)	bark	PE, BT, EA, Water	DPPH, TBARS	Mathisen et al. (2002)
Caesalpinia digyna Rottler (Caesalpiniaceae)	root	PE, ME, Water	TPC, ABTS, DPPH, H_2O_2, NO, SO, HO, in *vivo*	Srinivasan et al. (2007)
Caesalpinia sappan L. (Caesalpiniaceae)	heartwood	PE, C, EA, ME, Water	DPPH, NO, In *vivo*	Badami et al. (2003)
Camellia crassicolumna Var. multiplex (Theaceae)	leaves	C, EA, Water	DPPH	Liu et al., (2009)
Camellia sinensis var. sinesis (L.) Kuntz (Theaceae)	leaves	ET, Water	HO, SO	Chen et al., (2008)
Campanula alliariifolia Willd.(Campanulaceae)	whole plant	C, ME	DPPH, RP	Dumlu et al., (2008)
Campis grandiflora (Thunb.) K. Schum	flower	50% ET	DPPH, SO	Cui et al., (2006)
Careya arborea Roxb (Barringtoniaceae)	bark	PE, C, EA, ME	ABTS, DPPH, H_2O_2, NO, TPC, Total antioxidant capacity	Senthilkumar et al., (2007)
Cassia siamea Lam. (Caesalpiniaceae)	flower	95% ET	TPC, RP, DPPH, H_2O_2, NO, Protein oxidation, TBARSMetal chelating	Kaur et al., (2006)

Table 1. Contd.

Cassia tora L. (Caesalpiniaceae)	seeds	ME	RP, DPPH, Metal Chelating	Zhenbao et al., (2007)
Celtis africana Burm.f. (Ulmaceae)	stem, leaves	ME	TPC, TF, ABTS, DPPH, FRAP	Adedapo et al., (2009)
Chaerophyllum hirsutum L. (Apiaceae)	root, aerial parts	C, ME	DPPH	Acqua and Innocenti, (2004)
Chamaecyparis lawsoniana (A. Murr.) Parl.(Cupressaceae)	wood, bark	ME	ABTS, DPPH, TPC	Gao et al., (2007)
Chlorophytum tuberosum baker (Liliaceae)	whole plant	PE, 80% ET	DPPH, NO, TBARS	Narasimhan et al., (2006)
Cissus quadrangularis L. (Vitaceae)	stem	ME	SO, DPPH, TBARS	Jainu and Shyamala Devi, (2005)
Citrullus colocynthis L. (Cucurbitaceae)	fruit	ME	TPC, TF, DPPH, HO, NO, SO	Kumar et al. (2008)
Cocos nucifera L. (Arecaceae)	mesocarp	ME	DPPH, FRAP	Chakraborty and Mitra (2008)
Cordia gilletii De Wild (Boraginaceae)	root bark	HE, DM,EA, ME, Water	DPPH	Okusa et al. (2007)
Cydonia vulgaris Pers. (Rosaceae)	leaves	ET	RP, total antioxidant activity	Yildirim et al. (2001)
Cynara cardunculus L. (Compositae)	involucral bracts	C, BT , EA, ET	FRAP, DPPH	Kukic et al. (2008)
Cytisus scoparius Linn. (Leguminosae)	aerial parts	70% ET	DPPH, NO, SO, HO, RP, TPC	Sundararajan et al.(2006)
Dimocarpus Longan Lour (Sapindaceae)	peels	ET	DPPH, TPC, HO, RP, total antioxidant activity	Pan et al. (2008)
Dimocarpus Longan Lour (Sapindaceae)	seeds	PE, C, BT, ME	DPPH, SO	Zheng et al. (2009)
Diospyros ebenum Roxb. (Ebenaceae)	leaves	PE, EA, ME, Water	DPPH, TPC, TF	Baravalia et al. 2009
Dipsacus asper Wall (Dipsacaceae)	root	C, BT, EA, ME	DPPH	Hung et al. (2006)
Ecklonia cava (Alariaceae)	prothallus	HE, C, EA, 70% ME	DPPH, SO, HO, H_2O_2, RP, NO	Senevirathne et al. (2006)
Eclipta prostrata L. (Asteraceae)	whole plant	HE, EA, ET, Water	Antinociceptive activity	Karthikumar et al. (2007)
Elephantopus tomentosus L. (Asteraceae)	whole plant	ET	DPPH, total antioxidant activity, RP, HO, TPC	Yam et al. (2008)
Emblica officinalis Gaertn (Euphorbiaceae)	fruit	ME	TPC, DPPH, ABTS	Scartezzini et al.(2006)
Ferula szovitsiana DC (Umbelliferae)	aerial parts, root	HE, DE, EA, ME	TPC, DPPH FRAP, In *vivo*	Dehghan et al. (2007)
Garcinia kola Heckel (Guttiferae)	seeds	PE, EA, A	RP, DPPH, SO, H_2O_2, HO	Farombi et al. (2002)
Gracilaria changii (Gracilariaceae)	prothallus	80% ME	TPC, DPPH (TLC)	Sreenivasan et al. (2007)
Vitis vinifera L. (Vitaceae)	seeds, bagasse, berries	PE, A, AA, Water	total antioxidant activity, TPC, H_2O_2	Baydar et al. (2007)
Gynura procumbens (Merr.) (Compositae)	leaves	PE, C, BT, EA, ME, Water	ABTS, total, RP, TPC antioxidant activity, DPPH, Xanthin oxidase,	Rosidah et al. (2008)
Haplopappus baylahuen Remy (Asteraceae)	leaves	ME	DPPH (TLC)	Vogel et al. (2005)
Heracleum nepalense D Don (Apiaceae)	root	70 % ME	DPPH, HO, SO	Dash et al. (2005)

Table 1. Contd.

Hordeum vulgare L. (Poaceae)	seeds	HE, ME	TPC, DPPH, metal chelating	Madhujith and Shahidi (2008)
Hymenocardia acida Tul. (Hymenocardiaceae)	leaves	ME , Water	DPPH, RP, ABTS, TF	Sofidiya et al. (2009)
Hypericum venustum Fenzl (Hypericaceae)	flower	ET, Water	RP, SO, Metal chelating, H_2O_2	Spiteller et al. (2008)
Hyphaene thebaica (L.) Mart. (Arecaceae)	fruit	Water	TPC, RP, DPPH, Metal chelating, HO, SO	Hsu et al. (2005)
Hypsizigus marmoreus (Peck) Bigelow (Tricholomataceae)	prothallus	ET, Water	Total antioxidant activity, RP, DPPH, Metal chelating	Lee et al. (2008)
Ilex kudingcha C.J. Tseng (Aquifoliaceae)	leaves	C , BT, EA, Water	TPC, DPPH, TEAC, FRAP	Liu et al. (2009)
Inonotus obliquus (Hymenochaetaceae)	prothallus	EA, 80% ET, Water	DPPH, SO	Cui et al. (2005)
Ipomoea aquatica Forsk (Convolvulaceae)	leaves, veins	95% ET	DPPH, TPC, TF, RP, Ferric thiocynate	Huang et al. (2005b)
Jasminum sambac Linn. (Oleaceae)	leaves	BT, EA, Water	DPPH, NO, HO, β-carotene, RP	Tenpe et al. (2008)
Kadsura coccinea (Lem.) A.C. smith (Schisandraceae)	peels, pulp	EA, A, ME, Water	TPC, DPPH, FRAP, metal chelating	Sun et al. (2009)
Kappaphycus alvarezii (Doty) Doty (Solieriaceae)	prothallus	HE, EA, ET, ME, Water	TPC, DPPH, Metal chelating, RP, Total antioxidant activity	Suresh Kumar et al. (2008)
Lannea velutina A. Rich (Anacardiaceae)	root bark	ET, ME, Water	DPPH	Maiga et al. (2007)
Laurus nobilis L. (Lauraceae)	leaves	ET	Total antioxidant activity, Metal chelation, SO, DPPH, RP, TPC	Elmastas et. al. (2006)
Lawsonia inermis L. (Lythraceae)	leaves	HE, C, A, ET	TPC	Khodaparast et al. (2007)
Lecaniodiscus cupanioides Planch. (Sapindaceae)	leaves	ME	DPPH, ABTS, TPC, TF	Sofidiya et al. (2008)
Lithospermum erythrorhizon Sieb. & Zucc. (Boraginaceae)	root	PE, C	DPPH, RP, ABTS	Han et al. (2008)
Mahonia aquifolium (Pursh) Nutt. (Berberidaceae)	stem bark	ET	DPPH	Rackova et al. (2007)
Mangifera indica L. (Anacardiaceae)	fruits (pulp, seeds, peels, kernels)	ME, Water	TPC, DPPH, RP	Ribeiro et al. (2008)
Mangifera indica L. (Anacardiaceae)	seed kernels	95% ET	TPC, Metal chelating activity, DPPH, ABTS	Maisuthisakul and Gordon (2009)
Momordica dioica Roxb. (Cucurbitaceae)	leaves	PE, ET, Water	DPPH, In *vivo*	Jain et al. (2008)

Table 1. Contd.

Murraya koenigii L. (Rutaceae)	leaves	HE, C, ET, Water	SO, HO, DPPH, ferric reducing, Metal chelating	Ningappa et al. (2008)
Musa paradisiaca L. (Musaceae)	peels	70% A	DPPH,	Mokbel and Hashinaga (2005)
Nasturtium officinale R. Br. (Brassicaceae)	aerial parts	ET: Water (7:1)	TPC, TF, DPPH, FRAP, In *vivo*	Yazdanparast et al. (2008)
Nelumbo nucifera Gaerth. (Nymphaeaceae)	seeds	50% ET	DPPH, NO, In *vivo*	Rai et al. (2006)
Nelumbo nuficera Gaerth. (Nymphaeaceae)	rhizomes	PE, DM, A, ME, ET	TPC, DPPH, β carotene	Me et al. (2007)
Nicotina tabacum L.(Solanaceae)	leaves	80% ET	TPC, HO, SO, DPPH, RP	Wang et al. (2008)
Ocimum basilicum L. (Lamiaceae)	leaves	ET	Total antioxidant activity, RP, HO, DPPH	Gulcin et al. (2007)
Osbeckia aspera Blume (Melastomataceae)	leaves	A, ME, Water	Hepatoprotective activity	Grayer et al. (2008)
Parmelia saxatilis (L.) (Parmeliaceae)	prothallus	ME, Water	RP, SO, Ferric thiocynate , metal chelating, H_2O_2, TPC	Ozen and Kinalioglu (2008)
Paullinia cupana Mart. (Sapindaceae)	seeds	ME,35% A, 60% ET, Water	TPC, β carotene assay, DPPH	Majhenic et al. (2007)
Pedilanthus tithymaloides L. Poit. (Euphorbiaceae)	stem, leaves	30 % ET	SO, HO, H_2O_2, NO, DPPH	Abreu et al. (2006)
Perilla pankinesis decne (Labiatae)	leaves	EA , 1% AA	Ferric thiocynate , RP, Metal chelating, H_2O_2, DPPH, SO	Gulcin et al. (2005)
Peumus boldus Mol. (Monimiaceae)	leaves	EA, ME, Water	DPPH, SO, Xanthine oxidase, TBARS, TPC, TF	Schmeda-Hirschmann et al. (2003)
Phoenix dactylifera L. (Arecacea)	fruit	ME :Water (4:1)	ABTS, FRAP, TPC, TF	Biglari et al. (2008)
Phyllanthus emblica L. (Euphorbiaceae)	fruit	ME	DPPH, SO, HO, RP	Liu et al. (2008)
Phyllanthus niruri Linn. (Euphorbiaceae)	leaves , fruit	ME, Water	TPC, DPPH, SO, TBARS	Harish and Shivanandappa (2006)
Piper nigrum Linn. (Piperaceae)	seeds	ET, Water	DPPH, Total antioxidant activity, RP, Metal chelating H_2O_2, TPC	Gulcin (2005)
Piper nigrum Linn. (Piperaceae)	fruit	PE, EA	SO, HO, DPPH, NO, TPC	Singh et al. (2008)
Polygonum paleaceum Wall. ex Hook. f. (Polygonaceae)	rhizomes	BT, EA, A, Water	DPPH	Wang et al. (2005)
Psidium guajava L. (Myrtaceae)	leaves	50 % ET, Water	DPPH, TPC	He and Venant (2004)
Pyrrosia petiolosa Ching (Polypodiaceae)	whole plant	50% ET	DPPH, SO, TPC, TF	Hsu (2008)
Ramaria flava (Schaeff) Quel. (Ramariaceae)	prothallus	ET	DPPH, β carotene, TPC, TF	Gezer et al. (2006)

Table 1. Cont.

Randia hebecarpa Benth. (Rubiaceae)	leaves	HE, EA, ME, Water	DPPH, Total antioxidant activity	Nazari et al. (2006)
Rhizophora mangle L.(Rhizophoraceae)	bark	Water	In *vivo*	Berenguer et al. (2006)
Rhus succedanea L. (Anacardiaceae)	galls	Water	DPPH, NO	Baheti et al. (2005)
Rosmarius officinalis L. (Lamiaceae)	leaves	Essential oil	TPC, FRAP	Stefanovits-Banyai et al.(2003)
Rubus ulmifolius Schott (Rosaceae)	leaves	ME	TEAC, TPC, DPPH	Dall'Acqua et al. (2008)
Rumex crispus L. (Polygonaceae)	aerial parts	ME, Water	TPC, β carotene	Coruh et al. (2008)
Rumex ecklonianus Meissner (Polygonaceae)	whole plant	A, ME, Water	TPC, TF, ABTS, DPPH, FRAP	Jimoh et al. (2008)
Salvia mirzayani Rech. (Labiatae)	aerial parts	PE, C, BT, EA, ET, Water	RP, DPPH, β carotene, TPC	Moein et al. (2007)
Salvia verbenaca L. (Lamiaceae)	aerial parts	ME, Water	Total antioxidant activity, TPC, TBARS	Khlifi et al. (2006)
Sargassum Sp.(Sargassaceae)	prothallus	ME	HO, DPPH, In *vivo*	Patra et al. (2008)
Scoparia dulcis L.(Scrophulariaceae)	whole plant	Water	TBARS	Ratnasooriya et al. (2005)
Sida cordifolia Linn (Malvaceae)	root, stem, leaves, whole plants	90% ET	TPC, DPPH, RP, NO, SO	Dhalwal et al. (2005)
Sideritis raeseri Boiss et Heldr. subsp. raeseri (Lamiaceae)	aerial parts	BZ , C, ME	DPPH	Gabrieli et al. (2004)
Smilax china L. (Liliaceae)	root	HE, BT, DM, EA, ME	DPPH, In *vivo*	Lee et al. (2001)
Smilax glyciphylla Sm.(Smilaceae)	leaves, stem	Water	TBARS, SO, TRAP, HO	Cox et al. (2005)
Solanum pseudocapsicum L. (Solanaceae)	leaves	ME	DPPH, NO, ABTS, HO, H_2O_2	Badami et al. (2005b)
Soymida febrifuga (Roxb.) A. Juss. (Meliaceae)	leaves	ME	TPC, DPPH	Reddy et al. (2008)
Sphenocentrum jollyanum Pierre (Menispermaceae)	leaves, stem, root, bark	ME	DPPH, DPPH (TLC)	Nia et al. (2004)
Staphylea sp. L. (Staphyleaceae)	leaves	PE, C,EA, Water	TPC, DPPH	Lacikova et al. (2007)
Tagetes mendocina Phil. (Asteraceae)	aerial parts	HE, DM, ME	DPPH (TLC), DPPH, SO	Schmeda-Hirschmann et al. (2004)
Tamarindus indica L. (Fabaceae)	seed coat	ME	TPC, SO, Total antioxidant activity, DPPH, ABTS, FRAP	Siddhuraju (2007)
Tamus communis L. (Dioscoreaceae)	root	ME, Water	TPC, TF, xanthine oxidase, TRAP	Boumerfeg et al. (2009)
Terminalia chebula Retz. (Combretaceae)	fruit	Water	DPPH, xanthine oxidase, In *vivo*	Naik et al. (2004)
Toona sinensis Roem (Meliaceae)	leaves	Water	In *vivo*, RP, Metal chelating	Hseu et al. (2008)

Table 1. Contd.

Uncaria tomentosa Willd. DC. (Rubiaceae)	bark	Phosphate buffer (0.1M, pH 7.4), ET	TPC, TEAC, peroxyl radical trapping capacity	Pilarski et al. (2006)
Urtica dioica L. (Urticaceae)	nettle	Water	Total antioxidant activity, RP, SO, DPPH, Metal chelating, H_2O_2, TPC	Gulcin et al. (2004)
Vaccinium stamineum L. (Ericaceae)	fruit	80% A	TPC, TF, ORAC, ABTS, DPPH, SO, H_2O_2, HO	Wang and Ballington (2007)
Varthemia iphionoides Boiss. (Asteraceae)	aerial parts	HE, EA, ET, Water	TPC, TF, DPPH, RP	Al-Dabbas et al. (2006)

A – acetone, AA – acetic acid, BT – butanol, BZ – benzene, C – chloroform, DE – diethyl ether, DM – dichloromethane, EA – ethyl acetate, ET – ethanol, HE – hexane, ME – methanol, PE – petroleum ether.

Summary

This review provides information on a number of plants which show promising antioxidant activity. It lists various methods for evaluating antioxidant activity along with different standards so it will be easy for the experimenter. It is also recommended to use at least two different types of assays for antioxidant activity. It emphasizes that *in vitro* antioxidant assays have been carried out for most of the plants, but *in vivo* remains to be done in majority of them. Methanol as a solvent has priority for extraction of plants for evaluating their antioxidant activity.

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