Evaluation of radical scavenging activity of certain plant extracts using cell free assays

R.M. Samarth*[1] and Vivek Krishna[2]

[1]Radiation and Cancer Biology Laboratory, Department of Zoology, University of Rajasthan, Jaipur -302 004 (India).

[2]Department of Chemistry, University of Rajasthan, Jaipur-302004 (India).

Abstract:

Methanolic extracts of 35 plant were evaluated for their radical scavenging activity using $DPPH^{\bullet}$ and $ABTS^{+\bullet}$ assays. Out of 35 plant extract screened, 15 showed radical scavenging activity in both $DPPH^{\bullet}$ and $ABTS^{+\bullet}$ assays. In the present investigation, *Aleurites trispesma*, *Elaeocaspus multiforus, Livestona chinensis and Nagia nagi* showed the very strong radical scavenging activity in both DPPH and ABTS assays. However, extracts of plants such as *Syzygium jambos, Grevillea robusta, Terminalia bovinii, Elaeocarpus serratus, Tabebuia pentaphylla, Fraxinus griffithii, Machilus zuihoensis, Artocarpus incisus, Cajanus cajan* and *Corchorus aestuand* showed moderate antioxidant activity. The IC_{50} values of these plant extracts were also calculated and compared.

Key Words:

Radical scavenging activity, $DPPH^{\bullet}$ and $ABTS^{+\bullet}$, methanolic extract, antioxidant activity.

Corresponding author: rmsamarth@yahoo.co.in; samarth_ravindra@rediff.com

Introduction

The essential oils and extracts of many plant species have become popular in recent years, and attempts to characterize their bioactive principles have gained momentum in many pharmaceutical and food processing applications (Cowan, 1999). Numerous physiological and biochemical processes in the human body produce oxygen centered free radicals and other reactive oxygen species as byproducts. Over production of such free radicals cause oxidative damage to biomolecules leading to many chronic diseases (Halliwell, 1994; Niki, 1997; Paulson et al, 1998). Plants (fruits, vegetables, medicinal herbs etc.) contain a wide variety of free radical scavenging molecules, such as phenolic compounds, nitrogen compounds, vitamins, terpenoids, and some other endogenous metabolites, that are rich in antioxidant activity (Larson, 1988; Shahidi and Nalzk, 1995; Cotelle et al 1996: Velioglu et al; 1998; Zheng and Wang, 2001, Cai et al 2003). The intake of natural antioxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes and other diseases associated with ageing, but there is still controversy in this area (Hertog et al, 1995; Kuo, 1997; Mclarty 1997; Yang et al, 2001; Sun et al 2002). Antioxidant activity is a fundamental property important for human life and many of the biological functions including antimutagenicity, anticarcinogenicity, and antiageing. Therefore, it is important to select and employ a stable and rapid method to assay antioxidant activity. Several methods have been developed to assay of radical scavenging capacity and total antioxidant activity of plant extracts. The most common and reliable method involves the determination of the disappearance of free radicals using a spectrophotometer, such as 2, 2-azinobis (3- ethyl benzothiazolium -6-sulfonic) acid

radical ($ABTS^{\bullet+}$) and 1, 1-diphenyl -2-picrylhydrazyl radical ($DPPH^{\bullet}$) was employed in the present study. A large number of aromatic, spicy, medicinal and other plants were studied for antioxidant properties and resulted in a development of antioxidant formulation for food, cosmetic and other applications. However, scientific information on antioxidant properties of various plants, particularly those that are less widely used in culinary and medicine is still rather scarce. Therefore, the assessment of such properties remains new area for finding sources for natural antioxidants, functional foods and neutraceuticals. Chemical and biological diversity of aromatic and medicinal plants depending on factors such as collection area climatic conditions, vegetation place, genetic modifications and others is an important impetus to study flora present in different growing sites, countries and geographical zones. Present investigation is aimed for evaluation of radical scavenging activities of the certain plant extracts (Table 1).

Materials and Methods

1. **Plant materials and preparation of extracts-** Plant materials (fresh leaves) were collected from identified plants. The fresh leaves were cut into small pieces, dried under shade and ground. The air dried and finely ground samples were extracted with methanol at room temperature for 48 hrs. The extracts were filtered and concentrated in a rota vapor apparatus at 40^0 C. The extraction was repeated three times and the solvent was evaporated *in vacuo*.

2. **Evaluation of antioxidant activity**

   2.1 DPPH radical scavenging assay: Radical scavenging activity of plant extracts against stable DPPH (1, 1 diphenyl-2-picryl hydrazyl radical) was determined spectrophotometrically.

Table 1. Plant extracts screened for the radical scavenging activity

Name of the plant	Family
Liquidamber formosana	Hamamelidaceae
Araucaria cunninghamii	Araucasiaceae
Ixora duffii	Rubiaceae
Aglaia elliptifolia	Meliaceae
Acmena accuminatissima	Myrtaceae
Adenanthera pavonica	Mimosaceae
Macadamia ternifolia	Proteacaeae
Nageia nagi	Podocarpaceae
Phyllanthus acidus	Euphorbiaceae
Elacocarpus multiforus	Elaeocarpaceae
Pistacea Chinensis	Anacardiaceae
Spondias cythera	Anacardiaceae
Agathis dammara	Arauriaceas
Aleurites trispenna	Euphorbiaceae
Grevillea robusta	Proteaceae
Syzygium jambos	Myrtaceave
Terminalia boivinii	Combretaceae
Elaeocarpus serratus	Elaeocarpaceae
Podocarpus costalis	Podocarpaceae
Tabebuia pentaphylla	Bignoniaceae
Fraxinus griffithii	Oleacea
Samanea Saman	Mimosaceae
Livestoma chinensis	Arecaceae
Cinnamomum reticulatum	Lauraceae
Tabernaemontana subglobosa	Apocyanaceae
Machilus zuihoensis	Lauraceae
Artocarpus incisus	Moraceae
Podocarpus macrophyllus	Podocarpaceae
Myristica cagayanesis	Mysisticaceae
Gonocaryun calleryanum	Icacinaceae
Cajanus cajan	Leguminosae
Echinacea purpurea	Asteraceae
Corchorus acstuand	Tiliaceae
Michelia alba	Magnoliaceae
Machilus kusanoi	Lauraceae

The DPPH assay was carried out as described by Cuendet et al (1997). Stock solutions of crude extracts were prepared as 1mg/ml in methanol.

Fifty microlitre of different concentration samples were added to 5 ml of 0.004% methanol solution of $DPPH^{\bullet}$. After 30 min incubation in dark at room temperature, the absorbance was read against a blank at 517 nm. The assay was carried out in triplicate percentage of inhibition was calculated using the following formula.

$$\% \text{ Inhibition} = \frac{(AB-AA)}{AB} \times 100$$

Where AB= Absorption of blank

AA= Absorption of test

2.2 ABTS radical cation decolourization assay: $ABTS^{\bullet+}$, 2, 2 azinobis (3-ethyl;benzothiazolium-6-sulfonic acid) radical cation decolourization test is also a spectrophotometric method widely used for the assessment of antioxidant activity of various substances. ABTS assay was carried out using an improved ABTS decolourization assay of Re et al (1999). $ABTS^{\bullet+}$ was generated by oxidation of ABTS with potassium persulphate. ABTS was dissolved in deionized water to 7 Mm concentration, and potassium persulphate added to a concentration of 2.45 Mm. The reaction mixture was left to stand at room temperature overnight (12-16 hr) to dark before use for the study of plant extracts, the $ABTS^{\bullet+}$ solution was diluted with ethanol, to an absorbance of 0.700±.020 at 734 nm. After addition of 1 ml of diluted

ABTS solution (A 734 nm = .700±.020) to 10 μl of plant extracts the absorbance reading was taken at 30^0 C exactly 1 min., after initial mixing and upto 6 min. All determinations were carried out in implicates.

Results

The results of DPPH and $ABTS^+$ inhibition by different plant extracts are summarized in Table 2 and 3 respectively.

Table 2. IC_{50} (μg/ml) values and % inhibition of DPPH activity of plant extracts at of different concentrations

Plant Extract	IC 50 μg/ml	DPPH % inhibition at of different concern			
		1000 μg/ml	500 μg/ml	250 μg/ml	125 μg/ml
Pistacea chinensis	272	93.91 ± 0.68	82.48 ± 1.20	48.47 ± 1.21	30.12 ± 0.87
Aleurites trisperma	320	93.0 ± 1.87	77.12 ± 0.10	52.28 ± 1.04	25.10 ± 0.72
Elaeocanpus multiforus	325	92.9 ± 1.86	65.10 ± 1.20	50.30 ± 1.02	28.22 ± 1.00
Livostona chinensis	334	92.3 ± 1.75	60.20 ± 1.10	46.12 ± 1.40	30.12 ± 1.10
Nagia nagi	347	91.7 ± 1.55	58.12 ± 0.80	44.20 ± 1.20	26.11 ± 1.20
Syzygium jambos	456	75.8 ± 1.62	42.1 ± 0.88	38.2 ± 1.20	22.2 ± 0.80
Grevillea robusta	396	68.7 ± 0.41	48.2 ± 0.22	30.1 ± 0.42	20.2 ± 0.40
Terminalia bovinii	444	44.8 ± 1.82	32.4 ± 0.62	22.4 ± 0.44	15.5 ± 0.22
Elaeocarpus serratus	467	77.6 ± 1.28	41.2 ± 0.82	33.2 ± 0.32	25.1 ± 0.32
Tabebuia pentaphylla	454	66.9 ± 0.85	38.9 ± 0.72	28.4 ± 0.38	20.4 ± 0.34
Fraxinus griffithii	503	57.5 ± 0.56	44.2 ± 0.80	22.2 ± 0.40	18.4 ± 0.22
Machilus zuihoensis	533	48.9 ± 0.48	28.42 ± 0.40	18.2 ± 0.32	10.6 ± 0.22
Artocarpus incisus	561	44.7 ± 0.52	25.80 ± 0.42	17.8 ± 0.44	15.22 ± 0.18
Cajanus cajan	582	44.0 ± 1.28	31.2 ± 0.80	25.2 ± 0.62	18.22 ± 0.42
Corchorus aestuand	603	49.01 ± 1.72	28.8 ± 0.92	18.8 ± 1.22	14.20 ± 0.32

Fifty microlitre of different concentration of samples were added to 5 ml of 0.004% methanol solution of $DPPH^{\bullet}$. After 30 min incubation in dark at room temperature, the change in colorization from violet to yellow and subsequent fall in absorbance of the stable radical DPPH was measured at 517 nm for various concentrations i.e. 1000, 500, 250 and 125 μg/ml.

The change in colorization from violet to yellow and subsequent fall in absorbance of the stable radical DPPH was measured at 517 nm for various concentrations i.e.

1000, 500, 250 and 125 μg/ml and the results were presented in Table 2. The IC_{50} value for each plant extract was calculated and defined as the concentration of extract causing 50 percent inhibition of absorbance, was determined from the values of Table 2 since IC_{50} is a measure of inhibitory concentration, a lower IC_{50} value would reflect greater antioxidant activity of the sample. In the present investigation methanol extracts of the five plants were most effective DPPH radical scavengers and showed percent inhibition of DPPH activity *Pistacea* (93.9±1.68%), *Aleurites* (93.0±1.87%), *Elaeocarpus* (92.9±1.86%), *Livestona* (92.3±1.75%) and *Nageia* (91.7±1.55%). The extracts of *S. jambos*, (75.5±100), *A. robusta* (68.7±0.41%), *E. serratus* (77.6±1.28%), *I. pentaphylla* (66.9±0.85%), and *C. cajan* (64.0±0.25%) showed moderate radical scavenging activity, whereas the extracts of *T, bovinii* (44.8±1.52%), *F. griffithii* (57.5±0.55%), *M. Zuihoensis* (48.9±0.45%), *A. incisus* (54.7±0.52) and *C. actuand* (49.0±1.72) contained remarkable lower amounts of radical scavenging compounds. The IC_{50} values are given in Table 2. These results demonstrated that the most active radical scavengers were the methanolic extracts of *P. chinensis* (272 μg/ml), *A. trisperma* (320 μg/ml*) E. multiforus* (325 μg/ml) *L. chinensis* (334 μg/ml) and *N. nagi* (347 μg/ml).

ABTS radical cation decolorization assay showed the methanolic extracts of *P. chinensis, A. trisperma, E. multiforus, L. chinensis* and *N. nagi*, were the most active as they nearly fully scavenged $ABTS^{+}$ (Table 3).

Discussion

In the present investigation methanol extracts of the five plants were most effective DPPH radical scavengers and showed percent inhibition of DPPH activity as

Pistacea (93.9±1.68%), *Aleurites* (93.0±1.87%), *Elaeocarpus* (92.9±1.86%), *Livestona* (92.3±1.75%) and *Nageia* (91.7±1.55%). These percentages can be considered as a full absorption inhibition of DPPH, as after completing the reaction the final solution always possess some yellowish

Table 3. ABTS activity at different time intervals by plant extracts

Plant extract	0 min	1 min	2 min	4 min	6 min
Pistacea chinensis	0.704±0.002	0.041±0.003	0.035±0.002	0.022±0.001	0.014±0.002
Aleurites trisperma	0.764±0.017	0.062±0.003	0.061±0.002	0.056±0.002	0.050±0.002
Elaeocarpus multiforus	0.740±0.016	0.087±0.001	0.080±0.004	0.069±0.002	0.062±0.002
Livostona chinensis	0.712±0.012	0.089±0.008	0.063±0.008	0.040±0.002	0.035±0.002
Nageia nagi	0.732±0.018	0.150±0.006	0.121±0.017	0.104±0.002	0.095±0.002
Syzygium jambos	0.729±0.021	0.320±0.004	0.287±0.012	0.280±0.008	0.280±0.012
Grevillea robusta	0.741±0.014	0.242±0.001	0.232±0.012	0.213±0.002	0.196±0.002
Terminalia bavinii	0.740±0.013	0.178±0.011	0.178±0.010	0.174±0.011	0.173±0.032
Elaeocarpus serratus	0.747±0.022	0.415±0.028	0.390±0.019	0.365±0.012	0.345±0.022
Tabebuina pentaphylla	0.706±0.012	0.248±0.016	0.228±0.019	0.218±0.012	0.210±0.012
Fraxinus griffithii	0.750±0.038	0.202±0.024	0.196±0.018	0.185±0.012	0.177±0.022
Machilus zuihoensis	0.752±0.016	0.588±0.034	0.566±0.022	0.555±0.022	0.532±0.024
Artocarpus incisus	0.732±0.026	0.580±0.028	0.546±0.024	0.540±0.042	0.535±0.028
Cajanus cajan	0.742±0.022	0.428±0.022	0.392±0.021	0.354±0.032	0.382±0.022
Corchorus aestuand	0.705±0.013	0.581±0.025	0.562±0.021	0.542±0.012	0.523±0.032

After addition of 1 ml of diluted ABTS solution (A 734 nm = .700±.020) to 10 μl of plant extracts the absorbance reading was taken at 30^0 C exactly 1 min., after initial mixing and upto 6 min. All determinations were carried out in triplicates.

colour and therefore its absorption inhibition compared to colorless methanol solution can't reach 100% permanent residual absorption results in upto 7% of total absorption inhibition (Miliauskas et al, 2004). The extracts of *S. jambos*, (75.5±100), *A. robusta* (68.7±0.41%), *E. serratus* (77.6±1.28%), *I. pentaphylla* (66.9±0.85%), and *C. cajan* (64.0±0.25%) showed moderate radical scavenging activity, whereas the extracts of *T, bovinii* (44.8±1.52%), *F. griffithii* (57.5±0.55%), *M. Zuihoensis*

(48.9±0.45%), *A. incisus* (54.7±0.52) and *C. actuand* (49.0±1.72) contained remarkable lower amounts of radical scavenging compounds.

For further DPPH scavenging assessment and for determination of IC_{50} values these plants extracts were further diluted and % inhibition at different concentrations was evaluated. The IC_{50} values are given in Table 2. These results demonstrated that the most active radical scavengers were the methanolic extracts of *P. chinensis* (272 μg/ml), *A. trisperma* (320 μg/ml*) E. multiforus* (325 μg/ml) *L. chinensis* (334 μg/ml) and *N. nagi* (347 μg/ml). The methanolic extracts of these plants showed strong radical scavengers indicating that active compounds of different polarity could be present in these plants.

Another antioxidant activity screening method, applicable for both lipophilic and hydrophilic antioxidants, ABTS radical cation decolorization assay showed the similar results compared to those obtained from DPPH assay. Methanolic extracts of *P. chinensis, A. trisperma, E. multiforus, L. chinensis* and *N. nagi*, were the most active as they nearly fully scavenged $ABTS^+$. It was noted that the reaction with $ABTS^+$ was fast and almost in all cases was completed in a minute. During the remainder of the reaction time the changes in absorption were negligible in both the radical scavenging assays*, P. chinensis, A. trispema, E. multiforus, L. chinensis* and *N. nagi* showed the highest antioxidant activity similar observation were also obtained for other plant extracts.

Flavonoids as one of the most diverse and wide spread group of natural compounds are probably the most natural phenolics. Plant phenolics constitute one of the major groups of compounds acting as primary antioxidants free radical terminators (Agarwal, 1989).

These compounds process wide spectrum of chemical and biological activities including radical scavenging properties. Although, there are synthetic antioxidant compounds such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) that are commonly used in food industry, it has been reported that these compounds have side effects (Branien, 1975, Ito et al, 1983), Therefore, there is still need to focused on screening various plants for natural antioxidant source. Several studies have reported relationship between phenolic content and antioxidant activity. Veliaglu et al (1998) reported a strong relationship between total phenolic content and antioxidant activity in selected fruits, vegetables and grain products.

Conclusions

Both the ABTS and DPPH assay measure the total antioxidant activity of the plant extracts in an organic medium, therefore all extracts were prepared methanol. The results of both the assays are in agreement that the methonol extracts of *P. chinensis, A. trispema, E. multiforus, L. chinensis* and *N. nagi* displayed the highest antioxidant activity. The high antioxidant of these plants might be due to its flavonoids and phenolics contents.

Acknowledgement

Senior Research Associateship (Scientist's Pool Scheme) to Dr. R. M. Samarth from Council of Scientific & Industrial Research [CSIR], India [No. B-11007] is gratefully acknowledged.

Reference

Agrawal, P.K. (1989): Carbon-13 NMR of flavonoids New York: Elsevier.

Branien, A.L. (1975): Toxicology and biochemistry of butylated hydroxyanisole and butylated hydroxytoluene. Journal of the American Oil Chemists Society 52, 59-63.

Cai, Y.Z., Sun, M. and Corke, H., (2003): Antioxidant activity of betalains from plants of the Amaranthaceae. Journal of Agricultural and Food Chemistry 51 (8), 2288-2294.

Cotelle, N., Bernier, J.L, Catteau, J.P. Pommery, J., Wallet, J.C. and Gaydou, E.M. (1996): Antioxidant properties of hydroxy flavones, Free Radical Biology and Medicine 20 (1), 35-43.

Cowan, M. M. (1999): Plant Products as antimicrobial agents. Clin. Microbiol. Rev. 12: 564-582.

Cuendet, M., Hostettmann, K. and Potterat, O. (1997): Iridoid glucosides with free radical scavenging properties from Fagraea blumei. Helvetica Chimica Acta, 80, 1144-1152.

Halliwell, B. (1994): Free radicals, antioxidants and human disease: Curiosity, cause, or consequence? Lancet 344(8924), 721-724.

Hertog, M.G.L, Kromhout, D., Aravanis, C., Blackbur, H., Buziha, R., Fidanza, F., Giampaoli S., Jansen, A., Menotti, A., Nedeljkovic, S., Pekkarinen, M., Simic, B.S., Toshima, H., Feskens, E.J.M. Hollman, P.C.H. and Katan, M.B., (1995): Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. Archives of Internal Medicine 155 (11), 281-286.

Ito, N., Fukushima, S., Hassegawa, A., Shibata, M., and Ogiso, T., (1983): Carcinogenicity of butylated hydroxyanisole in F344 rats. Journal of The National Cancer Institute 70, 343-347.

Kuo, SM, (1997): Dietary flavonoid and Cancer prevention: Evidence and potential mechanism Critical Reviews in oncogenesis 8 (1), 47-69.

Larson, R.A. (1988): The antioxidants of higher plants. Phytochemistry 27 (4), 969-978.

Mclarty, J.W. (1997): Antioxidants and cancer: the epidemilogic evidence. In: Garewal, H.S. (Ed.), Antioxidants and Disease prevention. CRC Press, New York, PP. 45-66.

Miliauskas, G., Venskutonis, P.R. and Van Beek, T.A. (2004): Screening of radical scavenging activity of some medicinal and aromatic plant extracts, Food Chemistry, 85: 231-237.

Niki, E. (1997): Free radicals, antioxidant and Cancer. In: Ohigashi, H., Osawa, T., Terao, J., Watanabe, S., Yoshikawa, T (Eds.), Food Factors for Cancer Prevention, Springer, Tokyo, pp 55-57

Poulson, H.E., Prieme, H. and Loft, S. (1998): Role of oxidative DNA damage in cancer inhibition and promotion. European Journal of Cancer Prevention 7(1), 9-16.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M and Rice-Evans, C. (1999): Antioxidant Activity Applying An Improved ABTS Radical Cation Declorization Assay. Free Radical Biology and Medicine. 26, 1231-1237.

Shahidi, F. and Naczk M (1995): Food Phenolics: Sources, Chemistry, Effects and applications. Technomic Pub. Co, Basel, Switzerland.

Sun, J., Chu Y.F., Wu, X.Z. and Liu R.H., (2002): Antioxidant and antiproliferative activities of common fruits. Journal of Agricultural and Food Chemistry 50 (25), 7449-7454.

Velioglu, Y.S., Mazza, ., Gao, L and Oomah B.D. (1998): Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. Journal of Agricultural and Food Chemistry 46 (10), 4113-4117.

Yang, C.S., Landau, J.M., Huang M.T. and New mark H.L. (2001): Inhibition of carcinogenesis by dietary polyphenolic compounds. Annual Review Nutrition 21,381-406.

Zheng, W and Wang S.Y. (2001): Antioxidant activity and phenolic compounds in selected herbs. Journal of Agricultural and Food Chemistry 49 (11), 5165-5170.