Volume : 2 | Issue : 8 | August 2013 • ISSN No 2277 - 8179

Research Paper

Ferrous Ion Chelating Activity (FICA)- A Comparative Antioxidant Activity Evaluation of Extracts of Eleven Naturally Growing Plants of Gujarat, India

Pharma

KEYWORDS : Antioxidant, Free radicals, Phyto-chemicals, Extracts

Riddhi M. Patel

Department of Botany, University School of Sciences, Gujarat University, Ahmedabad, Gujarat, India- 380009

ABSTRACT

Plant based natural antioxidant phyto-chemicals are capable of exerting antioxidant effect by quenching various free radical species. Routinely used synthetic antioxidants in many products, medicines and as food preservative agent, impose long term harmful effects. Hence in the present investigation, considering the bio-safe and highly significant therapeutic role of antioxidant phyto-chemicals, an effort was made to test the antioxidant capacity from certain commonly found and naturally growing plants of Gujarat and to assess their natural defense system. A comparative evaluation of different solvent extracts was made to find the effective FICA fraction from selected plants. Plant extracts were prepared using polar to non-polar solvents like water, methanol, chloroform and petroleum ether. Study demonstrated an excellent FICA antioxidant activity from all the selected plants. A good amount of % FICA was observed in Polyalthia longifolia petroleum ether extract, followed by aqueous extract of Calotropis procera and Lantana camara, and Cassia tora methanol extract.

INTRODUCTION

In the living system during the normal metabolism process, oxidation reactions produce unstable free radicals which start off chain reactions and damage the cell. While antioxidants are the free radical scavenging compounds and are able to terminate these chain reactions by being oxidized themselves and forming a stable radical (Kitazurua *et al,* 2004; Aluyor and Ori-Jesu, 2008). Bio-chemically, antioxidant acts as a radical scavengers, singlet or triplet oxygen quenchers, peroxide decomposers, enzyme inhibitors and synergists (Politeo *et al,* 2006; Wu *et al,* 2009). The antioxidant activity is thus evaluated at various levels by using diverse *in vitro* assay protocols like, DPPH- Radical Scavenging method, Total Phenol and Flavanoid estimation, Ferrous ion Chelating activity, Ferric Reducing Antioxidant Power, Thiobarbituric acid Reactive Species, Automatic Determination of the Oxidative Stability of Fat etc.

Plant kingdom on the whole, constitute most widely distributed extremely heterogeneous groups of compounds, also called as PSMs (Plant Secondary Metabolites), generated as a byproduct of their metabolic process (Nickavar and Abolhasani, 2009; Patel and Jasrai, 2009). These PSMs doesn't have direct role in plant physiology but they impart plant, a protective job against microbial attack as well as protection from the herbivores. Whereas some plants naturally generate antioxidant defense mechanisms and in the course, synthesize numerous potent antioxidant compounds to overcome from the damage caused by active oxygen. Many fruits, vegetables and medicinal plants contain such potential antioxidant bioactive phyto-chemicals, such as vitamins A, C and E, β-carotene, α-tocopherol, carotenoids, flavonoids, isoflavones, flavones, flavonols, anthocyanins, proanthocyanidins, coumarins, lignans, polyphenols, catechins, isocatechins, tannins and other phenolic constituents etc. (Mandal *et al*, 2009; Ghasemi *et al*, 2009).

On use or consumption of such plants/ plant products, these antioxidant secondary metabolites provides antioxidative defense system and medicinal benefits to the body by reducing the oxidative stress, protecting against onset of various chronic and degenerative diseases, preventing cellular damage and slowing aging process (Aquil *et al,* 2006). These natural compounds are also bio-safe in nature and thus many researches are going on to find newer and newer sources of natural antioxidants with broad-spectrum actions (Patel and Jasrai, 2012). In the context, majority of the rich diversity of Indian medicinal plants are yet to be scientifically evaluated for such properties, there is an attempt made here to find the potential antioxidant plant sources and their effective solvent extracts/ fractions.

Metal ion chelating activity –Antioxidant mechanism of action: In living system, transition elements iron, copper etc. have one or more unpaired electrons, and thus act as free radicals and powerful catalyst of oxidation reactions. While, metal ion chelating activity of an antioxidant molecule be capable of inactivate, catalyse and inhibit the harmful transition metal ions responsible for the generation of oxygen free radicals in living organisms. More specifically, antioxidant as a chelating agent participate at various levels for instance, convert H_2O_2 (Hydrogen peroxide) in to -OH also called Fenton and Haber-Weiss reaction, form low risk redox potential complexes, decompose alkyl peroxides to the heavy reactive alkoxyl as well as hydroxyl radicals, and prevents oxyradical generation and the consequent oxidative damage (Rathee *et al*, 2006; Ghimeray *et al*, 2009).

Biochemically during the metal ion chelating assay, the extract and standard compounds interfered with the formation of ferrous and ferrozine complex and are able to capture ferrous ion before the formation of ferrozine by their chelating activity. Ferrozine can quantitatively form complexes with Fe^{2+}. Thus in presence of chelating agents, the complex formation is disrupted, resulting in drop off of the pinkish red colored complex. Thus reduction of the color intensity is in equivalence to the metal chelating activity (Singh *et al*, 2009).

Figure 1. Plants used for the study

MATERIALS AND METHODS

Collection and extraction of plant material: Plants used in the present study were *Ailanthus excelsa* Roxb, *Calotropis procera* Aiton, *Cassia tora* L, *Citrus limon* (L) Burm f, *Clerodendrum inerme* (L) Gaertn, *Lantana camara* L, *Ocimum canum* Sims, *Petunia violacea* Lindl, *Polyalthia longifolia* Benth & Hook F, *Pongamia pinnata* (L) Pierre, *Salvadora persica* L (Figure 1). The plant materials for the study were collected from the campus of Gujarat University, Ahmedabad and environs. Collected plant material

Volume : 2 | Issue : 8 | August 2013 • ISSN No 2277 - 8179

washed and air dried under shade (one week). The dried plant parts were finely powdered using electric grinder, sieved (mesh size 500µ) and subjected for the extraction. All plant samples were extracted in four solvents of different polarity viz water, methanol, chloroform and petroleum ether. For preparation of aqueous extracts, powdered plant material (50 g) extracted in 1000 ml of distilled water at 50°C temperature until the volume reduces to half. The content then filtered through whatman filter paper (no 1). The filtrate was evaporated till complete dryness in oven (40°C) (Harborne, 1984). While for the organic solvent extraction, solvents methanol, chloroform and petroleum ether were used. The finely powdered plant material (100 g), was soaked overnight in solvent (400 ml) in air tight erlenmeyer flask. The residues were repeatedly extracted (three times) in 200 ml of solvent (Khan and Nasreen, 2010). The flask content was filtered through a whatman filter paper (no 1). The filtrate was evaporated to dryness to yield a dark-residue also referred as extract. Each sample was then transferred to glass vials (6 × 2 cm) and % yield of extracts was calculated (Table 1). The obtained extracts were screened for finding possible antioxidant activity in FICA assay.

Table 1: % Extract yield of plant materials extracted in different solvents

Plants	Family	Plant Part Used	% Extract Yield *			
			WT	ME	CH	PE
Ailanthus excelsa Roxb	Simaroubaceae	Twigs	28.78	11.68	12.37	1.78
Calotropis procera Aiton	Apocynaceae	Leaves	28.73	9.57	8.85	4.22
Cassia tora L	Caesalpinaceae	Aerial part	13.25	10.19	1.72	0.63
Citrus limon (L) Burm f	Rutaceae	Leaves	25.68	16.10	8.32	2.04
Clerodendrum inerme (L) Gaertn	Verbenaceae	Leaves	23.24	12.87	3.79	5.35
Lantana camara L	Verbenaceae	Twigs	45.51	20.21	5.34	2.56
Ocimum canum Sims	Labiatae	Aerial part	17.72	16.40	3.30	4.10
Petunia violacea Lindl	Solanaceae	Leaves	20.83	29.60	10.08	1.93
Polyalthia longifolia Benth & Hook F	Annonaceae	Leaves	19.93	19.74	21.64	5.12
Pongamia pinnata (L) Pierre	Fabaceae	Leaves	24.24	24.45	6.83	0.94
Salvadora persica L	Salvadoraceae	Leaves	34.22	18.38	0.99	2.23

[**Note:** *represents g extract/100g dry powder**, WT**= Water extract; **ME**= Methanol extract; **CH**= Chloroform extract; **PE**= Petroleum ether extract]

Determination of %FICA of plant extracts: All the plant extracts were subjected for the antioxidant activity screening using standardized protocols for the %FICA (Ferrous ion chelating activity) assay (Dinis *et al*, 1994). The chemicals utilized were of pure and analytical grade. Readings were taken in six replicates for each sample and standard error was calculated.

In the present % FICA assay, 3 mg of extract was mixed with the 2 ml of 0.04 mM $FeCl_2$ and 2ml of 0.5 mM aqueous ferrozine solution. The mixture was shaken vigorously and left standing at room temperature for 10 min. and the OD taken at 562nm. Ascorbic acid was used as a reference compound IC_{50} value was calculated for standard, representing the concentration of the compounds that caused 50% inhibition/ antioxidant activity. The calculation was performed using following formula (Dinis *et al*, 1994).

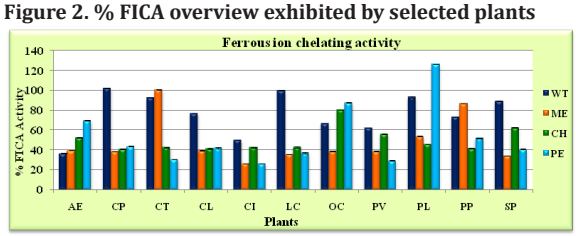
$$Fe^{2+}\ complex = \left(\frac{1\text{-}A_1\ sample}{A_0\ control}\right) \times 100$$

[**Note:** A_0 control = OD of $FeCl_2$ and Ferrozine solution without extract or standard, A1 sample= OD of $FeCl_2$ and Ferrozine solution with extract or standard]

RESULTS AND DISCUSSION

The antioxidant activity exhibited by plants is due to the presence of bioactive secondary metabolites, their amount, polar or non-polar composition and therefore solubility nature of such compounds in the solvent used for the extraction etc. The importance of conducting comparative bioactive antioxidant phyto-chemical evaluation, is not only for the chemical characterization but also important for linking the chemical contents with specific functional properties (Sacchetti *et al*, 2005). As above mentioned, antioxidant plant metabolites can be extracted by subjecting the plant material for organic solvent extraction. The percentage of extraction yields depends on the particle size of sample, temperature and the ratio of solvent and sample extraction (Huda-Faujan *et al*, 2009).

Figure 2. % FICA overview exhibited by selected plants



[**Note: AE**= *Ailanthus excelsa*; **CP**= *Calotropis procera*; **CT**= *Cassia tora*; **CL**= *Citrus limon*; **CI**= *Clerodendrum inerme*; **LC**= *Lantana camara*; **OC**= *Ocimum canum*; **PV**= *Petunia violacea*; **PL**= *Polyalthia longifolia*; **PP**= *Pongamia pinnata*; **SP**= *Salvadora persica*]

In the current study, % FICA (Ferrous ion chelating activity) IC_{50} value for standard Ascorbic acid was observed at 1.5 mg/ml concentration. Present comparative analysis efficiency study among different solvent extracts of plants, an excellent amount of % FICA was observed in *Polyalthia longifolia* (126.13 ± 0.27) PE extract; followed by *Calotropis procera* (101.93 ± 0.20) WE extract; *Cassia tora* (100.73 ± 0.14) and *Pongamia pinnata* (86.74 ± 0.09) ME extracts; *Ocimum canum* (87.54 ± 0.15) PE extract.

The aqueous extract of plants *Lantana camara* (99.83 ± 0.08), *Polyalthia longifolia* (92.94 ± 0.54), *Cassia tora* (92.52 ± 0.09), *Salvadora persica* (89.18 ± 0.14) and *Citrus limon* (76.66 ± 0.13) demonstrated significant amount of % FICA. Also an appreciable quantity of % FICA was exhibited by *Ailanthus excelsa* PE and CH; *Ocimum canum* WT; *Petunia violacea* WT and CH; *Polyalthia longifolia* ME; *Pongamia pinnata* PE and *Salvadora persica* CH extracts (Figure 2,3). The variation of relative radical scavenging capacity of individual extract in different assays can be explained by the different mechanisms of action involved with each group of secondary metabolites. Thus extraction methods, solvents used for the extraction and the assay used for antioxidant evaluation affects the capacity of secondary metabolites to scavenge radicals (Yoo *et al*, 2008). Concisely metal ion chelat-

ing activity of an antioxidant molecule can inactivate, catalyse and inhibit the harmful transition metal ions responsible for the generation of oxygen free radicals in living organisms (Rathee *et al*, 2006; Ghimeray *et al*, 2009).

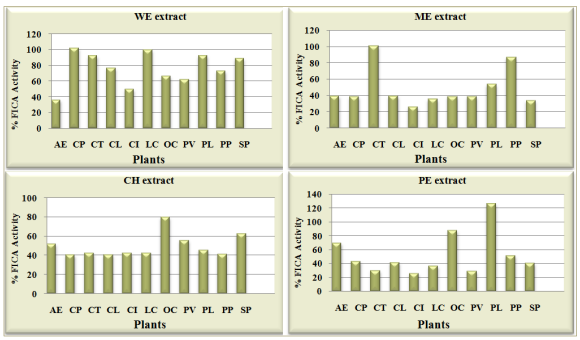


Figure 3. Comparative Ferrous ion chelating activity antioxidant activity exhibited by plant extracts

On the whole, plant extracts of *Polyalthia longifolia*, *Cassia tora*, *Calotropis procera*, *Pongamia pinnata*, *Ocimum canum* have demonstrated presence of an excellent antioxidant activity in the present study. The order for the high to low % FICA observed with different solvent extracts in the study, is the aqueous extract followed by chloroform, methanol and petroleum ether extracts (Figure 3). This shows that these naturally growing plants have a rich source of antioxidant compounds which proves their defensive and natural ability to survive in nature. Apart from this, these plants serves an important area of pharmacological evaluation and can be implemented for medicinal and disease curing purposes.

REFERENCE

• Aluyor, EO and Ori-Jesu, M 2008. The use of antioxidants in vegetable oils - A review. African Journal of Biotechnology, 7(25), 4836-4842. | • Aquil, F, Ahmad, I & Mehmood, Z 2006. Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. Turkish Journal of Biology, 30, 177-183. | • Dinis, TCP, Madeira, VMC & Almeida, LM 1994. Action of phenolic derivates (acetoaminophen, salycilate and 5- aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Archive Biochemistry Biophysics, 315, 161-169. | • Ghasemi, K, Ghasemi, Y & Ebrahimzadeh, MA 2009. Antioxidant activity, phenol and flavanoid contents of 13 citrus species peels and tissues. Pakistan Journal of Pharmaceutical Sciences, 22(3), 277-281. | • Ghimeray, AK, Jin, CW, Ghimire, BK & Cho, DH 2009. Antioxidant activity and quantitative estimation of azadirachtin and nimbin in Azadirachta indica A. Juss grown in foothills of Nepal. African Journal of Biotechnology, 8(13), 3084-3091. | • Harborne, JB 1984. A Guide to Modern Techniques of Plant Analysis. In: Phytochemical methods. 3rd edn, edited by Harborne JB (Chapman and Hall, Hong Kong). | • Huda-Faujan, N, Noriham, A, Norrakiah, AS & Babji, AS 2009. Antioxidant activity of plants methanolic extracts containing phenolic compounds. African Journal of Biotechnology, 8(3), 484 -489. | • Khan, ZS & Nasreen, S 2010. Phytochemical analysis, antifungal activity and mode of action of methanol extracts from plants against pathogens. Journal of Agricultural Technology, 6, 793-805. | • Kitazurua, ER, Moreirac, AVB, Mancini-Filhoc, J, Delinceed, H, & Villavicencio, ALCH (2004). Effects of irradiation on natural antioxidants of Cinnamon (Cinnamomum zeylanicum N.). Radiation Physics and Chemistry, 71, 37-39. | • Mandal, S, Yadav, S, Yadav, S, & Nema, RK 2009. Antioxidants: A Review. Journal of Chemical and Pharmaceutical Research, 1(1), 102-104. | • Nickavar, B and Abolhasani, FAS 2009. Screening of antioxidant properties of seven Umbelliferae fruits from Iran. Pakistan Journal of Pharmaceutical Sciences, 22(1), 30 -35. | • Patel, RM & Jasrai, YT 2009. Plant secondary metabolites and their commercial production. South Asian Journal of Social and Political Sciences, 9(2), 115-122. | • Patel, RM & Jasrai, YT 2012. Antioxidant activity of medicinal spices and aromatic herbs. Annals of Phytomedicine 1, 75-80. | • Politeo, O, Juki, M & Milo, M 2006. Chemical composition and antioxidant activity of essential oils of twelve spice plants. Croatica Chemica Acta, 79(4), 545-552. | • Rathee, JS, Patro, BS, Mula, S, Gamre, S & Chattopadhyay, S 2006. Antioxidant activity of Piper betel leaf extract and its constituents. Journal of Agriculture and Food Chemistry, 54, 9046-9054. | • Sacchetti, G, Maietti, S, Muzzoli, M, Scaglianti, M, Manfredini, S, Radice, M & Bruni, R 2005. Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food Chemistry, 91, 621 -632. | • Singh, R, Singh, B, Singh, S, Kumar, N, Kumar, S & Arora, S 2009. Investigation of ethyl acetate extract/fractions of Acacia nilotica willd. Ex Del as potent antioxidant. Records of Natural Products, 3(3), 131-138. | • Wu, N, Fu, K, Fu, YJ, Zu, YG, Chang, FR, Chen, YH, Liu, XL, Kong, Y, Liu, W & Gu, CB 2009. Antioxidant activities of extracts and main components of pigeonpea (Cajanus cajan (L.) Millsp.) leaves. Molecules, 14, 1032-1043. | • Yoo, K.M., Lee, C.H., Lee, H., Moon, B.K. & Lee, C.Y. 2008. Relative antioxidant and cytoprotective activities of common herbs. Food Chemistry, 106, 929-936.