The Bioscan 5 (2) : 225-229, 2010
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES

IN VITRO ASSESSMENT OF ALCOHOLIC LEAF EXTRACTS OF *ANNONA SQUAMOSA* AND *AEGLE MARMELOS*

V.VANITHA, K. J. UMADEVI AND K. VIJAYALAKSHMI*
Department of Biochemistry, Bharathi Women's College,
Chennai - 600 108, TN
E-mail: vrr.vanitha@gmail.com

KEY WORDS
Annona squamosa
Aegle marmelos
Antioxidant
Free radicals
DPPH, ABTS

Received on :
04.03.2010
Accepted on :
15.05.2010

***Corresponding author**

ABSTRACT
Antioxidant potential of leaves of *Annona squamosa* and *Aegle marmelos* were studied by using different *invitro* models like DPPH, ABTS and the scavenging activity was investigated by the production of nitric oxide, hydroxyl radical, super oxides and lipid peroxides. Leaves of *Annona squamosa* and *Aegle marmelos* are used in folklore medicine for the treatment of various diseases. It was observed that *Annona squamosa* (500 µg/mL) showed maximum activity using DPPH where as *Aegle marmelos* showed maximum scavenging activity using ABTS. Both of them reduced the production of free radicals in a dose dependent manner. This finding shows that the alcoholic leaf extract of *Annona squamosa* and *Aegle marmelos* may possess different compounds which have potent antioxidant activity exhibited differently.

INTRODUCTION

Natural remedies from medicinal plants are found to be safe and effective. Many plant species have been used in folkloric medicine to treat various ailments. Even today compounds from plants continue to play a major role in primary health care as therapeutic remedies in many developing countries. Oxygen is essential for survival of all living things and during the process of its utilization in normal physiological and metabolic process approximately 5% of oxygen gets univalently reduced to oxygen derived free radicals (Yu, 1994). Oxidative stress has been implicated in the pathology of many diseases such as inflammatory conditions, cancer, diabetes and ageing (Marx, 1987). Free radical induced peroxidation has gained much importance because of their involvement in several conditions like atherosclerosis, ischemia, liver disorder, neural disorders (Pandey *et al.,* 1994). Reactive oxygen species such as superoxide anions ($O_2^{\cdot -}$), hydroxyl radical ($OH^{\cdot}$) and nitric oxide (NO) inactivate enzymes and damages the important cellular components causing injury through covalent binding and lipid peroxidation (Geesin *et al*., 1990).

Annona squamosa Linn, commonly known as Sugar apple, belonging to the family Annonaceae, is said to show varied medicinal effects, including insecticide, antiovulatory and abortifacient. A bark decoction of this is used to prevent diarrhoea, while the root is used in the treatment of dysentery. A decoction of the leaves is used for cold and to clarify urine. Leaves are used to treat hysteria and fainting spells (Asolkar *et al.,* 1992). The fruits of *Annona* are haematinic, cooling, sedative, stimulant, expectorant, maturant tonic. They are useful in treating anemia and burning sensation. The seeds are abortifacient and insecticidal and are useful in destroying lice in the hair. Fruit is used in making of ice creams and milk beverages. The bark and leaves contain annonaine, an alkaloid (Vohar *et al.,* 1975) which is found to posses many of these properties.

Aegle marmelos, commonly known as bael, is a spiny tree belonging to the family Rutaceae. The leaves, roots, bark, seeds and fruits of *Aegle marmelos* are edible. The medicinal properties of this plant have been described in the Ayurveda. In fact, as per Charaka (1500 B.C.), no drug has been longer or better known or appreciated by the inhabitants of India than the bael (Chemexcil, 1992). The leaves of bael are astringent, a laxative, and an expectorant and are useful in treating ophthalmia, deafness, inflammations, cataract, diabetes and asthmatic complaints. The unripe fruits are bitter, acrid, sour, astringent, a digestive and stomachic and are useful in diarrhoea, dysentery and stomachalgia. The roots of *Aegle marmelos* are one of the ingredients of dashamula (10 roots), a medicine commonly used by ayurvedic practitioners. The leaves are bitter and are used as a remedy for ophthalmia, ulcers, dropsy, cholera and beri beri. Fresh aqueous and alcoholic leaf extracts of *Aegle marmelos* are reported to have a cardiotonic effect, like digitalis, and decrease the requirement for circulatory stimulants (Nadkarni, 1976). An aqueous decoction of the leaves has been shown to possess a significant hypoglycemic effect (Karunanayake *et al*., 1984). *Aegle* leaf extract has been reported to regenerate damaged pancreatic ß-cells in diabetic rats (Das *et al*., 1996). It is found to be as effective as insulin in the restoration of blood glucose and body weight to normal

levels (Seema *et al*., 1996).

Preliminary investigations have confirmed the antioxidant potential of *Annona squamosa* and *Aegle marmelos* in different *invitro* models. This has been attributed to the presence of flavonoids like rutin and hyperoside in *Annona squamosa* leaves (Shirwaikar *et al*., 2004) and flavon-3-ols, leucoanthocyanins and flavonoid glycosides in bale leaves (Maridonneau – Pairini *et al*., 1986). Due to variations in composition and content of both leaves, it was proposed to analyze their antioxidant capacities with different *invitro* models.

MATERIALS AND METHODS

Plant material

Fresh leaves of *Annona squamosa* and *Aegle marmelos* plants were collected locally during the month of November to January. The taxonomic identification of these plant materials were authenticated by Prof. P. Jayaraman, Director, Plant Anatomy Research Centre, Chennai, India.

Preparation of crude extract

10gms of air dried powder was macerated with 100 mL of absolute alcohol and stored for 72 hrs in ice cold condition. After 72 hrs the miscella was filtered using Whatmann No. 1 filter paper and the organic layer was allowed to evaporate. The resulted dark green extract was concentrated upto 100 mL on Rota vapour under reduced pressure. The concentrated crude extracts were lyophilized into paste (5 and 15 g respectively) and were taken for *invitro* assessment of free radical scavenging activity.

Invitro assesment of free radical scavenging activity of *Annona squamosa* and *Aegle marmelos*

Hydroxyl radical scavenging activity

Scavenging of the hydroxyl ($^{-}$OH) free radical was measured by the method of Halliwell *et al.,* (1987). Briefly, the reaction mixture contained deoxyribose (2.8 mM), KH_2PO_4–NaOH buffer, pH 7.4 (0.05 M), $FeCl_3$ (0.1 mM), EDTA (0.1 mM), H_2O_2 (1 mM), ascorbate (0.1 mM) *Annona squamosa and Aegle marmelos* (100–500 μg/mL) in a final volume of 2 mL. The reaction mixture was incubated for 30 min at ambient temperature followed by addition of 2 mL of trichloroacetic acid (2.8% w/v) and thiobarbituric acid. The reaction mixture was kept in a boiling water bath for 30 min, cooled and the absorbance was read at 532 nm in a UV double beam spectrophotometer (UV-260; Shimadzu Corp, Tokyo, Japan).

Superoxide anion scavenging activity

Scavenging of the superoxide (O_2^{-}) anion radical was measured as described by Hyland *et al.,* (1983). The reaction mixture contained various concentrations of *Annona squamosa* (100-500 μg/mL) and *Aegle marmelos* (100-500 μg/mL), nitroblue tetrazolium and alkaline DMSO. The blank consisted of pure DMSO instead of alkaline DMSO. The absorbance was read at 560 nm using a UV double beam spectrophotometer (UV-260).

DPPH scavenging activity

The principle for reduction of the DPPH free radical is that the antioxidant reacts with the stable free radical DPPH and converts it to 2, 2-diphenyl-1-picryl hydrazine. The ability to scavenge the stable free radical DPPH is measured as a decrease in absorbance at 517 nm (Mensor *et al*., 2001). To an alcoholic solution of DPPH (0.05 mM) was added an equal volume of *Annona squamosa* (100-500 μg/mL) and *Aegle marmelos* (100-500 μg/mL)dissolved in water, to a final volume of 1.0 mL. An equal amount of alcohol was added to the control. After 20 min, absorbance was recorded at 517 nm in a UV double beam spectrophotometer (UV-260).

Total antioxidant activity assay

Total Antioxidant potential was determined by the ABTS assay, as described by Miller *et al*., (1996). This technique measures the relative ability of antioxidant substances to scavenge the $ABTS^{+}$ cation radical generated in the aqueous phase. The reaction mixture contained ABTS (0.00017 M), *Annona squamosa* (100-500 μg/mL) and *Aegle marmelos* (100-500 μg/mL) and buffer in a total volume of 3.5 mL. The absorbance was measured at 734 nm in a UV double beam spectrophotometer (UV-260).

Nitric oxide scavenging activity

Nitric oxide was generated from sodium nitroprusside and measured by the Greiss reaction as described previously. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide (Marcocci *et al*., 1994; Sreejayan and Rao, 1997), which interacts with oxygen to produce nitrite ions that can be estimated by use of Greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide (Marcocci *et al*., 1994). Sodium nitroprusside (5 mM) in PBS was mixed with different concentrations of *Annona squamosa* (100-500 μg/mL) and *Aegle marmelos* (100–500 μg/mL) and incubated at 25°C for 150 min. The samples from the above were reacted with Greiss reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% naphthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during diazotization of nitrite with sulfanilamide and subsequent coupling with napthylethylenediamine was read at 546 nm and compared with that of standard solutions treated in the same way.

RESULTS

Several concentrations ranging from 100-500 μg/mL of alcoholic leaf extract of *Annona squamosa* and *Aegle marmelos* were tested for their antioxidant activity using different *invitro* models. It was observed that free radicals were scavenged by the test compounds in a dose dependent manner in the various methods.

Table 1 shows the dose dependent free radical scavenging activity of alcoholic leaf extract of *Annona squamosa*. It was observed from table that the impact of *Annona squamosa* extract on various *invitro* models was dose dependent. At 500 μg/mL (maximum concentration) the % inhibition was highest with DPPH and ABTS followed by hydroxyl, Superoxide and finally Nitric oxide.

Table 2 shows the impact of *Aegle marmelos* on the same *invitro* models. The percentage of inhibition with different free radicals was dose dependent. The table shows that at 500

Table 1: Effect of alcoholic leaf extract of *annona squamosa* on different *invitro* models

Conc (μg/mL)	DPPH (%)	ABTS (%)	NO (%)	OH (%)	SO (%)
100	75.22	*11.80*	35.69	37.56	10.86
200	79.16	*33.83*	40.71	45.60	25.47
300	85.23	*55.2 J*	44.96	52.46	51.10
400	86.51	*74.14*	5.60	69.78	66.91
500	88.11	*88.01*	68.44	78.93	77.21

Table 2: Effect of alcoholic leaf extract of *aegle marmelos* on different *invitro* models

Conc (μg/mL)	DPPH**(%)**	ABTS(%)	NO (%)	OH (%)	SO (%)
100	10.32	66.21	73.82	58.356	76.45
200	12.56	93.55	77.53	60.28	80.81
300	19.16	94.36	79.16	70.18	82.15
400	22.83	98.11	84.21	71.29	84.21
500	30.66	98.11	85.06	80.16	86.18

μg/mL concentration of *Aegle marmelos*, the % inhibition was minimum with DPPH. The *Aegle marmelos* extract was more efficient with ABTS. It was observed from the table that the alcoholic extract of *Aegle marmelos* is more efficient in scavenging Nitric oxide and Superoxide rather than hydroxyl anion. Thus the scavenging power of alcoholic leaf extract of *Aegle marmelos* was inferred to be dose dependent as shown in Table 2.

DISCUSSION

Free radicals are chemical entities that can exist separately with one or more unpaired electrons. The propagation of free radicals can bring about many adverse reactions leading to extensive tissue damage. Lipids, proteins and DNA are all susceptible to attack by free radicals. (Cotran *et al.,* 1999., Yu *et al.,* 1992). Antioxidants may offer resistance against oxidative stress by scavenging the free radicals. Antioxidants are compounds that can reduce or inhibits the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions. The antioxidant activity of phenolic compounds is mainly due to their redox properties in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides.

DPPH radical scavenging activity

DPPH is a relatively stable free radical and the assay determines the ability of alcoholic leaf extract of *Annona squamosa* and *Aegle marmelos* to reduce DPPH radical to corresponding hydrazine (Blois *et al.,* 1958). DPPH was used to determine the proton radical scavenging action of extracts of *Annona squamosa* and *Aegle marmelos* because it shows a characteristic absorbance at 517 nm. DPPH radical was scavenged by antioxidants through the donation of proton by forming reduced DPPH. The *Annona squamosa* extract showed significant antioxidant activity *invitro* in scavenging DPPH radical by 88% whereas *Aegle marmelos* showed only 30% inhibition was noted at 500 μg/mL concentration.

It has been reported by Rastogi and Mehrotra (1990) that the maximum activity noted in *Annona squamosa* could be attributed to high amounts of flavonoids like rutin and hypersides in leaves (Shirwaikar *et al.,* 2004). The study showed that the extract has the proton donating ability and could serve as free radical scavengers, acting possibly as primary antioxidant.

ABTS radical scavenging activity

ABTS assay is based on the inhibition of the absorbance of the radical cation $ABTS^{\cdot +}$ which has a characteristic long wavelength absorption spectrum (Sanchez – Moreno, 2002). ABTS, a protonated radical has characteristic absorbance maxima at 734nm which decreases with the scavenging of proton radicals (Mathew and Abraham, 2004).

From the present study, it may be concluded that the alcoholic leaf extract of *Aegle marmelos* was fast and effective in scavenging of ABTS radical at 500 μg/mL concentration. The scavenging of ABTS radical by the alcoholic leaf extract of *Aegle marmelos* was found to be signifanctly higher than that of *Annona squamosa*. Factors like stereoselectivity of the radicals or the solubility of the extract in different models has been attributed to affect the capacity of the extract to react and quench different radicals (Yu *et al.,* 2002).

It has been reported by Maridonneau-Pairini *et al.,* (1986) that the maximum activity noted in *Aegle marmelos* could be mainly due to the presence of flavonoids like leucoantocyanins, anthocyanins and flavonoid glycosides. Wang *et al.,* (1998) found that some compounds which have ABTS scavenging activity did not show DPPH activity. From the present study, it may be concluded that the alcoholic leaf extract of leaves of *Aegle marmelos* was fast and effective in scavenging ABTS radical at 500 μg/mL concentration.

Nitric oxide radical scavenging activity

NO is an important chemical mediator generated by endothelial cells, macrophages, neurons and is involved in the regulation of various physiological processes (Lata and Ahuja, 2003). Excess concentration of NO is associated with several diseases (Ialenti *et al.,* 1993 and Ross, 1993).Oxygen reacts with the excess nitric oxide to generate nitrite and peroxynitrite anions which acts as free radicals (Sainani *et al.,* 1997). Nitric oxide can react rapidly in the intracellular environment to form nitrate, nitrite and s-nitrosothiols. These metabolites play a key role in mediating many xenotoxic effects such as DNA damage. NO causes DNA damage via peroxynitrite.

It has been reported by Rastogi *et al.,* (1996 that the maximum activity noted in *Aegle marmelos* could be due to the flavonoids present in them. Bael has been reported to contain aegelinine, cineole which posses antioxidative and free radical scavenging activity (Korina and Afanasev, 1997). In the present study, the alcoholic leaf extract of *Aegle marmelos* showed better activity with nitric oxide radical and thus inhibiting the generation of anions. Thus the alcoholic leaf extract of *Aegle marmelos* scavenges the NO radical more effectively than that of *Annona squamosa* at 500 μg/mL concentration.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity is measured as the % inhibition of hydroxyl radicals generated in fenton's reaction mixture (Braugghler *et al.,* 1986) by studying the competition between deoxyribose and the extract for hydroxyl radicals

generated from Fe^{3+} / ascorbate/EDTA/H_2O_2 system. The hydroxyl radical attacks deoxyribose which eventually results in TBARS formation.

Ferrous salts can react with H_2O_2 and forms hydroxyl radical via Fenton's reaction. The iron required for this reaction is obtained from the pool of iron of heme containing proteins (Cotran *et al.,* 1999). The hydroxyl radical (OH^-) thus produced may attack the sugar of DNA base causing sugar fragmentation, base loss and DNA stand breakage(Kaneko *et al.,* 1996).In the present study the alcoholic extract of *Annona squamosa* leaf showed significant scavenging activity than *Aegle marmelos* at 500*µg*/mL concentration.

Superoxide radical scavenging activity.

Superoxide anion is produced from molecular oxygen due to oxidative enzymes (Sainani *et al.,* 1997) of body by non enzymatic reaction such as autooxidation by catecholamines (Hemmani and Parihar, 1998). The scavenging activity towards the superoxide radical is measured in terms of inhibition of generation of O_2^-.

In the present study superoxide radical reduces NBT to blue coloured formazan that is measured at 560 nm (Khanam *et al.,* 2004). It has been reported by Vidhya and Devaraj (1999) that *Aegle marmelos* has high concentration of eugenol, tannins and phlobatannins. Hence, the high scavenging activity of *Aegle marmelos* observed could be due to eugenol which in high concentration and it may inhibit lipid peroxidation.

REFERENCES

Asolkar, L., Kallar, K. K. and Chakre, O. J. 1992. Glossary of Indian Medicinal Plants With Active principles, Publications and Information Directorate, New Delhi. 2nd Edn, pp. 72-73.

Baumann J., Wurn, G. and Bruchlausen, F.V. 1979. Prostaglandin synthetase inhibiting O_2^- radical scavenging properties of some flavonoids and related phenolic conpounds. *Deutsche pharmakologische gesellschaft Abstracts of the 20th spring meeting. Naunyn-schmiedebergs Abstract No: R27 cited in Arch Pharmacol.* **307:** R1-R77.

Blois, M. S. 1958. Antioxidant determination by the use of stable free radicals. *Nature.* **26:** 199.

Braugghler, J. M., Duncan, C. A. and Chase, L. R. 1986. The involvement of iron in lipid peroxidation. Important of ferrous to ferric iron in initiation. *J. Biol. Chem.* **261:** 10282.

Chemexcil, 1992. Selected Medicinal plants of India. Basic chemicals, Pharmaceutical and cosmetic Export Promotion Council, Bombay, India.

Cotran, R. S., Kumar, V. and Collins, T. 1999. In *Robbin's pathological basis of diseases*, 6th Edn (Thomson press (I) Ltd, Noida, India). p.1.

Das, A. V. and Padayatti, P. S. 1996. Effect of leaf extract of *Aegle marmelos* (L.) Correa ex Roxb. On histological and ultrastructural changes in tissues of streptozotocin induced diabetic rats. *Indian J. Exp. Biol.* **34:** 341-345.

Geesin, J. G., Gordon, J. S. and Berg, R. A. 1990. Retinoids affect collagen synthesis through inhibition of ascorbate-induced lipid peroxidation in cultured human dermal fibroblasts. *Arch. Biochem. Biophys.* **278:** 352.

Halliwell, B. and Gutteridge, J. M. and Aruoma, O. I. 1987. The deoxyribose method: a simple "test-tube" assay for determination of rate constants for reactions of hydroxyl radicals. *Anal. Biochem.* **15:** 215–219.

Halliwell, B. and Gutteridge, J. M. C. 1998. *In Free radicals in biology and medicine*, 2nd Edn (Clarendan press, Oxford) p. 1

Hemmani, T. and Parihar, M. S. 1998. Reactive oxygen species and oxidative DNA damage. *India J. Physiol. Pharmacol.* **42:** 440.

Hyland, K., Voisin, E., Banoun, H. and Auclair, C. 1983. Superoxide dismutase assay using alkaline dimethylsulfoxide as superoxide anion-generating system. *Anal. Biochem.* **135:** 280–287.

Ialenti, A., Moncada, S. and Di Rosa, M. 1993. Modulation of adjuvant arthritis by endogenous nitric oxide, *Br. J. Pharmacognosy*. **110:** 701.

Kaneko, T., Tahara, S. and Matsu, M. 1996. Retarding effect of dietary restriction on the accumulation of 8-hydroxy-2-deoxyguanosine in organs of Fischer 344 rats during ageing. *J. Free Radic. Biol. Med.* **23:** 76.

Karunanayake, E. H., Welihinda, J., Sirimanne, S. R. and Sinnadorai, G. 1984. Oral hypoglycemic activity of some medicinal plants of Srilanka. *J. Etanopharmacol.* **11:** 223-231.

Khanam, S., Shivprasad, H. N. and Kshama, D. 2004. *In vitro* antioxidant screening models: a review. *Indian J. Pharm. Educ.* **38:** 180.

Korina, L. G. and Afanasev, I. B. 1997. Antioxidant and chelating property of flavinoids. *Adv. Pharmacol.* **38:** 151-163.

Lata, H. and Ahuja, G. 2003. Role of free radicals in health and disease. *Ind. J. Physio. and Allied Sci.* **57:** 124.

Marcocci, L., Packer, L., Dory-Lefaix, M. T., Sekaki, A. and Gardes-Albert, M. 1994. Antioxidant action of Ginkgo biloba extract EGB 761. *Methods Enzymol.* **234:** 462-475.

Maridonneau-Parini, I., Braquet, P. and Garay, R. P. 1986. Heterogenous effect of flavonoids on K^+ loss and lipid peroxidation induced by oxygen-free radicals in human red cells. *Pharm. Res. Commun.* **18:** 61–73.

Marx, J. L. 1987. Oxygen free radicals linked to many diseases, *Sci.* **235:** 529.

Mathew, S. and Abraham, E. T. 2004. Invitro antioxidant activity and scavenging effects of Cinnamomum verum leaf extract assayed by different methodologies. *Food Chem. Toxicol.* **44:** 198-206.

Mensor, L. L., Menezes, F. S., Leitao, G. G., Reis, A. S., Dos Santos, T. C., Coube, C. S. and Leitao, S. G. 2001. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytother. Res.* **15:** 127–130.

Miller, N. J., Castelluccio, C., Tijburg, L. and Rice-Evans, C. 1996. The antioxidant properties of theaflavins and their gallate esters—radical scavengers or metal chelators? *FEBS Lett.* **392:** 40–44. CrossRefi, Web of Science, Medline.

Nadkarni, A. K. 1976. Indian Materia Medica, 3rd Edn. Popular press, Mumbai, India.

Pandey, S., Sharma, Chaturved, P. and Tirupathi, B. 1994. Protective effect of Rubia cordifolia on lipid peroxide formation in isolated rat mice, *Indian J. Exp. Biol.* **32:** 180.

Rastogi, P., Ram. and Mehrotra, N. B. 1996. Compendium of India Medicinal Plants CDRI, and NISC, New Delhi. p. 371.

Rastogi, R. M. and Mehrotra, B. N. 1990. *Compendium of Indian medicinal plants.* Central drug Research Institute, Lucknow, India. **1:** 338-389.

Ross, R. 1993. The pathogenesis of atherosclerosis: a prospective for the 1990's. *Nature.* **36:** 801.

Sainani, G. S., Manika, J. S. and Sainani, R. G. 1997. Oxidative stress: a key factor in pathogenesis of chronic diseases, *Med. Update.* **1:** 1.

Sanchez-moreeno, C. 2002. Methods used to evaluate the free radical activity in foods and biological systems, *Food Sci. Tech. Int.* **8:** 122.

Seema, P. V., Sudha, B., Pandayatti, P. S., Abraham, A., Raghu, K. G. and Paulose, C. S. 1996. n Kinetic studies of purified malate dehydrogenase in liver of streptozotocin-diabatic rats anmd the effect

of leaf extract of *Aegle Marmelos(L.)* Correa ex Roxb. *Indian J. Exp. Biol*. **34:** 600-602.

Shirwaikar, A., Rajendran, K. and Dinesh kumar, C. 2004. *In vitro* antioxidant studies of *Annona squamosa* Linn leaves. *Indian J. Exp. Biol*. **42:** 803.

Sreejayan, N. and Rao, M. N. A. 1997. Nitricoxide scavenging by curcuminoids. *J. Pharm. Pharmacol*. **49:** 105

Vidhya, N. and Devraj, S. N. 1999. Antioxidant effect of eugenol in rat intestine. *Indian J. Exp. Biol*. **37:** 1192-1195

Vohar, S. B., Ishwar Kumar and Naquvi, S. A. H. 1975. Phytochemical, Pharmacological, antibacterial and anti-ovulatory studies on *Annona Squamosa*. *Planta Med.* **28:** 97-100.

Wang, M., Li, J., Rangarajan, M., Shao, Y., La Voie, E. J., Huang, T. and Ho, C. 1998. Antioxidative phenolic compounds from sage (*Salvia officinalis*). *J. Agr. Food Chem*. **46:** 4869-4873. dio: 10.1021/jf980614b.

Yu, B. P., Suescun, E. A. and Yang, S. Y. 1992. Effect of age- related lipid peroxidation on membrane fluidity and phospholipids A2: modulation by dietary restriction. *Mech. Ageing Dev*. **65:** 17.

Yu, B. P. 1994. Cellular defenses against damage from reactive oxygen species, *Physiol. Rev*. **74:** 139.

Yu, L., Haley, S., Perret, Harris, M., Wilson, J. and Qian, M. 2002. Free radical scavenging properties of wheat extracts. *J. Agr. Food Chem*. **50:** 1619-1624.

Announcing

The Second International Conference of

National Environmentalists Association, India

INTERNATIONAL CONFERENCE ON ENERGY, ENVIRONMENT AND DEVELOPMENT

(from Stokholm to Copenhagen and beyond)

(ICEED 2010)

December 10-12, 2010

Contact

PROF. P. C. MISHRA
D. Sc., FNEA,

Prof. and Head
Department of Environmental Sciences,
Sambalpur University,
Jyoti Vihar, Sambalpur
ORISSA

-:Important dates: -		
Last date of Abstract submission for oral presentation	-	31.08.10
Last date of Full paper submission for proceedings	-	31.08.10
Last date of Registration without late submission charges	-	31.08.10
Organisers will not be responsible for accommodation if not booked in advance		

Web site: www.iceed2010.in

E-mail: pcm_envsu@rediffmail.com; iceed2010@yahoo.in
Mobile no: 99437052301