Available online at www.ewijst.org

ISSN: 0975-7112 (Print)
ISSN: 0975-7120 (Online)

Environ. We Int. J. Sci. Tech. 4 (2009) 45-52

Environment & We an International Journal of Science & Technology

Phytochemistry and Free radical scavenging activities of *Oroxylum indicum*

T. Kalaivani* and Lazar Mathew
School of Bio Sciences and Technology
VIT University, Vellore- 632 014, TN. India
*Email: tkvani72@gmail.com
Phone +91914162202542; Mobile +919442994888; Fax +914162243092

Abstract

The present study estimates the free radical scavenging activity of the different extracts of *Oroxylum indicum* in different assays viz. total antioxidant assay and β-carotene bleaching assay. The stem bark powder of the plant was extracted with different solvents by sequential extraction method in the order of increasing polarity using soxhlet apparatus. The phytochemistry of the plant was qualitatively analyzed using various methods. The scavenging activity of ethanol and chloroform extracts showed high potency compared to the other extracts. The ethanol extract exhibited maximum antioxidant potential in β-carotene bleaching assays whereas chloroform extract showed maximum reducing power in total antioxidant activity. The variation amongst the antioxidant potential may be due to the phytochemical diversity. The maximum antioxidant potential of ethanol extract may be due to its free radical scavenging activities, while the chloroform extract may be due to its reducing potential. Studies are in progress to evaluate the effect of extract/ fractions in other antioxidant assays and identify the factors responsible for the activity.

Keywords: Oroxylum indicum, free radicals, β-carotene bleaching assay, total antioxidant assay, phytochemical analysis

Introduction

The plant *Oroxylum indicum* belonging to the family Bignoniaceae, selected in the present investigation, is evergreen or partly deciduous, small to medium sized tree

with light grayish brown, soft and spongy bark. The tree is often grown as an ornamental for its strange appearance. The long, podded fruits hang down from bear branches, looking like dangling sickles or swords in the night. The tree is also a night-bloomer and is pollinated naturally by bats. Additionally, after the large leaf stalks wither, they fall off the tree and collect near the base of the trunk, appearing to look like a pile of broken limb bones. It is distributed throughout India in Eastern, Western Ghats and North East region up to an altitude of 1200m and found mainly in ravine and moist places in forests. It has been categorized as vulnerable medicinal plant by the government of India (Ravikumar and Ved, 2000). The plant contains flavonoids like chrysin, oroxylin and baicalein as active principles (Chen *et al*., 2003). *Oroxylum indicum* is widely used by the Indians for the treatment of various ailments. In India, roots are used in Ayurvedic preparation called ñDasamoolaò i.e., used as an astringent, anti-inflammatory, anti-helminthic, anti-bronchitic, antileucodermatic, anti-rheumatic, anti-anorexic and for treatment of leprosy and tuberculosis (Raghbir *et al*., 2008). *Oroxylum* root bark is the part used in Ayurvedic medicine, administered as an astringent, bitter tonic, stomachic, and anodyne. It is included in famous tonic formulations, such as Chyawanprash.

The traditional knowledge of Maram Naga village of Senapati district, Manipur, reveals that the decoction of *O. indicum* bark can be used as a potent anticancer medicine, especially against nasopharyngeal cancer (Mao, 2002). The plant is also used in Asian folk medicine for the treatment of abdominal tumors (Soe and Myongure, 2004). It was also reported to possess anticancer properties (Lambertini *et al*., 2004; Costa-Lotufo *et al*., 2005). The plant was reported to possess various pharmacological activities, which may be due to its antioxidant potential.

Free radicals are known to have an important role in stimulation of phagocytosis, induction of drug detoxification pathways and stimulation of signal transduction pathways. However, the same radicals can be implicated in the pathogenesis of various diseases such as atherosclerosis, aging, ischemia, and reperfusion injury of many tissues, central nervous system injury, gastritis, including cancer (Costa *et al*., 2009).

Therefore, antioxidants with free radical scavenging activities may have great relevance in the prevention and therapeutics of these diseases. Phytocompounds like flavonoids and phenolic acids, commonly found in plants have been reported to have multiple biological effects, including antioxidant activity (Conforti *et al*., 2008; Kalaivani and Mathew, 2009). Therefore, medicinal plants can be a potential source of natural antioxidants (Cesquini *et al*., 2003)

Therefore, the aim of the present study is to analyze free radical scavenging potential of five different solvent extracts of different polarities obtained from the stem bark of *Oroxylum indicum* under *in-vitro* conditions. In this study, we analyzed the antioxidant activity of stem bark of *Oroxylum indicum* using different methods viz. total antioxidant assay and β-carotene bleaching assay. We also determined the phytochemical constituents by using qualitative methods. In addition, results would be compared with those of commercial antioxidants commonly used in the food industry e.g. catechin and BHT.

Materials and Methods

Preparation of extracts

Air-dried *Oroxylum indicum* stem bark was packed into a soxhlet apparatus and was extracted sequentially with petroleum ether (PE), benzene (BZ), chloroform (CF), ethanol (EA) and water (AQ). The organic extracts were dried in vacuum desiccator and the solvents were removed in vacuum (40°C). The extracts were dissolved in Di methyl sulphoxide (DMSO), ethanol or H_2O prior to analysis depending upon their solubility. The extracts were subjected to further analysis and all the assays were done in triplicates.

Phytochemical analysis

The extracts were tested by different tests to determine the presence of various phytochemicals: Wagner for alkaloids, Foam test for Saponins and Ferric chloride, gelatin and Lead acetate for the presence of phenolic compound and flavonoids. (Harborne and Harborne, 1998)

Determination of total antioxidant activity

The total antioxidant activity was done by modified method (Prieto et. al., 1999). This method is based upon the reduction of Mo (VI) to Mo (V) by the sample and the subsequent formation of green phosphate/ Mo (V) complex, which has an absorption optimum of 695nm. Reaction mixture 3ml containing 0.6M Sulphuric acid, 28mM Sodium phosphate and 1% ammonium molybdate was added to 0.5ml of water containing different aliquots of all the extracts of varying concentration (10, 50, 100, 250, 500µg). Absorbance at 695 nm was recorded after incubating 10 mins in 95°C water bath.

β - carotene bleaching test

This was done by the method of (Graven *et al*., 1992). Linoleic acid solution (10ml of 2mg/ ml solution in ethanol) and β - carotene solution (10ml, 2mg/ml solution in acetone) were added to the molten agar (10ml, 1.2% solution in boiling water). The mixture was then shaken to give an orange colour. The agar was then poured into Petri dishes (25ml per dish) and were excluded light and left standing to allow the agar to set. Holes (4mm diameter) were then punched into the agar, each extract (1mg) in DMSO were transferred into the holes, and the petri dishes were then incubated at 45°C for 4 h. A zone of colour retention around the hole after incubation indicated sample with antioxidant activities. The zone diameter was measured.

Statistical analysis

All experiments were repeated at least three times. Results were reported as Mean ± SE. The statistical significance between antioxidant activity values of the extracts was evaluated with one way ANOVA between the groups followed by Holm-Sidak test. P values less than 0.05 were considered statistically significant.

Results and Discussion

Phytochemical analysis

The qualitative analysis of *Oroxylum indicum* indicated the presence of phenolics and flavonoids in all the extracts but the contents were high in both chloroform and ethanol extract. Saponins were present only in water extract and alkaloids were found in benzene, chloroform and ethanol extracts (Table 1)

Total antioxidant activity

The assay is based on the reduction of Mo (VI) to Mo (V) by various extracts and subsequent formation of a green phosphate/ Mo (V) complex at acid pH (Prieto *et al*., 1999). The TAA was measured and compared among different extracts and with that of catechin, the positive control. The high absorbance values indicated that the sample possessed significant antioxidant activity. The results revealed, the chloroform extract had significant antioxidant activities and the effects increased with increasing concentration (Fig. 1). The order of total antioxidant activity of various extracts can be seen as chloroform > Ethanol > benzene > water> petroleum ether. There was significant difference among the extracts ($p<0.05$). The absorbance values of chloroform extract and catechin at 500µg concentration were 0.398± 0 and 1.19 ± 0.17 respectively.

Variations in antioxidant capacity of different extracts may be attributed to differences in their chemical composition such as phenolics, flavonoids ascorbic acid and carotenoids. It is clear that, all the extracts showed an increase in antioxidant capacity with increase in dose of sample. The same was also reported in fruit extracts from citron and blood orange (Jayaprakasha and Patil, 2007). The chloroform extract was significant as compared to other extracts but not with positive control catechin. This activity may be due to their reducing ability thereby donating its proton or electron to free radicals and terminating the chain reaction.

β-Carotene Bleaching Assay

The antioxidant activity of the *Oroxylum indicum* extracts as measured by the bleaching of ɓ-carotene (Table 2). The positive control BHT, and all the extracts were able to inhibit the discoloration of ɓ-carotene but none of the extracts were significant as compared to BHT. The order was BHT > Ethanol > water > chloroform> dichloromethane > benzene > petroleum ether. The zone of color retention of positive control BHT and ethanol extract was 15.1mm and 9.2mm respectively. In a β-carotene/ linoleic acid model system, ɓ-carotene undergoes rapid discoloration in the absence of an antioxidant.

In the ɓï carotene bleaching assay, one of the biologically relevant oxidizable substrate ɓ-carotene, gives direct information on the protective effect of extract (Koleva *et al*., 2002). Oxidation of linoleic acid produces hydro peroxide derived free radicals, which bleach the yellow color of β-carotene. The antioxidant activity (AA) was measured

on the based ability of the samples to prevent the bleaching of the ɓ-carotene. The presence of different antioxidants can hinder the extent of ɓ-carotene-bleaching by neutralizing the linoleate-free radical and other free radicals formed in the system (Jayaprakasha *et al*., 2001). Accordingly, the zone of discoloration increased rapidly in samples without antioxidant whereas, in the presence of an antioxidant, they retained their colour, and thus the diameter of the zone of color retention is greater. The result of this test corresponds to their free radical scavenging activity, which may be due to the presence of high amount of poly phenolics.

Present study confirmed that the different extracts had different degree of antioxidant potential in different antioxidant assays. This may be due to the diversity and complexity of the natural mixtures of antioxidant compounds in these plant extracts and it is not easy to characterize every compound and assess their antioxidant activities. Each extract contains generally different antioxidant compounds with different amount of antioxidant activity. Upon this study, we can state that in vivo studies are needed to confirm the advantageous quality of these extracts.

At last, to manage pathological disorders implicated in oxidative damage, detailed information on the structure of the most active compounds of the plant must be investigated and other flavonoids to be identified and further biological tests should be conducted. That work will be the core of the next scientific communications.

Acknowledgement

The authors are thankful to VIT University management for their providing infrastructure, constant support and encouragements. Special thanks to Dr. C.Rajasekaran for his critical comments.

Table 1. Qualitative phytochemical analysis of various extracts obtained from *O. indicum*

Test for phytocompound	Name of the test	Name of the extract				
		PE	**BZ**	**CF**	**EA**	**AQ**
Alkaloids	Wagner	-	++	+	+	-
Saponins	Foam	-	-	-	-	+
Phenolics & Flavonoids	Ferric chloride	+	+	+++	+++	+
	Gelatin	-	-	-	+	+
	Lead acetate	_	_	_	+	+

Table 2 β-carotene bleaching assay of various extracts obtained from *O. indicum*

Zone of color retention in mm					
PE	**BZ**	**CF**	**EA**	**AQ**	**BHT**
6.2	**7.3**	**8.5**	**9.2**	**8.7**	**15.1**

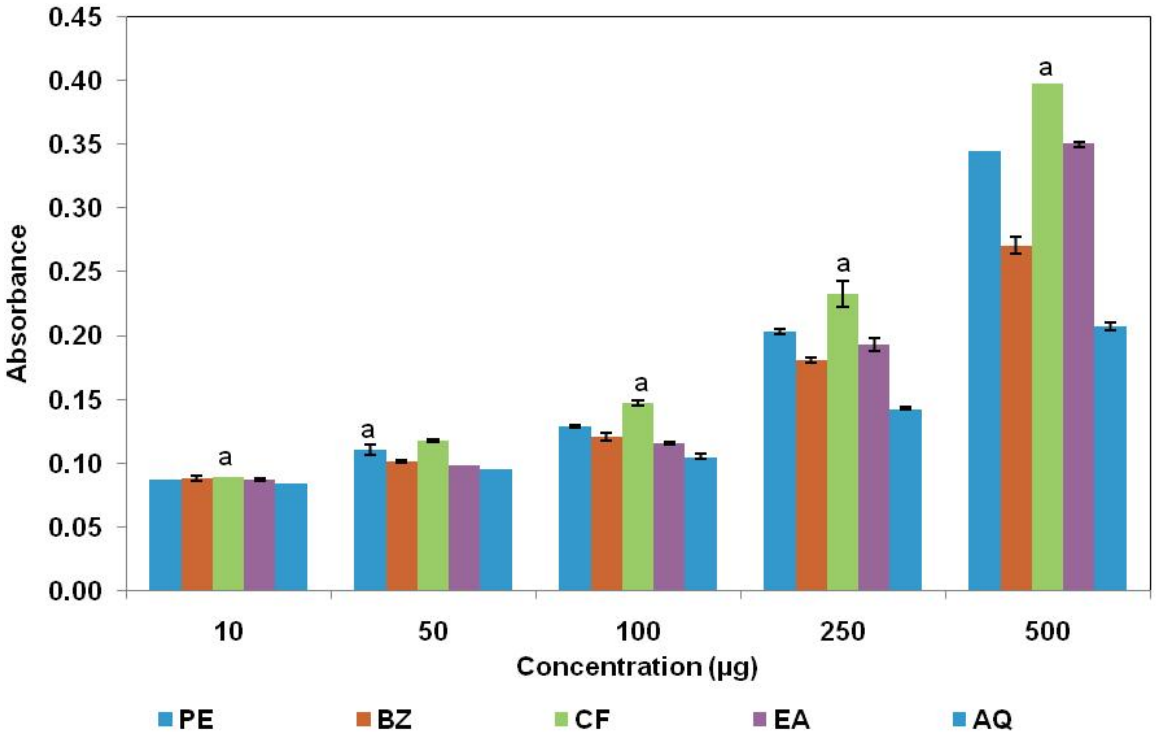

Figure 1. Total antioxidant activity of different extracts obtained from bark of *Oroxylum indicum*

Abbreviations

PE	ï	Petroleum ether
BZ	-	Benzene
CF	ï	Chloroform
EA	ï	Ethanol
AQ	ï	Water
TAA	ï	Total antioxidant activity
BHT	_	Butylated hydroxy toluene

REFERENCES

Cesquini, M., Torsoni, M.A., Stoppa, G.R., Ogo, S.H. T- BOOH, 2003. Induced Oxidative Damage in Sickle Red Blood Cells and the Role of Flavonoids. *Biomedical and Pharmacotheraphy 57*, 124-129.

Chen, L.J., Games, D.E., Jones, J., 2003. Isolation and identification of four flavonoid constituents from the seeds of *Oroxylum indicum* by high-speed counter-current chromatography. *Journal of Chromatography A* 988(1), 95-105.

Conforti, F., Ioele, G., Statti, G., Marrelli, M., Ragno, G., Menichini, F., 2008. Anti-proliferative activity against human tumor cell lines and toxicity test on Mediterranean dietary plants. *Food and Chemical Toxicology* 46, 3325-3332.

Costa, R., Magalhães, A., Pereira, J., Andrade, P., Valentão, P., Carvalho, M., Silva, B., 2009. Evaluation of free radical-scavenging and antihemolytic activities of quince (*Cydonia oblonga*) leaf: A comparative study with green tea (*Camellia sinensis*). *Food and Chemical Toxicology* 47, 860-865.

Costa-Lotufo, L., Khan, M., Ather, A., Wilke, D., Jimenez, P., Pessoa, C., de Moraes, M., de Moraes, M., 2005. Studies of the anticancer potential of plants used in Bangladeshi folk medicine. *Journal of Ethnopharmacology* 99, 21-30.

Graven, E.H., Deans, S.G., Svobodam, K.P., Mavi, S., Gundidza, M.G., 1992. Antimicrobial and antioxidative properties of the volatile (essential) oil of *Artemisia afra* Jacq. *Flavour and Fragrance Journal* 7(3), 121ï 123.

Gupta, R.C., Sharma, V., Sharma, N., Kumar, N., Singh, B., 2008. *In vitro* antioxidant activity from leaves of *Oroxylum indicum* (L.) Vent. - A North Indian highly threatened and vulnerable medicinal plant. *Journal of Pharmacy Research* 1(1), 65-72.

Harborne, J. and Harborne, A., 1998. Phytochemical methods: a guide to modern techniques of plant analysis: Springer.

Jayaprakasha, G.K., Patil, B.S., 2007. *In vitro* evaluation of the antioxidant activities in fruit extracts from citron and blood orange. *Food Chemistry* 101, 410ï 418.

Jayaprakasha, G., Singh, R., Sakariah, K., 2001. Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models *in vitro*. *Food Chemistry* 73, 285-290.

Kalaivani, T., Mathew, L., 2009. Free radical scavenging activity from leaves of *Acacia nilotica* (L.) Wild. *ex* Delile, an Indian medicinal tree. *Food and Chemical Toxicology Doi:10.1016/j.fct2009.10.013.* (In Press*) Available online 16 October 2009.*

Koleva, I., van Beek, T., Linssen, J., Groot, A., Evstatieva, L., 2002. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. *Phytochemical Analysis* 13, 8-17.

Lambertini, E., Piva, R., Khan, M.T.H., Lampronti, I., Bianchi, N., Borgatti, M., Gambari, R., 2004. Effects of extracts from Bangladeshi medicinal plants on *in vitro* proliferation of human breast cancer cell lines and expression of estrogen receptor alpha gene. *International Journal of Oncology* 24, 419ï 423.

Mao, A.A., 2002. *Oroxylum indicum Vent.* ï A potential anticancer medicinal plant. *Indian Journal of Traditional Knowledge* 1 (1), 17-21.

Prieto, P., Pineda, M., Aguilar, M., 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific

application to the determination of vitamin E. *Anals of Biochemistry* 269, 337-341.

Ravi kumar, K. and Ved, D.K., 2000. 100 Red listed medicinal plants of conservation concern in Southern India. pp.1-467. Foundation for revitalisation of local health traditions, Bangalore. India.

Soe, K. and Myo Ngwe, T., 2004. Medicinal plants of Myanmar. pp 255. Myanmar Forest Resource Environment Development & Conservation Association, Myanmar.

.