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A Comparative Assessment of Total Polyphenols, Antioxidant Activity and Free Radical Scavenging Activity of the Root Barks of *Oroxylum indicum* (L.) Vent. and Its Two Allied Species

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Abstract: ***Oroxylum indicum*** **(L.) Vent. is an important medicinal plant of Bignoniaceae family, whose root bark is authentically used in many Ayurvedic preparations. The increased demand for this plant in the herbal drug market resulted in the substitution of the original plant parts with the parts of two ethno medicinal plants-** ***Pajanelia longifolia*** **(Willd.) K. Schum and** ***Radermachera xylocarpa*** **(Roxb.) K. Schum., belonging to the same family. In the present study the total polyphenol content, DPPH free radical scavenging efficiency and total antioxidant activity of the root barks of the above mentioned three plant species were evaluated and compared. The estimation of total phenolic content revealed that** ***Oroxylum indicum*** **has the maximum phenolic content, than the other two species. Also there was a positive correlation between the total phenolic content and radical scavenging activity. It was observed that root bark of** ***Oroxylum idicum*** **has the highest DPPH radical scavenging activity (IC_{50} value 50), followed by** ***Radermachera xylocarpa*** **(IC_{50} value 150) and Pajanelia** ***longifolia*** **(IC_{50} value 253). The total antioxidant activity was also higher for** ***Oroxylum indicum,*** **equivalent to 0.34 mg of ascorbic acid, when compared to the other two species. Methanol found to be the best solvent for extraction.**

Index Terms: **Antioxidant activity, DPPH free radical, IC_{50} value,** ***Oroxylum indicum, Pajanelia longifolia,*** **Polyphenols,** ***Radermachera xylocarpa*****.**

I. INTRODUCTION

Free radicals are molecules possessing unpaired electrons. They include Superoxide radicals, Hydrogen peroxide (H_2O_2), Oxygen molecule (O_2), and Hypochlorous acid (HOCl). Free radicals are produced continuously in the cells as a result of metabolic activities. They can cause DNA damage, protein damage and peroxidation of lipids (Aruoma., 1999). Herbal medicines rich in antioxidants are commonly used for the treatment of ailments like atherosclerosis, rheumatism etc., and they can even prevent cancer and Alzheimer's disease (Devasagayam *et al*., 2004). Antioxidant compounds can scavenge these free radicals and there by prevent cell damage.

O. indicum is an important medicinal tree belonging to Bignoniaceae family, whose root and stem barks are used in many Ayurvedic formulations including 'Dasamularishta', 'Dhanwanthara taila', 'Dhanwanthara ghritha' etc. (Warrier *et al*., 1995). Several active compounds like Oroxylin, Baicalein, Chrysin etc. have been reported from this tree (Raghu *et al*., 2013). Overexploitation and uprooting has resulted in the large scale destruction of this tree in the natural population (Ravi kumar *et al*., 2000). Now this valuable indigenous tree has become vulnerable in the South Indian states, with an endangered status in Kerala and Maharashtra (Chaudhuri., 2007, Jain *et al*., 1981). *R. xylocarpa* and *P. longifolia* are used widely by the ethnic population throughout India for the treatment of various ailments like rheumatism, skin disorders, snake bite etc. (Jagtap *et al*., 2006, Jain *et al*., 2011, Kshirsagar *et al*., 2001, Natarajan *et al*., 2000, Singh *et al*., 2012). The root and stem bark of these two plants are often used as substitutes for the original species in herbal preparations. Antihelminthic, antibacterial and antioxidant activities were already reported from *P. longifolia* (Asha *et al*., 2013, Zainab *et al*., 2013). In the present study the total polyphenol content, DPPH free radical scavenging activity and total antioxidant activity of the root barks of these three species were evaluated and compared.

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II. Materials and Methods

A. Collection of Plant Material

The roots of the three species were collected from Attapadi reserve forest of Palakkad district in Kerala. The plants were identified with the help of Dr. P.S Udayan, Head of the PG and Research Dept. of Botany, Sree Krishna College, Guruvayur, Thrissur.

B. Preparation of Crude Samples

The root barks were cut into small pieces, shade dried and powdered in a grinder. About 10 grams of each powder was soaked separately in 100 ml of the solvents- methanol, ethyl acetate and water, in a conical flask and kept in a rotary shaker for 12 hour and allowed to stand for the next 12 hour. The extracts were filtered through Whatmann filter paper no.1 and solvents were removed by evaporation. The crude extracts obtained were stored in air tight borosil vials at 8°C for further study.

C. Estimation of total Polyphenols

The total polyphenols present in the crude extracts were estimated by Folin's ciocalteau method (Singleton *et al*., 1965). 1mg of the extract was dissolved in 1 ml methanol and made up to 8.5 ml with distilled water. To this 0.5 ml of Folin's ciocalteau reagent and 1 ml of 35% Sodium Carbonate was added. The mixture was kept at room temperature for 30 minutes and then the absorbance was measured at 760 nm against blank. Gallic acid was used as the standard. The total phenolic content of different concentrations of gallic acid (20µg to 100µg) was estimated by the same procedure and the standard graph was prepared. From the standard graph the total polyphenols present in each sample was calculated in gallic acid equivalents according to the equation $T=\frac{CxV}{M}$, where T= total phenolic content, C= concentration of gallic acid, V= volume of sample, M= mass of extract.

D. DPPH Radical Scavenging Assay

DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay was carried out according to the modified method (Braca *et al*., 2001). The scavenging of the DPPH free radicals by the antioxidant compounds of the sample will result in a colour change of the reaction mixture from purple to shades of yellow.

A 0.1 mM solution of DPPH is prepared by dissolving 3.94 mg DPPH in 100 ml methanol. Working stock solution of the sample (1mg/ ml) was prepared in methanol. From this stock solution different aliquots (50 µl, 100 µl, 150 µl etc.) were pipetted and made up to 1 ml with methanol. To this sample 1 ml of DPPH solution was added. Ascorbic acid was used as standard. From a 1 mg/ ml stock solution of ascorbic acid in methanol, different aliquots (10µl, 20µl, 30µl etc.) were pipetted out and each aliquot was made up to 1 ml with methanol. 1 ml of DPPH was added to this. After incubating all the samples in darkness for 30 minutes, the absorbance was read at 517 nm against blank, which consist of 1 ml methanol and 1 ml DPPH. The IC value (inhibitory concentration) of each sample was calculated as $\frac{(A-B)}{A}$, where A= OD of the blank, B= OD of the sample. The concentration of the sample (µg) required to scavenge 50% of the free radical in DPPH solution is represented as the IC_{50} value.

E. Evaluation of total Antioxidant Activity

The total antioxidant activity was estimated according to Phosphomolybdenum reduction method (Prieto *et al*., 1999). About 1 mg of the extract is dissolved in 1 ml distilled water and is added to 3 ml of reaction mixture containing 1 ml of 0.6 M Suphuric acid, 1 ml of 28 mM Sodium phosphate and 1 ml of 4 mM Ammonium molybdate. The mixture was incubated at 95°C for 90 minutes in water bath. The absorbance is measured at 695 nm against blank. The standard graph of ascorbic acid was prepared by plotting concentration on x- axis and absorbance on y-axis. From this graph the total antioxidant activity of 1 mg of each sample is determined in ascorbic acid equivalents.

All the experiments were repeated thrice and the values represent mean ± standard error. The significance between the mean values were evaluated with one way ANOVA.

III. Results and Discussion

The total polyphenols present in the root bark of all the three species were estimated in gallic acid eqivalents, with the help of standard graph of gallic acid ("Fig.1") and total antioxidant activity was calculated in ascorbic acid equivalents from the standard graph of ascorbic acid ("Fig.2"). The methanolic extract of *O. indicum* has the maximum polyphenol content- 0.095 mg, followed by *R. xylocarpa* and *P. longifolia* (0.092 mg and 0.064 mg respectively). Water and methanol found to be suitable solvents for eluting phenolic compounds, when compared to ethyl acetate.

There was earlier reports suggesting that the antioxidant property of plants are due to the presence of phenolic components, which include phenols, tannins etc. (Shahidi *et al*., 1992), and there exist a direct correlation between the phenolic content and antioxidant activity (Gardner *et al*., 2000, Tanaka *et al*., 1988). The DPPH free radical scavenging activity and phosphomolebdenum reducing capacity of the 3 species also reveals this co-relation. *O. indicum* has the maximum radical scavenging activity with IC_{50} value 50 µg, followed by *R. xylocarpa* and *P. longifolia* (IC_{50} value 150 µg and 253 µg respectively). The total antioxidant activity was also highest for methanolic extract of *O. indicum* (0.34 mg equivalents of ascorbic acid), whereas *R. xylocarpa* and *P. longifolia* has still lower values- 0.26 mg and 0.20 mg equivalents of ascorbic acid. The total poly phenol content, DPPH free radical scavenging activities and the antioxidant activities of all the samples in different solvents were summarized in "Table.1".

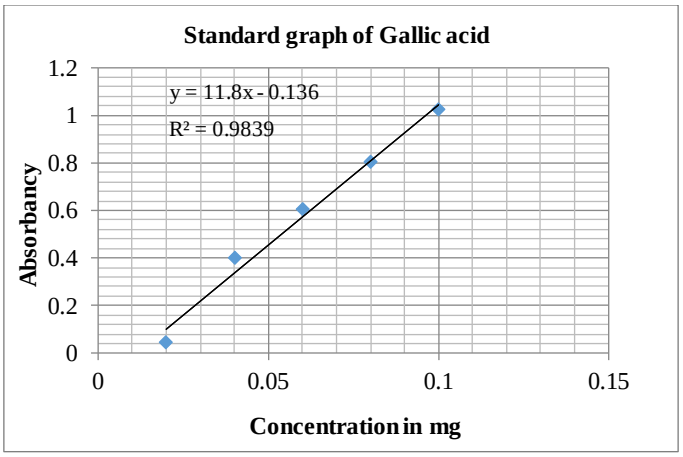

Fig 1. Standard graph of Gallic acid

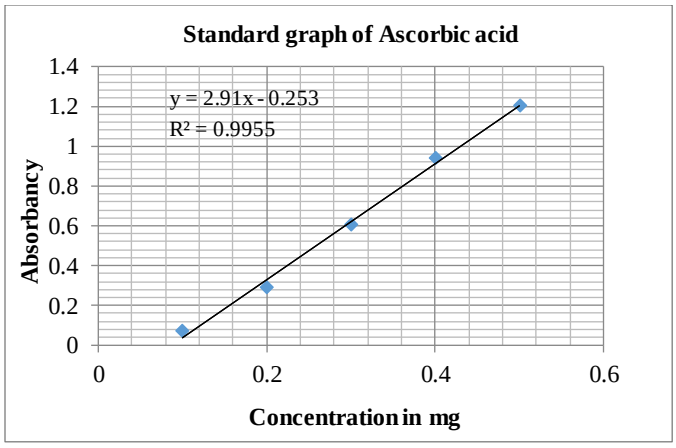

Fig 2. Standard graph of Ascorbic acid

Table 1. Total polyphenols, total antioxidant activity and DPPH free radical scavenging activity of the root extract of 3 species

Plants	Total Phenolic content in mg of GAE*			Total antioxidant activity in mg of AAE**			IC $_{50}$ value (µg)
	Methanol	Aqueous	Ethylacetate	Methanol	Aqueous	Ethyl acetate	
O.indicum	0.095±0.007^{a}	0.066±0.009^{a}	0.038±0.005^{a}	0.34±0.009^{a}	0.24±0.007^{a}	0.18±0.52^{a}	50±1.21^{a}
R. xylocarpa	0.092±0.008^{a}	0.052±0.005^{b}	0.028±0.005^{b}	0.26±0.008^{b}	0.21±0.008^{b}	0.14±0.63^{b}	150±1.45^{b}
P.longifoia	0.064±0.008^{b}	0.043±0.005^{c}	0.015±0.006^{c}	0.20±0.008^{b}	0.19±0.006^{c}	0.12±0.06^{c}	253±1.62^{c}

Note. Values represent mean ± standard error. Column values with different superscripts are significantly different ($p < 0.05$)
*Gallic acid Equivalents **Ascorbic acid equivalents

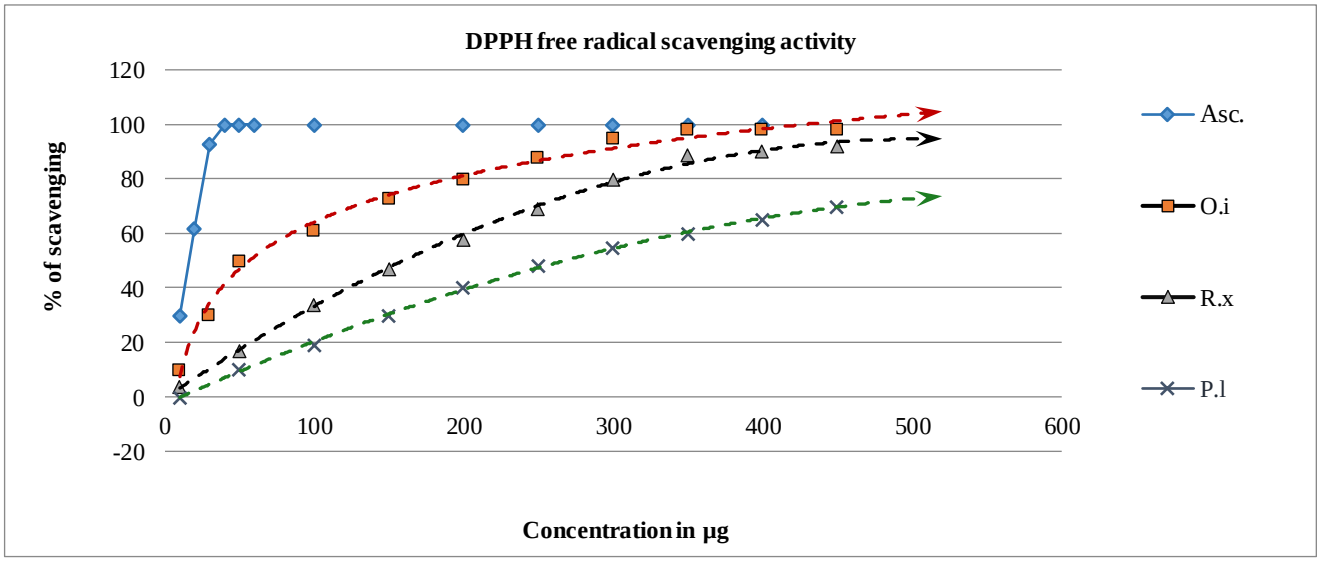

Fig 3. Comparison of DPPH radical scavenging activity of the 3 species with standard ascorbic acid

A comparative DPPH free radical scavenging activity of various concentrations of the methanolic extracts of the three species and ascorbic acid was given in "Fig 3". *O. indicum* exhibited 95% scavenging activity in 300 µg, but for *R. xylocarpa* and *P. longifolia* a concentration of 400 µg and 500 µg was required respectively for 90% scavenging of the radicals. The scarcity of authentic plant materials often leads to the substitution of the original plant with morphologically similar other plants with inferior quality (Kumar *et al*., 2014). Many of these substituted species may not be having pronounced bioactive properties at all; at the same time have adverse effect too. (De Smet., 1999).

Evaluation of the phytochemistry and the bioactivities of the substitute species are thus very important for ensuring the safety and quality of drugs. From the present study it was revealed that the original drug *O. indicum* is having the maximum phenolic content, DPPH free radical scavenging efficiency and total antioxidant activity. The other two species are with lesser efficacy when compared to *O.indicum***.** Therefore substitution of

R. xylocarpa and *P.longifolia* in the place of *O. indicum*, in the same quantity will not give sufficient results in herbal preparations. A phytochemical characterization of *P.longifolia* and *R. xylocarpa* are required for authentifying the recommendation of these two species as substitutes for *O. indicum.*

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