ISSN : 0974 - 7508 Volume 7 Issue 2

Trade Science Inc.

Natural Products

An Indian Journal

Full Paper

NPAIJ, 7(2), 2011 [51-56]

Antioxidant activity of *Plumbago zeylanica* and *Plumbago rosea* belonging to family plumbaginaceae

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Received: 8th April, 2011 ; Accepted: 18th April, 2011

ABSTRACT

Plumbago zeylanica (Chitraka) is a perennial herbs found throughout India. The root is used as laxative, expectorant, astringent, abortifacient and dysentery. *Plumbago rosea* (Lal chitraka) have plumbagin and napthoquinone which is highly antitumour and anticarcinogenic in animal studies. Few reports are available on *Plumbago rosea* which is a very important medicinal plant. In this study we have attempted to investigate the antioxidant activity and phenol content in the root extract in a comparative manner by DPPH and OH^- radical scavenging assay. In both the extracts ethanolic solvent gave maximum crude extract, phenol content and antioxidant activity. Highest antioxidant activity was demonstrated in *Plumbago rosea* (82.34%) in comparison to *Plumbago zeylanica* (74.65%) at 200µg/ml in DPPH assay. Similarly in OH^- radical assay ethanolic extract of *Plumbago rosea* at 200µg/ml demonstrated highest activity in comparision to *Plumbago zeylanica*. The total phenolic content of *Plumbago rosea* root extract is 235µg/gm in comparison to 204µg/gm in *Plumbago zeylanica* which indicates regarding the higher antioxidant activity in *Plumbago rosea*. The unexplored medicinal plant *Plumbago rosea* need more attention for detailed scientific investigation.

KEYWORDS

Plumbago zeylanica;
Plumbago rosea;
Antioxidant activity;
Hydroxyl radical;
DPPH.

INTRODUCTION

In the modern world it has been realized the herbal drugs strengthens the body system specifically and selectively without side effects. The importance of traditional herbal medicinal system has now gained vital importance in developed countries. In the Ayurvedic and Siddha medicine *Plumbago zeylanica* and *Plumbago rosea* has been assigned medical properties and is used in formulations for Ayurvedic medicines (Figure 1). *Plumbago zeylanica* (Family-*Plumbaginaceae*) mainly called as "Chitrak"[1] is a valuable Indian medicinal plant widely used in treatments of piles, diarrhea, leprosy and anasarca[2]. Roots of plant have potential therapeutic properties like anti-anthrogenic, cardoitonic, hepatoprotective, neuroprotective[3]. It is has been also reported that the plant have anticancer, antibacterial, antifungal and antitumor properties[4]. The root, root bark and seeds are used medicinally as a stimulant, caustic, digestion, antiseptic, anti-parasitic. Chitrak root helps improve digestion and it stimulates the appetite. Chitrak root is also an acronarcotic poison that can cause an abortion. The leaves

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and roots of *P.zeylanica* contains an alkaloid called plumbagin (2- methoxy-5hydroxy-1, 4-napthoquinone), which externally is a strong irritant but a powerful germicide; stimulates muscular tissue in smaller doses and paralyzes in larger ones; stimulates the contraction of the muscular tissues of the heart and intestines; stimulates the secretion of sweat, urine and bile; and also has a stimulant action on the nervous system[5].

Flowers of *Plumbago rosea*

Flowers of *Plumbago zeylanica*

Figure 1 : Flowers of *P.rosea* and *P.zeylanica*

The objective of this investigation was to determine the antioxidant activity and phenol content in the root extract of *Plumbago zeylanica* and *Plumbago rosea* in a comparative manner by DPPH scavenging and Hydroxyl radical assay. Results from this preliminary study will help to provide a better understanding of antioxidant properties of these plants and identify plants with high antioxidants for further investigation and development into pharmaceuticals and neutraceuticals.

MATERIALS AND METHODS

Plant materials

The roots both the species i.e. *Plumbago zeylanica* and *Plumbago rosea*, were collected from the Medicinal garden of B.J.B Autonomous College, Bhubaneswar, Odisha (India). The collected roots cleaned with properly with tap water followed by distilled water and then dried under sunlight, powdered, stored in a closed container for further use.

Chemicals and reagents

Folin-Ciocalteu reagent (Merck Pvt. Ltd, India), Sodium chloride (S.D. Fine Chem, India), Sodium carbonet (Merck Pvt. Ltd, India), Catechol (Himedia Lab., India), 2, 2-Diphenyle-2-picryl hydrazyl (DPPH) and Ascorbic acid are obtained from (Himedia Lab., India), Thiobarbituric acid (TBA) (Himedia Lab., India), Trichloroacetic acid (TCA) (Himedia Lab., India), EDTA and $FeSO_4.7H_2O$ (S.D. Fine Chem, India), Potassium hydroxide were purchased from Himedia Lab. and Ethanol, Methanol were obtained from SD-Fine Chem. Appropriate blanks were used for individual assays. All solutions, including freshly prepared doubled distilled water. Stock solutions of the test extracts were prepared in ethanol. Appropriate blanks were used for individual assays.

Instrumentations

Collection of multi-solvent extract was done by Soxhlet apparatus (J.S.G.W) with varying temperatures according to the B.P. of the solvents. The samples were evaporated through the Rotary vacuum evaporator at 60-100^{0}C according to the B.P. of supplied solvents. Absorbance spectrophotometery was carried out using a UV-vis spectrophotometer (EI, model-1371). Wavelength scans and absorbance measurements were in 1ml quartz cells of 1cm path length.

Preparation of crude extracts

The powdered root material of *Plumbago*

zeylanica and *Plumbago rosea* (each 50 g) was extracted with Water, Methanol and Ethanol by using Soxhlet extraction apparatus. The extract was filtered and concentrated by distilling of the solvent to obtain the crude extract. Then it was dried by rotary evaporator and stored in deep freezer at -18^{0}C for further study.

Phenolic estimation

The total phenolic content of plant extracts were determined by using Folin-Ciocalteu Spectrophotometric method according to the method described by[6]. Reading samples on a UV-Vis Spectrophotometer at 650nm. Results were expressed as catechol equivalents (µg/ml).

DPPH free radical scavenging assay

The antioxidant activity of the root extracts of plumbago on the basis of the scavenging activity of the stable 2, 2- diphenyl-2-picrylhydrazyl (DPPH) free radical was determined according to the method described by[7] with slight modification. The following concentrations of extracts were prepared 40ìg/mL, 80ìg/mL, 120ìg/mL, 160ìg/mL and 200ìg/mL. All the solutions were prepared with methanol. 5 ml of each prepared concentration was mixed with 0.5mL of 1mM DPPH solution in methanol. Experiment was done in triplicate. The test tubes were incubated for 30 min. at room temperature and the absorbance measured at 517nm. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. Ascorbic acid was used as a standard and the same concentrations were prepared as the test solutions. The different in absorbance between the test and the control (DPPH in ethanol) was calculated and expressed as % scavenging of DPPH radical. The capability to scavenge the DPPH radical was calculated by using the following equation.

Scavenging effect (%) = (1-As/Ac) ×100

As is the absorbance of the sample at t =0 min.
Ac is the absorbance of the control at t=30 min.

Hydroxyl radical scavenging assay

2-Deoxyribose is oxidized by OH^- radicals that are formed by Fenton reaction and degraded to malondialdehyde[8] which can be detected by reacting with Thiobarbituric acid (TBA). A reaction mixture composed of 0.1 ml of 10 mM $FeSO_4.7H_2O$; 0.1 ml of 10 mM EDTA, 0.2 ml of 10 mM 2-deoxyribose and 0.02 ml of sample (Multi-vitamin crude extract) in 1.38 ml of 0.1 mM phosphate buffer (pH 7.4) was made. The reaction was started by adding 0.2 ml of 10 mM H_2O_2 and incubating at 37 IC for 1 hr. After incubation, 1 ml each of 2.8 % Trichloroacetic acid (TCA) solution and 1 ml of 1 % Thiobarbituric acid (TBA) solution were added to the reaction mixture, which was then boiled for 10 min, cooled in ice and its absorbance recorded at 532 nm. All the analyses were done in triplicates and average values were taken. Inhibition (I%) of deoxyribose degradation in percent was calculated by the formula.

(I %) = (A_0-A_1/A_0) ×100

Where A_0 is the absorbance of the control reaction (without test compound) and A_1 is the absorbance of the test compound.

RESULTS AND DISCUSSION

There are variations in the yields of crude extracts of two plumbago species i.e. *P. rosea* and *P. zeylanica* obtained from 3 different solvents i.e. Ethanol, Methanol and Water. The yield of extracts using Soxhlet apparatus in case of *P. rosea* were 5.50gm, 4.35gm and 3.76gm and in case of *P. zeylanica* were 5.57gm, 3.65gm and 3.16gm respectively. The variation in yield may be due to the polarity of the solvents used in the extraction process (TABLE 1).

TABLE 1 : Crude extracts and phenol content in *P. rosea* and *P. zeylanica*

Solvent used	Crude Extracts (gm)		Phenol content (µg/ml)	
	P. rosea	*P. zeylanica*	*P. rosea*	*P. zeylanica*
Water	3.76	3.16	126	112
Methanol	4.35	3.65	147	134
Ethanol	5.50	5.57	235	224

It is reported that phenols are responsible for the variation in the antioxidant activity of the plant[9]. They exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals[10,11]. It has been determined that the antioxidant effect of plant products is mainly due to radical scavenging activity of phenolic compounds such as flavonoids, polyphenols, tannins, and phenolic terpenes[12]. There is not much difference in phenolic

Full Paper

contents between the two plumbago species as shown in (TABLE 1). The multi-solvent extracts of *P. rosea* showed a little bit higher value of phenol content in comparison to *P. zeylanica*. The ethanolic fraction of *P. rosea* exhibited the highest concentration of total phenolic content i.e. 235µg/ml followed by methanolic extract with 147µg/ml and aqueous extract with 126µg/ml respectively. Likewise same order was seen in case of *P. zeylanica* and they are ethanol (224µg/ml), methanol (134µg/ml) and aqueous (112µg/ml) respectively (TABLE 1). The relatively low level of total phenols in *P. zeylanica* in comparison to *Plumbago rosea* might account for the weak activity observed in the DPPH radical assay. A positive relationship is observed between phenol contents and antioxidants. These results indicated that all extracts have a noticeable effect on scavenging the free radicals.

Free radicals are chemical entities that can exist separately with one or more unpaired electrons. The propagation of free radicals can bring about thousands of reactions and thus may cause extensive tissue damage. Lipids, proteins and DNA are all susceptible to attack by free radicals. Antioxidant may offer resistance against oxidative stress by scavenging the free radicals[13,14]. DPPH scavenging assay is a commonly employed assay in antioxidant studies of specific compounds or extracts across a short time scale[15]. Highest scavenging was observed in DPPH method with *Plumbago rosea* root ethanolic extract i.e. 82.34% at 200µg/ml with an IC_{50} value of 0.018mg/ml as opposed to the IC_{50} value of ascorbic acid 0.008mg/ml, which is a well known antioxidant (Figure 2). The ethanolic extract of *P. zeylanica* showed 74.34% at 120µg/ml with an IC_{50} value of 0.020mg/ml. The Methanol and ethanol has been proven as effective solvent to extract phenolic compounds[16]. In the present study, the values of ethanolic and methanolic extracts were higher than those of aqueous ones. (TABLE 2) expresses a significant decrease in the concentration of DPPH radical (percentage inhibition) due to the scavenging ability of all extracts of these two plants. The scavenging effect of different solvent extracts on the DPPH radical decreased in the order Ethanolic > Methanolic > Aqueous and were 82.34%, 68.66%, and 63.36% in *Plumbago rosea* root and 74.34% (120µg/ml), 65.34% and 58.47% in case of *P.zeylanica* respectively, at a concentration of 200µg/ml. Among solvents used in this study ethanol has showed the best effectiveness in extracting phenolic components. Ethanol is preferred for the extraction of antioxidant compounds mainly because its lowers toxicity[17].

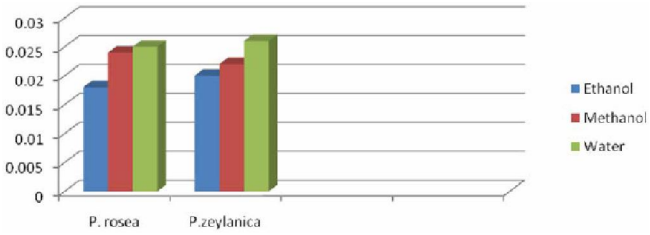

Figure 2 : IC_{50} Values of *P.rosea* and *P.zeylanica*

TABLE 2 : DPPH scavenging activity of *P. rosea* and *P. zeylanica*

Concentration of extracts (µg/ml)	Antioxidant activity (DPPH) (%)					
	Water		Methanol		Ethanol	
	P. rosea	*P.zeylanica*	*P. rosea*	*P. zeylanica*	*P. rosea*	*P. zeylanica*
40	52.45±0.02	48.56±0.07	57.84±0.02	54.60±0.05	63.19±0.05	60.30±0.05
80	54.63±0.10	51.50±0.09	62.88±0.10	58.11±0.04	68.43±0.05	65.19±0.05
120	58.33±0.07	54.42±0.03	67.33±0.07	60.43±0.05	72.44±0.01	74.34±0.06
160	61.77±0.02	56.22±0.01	71.23±0.02	63.44±0.11	75.21±0.06	68.43±0.05
200	63.36±0.07	58.47±0.08	68.66±0.07	65.34±0.07	82.34±0.04	71.65±0.06

Among the oxygen radicals, hydroxyl radical is one of the most reactive species and induces severe damage to adjacent biomolecules[18]. Formation of a highly reactive tissue damaging species like hydroxyl radical is caused by the interaction of iron ions with hydrogen peroxide in biological systems[19]. All of the extracts of both the plants showed higher activity in DPPH radical assay when compared with that of OH- scavenging assay (Figure 3). In OH^- methods we have also noticed similar results that ethanolic extract had higher activity than methanolic and aqueous extracts in both the plants. The scavenging effects of different extracts on the OH- radical were 70.34%, 63.34% and 48.47% in case of *P.rosea* and 64.65%, 60.66% and 46.36% respec-

Full Paper

tively (TABLE 3). A correlation between total phenolic content and antioxidant activity was observed in both the plants. These observations indicated a close linkage between phenolics and antioxidant activity. The phytochemical analysis of the crude extracts indicated the presence of major phytocompounds, including phenolics, alkaloids, glycosides, flavonoids, and tannins which may be responsible for the observed antioxidant activity. Our results further support the view that some traditionally used Indian medicinal plants are promising sources of potential antioxidants. In conclusion, our findings suggest *P.rosea* is a better antioxidant in comparison to *P.zeylanica*. However, further study will be aimed at isolating and identifying the substances responsible for the antioxidant activity of plant extracts, which may be further exploited in herbal formulations.

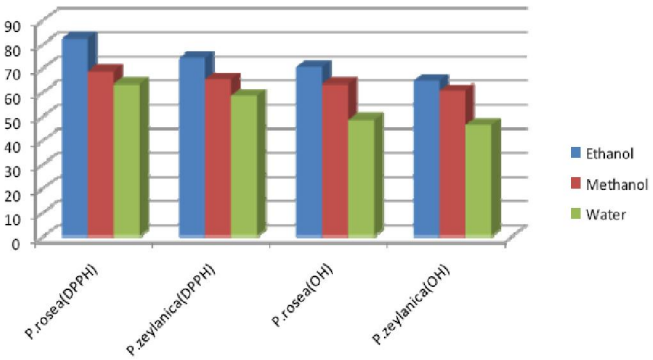

Figure 3 : DPPH radical OH^- radical scavenging assay in *P. rosea* and *P. zeylanica*

TABLE 3 : Hydroxyl scavenging activity of *P. rosea* and *P. zeylanica*

Concentration of extracts (µg/ml)	Antioxidant activity (OH^-) (%)					
	Water		Methanol		Ethanol	
	P. rosea	*P.zeylanica*	*P. rosea*	*P. zeylanica*	*P. rosea*	*P. zeylanica*
40	35.56±0.07	28.45±0.02	44.60±0.05	37.84±0.02	48.30±0.05	44.47±0.14
80	39.50±0.09	33.63±0.10	48.11±0.04	42.88±0.10	56.19±0.05	48.18±0.06
120	44.42±0.03	38.33±0.07	53.43±0.05	47.33±0.07	74.34±0.06	54.46±0.06
160	46.22±0.01	44.77±0.02	57.44±0.11	53.23±0.02	69.44±0.01	58.87±0.10
200	48.47±0.08	46.36±0.12	63.34±0.05	60.66±0.07	70.34±0.06	64.65±0.04

ACKNOWLEDGEMENTS

The authors are thankful to University Grants Commission New Delhi, for Financial Assistance in form of major research project to one of the author (R.K.S) we are also thankful to Head of the Department of Botany and principal B.J.B. (A) College for providing necessary facilities for carrying out the experimental work. Finally we are thankful to Sabitri Nahak for helping in computer work.

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Full Paper

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