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Research Article

In Vitro Antioxidant Activities of *Plumbago zeylanica* Linn. and *Plumbago rosea* Linn. : A Comparative Study

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ABSTRACT

Plumbago zeylanica Linn. is a useful medicinal plant. The root of the plant contains active constituent Plumbagin, a napthoquinone and other principles, hence constituents exhibits various potential therapeutic activities. *Plumbago rosea* Linn. is shrubby perennial which also contains same active constituents as *Plumbago zeylanica* Linn. .The aim of present study is to evaluate the physicochemical properties and the *in vitro* antioxidant activity of hydroehtanolic extracts of both the species of Plumbago. Determinations of their *in vitro* antioxidant activity were carried out by using 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity method and reducing power assay method. Results of the present study indicated that that the hydroethanolic extract of *Plumbago rosea* Linn. is better antioxidant in comparison to *Plumbago zeylanica* Linn.

Key words: Plumbago zeylanica Linn, Plumbago rosea Linn, Antioxidants, DPPH, Reducing power assay.

INTRODUCTION

"Antioxidants" are the substances that neutralize free radicals or their actions.[1]Reactive oxygen species (ROS), as well as reactive nitrogen species (RNS), are products of normal cellular metabolism and they are well recognized for playing a dual role as both deleterious and beneficial species, since they can be either harmful or beneficial to living systems.[2] Free radicals are produced in normal and/or pathological cell metabolism. Oxidation is essential to many living organisms for the production of energy to fuel biological processes. However, the uncontrolled production of oxygen derived free radicals is involved in the onset of many diseases such as cancer, rheumatoid arthritis, cirrhosis and arteriosclerosis as well as in degenerative processes associated with aging.[3]As individuals age, accumulation of oxidative damage may reduce functional capacity and increase the risk of disease.[4] Exogenous chemical and endogenous metabolic processes in the human body or in the food system might produce highly reactive free radicals, especially oxygen derived radicals, which are capable of oxidizing biomolecules, resulting in cell death and tissue damage.[5] Lack of antioxidants facilitates the development of degenerative disorders, cardiovascular diseases and cancer. One possible solution to this problem may be provided by the use of natural antioxidant compounds present in plant sources.[6]

There are various Indian medicinal plants which provide

Chitrak-With White Flowers
Botanical name- Plumbago zeylanica Linn

Chitrak – With Red Flowers
Botanical name- Plumbago rosea Linn

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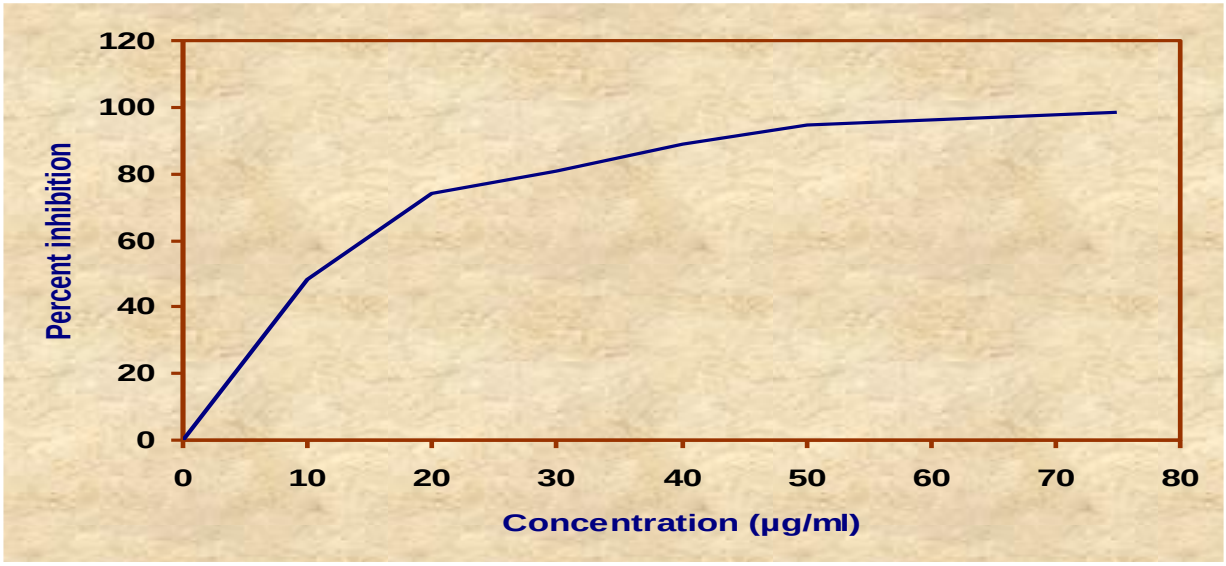

Fig. 1: Percent Inhibition curve of standard Ascorbic Acid

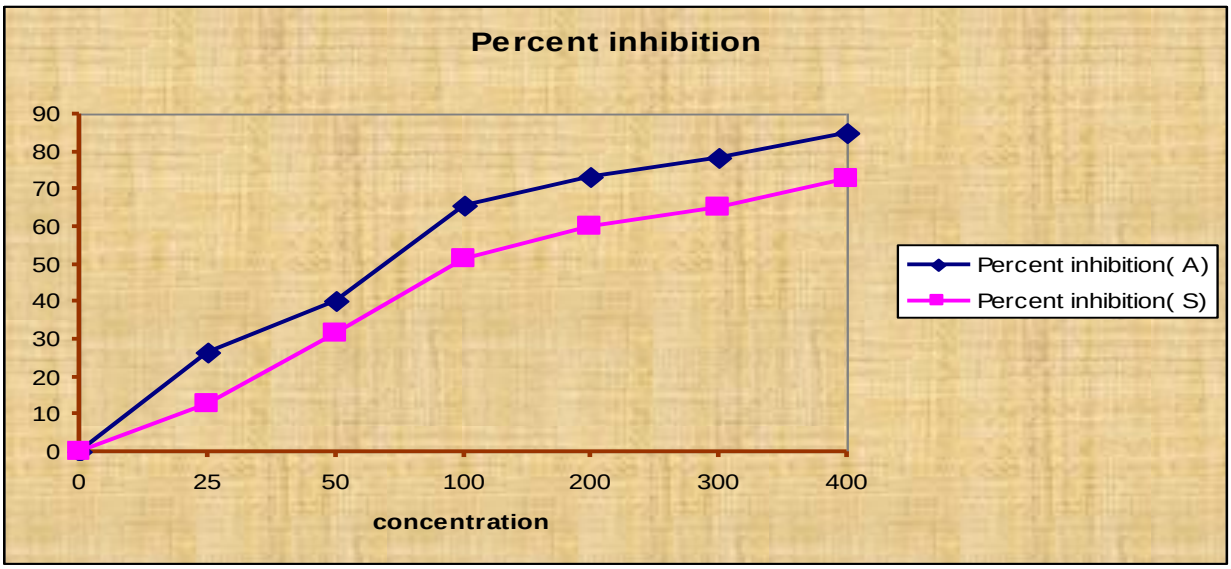

Fig. 2: Percent Inhibition curve of Extracts Plumbago zey;lanica(S) and Plumbago rosea (A)

Table 1: Physicochemical Properties

Plant species	*Plumbago zeylanica*	*Plumbago rosea*
Parameter	Determined value %w/w	Determined value %w/w
Moisture content	3.00%	2.80%
Total ash	2.30%	2.10%
Acid insoluble ash	<1.0%	<1.0%
Water soluble ash	10.20%	9.50%
Water soluble extractive	9.20%	9.90%
Ethanol soluble extractive	9.50%	9.70%

antioxidants such as Allium cepa (Onion), Allium sativum (Garlic), Momordica charantia (Bitter gourd), Tinospora cordifoloa(Guduchi), Ocimum sanctum (Tulsi), Glycyrrhiza glabra (Mulethi), Anrdographis paniculata (Kiryat), Curcuma longa(Turmeric), Emblica officinalis (Indian gooseberry), Terminalia bellarica(Baheda), Plumbago species (Chitrak).[1] The genus Plumbago includes 3 species, namely Plumbago indica L. (P. rosea L.) P. capensis L. and P. zeylanica L., which are distributed in several parts of India.[7]

Plumbago zeylanica Linn. is a perennial herb,subscandent, somewhat woody. The root and root bark are bitter. The root is said to increase the digestive power, to promote the appetite, useful in dyspepsis, piles,

Table 2: Determination of IC_{50} of Ascorbic acid (Positive Control test)

Concentration (µg)	Absorbance	Percent inhibition
Blank	1.23	-
10	0.92	48.2
20	0.6	74.2
30	0.52	80.7
40	0.42	88.8
50	0.35	94.5
75	0.3	98.6

IC_{50} of Ascorbic acid = 12 µg /ml

Table 3: Determination of IC_{50} of Plumbago zeylanica extract (S)

Concentration (µg/ml)	Absorbance	Percent inhibition
Blank	1.2	-
25	1.05	12.5
50	0.82	31.6
100	0.58	51.6
200	0.48	60
300	0.42	65
400	0.33	72.5

IC_{50} of Plumbago zeylanica extract S = 95 µg/ml

Table 4: Determination of IC_{50} of *Plumbago rosea* extract (A)

Concentration (µg)	Absorbance	Percent inhibition(A)
Blank	1.2	-
25	1.12	26.6
50	0.96	40
100	0.65	65.8
200	0.56	73.3
300	0.5	78.3
400	0.42	85

IC_{50} of Plumbago rosea extract A = 70 µg/ml

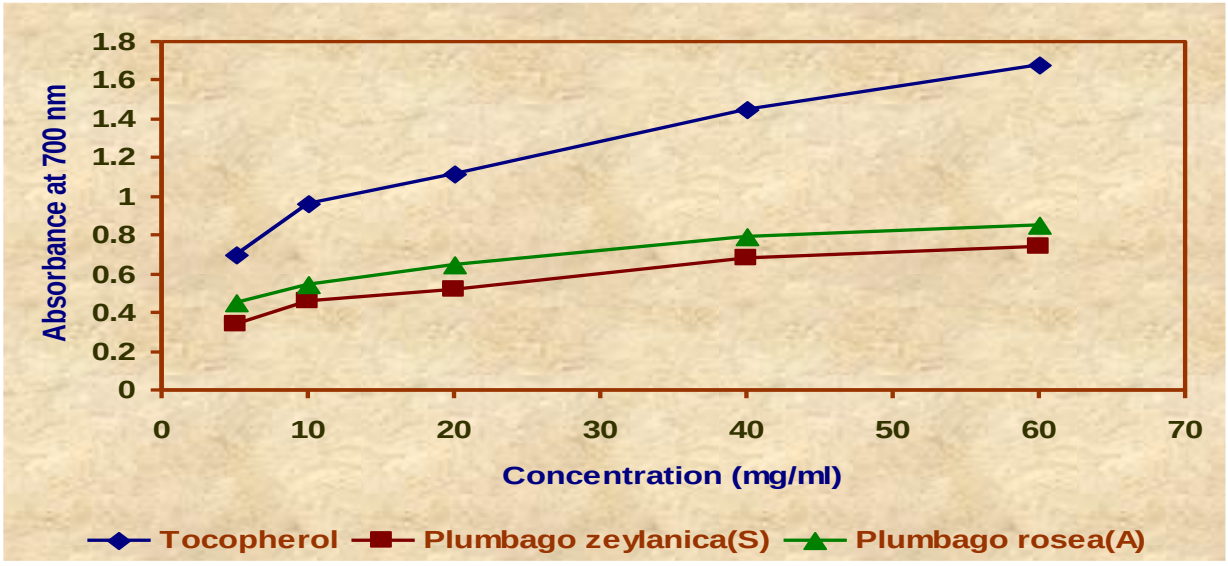

Fig. 3: Absorbance curve of Tochopherol and Extracts of Plumbago zeylanica (S) and Plumbago rosea (A)

diarrhoea.8. The root have been largely used as abortifacients in the indigenous practice.9 Roots and aerial parts principally contain an orange yellow pigment, plumbagin, (2-methoxy-5hydroxy-1, 4-napthoquinone) a naphthoquinone and a fatty alcohol. Its other constituents in roots are chitranone, zeylanone, dihydrosterone, 2-methyl naphthaquin, plumbazeylanone and terpenoids, lupeol and teraxesterol. The plant also contains alkaloids, glycosides, tannin, saponins and steroids.[10] The active principle is plumbagin and the pharmacological actions of the plant are due to the presence of this neutral principle.[8]

Plumbago rosea Linn is shrubby perennial; stems are herbaceous, erect slightly striate, simple .8. The root is mentioned as an abortifacient and vesicant .It is used as an external application in rheumatic affections of joints and in paralytic conditions.[9]. The root is used as sialogogue. In south India, the dried root is highly valued as remedy for secondary syphilis and leprosy.[8]

As *Plumbago zeylanica* the main compound in Plumbago rosea is plumbagin.[8]

Since *Plumbago zeylanica* contains the same active principle Plumbagin as the *Plumbago rosea* and has similar properties although in a smaller degree.[11]

MATERIALS AND METHODS

Pharmacognostical standardardization: The roots of *Plumbago zeylanica* Linn. were collected from the SIPS herbal garden and *Plumbago rosea* Linn. roots were bought from the Gwalior market. The roots of *Plumbago zeylanica* Linn. and *Plumbago rosea* Linn. were authenticated by Dr. Pradeep Tiwari, Deptt. Of Botany, Sagar University, Sagar. The roots of *Plumbago zeylanica* Linn.and *Plumbago rosea* Linn.were washed with water at the ambient temperature and dried under shed. They were preserved in the individual air tight containers after grinding to the requisite level.The various physicochemical analytical parameters like moisture content, ash values(total ash, acid insoluble ash, water soluble ash), extractive values(water soluble extractives, ethanol soluble extractive values)were performed for the powdered root of Chitrak with white flowers (*Plumbago zeylanica* Linn.) and Chitrak with red flowers (*Plumbago rosea* Linn*).*[12,13]

Chemicals: 1,1-Diphenyl-2-picrylhydrazyl (DPPH) [Sigma ,Ambernath,India] ,Ethanol [Qualigens, Ahemdabad,India] ,Tris-HCl Buffer [Qualigens, Ahemdabad,India] , L-ascorbic acid[Sigma Aldrich, NewDelhi,India] \,Sodium phosphate [CDH, New Delhi , India] Potassium ferricyanide [CDH, New Delhi , India] ,Trichloroacetic acid [Qualigens, Ahemdabad,India] Ferric chloride [CDH, New Delhi , India] , Alpha-Tochopherol [Piramal enterprise, India]

Sample Extraction: The 100 g. of dried and grounded herb was macerated with 50% Hydroethanolic solution and was macerated for 72 hours.Maceration was carried out with occasional shaking during the day time. Then the extract was filtered and the marc was pressed and the resultant extract was filtered and mixed in the earlier lot. Then it was distilled to recover the solvent (first ethanol at 55-60^{0}C, then water at 100^{0}C). The slightly moist extract was placed in a pre-weighed china dish and heated to get dry mass. (Plumbago zeylanica Linn.= Extract "S" and Plumbago rosea Linn.= Extract "A") .

Antioxidant activity:

DPPH radical scavenging activity: The antioxidant activity of each sample was expressed in terms of IC_{50} (micromolar concentration required to inhibit DPPH radical formation by 50%), calculated from the inhibition curve .1 mg extract was dissolved in 1 ml 50% ethanol solution to obtain 1000 µg/ml sample solution. The solution was diluted with 50% ethanol to contain 25 µg, 50 µg, 100 µg, 200 µg, 300 µg, and 400 µg in 0.05ml. In each reaction, the solutions were mixed with 1 ml of 0.1 µM 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 0.45 ml of 50 µM Tris-HCl buffer (pH 7.4) and 0.05 ml samples at room temperature and kept aside for 30 min. 50% ethanol solution was used as control. The reduction of the DPPH free radical was measured by reading the absorbance at 517 nm. L-ascorbic acid was used as positive control using concentration 10, 20, 30, 40, 50, and 75 µg in 0.05 ml.[14]

The percentage inhibition was calculated from the following equation:

% inhibition = [(absorbance of control-absorbance of test sample)/absorbance of control] x 100%.

Reducing Power Assay: The reducing power was determined according to the method of Oyaizu (1986). Various concentrations of extracts (2.5 ml) were mixed with 2.5 ml of 200 mmol/l sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50^{O}C for 20 min. After 2.5 ml of 10% trichloroacetic acid (w/v) were added, the mixture was centrifuged at 650 rpm for 10 min. The upper layer (5 ml) was mixed with 5 ml deionised water and 1 ml of 0.1% of ferric chloride, and the absorbance was measured at 700 nm: higher absorbance indicates higher reducing power. Alpha-tocopherol was used as standard.**[15]**

RESULT AND DISCUSSION

The physicochemical analysis was performed on *Plumbago zeylanica* Linn.and *Plumbago rosea* Linn. . The results obtained for the quantitative standards have been shown in table1. They indicate about the properties of the herb. The extractive values are used to indicate miscibility and presence of constituents in particular solvents. The total ash value shows the presence of inorganic matter in drug.

Antioxidant activity:

DPPH radical scavenging activity: The IC_{50} of *P.zeylanica* S extract and *P.rosea* A extract was 95 µg/ml & 70 µg/ml respectively, whereas the IC_{50} of Ascorbic acid (Standard) was 12 µg /ml.

Table 5: Reducing power assay of *P. zeylanica* and *P. rosea Extracts S and A*

Concentration	Absorbance at 700 nm	Absorbance at 700 nm	Absorbance at 700 nm
	Tocopherol	*Plumbago zeylanica(S)*	*Plumbago rosea (A)*
5 mg/ml	0.7	0.34	0.45
10 mg/ml	0.96	0.46	0.55
20 mg/ml	1.12	0.52	0.65
40 mg/ml	1.45	0.68	0.79
60 mg/ml	1.68	0.74	0.85

Reducing Power Assay: Absorbance was measured at 700 nm. Higher absorbance indicates higher reducing power .The reducing power was obtained as absorbance taken as 1.68 at 60 mg/ml for α-tocopherol (Standard), 0.74 at 60 mg/ml for S (*Plumbago zeylanica* extract) and 0.85 at 60 mg/ml for A (*Plumbago rosea* extract) respectively .

In *Plumbago zeylanica* Linn. and *Plumbago rosea* Linn. ingredient, plumbagin (napthoquinone) exhibit antioxidant property. The Antioxidant in- vitro testings that is DPPH assay and Reducing Power assay were performed with Plumbago *zeylanica* and Plumbago *rosea* hydroethanolic roots extracts S and A . Percent inhibition was calculated by plotting graph and Ascorbic acid (Vitamin C) was used as standard and Absorbance was taken at 700 nm and Tocopherol (Vitamin E) was used as standard indicated their Antioxidant property. **16** Hence, the present study concludes that the hydroethanolic extract of Plumbago rosea Linn is better antioxidant in comparison to Plumbago zeylanica Linn.

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