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Quantitative estimation of phenolic and flavonoid content and antioxidant activity of various extracts of different parts of *Plumbago zeylanica Linn*

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Abstract:
Plumbagozeylanica (Chitrak) is used as medicinal plant in India. The root of the plant and its constituents are credited with potential therapeutic properties including anti-atherogenic,cardiotonic, hepatoprotective and neuroprotective properties. In recent times, interest hasfocused on phytochemicals as new sources of natural antioxidants. Therefore in the present study methanolic crude extracts of stem, leaves, roots of *Plumbagozeylanica* were screened for their antioxidant property, phenolic content and flavonoid content. Free radical scavenging activity (antioxidant activity) was evaluated using 1, 1-diphenyl-2-picryl- hydrazyl (DPPH) and was measured as decolorizing activity followed by the trapping of the unpaired electron of DPPH. The plant roots extract reveled significant antioxidant activity as compared to standard flavonoid (quercetin). IC_{50} values were found to be 72.3µg/ml , 32.433µg/ml and 24.6µg/ml in stem, leaf and root extracts respectively for their antioxidant activity by DPPH assay. The phytochemical investigation showed presence of flavonoids, and phenolics. The total phenolic and total flavonoid content was found to be the maximum in leaf extracts (28.25±0.001 mg of GAE/g and 2.41±0.021 CE/g respectively). A weak linear correlation between total phenolic or flavonoid content and antioxidant activity was found (correlation coefficient, R2 =0.9989 and R2 = 0.9559, respectively). The findings indicated promising antioxidant activity of crude extracts of the plant and needs further exploration for their effective use in both modern and traditional system of medicines.

Keywords: *Plumbagozeylanica* , Phenolic, Flavonoids; DPPH,Antioxidant activity.

Introduction

Plumbagozeylanica (Family-Plumbaginaceae) mainly called as "Chitrak" (Nquyen, *et al.*, 2006) is a valuable Indian medicinal plant widely used in treatments of piles, diarrhea, leprosy and anasarca (Anonymous. 1989). Roots of plant have potential therapeutic properties like anti-anthrogenic, carditoxic, hepatoprotective, neuroprotective, anti-atherogenic, cardiotonic, hepatoprotective and neuroprotective properties (Tilak, *et al.*, 2004). It is has been also reported that the plant have anticancer, antibacterial, antifungal and antitumor properties (Kavimani, *et al.*, 1996). The leaves 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 (Chopra, *et al.*, 1996). Previously isolated constituents from P. zeylanica are Plumbagin, isoshinanolone, plumbagicacidbeta-sitosterol, 4-hydroxybenzaldehyde, Trans cinnamic acid, vanillic acid, 2, 5-dimethyl-7- hydroxychromone, indole-3-carboxaldehyde (Zhang, *et al.*, 2003). The plant has great potential for various diseases

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and disorders along with great antioxidant activity.

Natural antioxidants such as phenols, flavonoids and tannins are increasingly attracting because they are natural disease preventing, health promoting and anti-ageing substances (Ozyurt Detal 2004),. Antioxidants may serve the task of reducing oxidative damage in humans induced by free radicals and reactive oxygen species under oxidative stress conditions. These conditions can cause DNA and protein damage, lipid peroxidation, cancer, ageing and inflammatory activity (Blois MS *et al.*, 1958). Phenolics are an important class of secondary plant metabolites possessing an impressive array of pharmacological activity. One of the more prominent properties of the phenolics is their excellent radical scavenging ability. Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes (Frankel E *et al.,).* Some evidence suggests that the biological actions of these compounds are related to their antioxidant activity (Gryglewski RJ, *et al*). An easy, rapid and sensitive method for the antioxidant screening of plant extracts is free radical scavenging assay using 1,1-diphenyl-2-picryl hydrazyl (DPPH) stable radical spectro photometrically. In the presence of an antioxidant, DPPH radical obtains one more electron and the absorbance decrease (Kolevaetal). In the present study we have attempted to investigate the antioxidant activity (free radical scavenging activity), determination of the content of total phenolics, total flavonoids in plant extracts and to explore relationship between phenolic content, flavonoid content and antioxidant activity.

Materials and Method

Chemicals:

DPPH (1, 1-Diphenyl-2-picrylhydrazyl), Ascorbic acid, FolinCiocatteu'sreagent, Gallic acid, anhydrous sodium carbonate,Catechin, Quercetin, EDTA (Ethylene diamine tetra acetic acid),Aluminum chloride, , Methanol . All chemicals were of AR grade .

Plant Material

The plant was obtained from Botanical garden of University of Rajasthan. The plant material washed with water to remove dirt and oven dried at 45°C.The dried plant material was pulverized using electric blender. Weighed portion of the sample was refrigerated to cold extraction.

Preparation of plant extract:

Cold extraction method was employed for the extraction. 10gm of the powdered samples were weighed into conical flask ,90 ml of pure methanol was added and left for 72 hr .The mixture was filtered and this methanolic filtrate was concentrated under reduced pressure on rotary evaporator at 40 °C and then stored at 4 °C for further use. The filtrate was reconstituted in known amount of DMSO to obtain methanol extract of known concentration.

Determination of total phenolics

The determination of total phenolics based on Folin-Ciocalteu reagent assay (Singleton and Rossi., 1965). An aliquot (1ml) of extracts and standard solution of Gallic acid (100 mg/ml) was added to 25 ml volumetric flask, containing 9 ml distilled water. The distilled water itself was used as blank. One ml of Folin-Ciocalteu reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% Na_2CO_3 solution was added to the mixture. The solution was diluted to volume (25 ml) with distilled water and mixed. After

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incubation for 90 min at room temperature, the absorbance against prepared reagent blank was determined at 750 nm with an UV-Vis Spectrophotometer. The total phenolic content of root extracts expressed as mg Gallic acid equivalents (GAE)/100 G fresh weights. All samples were analyzed in triplicates.

Determination total flavonoids

Total flavonoid content was measured by the aluminum chloride colorimetric assay (Zhishen, *et al.*, 1999). An aliquot (1 ml) of extracts and standard solution of catechin (100 mg/ml) was added to 10 ml volumetric flask containing 4 ml of distilled water. To this 0.3 ml 5 % $NaNO_2$ were added. After 5 min, 0.3 ml 10 % $AlCl_3$ was added. Then after 1 min, 2ml of 1 M NaOH was added and the total volume was made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm. Total flavonoid content of root extracts expressed as mg catechin equivalents (CE)/100 G fresh weights. All samples were analyzed in triplicates.

DPPH radical scavenging assay: The free radical scavenging activities of the plant extracts were measured employing the modified method of Blois (1958). 1 ml each of the different concentrations (1000, 500, 250, 125 and 62.5 µg/ml) of extracts or standard (ascorbic acid) in a test tube was added 1 ml of 0.3 mM DPPH in methanol. The mixture was vortexed and then incubated in a dark chamber for 30 min after which the absorbance was measured at 517 nm against a DPPH control containing only 1 ml of methanol in place of the extract. Percentage scavenging activity was calculated using the expression (Gulcin, 2009):

% scavenging activity = (Absorbance of control - Absorbance of sample/Absorbance of control) ×100

Results

The results of the phenolic and flavonoid contents are shown in Table1. Experiments were done in triplicates and mean values and standard deviation were calculated.

Total Phenolic content

The phenolic content of the studied plant parts was found to be maximum in leaf (28.25±0.01 mg/g.d.wt.) followed by stem (24.95±0.02 mg/g.d.wt.) and root (14.26±0.01mg/g.d.wt.).

Total Flavonoid content

The total flavonoid content of the studied plant parts was found to be maximum in leaf (2.41±002 mg/g.d.wt.) followed by stem (2.025±0.001 mg/g.d.wt.) and root (1.20±0.015 mg/g.d.wt.).

DPPH Radical Scavenging Activity

The results of DPPH free radical scavenging activity on the methanolic extracts isolated from root, stem and leaves of the plant are shown in Table 2. IC_{50} values and Regression equation were calculated for each extract by statistical analysis. Plant extracts were evaluated at different concentration ranging from50 µg/ml to 400 µg/ml.

The methanolic extract of root exhibited maximum free radicle scavenging activity ranging from 56.42% to 96. 27% with an IC50 value of 24.67 µg/ml, followed by methanolic extract of leaf (51.86 to 90.13 %) with an IC50 value of 32.43 µg/ml and methanolic extract of stem (58.47 to 91.57 %) with an IC50 value of 72.31 µg/ml.

Discussion

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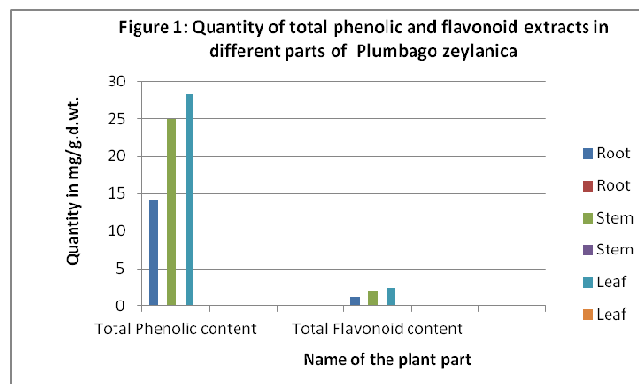
The results of the DPPH radical scavenging activities of the extracts revealed that the plant extracts contain free radical scavenging activity which could exert a beneficial action against pathological alterations caused by generation of free radicals. The IC_{50} value (the concentration required to inhibit radical formation) for stem, root and leaf extract is found to be72.3 µg/ml, 24.6µg/ml and 32.43µg/ml respectively. Polyphenols of plant kingdom are one of the most effective antioxidative constiuents . It is important to estimate phenolic contents of plant extracts so as to justify their controls with to antioxidant activity (Choi *et al* ., 2007) . In the present study we estimate total phenolic content of stem, root, leaf extracts by F.CR method. FCR method is one of the oldest and commonly used colorimetric techniques for estimating total phenolic content of a range of substances including plant extracts. The phenolic compounds react with FCR only under basic conditions to form blue complex having maximum absorption near 750 nm. Though the chemical nature of FCR is undefined, the total phenols assay by FCR is convenient, simple, and reproducible. A large data has been accumulated, and it has become a routine assay in studying the phenolic antioxidants (Dasgupta and De, 2004; Huang *et al.*, 2005; Chung *et al.*, 2006; Harish and Shivanandappa, 2006; Coruh *et al.*, 2007; Ardestani and Yazdanparast, 2007; Kekuda *et al.*, 2011; Rekha *et al.*, 2012; Junaid *et al.*, 2013). Total phenolic contents in stem, root, leaf was found to be 24.95 ± 0.021 mg, 14.26 ± 0.015 mg and 28.25 ± 0.01 mg respectively. Flavonoids as one of the most diverse and widespread group of natural compounds are probably the most important natural phenols. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties. Using the standard plot of quercetin (y = 0.0148x, R2 =0.975), the flavonoid contents of *Plumbago zeylanica* leaves, stem and root were found to be 2.41±0.021 mg, 2.025±0.001mg and 1.20±0.015 mg quercetin equivalent/g of dry sample respectively.

Table 1: Quantity of total phenolic and flavonoids in different parts of *Plumbagozeylanica*

Name of plant part	Total phenolic content (mg/ g.d.wt)	Total Flavonoid content (mg/ g.d.wt.)
Root	14.26±0.015	1.20±0.015
Stem	24.95±0.02	2.025± 0.001
Leaf	28.25±0.001	2.41±0.02

Table 2: Antioxidant acivity of methanolic extracts of different parts of *Plumbago zeylanica.*

Name of plant parts	Concentration (µg/ml)	% Scavanging activity	Regression equation	IC50 value (µg/ml)
Root	50	56.42	Y= 0.1047X+52.65	24.67
	100	66.98		
	200	71.62		
	300	84.76		
	400	96.27		
Stem	50	58.47	Y= 0.0932X+56.74	72.31
	100	66.42		
	200	79.55		
	300	85.54		
	400	91.57		
Leaf	50	51.86	Y= 0.1022X+46.68	32.43
	100	57.75		
	200	67.84		
	300	73.17		
	400	90.13		



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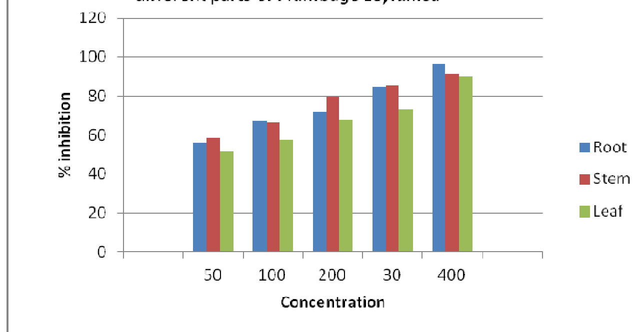

Conclusion

The present investigation revealed that the leaves, stem and root of *Plumbago zeylanica* contain significant amount of phenols and flavonoids and possess antioxidant property. The objective of this study was to get information of the amount of phenolics and flavonoids in the parts of Plumbagozeylanica. Further intention of this study is to correlate relationship between antioxidant activity and total phenolic content, total flavonoid content and evaluate *Plumbago zeylanica* as a potential source of natural bioactive chemicals.

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