Introduction

Plumbagozeylanica (Family-Plumbaginaceae) mainly called as “Chitrak” (Nyuyen, 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-atherogenic, carditoxic, hepatoprotective and neuroprotective properties. In recent times, interest has focused 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 revealed significant antioxidant activity as compared to standard flavonoid (quercetin). IC50 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.
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-picrylhydrazyl (DPPH) stable radical spectro photometrically. In the presence of an antioxidant, DPPH radical obtains one more electron and the absorbance decrease (Koleva etal). 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, FolinCiocatettu’s reagent, 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% Na2CO3 solution was added to the mixture. The solution was diluted to volume (25 ml) with distilled water and mixed. After...
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 % NaNO₂ were added. After 5 min, 0.3 ml 10 % AlCl₃ 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):

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\% \text{ scavenging activity} = \left( \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \right) \times 100
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**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±0.015 mg/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. IC₅₀ 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**
The results of the DPPH radical scavenging activity present study estimate total phenolic content of stem, leaf and root extracts by FCR method. FCR method is one of the oldest and commonly used colorimetric techniques for estimating total phenolic content and flavonoids. Estimation of total phenolic content based on the calibration curve of quercetin (y = 0.0148x, R² = 0.975). The IC₅₀ value of quercetin (y = 0.0148x, R² = 0.975), the quercetin equivalent/g of dry sample, is 24.67 µg/ml and 32.43 µg/ml respectively. These compounds possess a broad spectrum of chemical and biological activities including plant extracts. The phenolic compounds react with FCR 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 et al., 2005; Chung et al., 2006; Harish and Shivanandappa, 2006; Coruh et al., 2007; Ardestani and Yazdanparast, 2007; Junaid et al., 2013). Total phenolic content of a range of substances is important to estimate phenolic contents of plant extracts so as to justify their controls with to pathogens. The IC₅₀ value of quercetin (y = 0.0148x, R² = 0.975), the quercetin equivalent/g of dry sample, is 24.67 µg/ml and 32.43 µg/ml respectively. These compounds possess a broad spectrum of chemical and biological activities including plant extracts. The phenolic compounds react with FCR under basic conditions to form blue complex having maximum absorption near 750 nm. Though the chemical nature of FCR is undefined, the total phenolas act 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 et al., 2005; Chung et al., 2006; Harish and Shivanandappa, 2006; Coruh et al., 2007; Ardestani and Yazdanparast, 2007; Junaid et al., 2013). Total phenolic content of a range of substances is important to estimate phenolic contents of plant extracts so as to justify their controls with to pathogens. The IC₅₀ value of quercetin (y = 0.0148x, R² = 0.975), the quercetin equivalent/g of dry sample, is 24.67 µg/ml and 32.43 µg/ml respectively.

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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.

References


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