

Antioxidant activity and flavonoid derivatives of *Plumbago zeylanica*

Shivraj H. Nile and C.N.Khobragade*

Biotechnology Research Laboratory, School of Life Sciences, Swami Ramanand Teerth
Marathwada University, Nanded- 431606 (MH), India

* Corresponding author

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ABSTRACT

Plumbago zeylanica (Chitrak) is a useful Indian medicinal plant. The root of the plant and its constituents are credited with potential therapeutic properties. The isolation and spectral data for new flavonoid 2-(2, 4-Dihydroxy-phenyl)-3, 6, 8-trihydroxy-chromen-4-one from the roots of *Plumbago zeylanica* were determined and the antioxidant activity were studied by free radical scavenging and superoxide radical scavenging assays. The plant roots extract revealed significant antioxidant activity as compared to standard flavonoid (quercetin). The antioxidant activity by DPPH is 96µg/ml and by NBT is 4.6µg/ml which grater than that of standard (Quercetin) 45 µg/ml by DPPH and 10µg/ml by NBT assay. The phytochemical investigation showed presence of flavonoids, tannins and saponins. The total phenolic and total flavonoid content was found to be 260±48.0 and 45.5±5.2 mg of GAE/g and CE/g respectively.

Keywords: *Plumbago zeylanica*; Flavonoids; Antioxidant activity.

INTRODUCTION

Plumbago zeylanica (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, cardiotoxic, 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, plumbagic acid, beta-sitosterol, 4-hydroxy benzaldehyde, 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 and disorders along with great antioxidant activity. Nobody has not been discovered the plant compounds which are responsible for these activities mainly flavonoids. In this study we have attempted to investigate the antioxidant activity and the chemical compounds mainly flavonoids present in root extract.

MATERIALS AND METHODS

Plant material: Roots of *Plumbago zeylanica* were collected during the period of August to September from local forest of Nanded, India. It was identified by Dr. S.N.Shinde Department of Botany, R.S.M.Latur and deposited in department (Voucher No SRT/BT/P/SN/ 101).

Chemicals: All chemicals like DPPH (1, 1-Diphenyl-2-picrylhydrazyl), ABTS (2, 2-azinobis-3-ethyl benzothiazoline-6-sulfonic acid diammonium salt), Na₂EDTA, nitroblue tetrazolium chloride (NBT), hypoxanthine, xanthine oxidase, Catechin, Quercetin, EDTA (Ethylene diamine tetra acetic acid), Folin-ciocalteu reagent, Ferric chloride, Acetylsalicylic acid, Methanol were purchased from Hi-Media Laboratories Ltd. Mumbai, India and Sigma - Aldrich Chemicals, Co, U.S.A. All chemicals were of AR grade and used without further purification unless stated otherwise.

DPPH radical scavenging assay: In this method the stock solution 1 mg/L for root extract was prepared by using ethanol. The scavenging activity of the root extract was measured using the stable radical DPPH, according to this method. Briefly, each sample of stock solution of 100 μ L (0.06-1mg/ml in ethanol) was added to 1.5 mL of 0.1 mM ethanol solution of DPPH. The mixture was shaken vigorously and the absorbance was monitored at 515 nm after 45 min of incubation, when the reaction reached a steady state Quercetin was used as reference compound. The inhibition percentage (%) of radical scavenging activity was calculated by using following formula (Burda and Oleszek., 2001).

$$\% \text{ Inhibition} = [A_c (o) - A_A (t) / A_c (o)] \times 100$$

Where A_c (o) is the absorbance of control at t = 0 min and A_A (t) is the absorbance of antioxidant at t = 1 h. All measurement were done in triplicate

NBT assay: In this method 20 μ l of 15 mM NA₂EDTA in buffer (50 mM KH₂PO₄/KOH, pH 7.4), 50 μ l of the 0.6 mM nitroblue tetrazolium chloride (NBT) in buffer, 30 μ l of 3 mM hypoxanthine in 50 mM KOH, 5 μ l the root extract in 96-well microplates. The reaction was started by adding 50 μ l of xanthine oxidase in buffer (1 unit in 10 ml buffer) to the mixture. The reaction mixture was incubated at ambient temperature, and the absorbance at 570 nm was determined every 1 min up to 8 min using ELISA reader. Quercetins, well-known antioxidants, were used as positive controls. Three replicates were made for each test sample and the percent inhibition was calculated by using the following formula (Shang-Tzen Chang., 2001).

$$\% \text{ Inhibition} = [(\text{rate of control reaction} - \text{rate of sample reaction}) / \text{rate of control reaction}] \times 100$$

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 it self 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

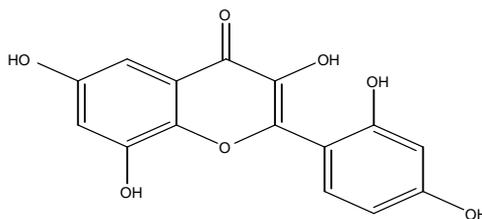
extracts expressed as mg Gallic acid equivalents (GAE)/100 G fresh weights. All samples were analyzed in triplicates.

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.

Isolation of flavonoids and structural elucidation: The UV and IR spectra were recorded on UV-Schimidzu and Shimadzu Japan spectrophotometers respectively. ¹H- and ¹³C-NMR spectrum was recorded at 400 MHz and 100 MHz, respectively, on a Bruker AM-400 spectrophotometer. TLC was performed on percolated silica gel plates: the detection was done at 254 nm and by spraying with ferric sulphate reagent. Silica gel (E-Merck, 230-400 mesh) was used for column chromatography (Hammami, et al., 2004).

RESULTS

The compound isolated and reported first time from *Plumbago zeylanica* root extract is 2-(2, 4-Dihydroxy-phenyl)-3, 6, 8-trihydroxy-chromen-4-one (yield: 0.082% on dry weight).



2-(2, 4-Dihydroxy-phenyl)-3, 6, 8-trihydroxy-chromen-4-one having Mp 312-314 °C, C₁₅H₁₀O₇; IR (Kbr) V_{max} =3431, 2926, 2854, 1745, 1612, 1014, 821, 721cm⁻¹ ¹H-NMR: (DMSO-d₆): δ 12.321-12.012 (s, 5H, Ar-OH), 7.7432 (s, 1H, Ar-OH), 7.6104 (d, 1H, Ar-H), 6.9277(d, 1H, Ar-H), 6.4170 (s, 1H, Ar-H), 6.4044 (s, 1H, Ar-H).

DISCUSSION

The previous study revealed the *Plumbago zeylanica* contains the bioactive compounds like Chitranone, Difuranonaphthoquinones, Plumbagin and β-Sitosterol (Lie-Chwen Lin, et al., 2003, A.T. Nguyena, et al., 2004, Akella V B, et al.,1976). The IC₅₀ value (the concentration required to inhibit radical formation by 50%) of *Plumbago zeylanica* root extract by DPPH assay is 96 µg/ml. The IC₅₀ value of root extract is less than that of the IC₅₀ value of standard (quercetin 45 µg/ml). While the IC₅₀ value of superoxide radical scavenging activity assays (NBT Assay) value is 4.6 µg/ml which is greater than standard (quercetin 10 µg/ml) (table 1).

Table-1: Antioxidant activities of the ethanolic extract of *Plumbago zeylanica* root extract.

Materials	Free radical scavenging activity IC ₅₀	Superoxide radical scavenging activity IC ₅₀
Root extract	96 µg/ml (N=5)	4.6 µg/ml (N=5)
Quercetin	45 µg/ml (N=5)	10 µg/ml (N=5)

- Values are expressed in mean±S.D
- ^aTotal Phenolic content is expressed as Gallic acid equivalent (mg of GAE/g).
- ^bTotal flavonoids are expressed as mg of total flavonoid content /g of samples based on quercetin as standard

The total phenolic content of *Plumbago zyleynica* root extract is 260±48.0 mg/gm where as the total flavonoid content is 45.5±5.2 (table 2) which may indicate that the antioxidant activity is due to this flavonoids and phenolic compounds present in root of *Plumbago zeylanica*. The results agree with the previous study carried by Tilak , et al., 2004, Burda and Oleszek., 2001.

Table-2: Total phenolic content of the ethanolic extract of *Plumbago zeylanica* root extract.

Material	Total phenolic content ^a	Total flavonoids ^b
Root extract	260±48.0 (N=10)	45.5±5.2(N=10)

Details are same as in table-1.

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