

# QUALITATIVE AND QUANTITATIVE DETERMINATION OF SECONDARY METABOLITES AND ANTIOXIDANT POTENTIAL OF *Plumbago indica* ROOT EXTRACTS

## ABSTRACT

Due to their well-known attribute of having minimal side effects as compared to medicines, natural items with medical potential are progressively gaining prominence in clinical research. The roots of *Plumbago indica* (*P. indica*, Plumbaginaceae) are commercially significant since they are the primary source of plumbagin and its derivatives. Plumbagin is well-known for its many pharmacological properties. *P. indica* roots yielded three naphthoquinones: plumbagin, 3, 30-biplumbagin, and elliptinone, which were employed as standard markers for quantitative HPLC analysis. The goal of this research was to screen phytochemicals, assess alkaloids, phenolic and flavonoid content, and measure the antioxidant potential of *P. indica* roots. The well-known test methodology was used to determine qualitative analysis of several phytochemical ingredients as well as quantitative analysis of total alkaloids, phenol, and flavonoids. The antioxidant activity of an ethanolic extract of *P. indica* roots was investigated in vitro using the 1,1-diphenyl, 2-picrylhydrazyl (DPPH) test technique. Alkaloids, glycosides, flavonoids, saponins, phenolics, proteins, and glucose were found in the ethanol and aqueous extracts, according to phytochemical study. *P. indica* roots ethanolic extract had 5.55, 0.930, and 3.940 mg of total phenolic, flavonoids, and alkaloids respectively. For comparative purposes, ascorbic acid was employed as a benchmark. In the investigated models, the extract showed dose-dependent free radical scavenging properties. For the DPPH technique, *P. indica* roots extract had an  $IC_{50}$  value of 23.02  $\mu\text{g/ml}$ , which was equivalent to that of ascorbic acid ( $IC_{50}=17.68 \mu\text{g/ml}$ ). These researches contributed to the accurate identification of this plant material. The plant's broad variety of phytochemicals implies that it has medicinal potential, which might be investigated in the pharmaceutical sector as well as in traditional medicine.

**Keywords:** *Plumbago indica*, Qualitative, Quantitative phytochemical, Antioxidant activity.

## INTRODUCTION

India is the world's greatest producer of medicinal plants and is properly known as the world's Botanical Garden [1]. Plants have been employed in traditional medicinal techniques for the treatment of many diseases since ancient times [2-5]. A lot of crude medications have yet to be scientifically recognized as to what plant they come from. A phytochemical is a natural

bioactive substance found in plants that protects against illness by interacting with nutrients and dietary fiber. According to several experts, phytochemicals function in tandem with nutrients present in fruits, vegetables, and nuts. In the body, they may have complimentary and overlapping mechanisms of action, such as an antioxidant effect. The qualitative examination of a medical plant's phytochemicals is considered a critical phase in any medicinal plant research. Chromatographic methods can be used to precisely screen the elements of plants [6]. Gravimetric and spectroscopic techniques are commonly used for quantification, however there are currently various sophisticated ways accessible [7]. Reactive oxygen species (ROS) capable of damaging DNA, such as hydrogen peroxide, superoxide anion, and hydroxyl radical, have been linked to carcinogenesis, coronary heart disease, and a slew of other age-related health issues [8]. Antioxidants that scavenge free radicals are advantageous for these illnesses because they protect cell proteins, lipids, and carbohydrates from damage [9]. Erythrocytes, the most common cells in the human body with good physiological and morphological properties, are frequently used in drug delivery [10]. Haemolysis associated with various haemoglobinopathies, reactive medications, transition metal excess, radiation, and abnormalities in specific erythrocyte antioxidant systems may all be linked to oxidative damage to the erythrocyte membrane (lipid/protein) [11]. This assay may be used for screening investigations on a variety of compounds and their metabolites, particularly those with oxidizing or antioxidant activity on the one hand, and long-acting molecules on the other [12]. Flavonoid, one of several herbal secondary metabolites, has been discovered to protect cells against oxidative damage [13]. By scavenging free radicals and decreasing lipid peroxidation, these chemicals have been shown to stabilize RBC membranes [14-15]. *Plumbago indica*, *Plumbago capensis* and *Plumbago zeylanica* are three species of the genus *Plumbago*, which belongs to the Plumbaginaceae family. The pharmacological characteristics (anticancer, antitumor, anti-inflammatory, antioxidant, and antibacterial capabilities) of *P. zeylanica* have been extensively studied, and the entire plant is used to cure a variety of illnesses. Rheumatic pain, sprains, scabies, skin problems, and wounds are all treated by *P. zeylanica* [16]. Plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone), a pharmacologically active compound isolated from the roots and leaves of *P. zeylanica*, has been shown to have anti-fertility, anti-parasitic, anticancer, and anti-inflammatory properties [17-18]. *P. indica* L is a sub scandent perennial plant or small shrub that grows up to 2 meters tall. The species is only found in Southeast Asia, including Sri Lanka and portions of India [19]. Concentrated decoctions, medicinal wines, and pills are among the treatments recommended [20]. The root of *P. indica* is used in Sri Lankan traditional medicine to treat

dyspepsia, colic, cough, and bronchitis [21]. As a rubefacient, a liniment made from the roots and vegetable oil of this plant is used to cure rheumatism and headaches [22]. The standard markers for quantitative HPLC determination were three naphthoquinones, plumbagin, 3, 30-biplumbagin, and elliptinone, which were isolated from *P. indica* roots [23]. The purpose of this study was to determine the phytochemical analysis and antioxidant activity of *P. indica* root.

## **MATERIAL AND METHOD**

### ***Plant material***

In the month of January 2020, medicinal plants *P. indica* were obtained in the local region of Bhopal. The plant material (root section) chosen for the study was properly cleaned under running tap water and then rinsed in distilled water before being allowed to dry at room temperature for a period of time. The plant material was then shade dried for 3 to 4 weeks without being contaminated. An electric grinder was used to ground dried plant material. Color, odor, taste, and texture of powdered plant material were evaluated. For phytochemical and biological experiments, dried plant material was placed in an airtight container and preserved.

### ***Chemical reagents***

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). All the chemicals and solvent used in this study were of analytical grade.

### ***Defatting of plant material***

*P. indica* powdered roots were shade dried at room temperature for ;incomplete sentence?. The shade-dried plant material was coarsely pulverized and macerated in petroleum ether for extraction. The extraction process was maintained until the material had been defatted.

### ***Extraction by soxhlation method***

By using the soxhlation technique, defatted powdered *P. indica* was extracted with several solvents (chloroform, ethyl acetate, ethanol, and water). The extract was evaporated at temperatures higher than their boiling points. Finally, the dried extracts' % yields were determined [24].

### ***Phytochemical screening***

Standard phytochemical screening protocols were used to detect the presence of bioactive compounds [25-26]. The tests were identified by visual inspection of color change or precipitate formation after the addition of particular reagents to the solution.

### ***Total phenol determination***

Parkhe and Bharti [27] developed a technique for determining total phenolic content. 1 ml Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5 g/l) sodium carbonate were combined with 2 ml extracts or standards. For color development, the mixture was vortexed for 15 s before being set aside for 10 min. A UV/visible spectrophotometer was used to detect the absorbance at 765 nm. The total phenolic content was determined using the gallic acid standard graph, and the findings were represented in milligrams per 100 mg of gallic acid.

### ***Total flavonoids determination***

Parkhe and Bharti [28] developed a technique for determining total flavonoid content. The absorbance of the reaction mixture was measured at 420 nm using a UV/visible spectrophotometer after 1 ml of 2 percent AlCl<sub>3</sub> solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature. The flavonoid content was determined using a standard graph of quercetin and represented as quercetin equivalent (mg/100 mg).

### ***Total alkaloids determination***

The plant extract (1 mg) was diluted in methanol, then filtered after adding 1 ml of 2 N HCl [29]. 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added to this solution in a separating funnel. The mixture was vigorously agitated with 1, 2, 3, and 4 ml chloroform before being collected in a 10-mL volumetric flask and chloroform diluted to volume. The same procedure was used to make a series of atropine reference standard solutions (40, 60, 80, 100, and 120 µg/ml). An UV/Visible spectrophotometer was used to measure the absorbance of the test and standard solutions against the reagent blank at 470 nm. The total alkaloid concentration was measured in milligrams of AE per 100 mg of extract.

### ***Antioxidant activity***

#### ***DPPH radical scavenging assay***

Parkhe and Jain [30] used a modified approach to measure the DPPH scavenging activity. The spectrophotometer was used to test the DPPH scavenging activity. The stock solution (6 mg in 100 ml methanol) was produced to give an initial absorbance of 1.5 ml in 1.5 ml methanol. After 15 min there was a decrease in absorbance in the presence of sample extract

at various concentrations (10-100 µg/ml). 1.5 ml of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of varying concentrations of the test sample were placed in a succession of volumetric flasks, and the final volume was adjusted to 3 ml using methanol. Three test samples were collected and processed in the same way. Finally, the average was calculated. For each concentration, the absorbance at zero time was measured. After 15 min at 517 nm, the absorbance of DPPH with varied concentrations showed a final reduction. The percentage inhibition of free radical DPPH was calculated from the following equation: % inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%. Though the activity is expressed as 50% inhibitory concentration (IC<sub>50</sub>), IC<sub>50</sub> was calculated based on the percentage of DPPH radicals scavenged. The lower the IC<sub>50</sub> value, the higher is the antioxidant activity.

## Results and Discussion

To achieve the real extraction yield, the crude extracts produced after each consecutive soxhlation extraction step were concentrated over a water bath by fully evaporating the solvents. Table 1 shows the yield of extracts produced from *P. indica* roots using chloroform, ethyl acetate, ethanol, and water as solvents. Table 2 shows the findings of a qualitative phytochemical study of the crude powder roots of *P. indica*. Flavonoids, glycosides, flavonoids, saponins, phenolics, proteins, and carbohydrate were found in ethanolic and aqueous extracts of *P. indica* roots, but only flavonoids and protein were found in chloroform extracts, and alkaloids, flavonoids, proteins, and carbohydrate were found in ethyl acetate extract. Total phenolic compounds (TPC) was expressed as mg/100 mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve:  $Y = 0.011X + 0.011$ ,  $R^2 = 0.998$ , where X is the gallic acid equivalent (GAE) and Y is the absorbance. Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve:  $Y = 0.032X + 0.018$ ,  $R^2 = 0.998$ , where X is the quercetin equivalent (QE) and Y is the absorbance. Total alkaloid content was calculated as atropine equivalent mg/100 mg using the equation based on the calibration curve:  $Y = 0.007X + 0.024$ ,  $R^2 = 0.995$ , where X is the Atropine equivalent (AE) and Y is the absorbance. Table 3 shows that total phenolic, flavonoids, and alkaloid concentrations of ethanolic extracts of *P. indica* roots were 5.55, 0.930, and 3.940 mg/100 mg respectively. The hydrogen donating nature of extracts was evaluated using the DPPH radical scavenging assay [31]. The inhibitory concentration 50 percent (IC<sub>50</sub>) value of *P. indica* ethanolic roots extract was reported to be 23.02 µg/ml when compared to ascorbic acid (17.68 µg/ml) in DPPH

radical scavenging activity. Table 4 shows a dose-dependent action with regard to concentration.

**Table 1: % Yield of *P. indica* extracts**

S. No.	Extracts	% Yield (W/W)
1.	Chloroform	1.162
2.	Ethyl acetate	0.925
3.	Ethanol	5.050
4.	Aqueous	3.280

**Table 2: Result of phytochemical screening of *P. indica* extracts**

S. No.	Constituents	Chloroform extract	Ethyl acetate extract	Ethanol extract	Aqueous extract
1.	<b>Alkaloids</b> Hager's Test:	-ve	+ve	+ve	+ve
2.	<b>Glycosides</b> Legal's Test:	-ve	-ve	+ve	-ve
3.	<b>Flavonoids</b> Lead acetate Test:	+ve	+ve	+ve	+ve
4.	<b>Diterpenes</b> Copper acetate Test:	-ve	-ve	-ve	-ve
5.	<b>Phenol</b> Ferric Chloride Test:	-ve	-ve	+ve	-ve
6.	<b>Proteins</b> Xanthoproteic Test:	+ve	+ve	+ve	+ve
7.	<b>Carbohydrate</b> Fehling's Test:	-ve	+ve	+ve	-ve
8.	<b>Saponins</b> Froth Test:	-ve	-ve	+ve	+ve

**Table 3: Estimation of total phenolic, flavonoids and alkaloid content of *P. indica* extracts**

S. No	Extracts	Total phenolic content	Total flavonoids content	Total alkaloid content
		mg/ 100 mg of dried extract		
1	Chloroform	-	0.74	-
2	Ethyl acetate	-	0.68	3.38
3	Ethanol	5.55	0.93	3.94
4	Aqueous	-	0.59	3.50

**Table 4: % Inhibition of ascorbic acid and *P. indica* extract using DPPH method**

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Ethanol extract
1	10	44.65	19.64
2	20	48.62	65.17
3	40	65.34	66.18

4	60	69.65	80.35
5	80	77.41	87.05
6	100	84.13	90.17
<b>IC 50</b>		<b>17.68</b>	<b>23.02</b>

## CONCLUSION

The presence of a significant amount of flavonoid, alkaloid, and phenolic content, as well as significant amounts of secondary metabolites in the roots of the plant investigated here, suggests that the plant might be a source of effective medications. The presence of phytoconstituents in significant quantities may help to recognize the plant's potential pharmacological value in disease management. Plants have medical value because they contain chemical compounds that have a specific physiological effect on the human body. It also supports folkloric medical usage and assertions regarding the plant's therapeutic properties as a cure-all. We recommend that bioactive chemicals from the leaf, stem, flower, and root of *P. indica* be isolated, purified, and characterized further in order to develop viable chemotherapeutic drugs.

## COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## REFERENCES

1. Ahmedulla M. and Nayar M.P. Red data book of Indian plants. 1999, Vol-4, Calcutta: Botanical survey of India.
2. Balakumar S, Rajan S, Thirunalasundari T, Jeeva S. Antifungal activity of *Aegle marmelos* (L.) Correa (Rutaceae) leaf extract on dermatophytes. *Asian Pac J Trop Biomed*, 2011; 1(3): 169-172.
3. Mohamed Saleem TK, Azeem AK, Dilip C, Sankar C, Prasanth NV, Duraisami R. Anti-inflammatory activity of the leaf extracts of *Gendarussa vulgaris* Nees. *Asian Pac J Tropical Biomed*, 2011; 1(2):147- 149.
4. Pour BM and Sasidharan S. In vivo toxicity study of *Lantana camara*. *Asian Pac J Trop Biomed*, 2011; 1(3): 189-191.

5. Paulraj K, Irudayaraj V, Johnson M, Patric Raja D. Phytochemical and anti-bacterial activity of epidermal glands extract of *Christella parasitica* (L.) H. Lev. *Asian Pac J Trop Biomed*, 2011; 1(1): 8-11.
6. Sneader W, The discovery of aspirin: A reappraisal, *BMJ (Clinical research ed.)*, 2000; 321(7276):1591-1594.
7. Aguruo CU, Ameh SJ, Olasan O, Comparative phytochemical studies on the presence and quantification of various bioactive compounds in the three major organs of okoko plant (*Cissampelos pareira*) in Benue State, North Central Nigeria, West Africa, *European Journal of Advanced Research in Biological and Life Sciences*, 2014; 2(2):16-22.
8. Beris H. Antioxidant affects a basis of drug selection. *Drugs*. 1991; 42: 569- 605.
9. Marnett L. Oxyradicals and DNA damage. *Carcinogenesis*. 2000; 21: 361- 370.
10. Hamidi H, Taferzadeh H. Carrier erythrocytes: an overview. *Drug Delivery*. 2003; 10: 9-20.
11. KO, Hsiao F, Kuo F. Protection of oxidative haemolysis by demethyldineisoeugenol in normal and beta –thalassemia red blood cells. *Free Radic Biol Med*. 1997; 22: 215-222.
12. Djeridane A, Yous M, Nadjemi B, Vidal N, Lesgards J, Stocker P. Screening of some Algerian medicinal plants for the phenolic compounds and their antioxidant activity. *European Food Res Technol*. 2007; 224: 801-809.
13. Kumar S, Pandey A. Chemistry and Biological Activities of Flavonoids: An Overview. *SCI World J*. 2013; 1-16.
14. Yu L. Free radical scavenging properties of conjugated linoleic acids. *J Agric Food Chem*. 2001; 49: 3452-3546.
15. Ebrahimzadeh M, Nabavi S, Nabavi S, Eslami B. Antihemolytic and antioxidant activities of *Allium paradoxum*. *Cent Eur J Biol*. 2010; 5: 338-345.
16. Tripathi V, Mishra B, Kishore N, Tiwari V. An account of phytochemicals from *Plumbago zeylanica* (Family: Plumbaginaceae): A natural gift to human being. *Chronicles of Young Scientists*, 2012; 3: 178.
17. Chauhan M. A review on morphology, phytochemistry and pharmacological activities of medicinal herb *Plumbago zeylanica* Linn. *Journal of Pharmacognosy and Phytochemistry*, 2014; 3:95–118.
18. Sand JM, Bin Hafeez B, Jamal MS, Witkowsky O, Siebers EM, Fischer J, Verma AK. Plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone), isolated from

- Plumbagozeylanica*, inhibits ultraviolet radiation-induced development of squamous cell carcinomas. *Carcinogenesis*, 2012; 33(1):184–190.
19. Devi PU, Solomon FE, Sharada AC. In vivo tumor inhibitory and radiosensitizing effects of an Indian medicinal plant, *Plumbago rosea* on experimental mouse tumors. *Indian Journal of Experimental Biology*, 1994; 32(8):523–528.
  20. Cheng C, Wu T, Shang H, Li Y, Altman DG, Moher D, Bian Z. Consort extension for Chinese herbal medicine formulas 2017: Recommendations, explanation, and elaboration (traditional Chinese version). *Annals of Internal Medicine*, 2017; 167: W7–W20.
  21. Lenora RDK, Dharmadasa RM, Abeysinghe DC, Arawwawala LDAM. Investigation of plumbagin content in *Plumbago indica* Linn. grown under different growing systems. *Pharmacologia*, 2012; 3(2):57–60.
  22. Ravindran A, Gayan J, Das BN. A standardized protocol for genomic DNA isolation from the species of *Plumbago* Linn. *IJLSSR*, 2017; 3:1345–1349.
  23. Kaewbumrung S, Panichayupakaranant P. Isolation of three antibacterial naphthoquinones from *Plumbago indica* roots and development of a validated quantitative HPLC analytical method. *Nat Prod Res*. 2012; 26:2020–2023.
  24. Mukherjee PK. Quality control of herbal drugs. 2<sup>nd</sup> Ed. Business Horizons; 2007.
  25. Khandelwal KR. Practical pharmacognosy technique and experiments. 23<sup>rd</sup> Ed. Nirali Prakashan; 2005.
  26. Kokate CK. Practical pharmacognosy. 4<sup>th</sup> Ed. Vallabh Prakashan; 1994.
  27. Geeta Parkhe, Deepak Bharti. Phytochemical Investigation and Determination of Total Phenols and Flavonoid Concentration in Leaves Extract of *Vitex trifolia* Linn. *Journal of Drug Delivery & Therapeutics*. 2019; 9(4-A):705-707.
  28. Geeta Parkhe, Deepak Bharti. In vitro antioxidant activity, total phenolic and flavonoid contents of hydroalcoholic extract of leaves of *Lagerstroemia Parviflora* Roxb. *Journal of Drug Delivery & Therapeutics*. 2019; 9(4-A):708-711.
  29. Fazel S, Hamidreza M, Rouhollah G, Mohammadreza V. Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. *Thai J Pharm Sci*. 2008; 32: 17-20.
  30. Geeta Parkhe, Prabhat Jain. Study of Antioxidant Potential of Hydroalcoholic Extract of *Anethum Graveolens*. *Career Int. J. Sci. & Tech*. 2018; 1(2): 39-45.
  31. Hudson BJ. Food antioxidants. In: Gordon MH, editor. *The Mechanism of Antioxidant Action in Vitro*. London: Elsevier Applied Science; 1990.