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-picryl hydrazyl (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 IC50 value of 23.02µg/ml, which was equivalent to that of ascorbic acid (IC50=17.68µg/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, super oxide 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 antioxidizing 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. 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 Ciocalteau reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) sodium carbonate were combined with 2 ml extracts or standards. For color development, the mixture was vortexed for 15 seconds before being set aside for 10 minutes. 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 milligrams 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 AlCl3 solution was added to 3 ml of extract or standard and allowed to stand for 15 minutes at room temperature. The flavonoid content was determined using a standard graph of quercetin and represented as quercetin equivalent (mg/100mg).

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 minutes, 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 minutes 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 (IC50), IC50 was calculated based on the percentage of DPPH radicals scavenged. The lower the IC50 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/100mg 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/100mg 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 3.940mg/100mg respectively. The hydrogen donating nature of extracts was evaluated using the DPPH radical scavenging assay [31]. The inhibitory concentration 50 percent (IC50) value of *P. indica* ethanolic roots extract was reported to be 23.02µg/ml when compared to ascorbic acid (17.68µg/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.	Ethylacetate	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	Ethylacetate extract	Ethanol extract	Aqueous extract
1.	**Alkaloids** Hager's Test:	-ve	+ve	+ve	+ve
2.	**Glycosides** Legal's Test:	-ve	-ve	+ve	-ve
3.	**Flavonoids** Lead acetate Test:	+ve	+ve	+ve	+ve
4.	**Diterpenes** Copper acetate Test:	-ve	-ve	-ve	-ve
5.	**Phenol** Ferric Chloride Test:	-ve	-ve	+ve	-ve
6.	**Proteins** Xanthoproteic Test:	+ve	+ve	+ve	+ve
7.	**Carbohydrate** Fehling's Test:	-ve	+ve	+ve	-ve
8.	**Saponins** 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	Ethylacetate	-	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
IC 50		**17.68**	**23.02**

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.

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