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UV AND HPLC ESTIMATION OF SECONDARY METABOLITE PRESENT IN EXTRACT OF PLUMBAGO ZEYLANICA ROOT

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ABSTRACT

Plumbago zeylanica (Ceylon leadwort) is a medicinal plant traditionally used for its therapeutic properties. The roots of *Plumbago zeylanica* are known to contain various bioactive compounds with pharmacological activities. This study aimed to evaluate the hydroalcoholic extract of *Plumbago zeylanica* root for its phytochemical composition and quantify the presence of quercetin using high-performance liquid chromatography (HPLC). The hydroalcoholic extract of *Plumbago zeylanica* root was prepared and subjected to phytochemical screening for alkaloids, glycosides, flavonoids, phenols, proteins, carbohydrates, saponins, diterpenes, and tannins. Total phenol and flavonoid content was quantified spectrophotometrically. HPLC was employed to quantify quercetin in the extract. The hydroalcoholic extract showed the presence of flavonoids, phenols, proteins, saponins, diterpenes, and tannins, while alkaloids and glycosides were absent. The extract contained 0.674 mg of total phenols and 0.793 mg of total flavonoids per 100 mg of extract. Quercetin was identified and quantified with a retention time of 2.569 minutes and an assay percentage of 0.075%.The study confirms that the hydroalcoholic extract of *Plumbago zeylanica* root is rich in bioactive compounds, particularly flavonoids and phenols. Quercetin, a known antioxidant and anti-inflammatory compound, was identified in the extract. These findings support the traditional use of *Plumbago zeylanica* in herbal medicine and suggest its potential for further therapeutic applications.

Keywords: *Plumbago zeylanica*, Hydroalcoholic extract, Phytochemical screening, Flavonoids, Quercetin, High-performance liquid chromatography (HPLC).

INTRODUCTION

Plumbago zeylanica, commonly known as Ceylon leadwort, is a medicinal plant traditionally used in various parts of the world for its therapeutic properties. The roots of *Plumbago zeylanica* have been of particular interest due to their rich content of secondary metabolites, which possess significant pharmacological activities. These metabolites include plumbagin, a naphthoquinone derivative, which is known for its antimicrobial, anti-inflammatory, antioxidant, and anticancer properties (Bhaskara Reddy *et al.,* 2017; Mishra *et al.,* 2018). *Plumbago zeylanica* root extract is rich in secondary metabolites, including plumbagin, which has garnered attention for its bioactive properties. Plumbagin has been reported to exhibit cytotoxic effects against various cancer cell lines, making it a potential candidate for

anticancer therapy (Bhaskara Reddy *et al.,* 2017). Additionally, the extract contains other secondary metabolites such as flavonoids, alkaloids, and phenolic compounds, which contribute to its medicinal properties (Mishra *et al.,* 2018).

UV-visible spectroscopy and high-performance liquid chromatography (HPLC) are widely used analytical techniques for the estimation of secondary metabolites in plant extracts. UV spectroscopy is useful for the qualitative and quantitative analysis of compounds that absorb UV light, such as flavonoids and phenolic compounds. HPLC, on the other hand, offers high sensitivity and specificity, allowing for the separation, identification, and quantification of individual compounds in complex mixtures like plant extracts (Santos *et al.,* 2020).

The aim of this study is to estimate the content of secondary metabolites, particularly plumbagin, in *Plumbago zeylanica* root extract using UV spectroscopy and HPLC. These analytical techniques will provide valuable information about the composition and concentration of bioactive compounds in the extract, which is essential for understanding its pharmacological potential and developing therapeutic applications.

MATERIAL AND METHODS

Collection of plant material

Roots of *Plumbago zeylanica* was collected from local area of Bhopal (M.P), India in the months of December, 2023.

Extraction procedure by maceration process

The extraction was continued till the defatting of the material had taken place. Defatted dried plant material of *Plumbago zeylanica* were extracted with hydroalcoholic solvent (ethanol: water: 25:75v/v) (Mukherjee; 2007). The extract was evaporated above their boiling points. Finally the percentage yields were calculated of the dried extract.

Determination of Percentage yield

The percentage yield of yield of each extract was calculated by using formula:

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of powdered drug taken}} \times 100$$

Qualitative phytochemical tests

1. Detection of alkaloids: Extract were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's Test: Filtrates was treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Wagner's Test: Filtrates was treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Dragendroff's Test: Filtrates was treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Hager's Test: Filtrates was treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of carbohydrates: Extract was dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Fehling's Test: Filtrates was hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides: Extract was hydrolysed with dil. HCl, and then subjected to test for glycosides (Kokate, 1994).

Legal's Test: Extract was treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

4. Detection of saponins

Froth Test: Extract was diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

5. Detection of phenols

Ferric Chloride Test: Extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

6. Detection of tannins

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

7. Detection of flavonoids

Alkaline Reagent Test: Extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate Test: Extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

8. Detection of proteins

Xanthoproteic Test: The extract was treated with few drops of conc. nitric acid. Formation of yellow colour indicates the presence of proteins.

9. Detection of diterpenes

Copper acetate Test: Extract was dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

Quantitative studies of bioactive constituents

Estimation of total phenolic content

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method (Parkhe and Bharti; 2019). 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10- 50µg/ml was prepared in methanol**.** 1gm of dried powder of drug was extracted with 100 ml methanol, filter, and make up the volume up to 100 ml. One ml (1mg/ml) of this extract was for the estimation of Phenol. 1 ml of extract or standard was mixed with 5 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 4 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 30min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method (Acharya *et al.,* 2019). 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids.1 ml of 2% $AlCl_3$

solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

Identification of marker compound (Quercetin) by HPLC

Reagents and chemicals

Quercetin was kindly provided by Scan Research Laboratories, Bhopal (India). Methanol and acetonitrile were of HPLC grade and purchased from Merck Ltd, New Delhi, India. Water used was of HPLC grade water from Merck Ltd, New Delhi, India.

Instrumentation

A thermospectronic model of Labindia 3000 + UV/VIS Spectrophotometer with 1cm. matched quartz cells was used for determination of λ_{max}. The HPLC system (Waters) consisted of a pump, a U.V. Visible detector, a Thermo C_{18} (250 X 4.6 mm, 5µm) column, a Data Ace software.

Chromatographic conditions

The chromatographic analysis was performed at ambient temperature on a RP-C18 analytical column with a mobile phase composed of Acetonitrile: Methanol (50:50 v/v) and was isocratically eluted at a flow rate of 1 mL min-1. A small sample volume of 20 µL was used for each sample run, being injected into the HPLC system. The chromatogram was monitored with UV detection at a wavelength of 256 nm (Acharya *et al.,* 2019).

Preparation of standard stock solution

10mg of Quercetin was weighed accurately and transferred to a 10ml volumetric flask, and the volume was adjusted to the mark with the methanol to give a stock solution of 1000ppm. From stock solutions of Quercetin 1 ml was taken and diluted up to 10 ml. from this solution 0.5, 1.0, 1.5, 2.0, 2.5 ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 10 ml with mobile phase, gives standard drug solution of 5, 10, 15, 20, 25µg/ ml concentration.

Analysis of extract

10 mg each extract was taken in 10 ml volumetric flask and dilute upto the mark with Methanol; resultant solution was filtered through Whatmann filter paper and finally volume made up to mark with same solvent to obtain concentration of 1000 µg/ml. The resulting solution was again filtered using 0.45µ membrane filter and then sonicated for 10 min.

RESULTS AND DISCUSSION

The percentage yield of the hydroalcoholic extract of *Plumbago zeylanica* root was determined to be 11.8%, as shown in Table 1. This yield indicates the efficiency of the extraction process in obtaining the bioactive constituents from the plant material.

Table 2 summarizes the results of phytochemical screening of the hydroalcoholic extract of Plumbago zeylanica root. The extract tested positive for flavonoids, phenols, proteins, saponins, diterpenes, and tannins, while alkaloids and glycosides were absent. These phytochemicals contribute to the medicinal properties associated with *Plumbago zeylanica*, such as antioxidant and antimicrobial activities.

Table 3 presents the quantitative analysis of total phenol and flavonoid content in the hydroalcoholic extract of *Plumbago zeylanica* root. The extract contained 0.674 mg of total phenols and 0.793 mg of total flavonoids per 100 mg of extract. These bioactive constituents are known for their antioxidant properties and contribute to the therapeutic

potential of the extract. Figure 1 shows the chromatogram of the standard quercetin used for comparison, while Figure 2 displays the chromatogram of the hydroalcoholic extract of *Plumbago zeylanica* root. Table 4 details the quantitative estimation of quercetin in the extract, with a retention time (RT) of 2.569 minutes and an assay percentage of 0.075%.

The HPLC analysis confirms the presence of quercetin, a flavonoid compound, in the hydroalcoholic extract. Quercetin is known for its various pharmacological activities, including antioxidant and anti-inflammatory effects. Its presence in the extract supports the traditional uses of *Plumbago zeylanica* in herbal medicine.

Table 1: Result of percentage yield of *Plumbago zeylanica* extract

S. No.	Solvents	Percentage Yield (%)
1.	Hydroalcoholic	11.8%

Table 2: Result of phytochemical screening of *Plumbago zeylanica* extract

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids**	
	Wagner's test	-ve
	Hager's test	-ve
2.	**Glycosides**	
	Legal's test	-ve
3.	**Flavonoids**	
	Lead acetate	+ve
	Alkaline test	+ve
4.	**Phenol**	
	Ferric chloride test	+ve
5.	**Proteins**	
	Xanthoproteic test	+ve
6.	**Carbohydrates**	
	Fehling's test	-ve
7.	**Saponins**	
	Foam test	+ve
8.	**Diterpenes**	
	Copper acetate test	+ve
9.	**Tannins**	
	Gelatin Test	+ve

Abbreviation: +ve indicate presence, -ve indicate absence of phytochemicals

Table 3: Total bioactive constituents content of *Plumbago zeylanica*

S. No.	Extract	Total phenol (mg/100mg)	Total Flavonoid (mg/100mg)
1.	Hydroalcoholic extract	0.674	0.793

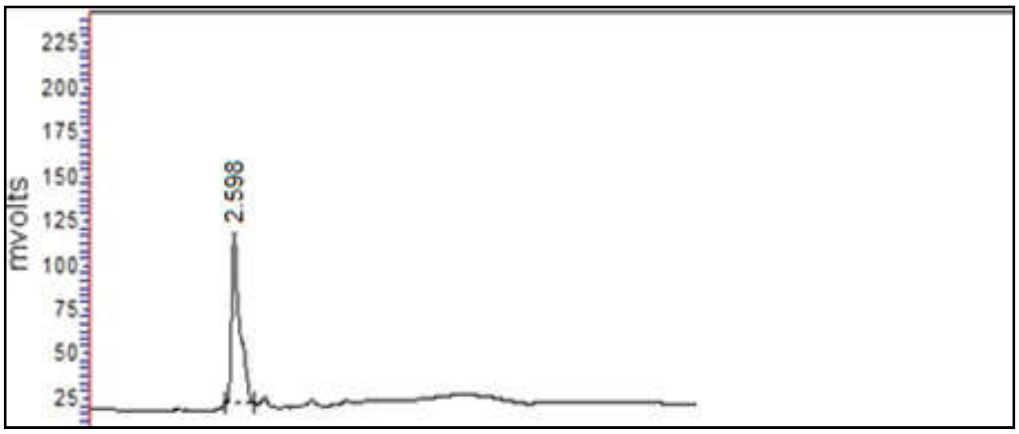

Figure 1: Chromatogram of standard Quercetin

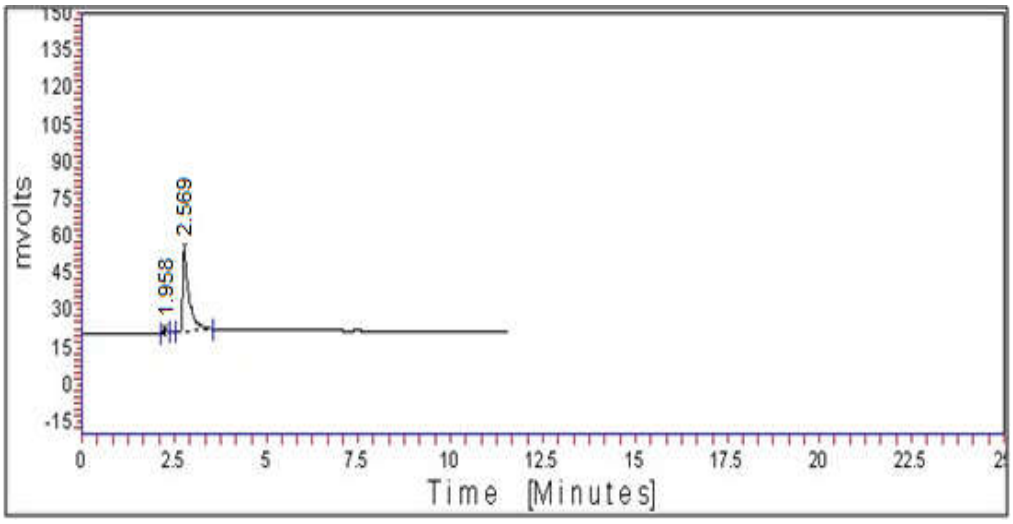

Figure 2: Chromatogram of hydroalcoholic extract of *Plumbago zeylanica*

Table 4: Quantitative estimation of Quercetin in *Plumbago zeylanica* extract

S. No.	Extract	RT	% Assay
1.	Hydroalcoholic extract	2.569	0.075%

CONCLUSION

The results of this study indicate that the hydroalcoholic extract of *Plumbago zeylanica* root is rich in bioactive compounds, including flavonoids and phenols. The presence of these phytochemicals, along with the identified quercetin, validates the traditional use of *Plumbago zeylanica* in treating various ailments. The findings suggest that the extract has potential therapeutic applications, particularly in antioxidant and anti-inflammatory treatments. Further research into the specific mechanisms and bioactivities of these compounds could lead to the development of novel therapeutic agents derived from Plumbago zeylanica.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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