Comparative Estimation of Plumbagin in Aerial and Root Part of Plumbago zeylanica Using UV-Visible Spectrophotometric

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Abstract

Plumbago zeylanica is one such important medicinal plant which is being used the world over in the traditional system of medicines. The plant is being used extensively in commercial preparations of medicines. The plumbagin present in different parts of Plumbago zeylanica as active constituent and it imparts wide range of biological activities. In the present study we planned to check the concentration of plumbagin present in aerial and root of Plumbago zeylanica. The petroleum ether, ethanol and aqueous extracts were prepared from the aerial and root of Plumbago zeylanica by successive extraction method. The phytochemical investigation of Plumbago zeylanica exhibited maximum phytoconstituents along flavonoids and polyphenol present in ethanol and aqueous extracts. While the petroleum ether extract of aerial and root contains steroid. The Plumbagin content in ethanol extract of aerial and root of Plumbago zeylanica was estimated from the calibration graph plotted from pure plumbagin. The findings of study indicate that concentration of plumbagin in aerial part of Plumbago zeylanica is maximum compared to root.

Keywords: Plumbago zeylanica, Plumbagin, Aerial, Root

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1 Introduction

India is one of the 12 mega biodiversity centers having over 45,000 plant species. About 1500 plants with medicinal uses are mentioned in ancient texts and around 800 plants have been used in traditional medicine¹. Plants produce thousands of specialized Secondary metabolites, many of which have medicinal uses. More than half of the top 150 prescribed drugs in the US have at least one compound derived from plants, and about 80% of the world’s population depends on plants or plant extracts as a major source of healthcare.

Secondary metabolites are those metabolites which are often produced in a phase of subsequent to growth, have no function in growth (although they may have survival function), are produced by certain restricted taxonomic groups of microorganisms, have unusual chemicals structures, and are often formed as mixtures of closely related members of a chemical family. They have a wide range of chemical structures and biological activities. They are derived by unique biosynthetic pathways from primary metabolites and intermediates. Secondary metabolites are beneficial, and others can be toxic. Secondary metabolites are produced in plants and serve survival functions for the organisms producing them.

In India, Plumbago zeylanica commands an important place among medicinal herbs in India since ancient times. The plant species Plumbago zeylanica is commonly known as Chitraka or “Lead white flowered” and “Ceylon Lead wort”, belongs to family Plumbaginaceae. The species is also known by several names in different parts of the world viz. bleiwurz/zahnkraut (in German), sanza (in Swahili), mosikomabe (in Tswana) and ensain (in Arabia)². Plumbago zeylanica is perennial, sub-scandant shrub with semi woody stems and numerous branches³,4. The family Plumbaginaceae consists of 10 genera and 280 species. The genus Plumbago includes 3 species, namely Plumbago indica L. (P. rosea L.) P. capensis L., and P. zeylanica L., which are distributed in several parts of India. In India Plumbago zeylanica grows in different parts as wild species but it is also cultivated due to its wide therapeutic applications.
Plumbago zeylanica contains a variety of important chemical compounds. Different plant parts of the plant possess naphthaquinones, alkaloids, glycosides, steroids, triterpenoids, tannins, phenolic compounds, flavanoids, saponins, coumarins, carbohydrates, fixed oil and fats and proteins. Of all the chemical constituents, plumbagin is the principal active compound. Plumbagin (5-hydroxy-2-methyl-1, 4-naphthaquinone- C11H8O3) is primarily present in roots in higher amounts with only about 1% in the whole plant5-10.

Fig 1: Chemical structure of Plumbagin

Plant pacifies vitiated vata, kapha, diarrhea, inflammation, fever, nervous palsy, hemorrhoids, skin diseases, acne, irritable bowel disease, epilepsy, digestant, astringent, abortifacient, dysentery, cirrhosis, arthritsetc, ammenorrhoea, anemia and in the treatment of muscular pain11 - 12. Root is bitter, laxative, expectorant, tonic, abortifacient, good appetizer, useful in rheumatism, laryngitis, scabies, filariasis, depigmentation of the skin, anasarca, generalized swelling all over the body, non-bleeding piles, relieves constipation, alleviates the urticaria - the allergic skin rashes, leprosy, ulcers and disease of spleen.

Ayurveda, the Indian indigenous system of medicine dating back to the Vedic ages (1500-8000 BC), has described chitraka as tumor-negating and anti-dyspeptic. In Charaka Samhita (an important work on Ayurvedic system of medicine) Plumbago zeylanica has been categorized as an appetizer, anti-saturative, antianorexic, anti-haemorrhoidal and pain-reliever (Vishnukanta et al, 2010). Herbal medicines such as Dabur Chitrak Haritaki, Medohar Guggulu, Morslim-Z, Divya Chandraprabhavati etc. use P. zeylanica extracts in different amounts. The plumbagin present in different parts of Plumbago zeylanica as active constituent and it imparts wide range of biological activities. In the present study we planned to check the concentration of plumbagin present in aerial and root of Plumbago zeylanica.

2 Materials and Methods

2.1 Collection and identification of plant material

The plant of Plumbago zeylanica was collected from the Sanjeevni botanical garden, Bhopal, Madhya Pradesh, India. The above plant materials were shade dried, reduced to coarse powder and stored in airtight container till further use.

2.2 Preparation of extracts

500 gram of powdered drug of Plumbago zeylanica were packed in soxhlet apparatus separately and extracted with different polarity of solvent.

2.2.1 Petroleum ether extract

500 gram of powdered drug was packed in soxhlet apparatus and extracted with petroleum ether (60-80°C) until the extraction was completed which was confirmed by the colour of the siphoned liquid. The extract was filtered while hot, and the solvents were removed by distillation and the last traces of solvent being removed under reduced pressure. The petroleum extracts were stored in refrigerator for further experimental work.

2.2.2 Ethanol extract

The marc left after petroleum ether extraction was dried in hot air oven below 50°C and packed well in soxhlet apparatus and extracted with ethanol (90%) until the completion of the extraction. The extract was filtered while hot, and the solvents were removed by distillation and the last traces of solvent being removed under reduced pressure. The ethanol extracts were stored in refrigerator for further experimental work.

2.2.3 Aqueous extract

The marc left after ethanol extraction was dried in hot air oven below 50°C and packed well in soxhlet apparatus extracted with distilled water until the completion of the extraction. The extract was filtered while hot, and the solvents were removed by distillation and the last traces of solvent being removed under reduced pressure. The aqueous extracts were stored in refrigerator for further experimental work.

2.3 Qualitative chemical tests

Qualitative chemical tests were performed to determine the presence of alkaloids, carbohydrates, cardiac glycosides, polyphenols, saponins, tannins and terpenoids13 - 16.

2.3.1 Test for alkaloids

2.3.1.1 Dragendorff’s test

To 1 ml of the extract, add 1 ml of dragendorff’s reagent (Potassium Bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.

2.3.1.2 Mayer’s test: To 1 ml of the extract, add 1 ml of Mayer’s reagent (Potassium mercuric iodide solution). Whitish yellow or cream coloured precipitate indicates the presence of alkaloids.

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2.3.1.3 Hager’s test

To 1 ml of the extract, add 3 ml of Hager’s reagent (Saturated aqueous solution of picric acid), yellow coloured precipitate indicates the presence of alkaloids.

2.3.1.4 Wagner’s test

To 1 ml of the extract, add 2 ml of Wagner’s reagent (Iodine in Potassium Iodide) was added, Formation of reddish brown precipitate indicates the presence of alkaloids.

2.3.2 Test for saponins

Take small quantity of alcoholic and aqueous extract were taken separately and add 20 ml of distilled water and shake in a graduated cylinder for 15 minutes lengthwise. A 1cm layer of foam indicates the presence of saponins.

2.3.3 Test for Glycosides

2.3.3.1 Legal test

The extract was dissolved in pyridine and sodium nitroprusside solution was added to make it alkaline. The formation of pink red to red colour shows the presence of glycosides.

2.3.3.2 Baljet test

To 1ml of the test extract, 1ml of sodium picrate solution is added and the yellow to orange colour reveals the presence of glycosides.

2.3.3.3 Keller-Killiani test

Powdered drug 1gm was extracted with 10ml of 70% alcohol for 2 minutes, filtered, to the filtrate, 10ml of water and 0.5ml of strong solution of lead acetate were added and filtered and the filtrate was shaken with 5ml of chloroform. The chloroform layer was separated in a porcelain dish and the solvent was removed by gentle evaporation. The cooled residue was dissolved in 3ml of glacial acetic acid containing 2 drops of 5% ferric chloride solution. This solution was carefully transfer to the surface of 2ml of concentrated sulphuric acid. A reddish brown layer forms at the junction of the two liquids and the upper layer slowly becomes bluish green, darkening with standing indicator the presence of glycoside.

2.3.3.4 Borntrager’s test

A few ml of dilute Sulphuric acid was added to 1ml of the extract it was boiled, filtered and extract the filtrate is extracted with chloroform. The chloroform layer was treated with 1ml of ammonia. The formation of red colour of the ammonical layer shows the presence of anthraquinone glycosides.

2.3.4 Test for carbohydrates and sugars

2.3.4.1 Molisch’s test

To 2ml of the extract, add 1ml of α-naphthol solution was added concentrated sulphuric acid through the side of the test tube. Purple or reddish violet colour at the junction of the two liquids reveals the presence of Carbohydrates.

2.3.4.2 Fehling’s test

To 1ml of the extract, equal quantities of Fehling solution A and B were added, upon heating formation of a brick red precipitate indicates the presence of sugars.

2.3.4.3 Benedict’s test

To 5ml of Benedict’s reagent, add 1ml of extract solution was added and boiled for 2 minutes and cool. Formation of red precipitate shows the presence of sugars.

2.3.5 Test for tannins and phenolic compounds:

Take the little quantity of test solution was taken and mixed with basic lead acetate solution. Formation of white precipitates indicates the presence of tannins.

To 1ml of the extract, Ferric chloride solution was added, formation of a dark blue or greenish black colour product shows the presence of tannins.

A little quantity of test extract was treated with Potassium ferric cyanide and ammonia solution. A deep red colour indicates the presence of tannins.

2.3.6 Test for flavonoids

2.3.6.1 Shinoda’s test

The alcoholic extract of powder treated with Magnesium foil and concentrated HCl give intense cherry red colour it indicates the presence of flavonones or orange red colour indicates the presence of flavonoids.

The drug in alcoholic and aqueous solution with few ml of ammonia was seen in U.V. and visible light, formation of fluorescence indicates the presence of flavonoids.

Little quantity of extract was treated with amyl alcohol, sodium acetate and ferric chloride. A yellow colour solution was formed, which disappears on addition of an acid it indicates the presence of flavonoids.

The extract is treated with sodium hydroxide, formation of yellow colour indicates the presence of flavones.

The extract was treated with concentrated H2SO4, formation of yellow or orange colour indicates flavones.

2.3.7 Test for steroids

2.3.7.1 Libermann-Burchard test
One gram of the Test substance was dissolved in a few drops of chloroform, 3ml of acetic anhydride, 3ml of glacial acetic acid were added, warmed and cooled under the tap and drops of concentrated sulphuric acid were added along the sides of the test tube. Appearance of bluish-green colour shows the presence of sterols.

2.3.7.2 Salkowski test

The extract was dissolved in chloroform and adds equal volume of concentrate H₂SO₄ was added. Formation of bluish red to cherry colour in chloroform layer and green fluorescence in the acid layer represents the steroidal components in the tested extract.

2.3.8 Test for triterpenoids

2.3.8.1 Noller's test

Two or three granules of tin metal were dissolved in 2ml thionyl chloride solution. Then added 1ml of the extract into test tube and warmed, the formation of pink colour indicates the presence of triterpenoids.

2.4 Estimation of Plumbagin content

2.4.1 Determination of wavelength of maximum absorbance

To 1ml of standard stock solution (1mg/ml) of Plumbagin, 1ml alcoholic KOH (10%) was added. The volume was then adjusted to the 5ml with absolute alcohol. The absorbance of the colored solution was scanned by Hitachi UV/Visible spectrophotometer in the range of 400 to 800 nm against reagent blank. The blank was prepared similarly in which volume of standard Plumbagin was replaced by an equal volume of absolute alcohol. The maximum absorbance was obtained at 520nm.

2.4.2 Calibration curves of pure plumbagin

Accurately weighed 100 mg of Plumbagin was dissolved in 100 ml of ethanol which gives the concentration of 1000 µg/ml. 10 ml of this solution was taken and made up to 100 ml with Plumbagin acid which contains the concentration of 100 µg/ml. Further 10 ml of this solution was taken and made up to 100 ml with Plumbagin acid which contains the concentration of 10 µg/ml. 1 to 10 ml were taken from this solution and made up to 10 ml to get the concentration ranges of 1 to 10 µg/ml. The absorbance was measured at 520 nm and calibration curve of Plumbagin was plotted.

2.4.3 Estimation of Plumbagin content

Estimation of Plumbagin content in ethanol extract of arial part and root of Plumbago zeylanica carried out by dissolving the 100 mg extract in required quantity of ethanol and analyzed spectrophotometrically at a particular wavelength using the calibration curve. Each experiment examined for Plumbagin content in a triplet manner. The Plumbagin content in extract was calculated by computing from the calibration graph or from the regression equation.

3 Results

3.1 Phytochemical analysis

3.1.1 Extraction of Plumbago zeylanica

Preliminary phytochemical investigations of the successive whole plant extracts of Plumbago zeylanica revealed the presence of flavonoids, terpenoids, tannins, phenolic compounds, saponins, steroids, glycosides and carbohydrates. The details are presented in table 1.

From the result of phytochemical screening, the petroleum ether extract of whole plant of Plumbago zeylanica exhibited the presence of steroids. Alkaloids, glycosides, carbohydrates, flavonoids, polyphenol and saponins were found in ethanol extracts. Similarly glycosides, flavonoids, polyphenol and saponins were investigated in aqueous extracts. The maximum phytocostituents were observed in ethanol extracts of Plumbago zeylanica (Table 2). Now ethanol extracts of Plumbago zeylanica were selected for further pharmacological evaluation as this extract revealed the presence of flavonoids and phenolic compounds.

Table 1: Phytochemicals present in aerial part of Plumbago zeylanica extracts

<table>
<thead>
<tr>
<th>Test for</th>
<th>Petroleum ether extract</th>
<th>Ethanol extract</th>
<th>Aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Triterpeoids</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ = Present, – = Absent

3.1.2 Plumbagin content
The percentage of plumbagin in extracts of aerial and root of *Plumbago zeylanica* was calculated from the calibration curve is shown in Table 3 and Fig 2. The plumbagin content found in Aerial extract was 2.4%, while in Root extract it was 1.9% (Table 4).

### Table 2: Phytochemicals present in root of *Plumbago zeylanica* extracts

<table>
<thead>
<tr>
<th>Test for</th>
<th>Petroleum ether extract</th>
<th>Ethanol extract</th>
<th>Aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Triterpeoids</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ = Present, - = Absent

### Table 3: Absorbance by plumbagin at different concentration

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.021</td>
</tr>
<tr>
<td>2</td>
<td>0.045</td>
</tr>
<tr>
<td>3</td>
<td>0.061</td>
</tr>
<tr>
<td>4</td>
<td>0.078</td>
</tr>
<tr>
<td>5</td>
<td>0.11</td>
</tr>
<tr>
<td>6</td>
<td>0.123</td>
</tr>
<tr>
<td>7</td>
<td>0.146</td>
</tr>
<tr>
<td>8</td>
<td>0.175</td>
</tr>
<tr>
<td>9</td>
<td>0.197</td>
</tr>
<tr>
<td>10</td>
<td>0.214</td>
</tr>
</tbody>
</table>

### Fig 2: Calibration curve of plumbagin at different concentration

![Calibration curve](image)

### Table 4: Estimation of plumbagin in extracts of Aerial extract and Root extract of *Plumbago zeylanica*

<table>
<thead>
<tr>
<th>Sample</th>
<th>%Plumbagin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerial extract</td>
<td>2.4</td>
</tr>
<tr>
<td>Root extract</td>
<td>1.9</td>
</tr>
</tbody>
</table>

4 Discussions

From the result of phytochemical screening, the petroleum ether extract of aerial part and root of *Plumbago zeylanica* exhibited the presence of steroids. Alkaloids, glycosides, carbohydrates, flavonoids, polyphenol and saponins were found in ethanol extracts. Similarly glycosides, flavonoids, polyphenol and saponins were investigated in aqueous extracts. The maximum phytocostituents were observed in ethanol extracts of *Plumbago zeylanica*. Now ethanol extracts of *Plumbago zeylanica* were selected for further estimation of plumbagin content.

The free radicals are associated in diverse irrational conditions like cell and tissue injury, inflammatory situation, neurodegenerative disorder, aging and malignant growth. The antioxidant present in dietary supplement can neutralize the free radical compounds and protect body from various diseases. Moreover, the antioxidant play vital role in prevention of human body from diseases.

Recently herbal products and synthetic drugs comprising of antioxidant substances attract researcher to develop new formulation. It is recorded that numerous crude extracts obtained from plant, and herbal products obsessed noteworthy antioxidant activity. The ethanol extracts of aerial part and root of *Plumbago zeylanica* selected for the present study depending on the presence of valuable phytocostituents possess several pharmacological action associated to its antioxidant pursuit.

The percentage of plumbagin in ethanol extracts of aerial part and roots were found to be 2.4% and 1.9%, respectively. The plumbagin imparts various pharmacological activities such as Hepatoprotective activity, antidiabetic activity, antioxidant activity, antitumor activity,
antifertility activity etc. The plumbagin content in aerial part of *Plumbago zeylanica* is maximum compared to root.

5 Conclusion

Medicinal plants continue to play a central role in the healthcare system of large proportions of the world’s population. The phytochemical investigation of *Plumbago zeylanica* exhibited maximum phytoconstituents along flavonoids and polyphenol present in ethanol and aqueous extracts. The plumbagin present in different parts of *Plumbago zeylanica* as active constituent. The findings of study indicate that concentration of plumbagin in aerial part of *Plumbago zeylanica* is maximum compared to root.

6 Competing interest

None

7 Author’s contributions

BD, BJ and SK carried out literature review and draft the manuscript. KJ participated in collection of data. All authors read and approved the final manuscript.

8 References