SPECTROPHOTOMETRIC ESTIMATION OF TOTAL ALKALOIDS IN CHITRAKA (PLUMBAGO ZEYLANICA LINN) USING BROMOCRESOL GREEN

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ABSTRACT

Background: Plumbago zeylanica L. (Plumbaginaceae) commonly known, as Chitraka is pharmacologically important plant. Various studies have been undertaken to assess the pharmacological potential of different parts of the plant namely like roots, stem, flower, and leaves as antimicrobial, hepatoprotective, anticancer, antifertility, antiulcer, antifungal and wound healing. Chitraka is rich in alkaloids hence present study is taken.

Objective: The objective of the present study was to determine the total alkaloid content in Chitraka.

Materials & Methods: The presence of alkaloids was confirmed by qualitative dragendroffs method subjected to quantification, the total alkaloid content (TAC) was estimated spectrophotometric ally using Bromocresol green method with Atropine as standard.

Results: The results showed that root of Chitraka are rich source of alkaloids. The root extract of chitraka showed highest alkaloid content 83.4mg/g Atropine equivalent.

Conclusion: The total alkaloid content of Chitraka was well established by spectrophotometric studies. And it is direct, simple & sensitive spectrophotometric method for determination of total alkaloid based on the reaction with Bromocresol green.

Keywords: Chitraka, Alkaloids, Bromocresol Green.

INTRODUCTION:

Chitraka has been botanically identified us Plumbago zeylanica Linn. belongs to family Plumbaginaceae. It is perennial, sub-scandant shrub, herb one of the common plants used in Indian traditional system of medicine. The family Plumbaginaceae consists of 10 genera and 280 species. P. zeylanica common, wild in cultivation due to Its more therapeutic uses. P. zeylanica roots were reported to possess antioxidant, hypolipidemic, artherosclerotic, central nervous system stimulant and anti-fertility

Access the article online

WWW.Avishkara.in
DOI:
https://doi.org/10.56804/Avishkara.2022.1102
Properties. The root is used as laxative, expectorant, astringent, abortifacient, and in dysentery. In traditional system of medicine to cure various ailments like body pain, headache, fever and inflammation. Alkaloids are a diverse group of low-molecular-weight, nitrogen containing compounds found in about 20% of plant species. The potent biological activity of some alkaloids has also led to their exploitation as pharmaceuticals, stimulants, narcotics, and poisons. Plant-derived alkaloids currently in clinical use include the analgesics morphine and codeine, the muscle relaxant (C)-tubocurarine, the antiarrhythmic ajmaline, the antibiotic sanguinarine, and the sedative scopolamine. Other important alkaloids of plant origin include caffeine, nicotine, cocaine, and the synthetic O, O-acetylated morphine derivative heroin Based on the strong evidence of biological activities of plant alkaloids, the study was focused on determination of total alkaloid contents of chitraka.

**MATERIALS & METHODS:**

A Simple spectrophotometric method is described for determination of total alkaloids based on the reaction with Bromocresol green. A yellow complex forms and is easily extractable by chloroform at pH 4.7, this procedure can be carried out in the presence of other compounds without interference.

**Plant Material:** The plant materials were collected from Shree Jagadguru Gavisiddeshwara Ayurveda Medical College Pharmacy, Koppal, Karnataka and authenticated by taxonomist.

**Extraction:**

The plant materials (100g) were ground and then extracted with ethanol for 24 h in a continuous extraction (soxhlet) apparatus. The extract was filtered and ethanol was evaporated on water bath, and dried in hot air oven up to constant weight. [1]

**Qualitative estimation (Test for Alkaloid)**

Dragendroffs test: To 2 to 3ml of extract add few drops of Dragendroffs reagents orange brown precipitate is formed. The sample which showed positive alkaloid was then subjected to further quantitative analysis.

**Preparation of Reagents:**

**BCG Solution:** Bromocresol green solution was prepared by heating 69.8 mg bromocresol green with 3 ml of 2N NaOH and 5 ml distilled water until completely dissolved and the solution was diluted to 1000 ml with distilled water.

**Phosphate Buffer Solution (pH 4.7):** It was prepared by adjusting the pH of 2M sodium phosphate (71.6gm of Na₂HPO₄ in 1L distilled water ) to 4.7 with 0.2M citric acid (42.02gm of citric acid in 1L distilled water).

**Atropine Standard Solution:** It was made by dissolving 1mg of pure Atropine in 10ml distilled water.[2]

**Separation of Alkaloids:***

A part of this residue was dissolved in 2 N HCl and then filtered. One ml of this solution was transferred to a separatory funnel and washed with 10 ml chloroform (3 times). The pH of this solution was adjusted to neutral with 0.1N NaOH. Then 5 ml of BCG solution and 5 ml of phosphate buffer were added to this solution. The mixture was shaken and the complex formed was extracted with 1, 2, 3, and 4 ml chloroform by vigorous shaking. The extracts were collected in a 10-ml volumetric flask and diluted to volume with chloroform.

**Preparation of standard curve:** Accurately measure aliquots (0.4, 0.6, 0.8, 1 and 1.2 ml) of Atropine standard solution and transfer each to different separatory funnels. Then, add 5 ml pH 4.7 phosphate buffers and 5 ml BCG solution and shake a mixture with 1, 2, 3 and 4 ml of chloroform. The extracts were collected in a 10ml volumetric flask and then diluted to adjust volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm against
blank prepared as above but without
Atropine. [2]

<table>
<thead>
<tr>
<th>Atropine in ml</th>
<th>pH 4.7 Phosphate buffer in ml</th>
<th>BCG Solution in ml</th>
<th>Taken in separating funnel &amp; mixture was shaken with extract &amp; BCG solution in ml</th>
<th>Concentration in ml</th>
<th>Absorbance at 470nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4</td>
<td>5</td>
<td>5</td>
<td>40</td>
<td>0.0927</td>
<td></td>
</tr>
<tr>
<td>0.6</td>
<td>5</td>
<td>5</td>
<td>60</td>
<td>0.114</td>
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<tr>
<td>0.8</td>
<td>5</td>
<td>5</td>
<td>80</td>
<td>0.1533</td>
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</tr>
<tr>
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<td>5</td>
<td>5</td>
<td>100</td>
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</tr>
<tr>
<td>1.2</td>
<td>5</td>
<td>5</td>
<td>120</td>
<td>0.199</td>
<td></td>
</tr>
<tr>
<td>Sample</td>
<td>5</td>
<td>5</td>
<td></td>
<td>0.1472</td>
<td></td>
</tr>
</tbody>
</table>

Table 1 : Standard Atropine Curve

RESULTS AND DISCUSSION:
The results for total alkaloid content (TAC) in the Chitraka species are presented in the table 2. TAC was expressed in milligram Atropine equivalent.

<table>
<thead>
<tr>
<th>Extract</th>
<th>mg of Atropine Equivalent of extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>83.42</td>
</tr>
</tbody>
</table>

Table 2: Total Alkaloid content in the plant extract expressed in terms of Atropine equivalent of (mg /g of extract). The Chitraka root showed 83.42 mg/g of Atropine, the higher alkaloid content was revealed in the roots of Chitraka.

A few methods with different sensitivities have been developed for the determination of alkaloids in plant materials for example gravimetric, titrimetric methods , But these methods lack the adequate sensitivity and have some problems, Spectrophotometric determination of total Alkaloids with bromo cresol green is a simple and sensitive method and do not need very special equipment . The proposed procedure has the advantage of being less time consuming with assay requiring an average of 2hrs. The present study revealed the highest alkaloid contents in chitrakamula containing 83.42mg/g. The higher content of Alkaloids in plants protect against chronic diseases.

REFERENCE

