Study on quantification of Anti-diabetic Molecule - Pinitol present in *Pisoniagrandis* (R.Br) by HPTLC method

Pavithra R, Karthiayani A, Udhaya Ganga M and Baskaran D

Abstract

Phytochemical investigations of leaves of *Pisoniagrandis* of Pinitol, which is a common constituent of this plant and earlier isolated from *Pinus lambertiana*, *Gliricidia sepium* and *Bougainvillea spectabilis*; more recently pinitol has been reported from many other plants also. The hypoglycemic action and antihyperlipidemic effect of pinitol have been well established. The antidiabetic nature of *Pisoniagrandis* may be largely due to the presence of this particular compound in this plant. High-performance Thin-Layer Chromatography (HPTLC) is an important method which utilizes the conventional techniques of Thin Layer Chromatography (TLC) in a more optimized way. A stabilized HPTLC method for identifying and quantifying pinitol was adopted for this study and revealed the limit of quantification (LQD) and the limit of detection (LOD) of the biomarker molecule pinitol as 1.5 and 1.2 µg respectively. This HPTLC method is a simple, accurate and cheap method and can be utilized for the routine analysis and quantitative determination of pinitol in *pisoniagrandis* (R.Br) and showed that more than 8µg of pinitol per milligram of extract can be obtained using suitable solvents. Also the preliminary phytochemical analysis revealed the presence of many components viz., tannins, flavonoids, phenols, steroids, carbohydrates and proteins.

Keywords: Pinitol, *Pisoniagrandis*, HPTLC, phytochemical screening and quantification

1. Introduction

In our traditional system of medicine, many leaves are used as analgesic, anti-diabetic, anti-inflammatory, diuretic and hypoglycemic agents; some roots are used as purgative [1]. Some leaves are useful in chronic rheumatism, wound healing, arthritis, and diabetes and also used as vegetable. *Pisoniagrandis* R. Brisa wide spread evergreen foliage shrub or tree species that’s preads throughout the Western Indian and Eastern Pacificoceans. The plant *pisoniagrandis* (synonym: *pisonia alba*, *pisoniamorindifolia*) commonly known as `Leechikottaikerai` in Tamil, `Velatisal` in Hindi is widely used to treat rheumatism and diabetics due to the presence of many phytochemicals, especially pinitol. The isolation of antidiabetic molecule, pinitol from *Pisoniagrandis* validates its traditional use as an anti diabetic plant [2]. Hence, an attempt has been made to quantify the pinitol content present in *pisoniagrandis* which will give firsthand information in isolation and further utilization of the component in various food and Pharmaceutical application.

2. Materials and methods

2.1 Processing of plants samples

Leaves of *Pisoniagrandis* were cleaned and dried in shade. The shade dried leaves were cut into small pieces and then used for the study. Preparation of *Pisonia grandis* Leaf Ethanol (PGLE) Extract were prepared with air dried pieces of leaves of *Pisoniagrandis* extracted with 100% ethanol at reflux temperature for 6 hours. The extract was filtered; the filtrate was evaporated under reduced pressure to get a greenish black pasty solid. Sequential Soxhlet Extracts (PGSX1 to PGSX6) [3] of leaves of *Pisoniagrandis* were done with petroleum ether, chloroform, chloroform: methanol mixture (9:1), ethanol, ethanol: water mixture (2:1) and water. The extracts were concentrated and the concentrated residues were designated as PGSX1, PGSX2, PGSX3, PGSX4, PGSX5 and PGSX6 respectively.

2.2. Preparation of stock solutions

Standard D-Pinitol (1mg) was dissolved in 200 µl of dimethyl sulfoxide and diluted to get concentrations of 1.5µg, 3µg, 4.5µg, 6µg, 7.5µg. Samples (PGLE, PGSX1, PGSX2, PGSX3, PGSX4, PGSX5 and PGSX6) of known quantity were dissolved in known volume of dimethyl sulfoxide and centrifuged at 3000rpm for 2min. The solutions were use das test solutions for HPTLC Analysis.
2.3 High performance thin layer Chromatography (HPTLC)
HPTLC instrument is controlled by software and the entire concept that includes a widely standardized methodology [4] based on scientific facts as well as the use of validated methods for qualitative and quantitative analysis. Sophisticated instruments, controlled by an integrated software platform ensure to the highest possible degree of usefulness, reliability, and reproducibility of generated data. The samples of the extracts were fed into the instrument for the quantification of pinitol present. A stabilized HPTLC method for identifying and quantifying pinitol described here utilized 60F254 TLC aluminium sheet plates as stationary phase and chloroform: methanol: water (6:3.5:0.5v/v/v) as mobile phase.

2.4 Preliminary phytochemical screening
The ethanolic extract of *Pisonia grandis* (R.Br) PGLEandrosoxhletextractsPGSX1, PGSX2, PGSX3, PGSX4, PGSX5 and PGSX6 were subjected to preliminary phytochemical screening [5] for the presence of secondary metabolites such as alkaloids, flavonoids, triterpenoids steroids, phenols, tannins.

2.4.1 Test for alkaloids
1 g of powdered sample of each specimen was separately boiled with water and 10 ml hydrochloric acid was added and then filtered. The pH of the filtrate was adjusted with ammonia to about 6-7. A very small quantity of the following reagents was added separately to about 0.5 ml of the filtrate in a different test tube and observed.
- Picric acid solution.
- 10% tannic solution.
- Mayer’s reagent (Potassium mercuric iodide solution). The test tubes were observed for colored precipitates or turbidity for the presence of alkaloids [5].

2.4.2 Test for tannins
1 g of each powdered sample was separately boiled with 20 ml distilled water for five minutes in a water bath and was filtered while hot. 1 ml of cool filtrate was distilled to 5 ml with distilled water and a few drops (2-3) of 10% ferric chloride were observed for any formation of precipitates and any color change. A bluish-black or brownish-green precipitate indicated the presence of tannins [6].

2.4.3 Test for flavonoids
1 g of the powdered dried leaves of each specimen was boiled with 10 ml of distilled water for 5 minutes and filtered while hot. Few drops of 20% sodium hydroxide solution were added to 1 ml of the cooled filtrate. A change to yellow colour which on addition of acid changed to colourless solution depicted the presence of flavonoids [6].

2.4.4 Test for terpenoids
A little of each portion was dissolved in ethanol. To it 1 ml of acetic anhydride was added followed by the addition of concentrated sulphuric acid. A change in colour from pink to violet showed the presence of terpenoids [6].

2.4.5 Test for Phenols
1 ml of each of the concentrated extractives were heated to remove the solvent and the residues were taken in a little of aqueous methanol. To the methanolic solution was added 0.5% ferric chloride solution and the change in colour was marked in alcoholic extract indicating the presence of phenolic compounds [7].

2.4.6 Test for Steroids
Few drops of conc. H₂SO₄ is added to the plant extract shaken and on standing if the lower layer turns red in color then it indicates the presence of steroids (Salkowski test) [8].

2.4.7 Test for Carbohydrates (Molisch’s Test)
Few drops of Molsich’s reagentis added to extracts which was then followed by addition of 1 ml of concentrated sulphuric acid by the side of the test tube. The mixture was then allowed to stand for two minutes and then diluted with 5 ml of distilled water. Formation of a red or dull violet colour/ring at the interphase of the two layers is a positive test [9].

2.4.8 Test for Proteins
The extract (100 mg) is dissolved in 10 ml of distilled water and filtered through Whatmann No. 1 filter paper and the filtrate is subjected to test for Amino acids [10].

3. Results and Discussion
Different concentrations of standard solution1.5µg, 3µg, 4.5µg, 6µg and 7.5µg represented by S1, S2, S3, S4, S5 and S6 respectively were spotted TLC plate. The peak area was plotted against corresponding concentration (Table 1). The results of HPTLC analysis are presented in Table 1 and Table 2. The calibration curve was linear in the range of 1.5 to 7.5 µg per spot and the correlation coefficient [11] was found to be 0.9718, thus exhibiting good linearity between concentration and area. The limit of quantification (LOQ) was found to be 1.5 µg and the limit of detection (LOD) was 1.2 µg for standard. The present study revealed that among all the extracts, PGSX3 [Chloroform: methanol mixture (9:1)] and PGSX4 (100% ethanol) contain more than 8 µg of pinitol per extract with 100% ethanol alone showed the presence of terpenoids and the results are shown in Table 2.

Table 1: Calibration table for Standard

<table>
<thead>
<tr>
<th>Standard</th>
<th>RF</th>
<th>Amount per fraction (µg)</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>0.65</td>
<td>1.500</td>
<td>32.53</td>
</tr>
<tr>
<td>S2</td>
<td>0.65</td>
<td>3.000</td>
<td>1672.94</td>
</tr>
<tr>
<td>S3</td>
<td>0.65</td>
<td>4.500</td>
<td>6860.60</td>
</tr>
<tr>
<td>S4</td>
<td>0.65</td>
<td>6.000</td>
<td>17619.35</td>
</tr>
<tr>
<td>S5</td>
<td>0.65</td>
<td>7.500</td>
<td>22616.00</td>
</tr>
</tbody>
</table>

![Figure 1: Quantity of Pinitol present in the extracts](image)
Table 2: Phytochemical analysis

<table>
<thead>
<tr>
<th>Sample Id</th>
<th>Al</th>
<th>Ta</th>
<th>Fl</th>
<th>TTr</th>
<th>Ph</th>
<th>St</th>
<th>Cho</th>
<th>Pr</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGLE</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>PGSX1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PGSX2</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PGSX3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>PGSX4</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PGSX5</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PGSX6</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

4. Conclusion

Pinitol is a completely non-toxic and common constituent of legume plants and is a major component of soybean (Glycine max L Merr). Earlier isolated from pine tree (Pinus lambertiana), Gliricidiaspectabilis and Bougainvillea spectabilis, more recently pinitol has been reported from many other plants also. The hypoglycemic action and anti-hyperlipidemic effect of pinitol have been well established.[12] Clinical trials on the effect of pinitol on glycemic control and lowering cardiovascular risk factors in patients with type II diabetes mellitus have been proved. Pinitol is reported safe and nontoxic as an anti-diabetic agent even at high levels. It is noteworthy that pinitol, with such pharmacological importance is isolated for the first time from Pisonia genus and from the medicinal plant Pisoniagrantis. The anti-diabetic nature of Pisoniagrantis may be largely due to the presence of this particular compound in this plant. [2, 13] HPTLC is an important method of TLC which utilizes the conventional techniques of TLC in a more optimized way. A stabilized HPTLC method for identifying and quantifying pinitol was adopted for this study.[14] The study revealed the limit of quantification (LOQ) and the limit of detection (LOD) of the biomarker molecule pinitol as 1.5 and 1.2 µg respectively and the study revealed that more than 8µg of pinitol per milligram of extract can be obtained using suitable solvents. Also the preliminary phytochemical analysis revealed the presence of many components viz, tannins, flavonoids, phenols, steroids carbohydrates and proteins and extract with 100% ethanol alone showed the presence of triterpenoids.

5. References