CHARACTERIZATION OF CAMPESTEROL, STIGMASTEROL AND β-SITOSTEROL FROM VINCA ROSEA LEAVES BY GC-MS AND BIOLOGICAL EVALUATION

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ABSTRACT

Vinca rosea, perennial shrub, grows throughout India. The species has long been cultivated and used for various treatments like diabetes mellitus, high blood pressure and infection. Various fractions of leaves extract of vinca rosea were evaluated to study the effect on glucoamylase, in vitro. Amongst the fractions, Petroleum ether: Chloroform (50:50) showed crystalline component and further, GC-MS revealed it comprised of campesterol, stigmasterol and β-sitosterol. It is observed that the fraction in total was moderate activator of glucoamylase with 51.87% activation. We report that this fraction would be used in the treatment of hypoglycemia and chronic diarrhea caused due to deficiency of glucoamylase in children.

KEYWORDS: Vinca rosea, glucoamylase, activator.

INTRODUCTION

Vinca rosea Linn or Catharanthus rosea (G. Don) belonging to the Apocynaceae family has been used both in Ayurvedic and Chinese medicine as the extracts of the plant for diseases such as diabetes,[1] menstrual regulators, hypertension, diarrhea,[2] malaria, leukemia a Hodgkin disease etc. In India, juice from the leaves were used to treat wasp stings. In Mexico, infusion of whole plant is taken orally for gastric problems,[3] ethanol extract of dried leaves show anti-inflammatory activity,[4] hot water extract of dried leaves show anti-hypercholesterolemic activity.[5] More than 130 alkaloids have been found in this plant of which vincristine and vinblastine have found extensive application in the treatment of human
METHODS AND MATERIALS

Glucoamylase Assay

0.5mL of the reaction mixture containing, 0.1mL modulator, 0.3mL of 100mM acetate buffer (pH 4.5), and 0.1mL of glucoamylase (1.3µg) were incubated at 37°C for 30 min. Then, 0.5mL of starch solution (5mg/mL prepared in 100mM acetate buffer pH 4.5) was added and incubated further at 37°C for 30 min. The reaction was terminated by keeping the test-tubes in boiling water bath for 1-2 minutes, cooled under running tap water; the test-tubes were cooled and diluted with 7mL of distilled water. The absorbance was recorded at 530nm using spectrophotometer and liberated glucose was estimated. The activity was calculated as,

\[ \text{Activity} = \frac{(A_s - A_c)}{A_c} \times 100 \]

where Ac and As are the absorbance of the control and sample, respectively. A unit (U) of enzyme activity is defined as the amount of enzyme required to release the mg of glucose liberated per mg of protein per minute. Vinca rosea leaves were collected from the campus of University of Mumbai. The leaves of the plant were shade dried and pulverized. 550 g of powder of air dried leaves was immersed in 2.5 liters of methanol for 7 days replacing the solvent after every 24 hours. The solvent was removed by distillation under reduced pressure giving 150 g (27.27%) of solid methanol extract. The methanolic extract was subjected for further fractionation on silica gel column using solvents in their increasing polarities from petroleum ether (Pet Ether) (A), petroleum ether: chloroform (Pet Ether: CHCl₃) (50:50) (B), Chloroform (CHCl₃) (C), Ethyl acetate (EtOAc) (D) and Methanol (MeOH) (E). Table 1.

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Amount obtained (gm)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Pet Ether</td>
<td>1.08</td>
</tr>
<tr>
<td>B</td>
<td>Pet Ether: CHCl₃ (50:50)</td>
<td>6.78</td>
</tr>
<tr>
<td>C</td>
<td>CHCl₃</td>
<td>47.78</td>
</tr>
<tr>
<td>D</td>
<td>EtOAc</td>
<td>32.56</td>
</tr>
<tr>
<td>E</td>
<td>MeOH</td>
<td>33.40</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>121.60</td>
</tr>
</tbody>
</table>

It was revealed by the TLC that the fraction B exhibited less number of compounds with proper separation. Therefore, fraction B was subjected for Silica gel Column Chromatography for separation of compounds using Pet. Ether: CHCl₃ in different
proportions. Consequently, fraction obtained from Pet. Ether: CHCl₃ (4:1), showed orange coloured mother liquor along with the crystalline solid. This coloured solution was washed with petroleum ether yielded crystalline solid compound (TRS-1). The effect of this isolated crystalline solid fraction on glucoamylase was studied in vitro. Table 2

**Table 2: Effect of TRS-1 with varying concentrations on glucoamylase in vitro**

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>TRS-1 (mg/ml)</th>
<th>Activity (U)</th>
<th>% Activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control</td>
<td>6.92±0.02</td>
<td>--</td>
</tr>
<tr>
<td>2.</td>
<td>0.2</td>
<td>7.94±0.02</td>
<td>14.73</td>
</tr>
<tr>
<td>3.</td>
<td>0.4</td>
<td>8.46±0.02</td>
<td>22.22</td>
</tr>
<tr>
<td>4.</td>
<td>0.6</td>
<td>9.48±0.02</td>
<td>36.99</td>
</tr>
<tr>
<td>5.</td>
<td>0.8</td>
<td>10.25±0.02</td>
<td>48.12</td>
</tr>
<tr>
<td>6.</td>
<td>1.0</td>
<td>10.51±0.02</td>
<td>51.87</td>
</tr>
</tbody>
</table>

**RESULTS AND DISCUSSION**

Various concentrations of TRS-1 were prepared ranging from 0.2 mg/ml to 1 mg/ml and was observed the increase in activity of glucoamylase with the increase in the concentration.

**Tests for steroids** [8]: Salkowski reaction

A few crystals of TRS-1 were dissolved in chloroform and a few drops of concentrated sulfuric acid were added to the solution. A reddish color was seen in the upper chloroform layer confirmed the presence of steroids. A white crystalline solid, TRS-1 was observed as a single spot on TLC, but GC-MS spectra

![GC chromatogram of TRS-1](image)

**Fig. 1: GC chromatogram of TRS-1**

Fig. 1, revealed it to be the mixture of three compounds viz. campesterol, stigmasterol, β-sitosterol. The effect of varying concentrations of this fraction showed that it is moderate activator of glucoamylase in vitro. The mass spectrometry of each compound showed the molecular ion peaks at m/e 400, 412 and 414 corresponds the molecular formulae as
Compound 1: Campesterol (C_{28}H_{48}O), Compound 2: Stigmasterol (C_{29}H_{48}O), and Compound 3: β-sitosterol (C_{29}H_{50}O). MS data of compound 1 Campesterol (C_{28}H_{48}O): Molecular ion (M^+) m/e = 400, m/z = 382, 340, 315, 289, 273, 255, 213, 199, 159, 145, 105, 91, 55, Base Peak= 43 MS data of compound 2 Stigmasterol (C_{29}H_{48}O): Molecular ion (M^+) m/e = 412, m/z= 379, 351, 327, 300, 271, 255, 213, 199, 161, 159, 133, 81, 55, Base Peak= 55 MS data of compound 3 β-sitosterol (C_{29}H_{50}O):Molecular ion (M^+) m/e = 414, m/z= 396, 329, 303, 273, 255, 231, 213, 161, 145, 107, 105, 55, Base Peak= 43

CONCLUSION

The spectral data revealed the isolated fraction to be the mixture of campesterol, stigmasterol and β-sitosterol in the ratio of 1:0.75:1 respectively. As per the literature survey these steroidal components have been found previously from the seeds.[9] For the first time the effect of this steroidal mixture on glucoamylase enzyme, in vitro has been studied and the fraction found to be the moderate activator of glucoamylase. The deficiency of glucoamylase in the duodenum results in a range of gastrointestinal symptoms. The recent study has determined the starch malabsorption caused by small intestinal glucoamylase deficiency in children with chronic diarrhea[10] and is also used in the treatment of hypoglycemia,[11,12] low blood sugar which occurs when blood glucose drops below normal levels. Therefore, we report that, the fraction TRS-1, isolated from Vincarosea can proved to be useful in the treatment of deficiency of glucoamylase.

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REFERENCES


