In Vitro α-Amylase Inhibitory Activity and GC-MS Analysis of Petrea volubilis

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Abstract: One of the therapeutic approaches which involve decreasing hyperglycemia aims at inhibiting the enzyme α-amylase. The aim of the present study was to investigate the in vitro anti-diabetic activity of the methanolic extract of Petrea volubilis. The assay results showed that the extract exhibit the dose-dependent increase in inhibitory effect on alpha-amylase enzyme up to 81.57% with IC 50 value 24.75±0.294 µg/ml. GC-MS analysis of root sample suggests that 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester compound may be responsible to decrease hyperglycemia. This study shows that the roots of Petrea volubilis were most effective in inhibiting a-amylase, thereby proving to be potential anti-diabetic agents.

Keywords: α-amylase enzyme, GC-MS analysis, IC 50 value, Petrea volubilis.

1. Introduction

Diabetes mellitus is a metabolic disorder with increasing incidence throughout the world. Insulin is a key player in the control of glucose homeostasis. Lack of insulin affects carbohydrate, fat and protein metabolism (1). Management of diabetes without side effects is still challenging for the medical community. The number of diabetes mellitus cases has been increasing worldwide in recent years. In 2000, the world health organization estimated a total of 171 million of people with diabetes mellitus from the global population, and this report projected to increase to 366 million by 2030 (2).

The treatment of DM is based on parenteral insulin and oral antidiabetic drugs. Oral hypoglycemic agents include sulphonylureas, biguanides, and other drugs like acarbose. These drugs have serious side effects and deleterious contraindications (3). Hence herbal remedies having high therapeutic efficacy with minimal side effects are favoured. A therapeutic approach to treat diabetes is to decrease postprandial hyperglycemia (4). This can be achieved by the inhibition of carbohydrate hydrolyzing enzymes like alpha amylase and alpha glucosidase (5). Alpha glucosidase and alpha amylase are the important enzymes involved in the digestion of carbohydrates. Alpha Amylase is involved in the breakdown of long chain carbohydrates and alpha glucosidase breaks down starch and disaccharides to glucose. They serve as the major digestive enzymes and help in intestinal absorption. Alpha amylase and glucosidase inhibitors are the potential targets in the development of lead compounds for the treatment of diabetes (6).

The principle of gas chromatography is adsorption and partition. Within the family of chromatography based methods gas chromatography (GC) is one of the most widely used techniques and has become one of the most important tools for the separation of volatile compounds. Gas chromatography has a very wide field of applications. It is widely used for quantitative and qualitative analysis of mixtures, for the purification of compounds, and for the determination of such thermo chemical constants as heats of solution and vaporization, vapor pressure, and activity coefficients.

2. Materials and Methods

2.1 Collection of Plant Material

The plant material (leaf; stem and root) was collected from University of Rajasthan, Jaipur. The plant material was duly authenticated by herbarium, Department of Botany, University of Rajasthan, Jaipur. (Voucher number: PARC/2011/742).

2.2 Preparation of Extract

The stem, root and leaves, were air-dried, pulverized by grinding using mortar and pestle. Thereafter, the coarse powder of air-dried plant parts were subjected to methanolic solvent extraction by maceration for 72 h.

2.3 Assay of Amylase Inhibition

In vitro amylase inhibition was studied by the method of Bernfeld [14]. In brief, 100 µL of the test extract was allowed to react with 200 µL of α- amylase enzyme (Hi media Rm 638) and 100 µL of 2mM of phosphate buffer (pH-6.9). After 20-minute incubation, 100 µL of 1% starch solution was added. The same was performed for the controls where 200 µL of the enzyme was replaced by buffer. After incubation for 5 minutes, 500 µL of dinitrosalicylic acid reagent was added to both control and test. They were kept in boiling water bath for 5min. The absorbance was recorded at 540nm using spectrophotometer and the percentage inhibition of α-amylase enzyme was calculated using the formula

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2.4 GC-MS Analysis

Gas chromatography analysis was carried out at advanced instrumentation research facility, Jawahar Lal Nehru University, New Delhi. It is one of the key techniques, generally used for screening/identification of different groups of plant phytochemicals. The high attainable separation power in combination with wide range of the detectors employing various detection principles to which it can be coupled makes GC an important, often irreplaceable tool in the analysis at trace level of plant phytochemical compounds.

| Table 1: α-Amylase inhibitory activity of the methanolic extracts of *Petrea volubilis* |
|---------------------------------------------------|---------------------------------------------------|---------------------------------------------------|---------------------------------------------------|
| **Concentration (µg/ml)** | **% Inhibition** | **IC-50 (µg/ml)** | **Concentration (µg/ml)** | **% Inhibition** | **IC-50 (µg/ml)** | **Concentration (µg/ml)** | **% Inhibition** | **IC-50 (µg/ml)** |
| 42 | 44.6 | 47.42±0.143 | 30 | 29.13 | 20 | 44.26 | 30 | 45.23±0.215 | 40 | 71.57 | 40 | 24.75±0.294 |
| 44 | 46.2 | 30 | 35.43 | 40 | 55.26 | 40 | 63.84 | 50 | 81.57 |
| 46 | 48.4 | 50 | 63.84 | 50 | 45.23±0.215 | 50 | 81.57 |
| 50 | 52.8 | 60 | 70.20 | 60 | 24.75±0.294 |

3. Results and Discussion

Hyperglycemia has been a classical risk in the development of diabetes and the complications associated with diabetes. Therefore control of blood glucose levels is critical in the early treatment of diabetes mellitus and reduction of macro and micro vascular complications. One therapeutic approach is the prevention of carbohydrate absorption after food intake, which is facilitated by inhibition of enteric enzymes including α-glucosidase and α-amylase present in the brush borders of intestine.

In this study, the α-amylase inhibitory activity of the leaves, stem and root of *P. volubilis* was investigated. The percentage inhibition of α-amylase by the root extracts was highest (81.57%) at 50 µg/mL concentration. The IC50 of leaf, stem and root was 47.42±0.143, 45.23±0.215 and 24.75±0.294 µg/ml, respectively (table-1, fig:1-3).

Methanolic root extract of *P. volubilis* showed maximum amylase inhibitory effect hence GC-MS analysis was done for the separation of compounds. The GC-MS analysis of *P. volubilis* root revealed the presence of thirty one compounds (phytochemical constituents) that could contribute the medicinal quality of the plant. Out of them 1, 2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester may be responsible for antidiabetic activity. (table-2, fig:3)
Figure 3: Chromatogram of methanolic root extract *P. volubilis* by GC-MS

Table 2: GC-MS analysis showed phytochemical compounds and their biological activities of methanol leaf extract of *P. volubilis*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>R.Time</th>
<th>Name of compounds</th>
<th>Molecular Formula</th>
<th>Molecular Weight</th>
<th>Peak Area (%)</th>
<th>Biological activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>10.466</td>
<td>Dodecanal</td>
<td>C_{12}H_{24}O</td>
<td>184</td>
<td>0.13</td>
<td>antibacterial activity against both of gram positive and gram negative bacteria [10]</td>
</tr>
<tr>
<td>2.</td>
<td>14.203</td>
<td>Dodecanoic acid</td>
<td>C_{12}H_{24}O_{2}</td>
<td>200</td>
<td>1.15</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>15.819</td>
<td>DODECANAL DIMETHYLACETAL</td>
<td>C_{14}H_{30}O_{2}</td>
<td>230</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>18.509</td>
<td>Tetradecanoic acid</td>
<td>C_{14}H_{26}O_{2}</td>
<td>228</td>
<td>0.82</td>
<td>antibacterial, antifungal [13] Antioxidant, Cancer preventive, Nematicide, Lubricant, Hypcholesterolemic [12]</td>
</tr>
<tr>
<td>5.</td>
<td>20.509</td>
<td>PENTADECANOIC ACID</td>
<td>C_{16}H_{30}O_{2}</td>
<td>242</td>
<td>0.12</td>
<td>Antimicrobial, antioxidant activity [13]</td>
</tr>
<tr>
<td>6.</td>
<td>20.653</td>
<td>1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester</td>
<td>C_{16}H_{28}O_{3}</td>
<td>278</td>
<td>1.90</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>21.680++</td>
<td>Hexadecanoic acid, methyl ester</td>
<td>C_{17}H_{34}O_{2}</td>
<td>270</td>
<td>0.42</td>
<td>Antioxidant, Hypcholesterolemic, Nematicide, Pesticide, Antiandrogenic flavor, Hemolytic, 5-Alpa reductase inhibitor [14]</td>
</tr>
<tr>
<td>8.</td>
<td>22.140</td>
<td>Oleic Acid</td>
<td>C_{18}H_{34}O_{2}</td>
<td>282</td>
<td>0.81</td>
<td>Antitumor, anticancer [15]</td>
</tr>
<tr>
<td>9.</td>
<td>22.701</td>
<td>n-Hexadecanoic acid</td>
<td>C_{18}H_{34}O_{2}</td>
<td>256</td>
<td>12.19</td>
<td>Antioxidant, Hypcholesterolemic Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic, 5-Alpa reductase inhibitor [16,17]</td>
</tr>
<tr>
<td>10.</td>
<td>24.890</td>
<td>9,12-Octadecadienoic acid, methyl ester</td>
<td>C_{19}H_{36}O_{2}</td>
<td>294</td>
<td>0.55</td>
<td></td>
</tr>
<tr>
<td>11.</td>
<td>25.024</td>
<td>9-OCTADECENOIC ACID (Z), METHYL ESTER</td>
<td>C_{19}H_{36}O_{2}</td>
<td>296</td>
<td>3.35</td>
<td>Anti fungal Antioxidant, anticancer [18]</td>
</tr>
</tbody>
</table>

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12. \[ 25.451 \] OCTADECANOIC ACID, METHYL ESTER \[ C_{16}H_{33}O_2 \] 298 0.10 Potent Antifungal, antimicrobial, antibacterial and at low PH. (19)

13. \[ 26.351 \] 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester \[ C_{16}H_{22}O_4 \] 278 61.97 Antifungal, anti retroviral, anti tumor, anti diabetic antibiotic, anti cancer, antioxidant, anti scabies anti inflammatory, potent antimicrobial agent (20,21)

14. \[ 26.549 \] Octadecanoic acid \[ C_{16}H_{33}O_2 \] 284 3.32 Cancer preventive Insectifuge (22)

15. \[ 26.779 \] 4,7-Octadecccinoic acid, methyl ester \[ C_{16}H_{34}O_2 \] 294 0.23

16. \[ 27.360 \] 3,6-OCTADECADIENOIC ACID, METHYL ESTER \[ C_{16}H_{34}O_2 \] 294 0.49

17. \[ 28.578 \] 2-Nonenal, 2-pentyl \[ C_{16}H_{34}O_2 \] 210 0.46

18. \[ 28.846 \] E,E,Z-1,3,12-Nonadecatriene-5,14-diol \[ C_{16}H_{34}O_2 \] 294 0.17

19. \[ 29.083 \] BICYCLO[3.3.1]NONAN-9,ONE, 2-BUTYL-7-(1,1-DIMETHYLETHYL)-3-NITRO-, [1S-(2-EXO,3-EXO,7-ENDO)]- \[ C_{17}H_{23}N_2O_3 \] 295 0.58

20. \[ 29.210 \] 2H-Pyrano, 2-(2-heptadecynoxy)tetrahydro- \[ C_{22}H_{40}O_2 \] 336 1.18

21. \[ 29.408 \] BICYCLO[3.3.1]NONAN-9,ONE, 2-BUTYL-7-(1,1-DIMETHYLETHYL)-3-NITRO-, [1S-(2-EXO,3-EXO,7-ENDO)]- \[ C_{23}H_{30}N_2O_3 \] 295 2.18

22. \[ 29.504 \] 9-Octadecenal, (Z)- \[ C_{18}H_{34}O \] 226 2.66 anti-alopecia, Antitumour, Choleretic, Dermatitigenic, immunostimulant, Anti leukotriene, anti- androgenic, Haemolytic, Hypercholesterolemic, (21)

23. \[ 30.182 \] Cyclohexane, 1',dodecylidenedis[4-methyl- \[ C_{20}H_{30} \] 362 0.93

24. \[ 30.308 \] 1-Cyclohexanol, 4-tert butyl-1-methyl- \[ C_{16}H_{32}O \] 170 0.74

25. \[ 30.870 \] CYCLOHEXANONE OXIME \[ C_{10}H_{10}N \] 114 0.54

26. \[ 31.414 \] Z-5-Methyl-6-heneicosen-1-one \[ C_{32}H_{50} \] 322 0.54

27. \[ 32.519 \] 1,2-BENZENEDICARBOXYLIC ACID \[ C_{16}H_{30}O_4 \] 390 0.29 Antimicrobial Antifouling (12)

28. \[ 35.208 \] 9-Octadecenoic acid (Z), 2,3-dihydroxypropyl ester \[ C_{17}H_{24}O_4 \] 356 0.66

29. \[ 36.299 \] 6-Octadecenoic acid, (Z)- \[ C_{18}H_{34}O_2 \] 282 0.25

30. \[ 49.316 \] Biphenylene, 1,2,3,6,7,8,8a,8b-octahydro-4,5-dimethyl- \[ C_{18}H_{18} \] 188 0.37 Wound healing activity,cholesterol lowering action, anti-inflammatory,cholesterolic and liver protecting activity (25) hypolipidic properties

31. \[ 50.026 \] Betulin \[ C_{28}H_{39}O_2 \] 442 0.44

Reference