

# In Vitro $\alpha$ -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  $\alpha$ -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 upto 81.57% with IC 50 value  $24.75 \pm 0.294 \mu\text{g/ml}$ . 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  $\alpha$ -amylase, thereby proving to be potential anti-diabetic agents.

**Keywords:**  $\alpha$ -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<sup>(1)</sup>. Management of diabetes without side effects is still challenge to 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<sup>(2)</sup>.

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<sup>(3)</sup>. Hence herbal remedies having high therapeutic efficacy with minimal side effects are favoured. A therapeutic approach to treat diabetes is to decrease postprandial hyperglycemia<sup>(4)</sup>. This can be achieved by the inhibition of carbohydrate hydrolyzing enzymes like alpha amylase and alpha glucosidase<sup>(5)</sup>. 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<sup>(6)</sup>.

*Petrea volubilis* L. (family Verbenaceae), commonly known as Queen's Wreath, is an ornamental perennial woody climber, subshrub or shrub. Traditionally, it is used for treatment of burns, wounds, inflammation and abscess<sup>(7)</sup>. *P. volubilis* is used in the folk medicinal system of Bangladesh for treatment of diabetes and it can be used as a source of antibiotic substances for possible treatment of bacterial and fungal infections, including gonorrhoea, pneumonia, urinary tract and some mycotic infections<sup>(8)</sup>.

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  $\mu\text{L}$  of the test extract was allowed to react with 200  $\mu\text{L}$  of  $\alpha$ - amylase enzyme (Hi media Rm 638) and 100  $\mu\text{L}$  of 2mM of phosphate buffer (pH-6.9). After 20-minute incubation, 100  $\mu\text{L}$  of 1% starch solution was added. The same was performed for the controls where 200  $\mu\text{L}$  of the enzyme was replaced by buffer. After incubation for 5 minutes, 500  $\mu\text{L}$  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  $\alpha$ -amylase enzyme was calculated using the formula

$$\text{Inhibition (\%)} = 100 \left( \frac{\text{control} - \text{test}}{\text{control}} \right) \quad (9)$$

### 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.

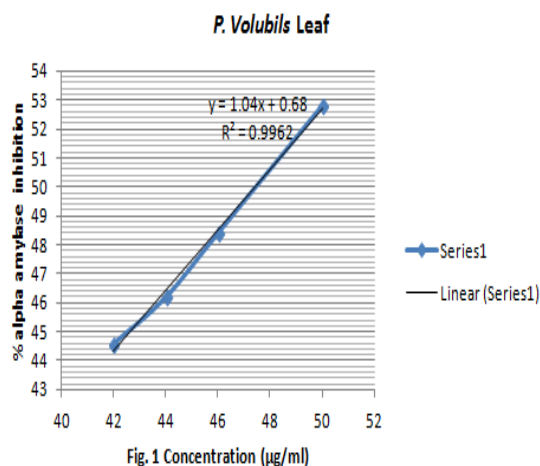
**Table 1:**  $\alpha$ -Amylase inhibitory activity of the methanolic extracts of *Petrea volubilis*

Leaf			Stem			Root		
Concentration	% Inhibition	IC-50 ( $\mu\text{g/ml}$ )	Concentration	% Inhibition	IC-50	Concentration	% Inhibition	IC-50 ( $\mu\text{g/ml}$ )
42	44.6	47.42 $\pm$ 0.143	30	29.13	45.23 $\pm$ 0.215	20	44.26	24.75 $\pm$ 0.294
44	46.2		40	35.43		30	55.26	
46	48.4		50	63.84		40	71.57	
50	52.8		60	70.20		50	81.57	

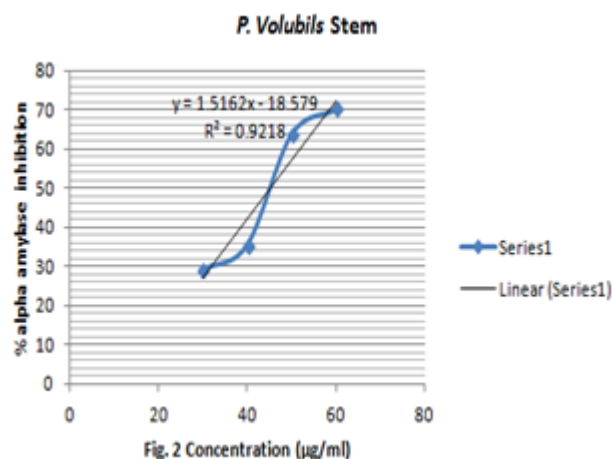
### 3. Results and Discussion

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  $\alpha$ -glucosidase and  $\alpha$ -amylase present in the brush borders of intestine.

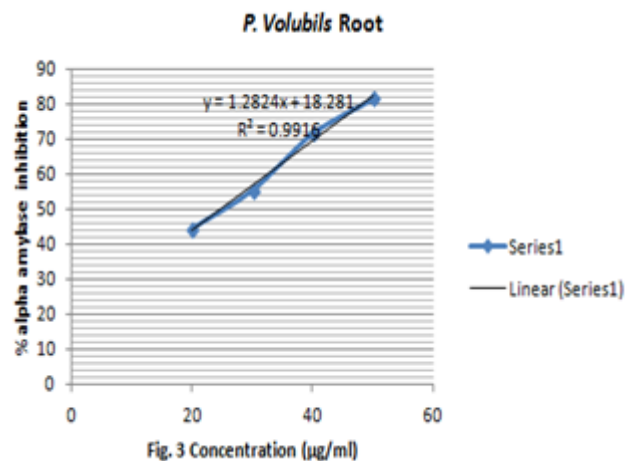
In this study, the  $\alpha$ -amylase inhibitory activity of the leaves, stem and root of *P. volubilis* was investigated. The percentage inhibition of  $\alpha$ -amylase by the root extracts was highest (81.57%) at 50  $\mu\text{g/ml}$  concentration. The IC50 of leaf, stem and root was 47.42 $\pm$ 0.143, 45.23 $\pm$ 0.215 and 24.75 $\pm$ 0.294  $\mu\text{g/ml}$ , respectively ( table- 1, fig:1-3 ).



**Figure 1:** % inhibition of alpha amylase enzyme by methanolic leaf extracts of *P. volubilis* (values are expressed as mean  $\pm$  SD, n = 3)



**Figure 2:** % inhibition of alpha amylase enzyme by methanolic stem extracts of *P. volubilis* (values are expressed as mean  $\pm$  SD, n = 3)



**Figure 3:** % inhibition of alpha amylase enzyme by methanolic root extracts of *P. volubilis* (values are expressed as mean  $\pm$  SD, n = 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)

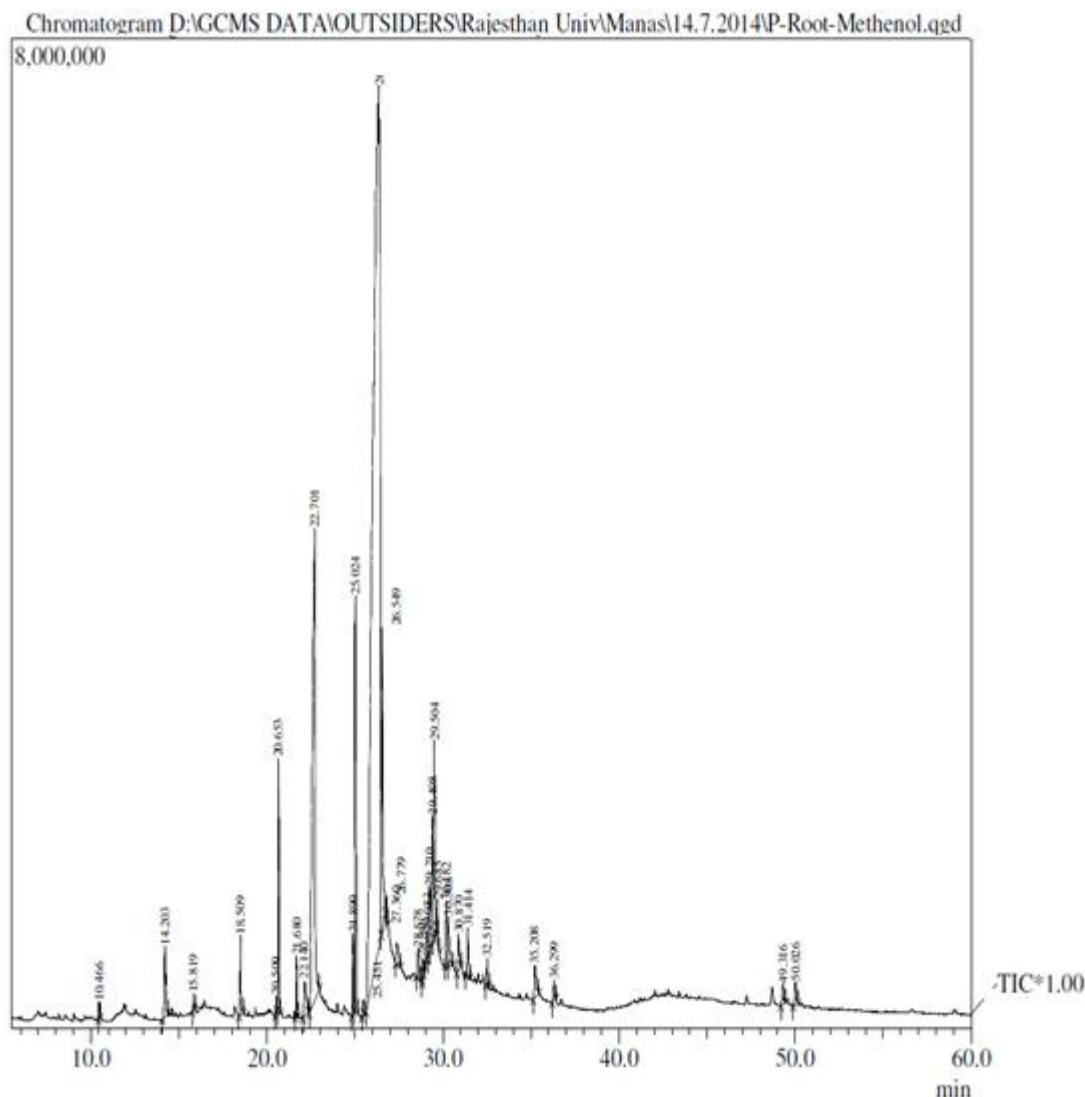


Figure 3: Chromatogram of methanolic root extract *P. volubilis* by GC-MS

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

S. No.	R.Time	Name of compounds	Molecular Formula	Molecular Weight	Peak Area (%)	Biological activity
1.	10.466	Dodecanal	C <sub>12</sub> H <sub>24</sub> O	184	0.13	antibacterial activity against both of gram positive and gram negative bacteria <sup>(10)</sup> .
2.	14.203	Dodecanoic acid	C <sub>12</sub> H <sub>24</sub> O <sub>2</sub>	200	1.15	
3.	15.819	DODECANAL DIMETHYLACETAL	C <sub>14</sub> H <sub>30</sub> O <sub>2</sub>	230	0.15	
4.	18.509	Tetradecanoic acid	C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>	228	0.82	antibacterial, antifungal <sup>(11)</sup> Antioxidant, Cancer preventive, Nematicide, Lubricant Hypocholesterolemic <sup>(12)</sup>
5.	20.509	PENTADECANOIC ACID	C <sub>15</sub> H <sub>30</sub> O <sub>2</sub>	242	0.12	Antimicrobial, antioxidant activity <sup>(13)</sup>
6.	20.653	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	278	1.90	
7.	21.680-++	Hexadecanoic acid, methyl ester	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270	0.42	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Antiandrogenic flavor, Hemolytic, 5-Alpha reductase inhibitor <sup>(14)</sup>
8.	22.140	Oleic Acid	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282	0.81	Antitumor, anticancer <sup>(15)</sup>
9.	22.701	n-Hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256	12.19	Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic, 5-Alpha reductase inhibitor <sup>(16,17)</sup>
10.	24.890	9,12-Octadecadienoic acid, methyl ester	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294	0.55	
11.	25.024	9-OCTADECENOIC ACID (Z)-, METHYL ESTER	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296	3.35	Antifungal Antioxidant, anticancer <sup>(18)</sup>

12.	25.451	OCTADECANOIC ACID, METHYL ESTER	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	298	0.10	Potent Antifungal, antimicrobial, antibacterial and at low PH. <sup>(19)</sup>
13.	26.351	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	278	61.97	Antifungal, anti retroviral, anti tumor, <b>anti diabetic</b> anti cancer, antioxi antioxidant, anti scabies anti inflammatory, potent antimicrobial agent <sup>(20,21)</sup>
14.	26.549	Octadecanoic acid	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284	3.32	Cancer preventive Insectifuge <sup>(22)</sup>
15.	26.779	4,7-Octadecadienoic acid, methyl ester	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294	0.23	
16.	27.360	3,6-OCTADECADIENOIC ACID, METHYL ESTER	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294	0.49	
17.	28.578	2-Nonenal, 2-pentyl-	C <sub>14</sub> H <sub>26</sub> O	210	0.46	
18.	28.846	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	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 <sub>17</sub> H <sub>29</sub> NO <sub>3</sub>	295	0.58	
20.	29.210	2H-Pyran, 2-(2-heptadecyloxy)tetrahydro-	C <sub>22</sub> H <sub>40</sub> O <sub>2</sub>	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 <sub>17</sub> H <sub>29</sub> NO <sub>3</sub>	295	2.18	
22.	29.504	9-Octadecenal, (Z)-	C <sub>18</sub> H <sub>34</sub> O	226	2.66	anti-aloptic, Antitumour, Cholerectic, Dermatigenic, immunostimulant, Anti leukotriene, anti- androgenic, Haemolytic, Hypercholesterolemic, <sup>(23)</sup>
23.	30.182	Cyclohexane, 1,1'-dodecylidenebis[4-methyl-	C <sub>26</sub> H <sub>50</sub>	362	0.93	
24.	30.308	1-Cyclohexanol, 4-tert.butyl-1-methyl-	C <sub>11</sub> H <sub>22</sub> O	170	0.74	
25.	30.870	CYCLOHEXANONE OXIME	C <sub>6</sub> H <sub>10</sub> DNO	114	0.54	
26.	31.414	Z-5-Methyl-6-heneicosen-11-one	C <sub>22</sub> H <sub>42</sub> O	322	0.54	
27.	32.519	1,2-BENZENEDICARBOXYLIC ACID	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	390	0.29	Antimicrobial Antifouling <sup>(12)</sup>
28.	35.208	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	C <sub>21</sub> H <sub>40</sub> O <sub>4</sub>	356	0.66	
29.	36.299	6-Octadecenoic acid, (Z)-	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282	0.25	
30.	49.316	Biphenylene, 1,2,3,6,7,8,8a,8b-octahydro-4,5-dimethyl-	C <sub>14</sub> H <sub>20</sub>	188	0.37	
31.	50.026	Betulin	C <sub>30</sub> H <sub>50</sub> O <sub>2</sub>	442	0.44	Wound healing activity, cholesterol lowering action, anti-inflammatory, cholerectic and liver-protecting activity <sup>(25)</sup> hypolipidemic properties

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