Invitro Alpha Amylase and Alpha Glucosidase Inhibitory Activity of Garcinia Gummi-Gutta Leaves and Fruit Rind Extracts

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ABSTRACT
The present study was to provide an in-vitro evidence for the potential inhibitory activity of extracts and fractions of Garcinia gummi-gutta on α-amylase and α-glucosidase enzymes. The G.gummi-gutta leaves and fruit rind extracts were prepared utilising Soxhlation techniques (pet ether, choroform, ethyl acetae, acetone, ethanol as solvents). Subsequently, the combined extracts were subjected for fractionation. Different concentrations (10,20,40,60,80,100 mg/ml) of various extract of solvents and fractions were subjected to α-amylase and α-glucosidase inhibitory assay. The absorbance was measured at 540 nm using multiplate reader and the percentage of α-amylase and α-glucosidase inhibitory activity and IC50 values of extract and fractions were evaluated. In the case of leaf extract of garcina gummigutta ethylacetate showed better alpha amylase activity with IC50 values GLEA 9.81±1.17 and alpha glucosidase inhibitory with IC50 value 18.54±1.36 with respect to acarbose (IC50 value of 4.50±0.70), where in case of fruit rind extract ethanol extract has shown better inhibition on both alpha amylase with IC50 value 11.45±1.11 and alpha glucosidase inhibitory activity with IC50 value 16.48±1.69 which was comparable with acarbose (8.80±0.41 μg/ml).

KEY WORDS: α- amylose, α-glucosidase, G.gummi-gutta type 2 diabetes

I. INTRODUCTION
The term ‘medicinal plant’ refers to plants that have therapeutic value. The plants exhibits a valuable source of compounds which can be used to design drug synthesis. The parts of medicinal plants that may be used include seeds, root, leaf, fruit, skin, flowers or even the whole plant. The bioactive compounds on the whole of the medicinal herbs must have collateral pharmacological effects and hence used as medicinal preparation. They exert substantial effects on the living beings. Plant-based compounds can significantly improve hard-to-treat illnesses or prevent the build up of specific conditions such as cancer, diabetes, neurodegenerative diseases [1,2]. Phytochemicals, the major active ingredients that possess therapeutic prospects which might be treated as a medicine or drug. Plant-metabolites are mainly organic compounds which perhaps classified into primary and secondary metabolites. Organic compounds in the primary metabolites include lipids, polysaccharide, protein, starch glucose, and nucleic acid which are beneficial for development and growth of the human body. Alkaloids, terpenoids, flavonoids, saponins, steroids, tannins, glycosides, volatile oils etc, are the secondary metabolites synthesized by plants. The therapeutic effectiveness of plants in place of these secondary natural products for healing many diseases.[1]

Diabetes mellitus is rising to an alarming epidemic level. It is probably one of the oldest diseases which were first reported in Egyptian manuscript about 3000 years ago. Diabetes mellitus is group of metabolic diseases with chronic hyperglycemia resulting from defective insulin release, effect, or both. Management is glucocentric. Many metabolic imbalance along with other complications (premature cardiovascular illnesses, renal illnesses, retinopathy and neuropathy) may be there[2].

Garcinia gummigutta also known as Kodampuli belonging to the family Clusiacea which has been utilised for the present study. Here the leaves as well as the fruit rind has been encountered for their invitro diabetic screening[1].

II. MATERIALS AND METHOD
Chemicals
α-amylase enzyme, α-glucosidase and 3,5-dinitro salicylic acid purchased from Sigma aldrich, Bangalore. P-nitro-phenyl- α-D-glucopyranoside(p-NPG), Sodium carbonate(Na2CO3), Sodium carbonate(Na2CO3),
Plant material

Leaves and fruit rind of Garcinia gummigutta were collected from the month of April-May. They were authenticated from the University of Kerala, Karyavattom campus Voucher no: KUBH 10884. The required materials were washed in distilled water and shade dried in case of leaves and sundried in case of fruit rind.[6]

Extraction

Extraction was carried out using Soxhlet apparatus with solvents were selected based on polarity. Petroleum, Chloroform, Ethylacetate, Acetone and Ethanol were selected as solvent system. The extract obtained was dried using rotatory evaporator and the yield was calculated.[6]

Invitro Assay

\[
\text{Inhibition (\%)} = \frac{\text{control-test}}{\text{control}} \times 100
\]

A suitable reagent blank and controls were also carried out at the same time. All of the tests were done thrice.

Inhibition of Alpha Glucosidase Activity

The test extract solution (20 µl in DMSO) was reacted with 20µl of α-glucosidase enzyme in 2 mM phosphate buffer (pH-6.9) in a 96 well microtiter plate (Kenjiro Tadeja K. et al., 2006, Nilufer Orhan N et al., 2013, with modifications). The substrate solution (20 ul of 10mM PNGP solution) added after 15 minutes of incubation at about 37 °C, mixed, and allowed to react for another 30 minutes at 37 °C. A micro titer plate reader was used to compared the increase in absorbance at 405 nm caused by PNPG hydrolysis by α-glucosidase to the equivalent blank. The control was carried out in the same way as the test but without the test extract. In a similar approach, positive control was carried out using acarbose, at the same time.[3,6] All of the tests were done in triplicate. The enzyme's percent inhibition computed as follows:

\[
\% \text{ inhibition} = \frac{\text{Absorbance (control) -Absorbance (test)}}{\text{Absorbance (control)}} \times 100
\]

III. RESULTS

In vitro alpha glucosidase inhibition

The α-glucosidase of S. cerevisiae is used to investigate the inhibitory activity of the crude extracts. Alpha-glucosidase inhibitory activity of crude extracts of G. gummigutta was determined using α-nitrophenyl- α -D-glucopyranoside (p-NPG) as a substrate and these were compared with Acarbose[3].

Invitro alpha glucosidase Inhibitory activity of leaves of G.gummigutta Leaves

Table 1 IC₅₀ values of α-glucosidase and α amylase inhibitory activity of leaves extracts of G. gummigutta and Acarbose

<table>
<thead>
<tr>
<th>Extracts of Garcinia gummigutta</th>
<th>IC₅₀ value of α amylase</th>
<th>IC₅₀ value of α glucosidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACARBOSE</td>
<td>4.50±0.70</td>
<td>8.807±0.41</td>
</tr>
</tbody>
</table>

Among five different extracts G. gummi-gutta leaves, ethyl acetate extract was found to be more active due to its low IC$_{50}$ value, followed by ethanol and acetone extract which was 18.54±1.36 µg/ml, 31.92±0.24 µg/ml and 50.14±3.02 µg/ml respectively (Table.1).[9] It was observed that the percentage inhibitory activity for alpha glucosidase enzyme was high for ethyl acetate (78.49±1.167 %) at its highest concentration (100 µg/ml). Acarbose showed 84.28±1.81 % inhibition at highest concentration and its IC$_{50}$ value was found to be 8.807±0.41 µg/ml. The percentage inhibitory activity of chloroform and pet ether extract were 59.9±1.49 % and 53.98±1.25 % at 100 µg/ml (Figure.1). When compared to Acarbose, there was significant difference in IC$_{50}$ values of different extracts of G.gummi-gutta. The IC$_{50}$ value of different extracts of G. gummi-gutta leaves and Acarbose was found in the order: Acarbose<ethyl acetate<ethanol<acetone< chloroform < pet ether (Figure .1).

<table>
<thead>
<tr>
<th>Extract</th>
<th>IC50 Value (µg/ml)</th>
<th>Percentage Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLPE</td>
<td>46.68±1.37</td>
<td>92.22±3.28</td>
</tr>
<tr>
<td>GLCF</td>
<td>25.76±2.60</td>
<td>67.79±2.96</td>
</tr>
<tr>
<td>GLEA</td>
<td>9.81±1.17</td>
<td>18.54±1.36</td>
</tr>
<tr>
<td>GLAC</td>
<td>20.02±4.46</td>
<td>50.14±3.02</td>
</tr>
<tr>
<td>GLET</td>
<td>15.56±1.27</td>
<td>31.92±0.24</td>
</tr>
</tbody>
</table>

Figure.1 The percentage inhibition α-glucosidase enzyme by different extracts of G. gummi-gutta leaves. The average of % inhibition is presented. Data are mean±SEM (n=3). AB: Acarbose; GLPE: G. gummi-gutta leaves pet ether extract; GLCF: G. gummi-gutta leaves chloroform extract; GLEA: G. gummi-gutta leaves ethyl acetate extract; GLAC: G. gummi-gutta leaves acetone extract; GLET: G. gummi-gutta leaves ethanol extract.
Among five different extracts G. gummigutta leaves, ethyl acetate extract was found to be more active due to its low IC\textsubscript{50} value, followed by ethanol and acetone extract which was 18.54±1.36 µg/ml, 31.92±0.24 µg/ml and 50.14±3.02 µg/ml respectively (Table.1). It was observed that the percentage inhibitory activity for alpha glucosidase enzyme was high for ethyl acetate (78.49±1.167 %) at its highest concentration (100 µg/ml). Acarbose showed 84.28±1.81 % inhibition at highest concentration and its IC\textsubscript{50} value was found to be 8.807±0.41 µg/ml. The percentage inhibitory activity of chloroform and pet ether extract were 59.9±1.49 % and 53.98±1.25 % at 100 µg/ml (Figure.1). When compared to Acarbose, there was significant difference in IC\textsubscript{50} values of different extracts of G.gummigutta. The IC\textsubscript{50} value of different extracts of G. gummigutta leaves and Acarbose was found in the order: Acarbose<ethyl acetate<ethanol<acetone< chloroform < pet ether (Figure.1).

Table 2: The values presented as mean ± SEM (n=3). ANOVA One way with Dunnett’s multiple comparison test was conducted to evaluate statistical significance of IC\textsubscript{50} value. * represents p<0.05, ** represents p<0.01, *** represents p<0.001 vs Acarbose. AB: Acarbose; GLPE: G. gummigutta leaves pet ether extract; GLCF: G. gummigutta leaves chloroform extract; GLEA: G. gummigutta leaves ethyl acetate extract; GLAC: G. gummigutta leaves acetone extract; GLET: G. gummigutta leaves ethanol extract.

![Figure 2](image-url)
ALPHA AMYLASE ENZYME INHIBITION OF G. gummi-gutta

α-amylase is the carbohydrate hydrolysing enzyme involved in hydrolysis of α-linked polysaccharides.[7] This enzyme begins the process of carbohydrate digestion by breaking the 1,4-glycosidic linkages of polysaccharides to disaccharides such as maltose which is mainly responsible for post prandial hyperglycemia. [10]

Thus inhibitors of α-amylase may be useful in the control of hyperglycemia which delays carbohydrate digestion and thereby reduce the post prandial plasma glucose level.

The leaves extracts of G. gummi-gutta inhibited α-amylase activity in a dose-dependent manner. Ethyl acetate extract G. gummi-gutta leaves exhibited more antioxidant property due to its low IC₅₀ value, followed by ethanol and acetone extract which was 9.81±1.17 µg/ml, 15.58±1.27 µg/ml and 20.02±4.46 µg/ml respectively (Table.1). It was observed that the percentage inhibitory activity for alpha amylase enzyme was high for ethyl acetate (95.67±0.88 %) at its highest concentration (100 µg/ml). Acarbose showed 94.51±1.94 % inhibition at highest concentration and its IC₅₀ value was found to be 4.50±0.70 µg/ml. The percentage inhibitory activity of chloroform and pet ether extract were 88.87±1.32 % and 85.67±2.02 % at 100 µg/ml (Figure.3 ). When compared to Acarbose, there was significant difference in IC₅₀ values of different extracts of G. gummi-gutta expect for GLEA. The IC₅₀ value of different extracts of G. gummi-gutta leaves and Acarbose was found in the order: AB<GLPE<GLCF<GLET<GLAC<GLCH<GLAC. The IC₅₀ value of different extracts for inhibiting the α-amylase activity is represented in Figure 4.

![Figure 4](image-url)
Figure 5 The values are mean of three replicates ± SEM. Data were analyzed by one-way ANOVA regulated utilising Dunnett’s collective analogy test. * represents p<0.05, ** represents p<0.01, *** represents p<0.001 when compared to Acarbose. AB: Acarbose; GLPE: G. gummi-gutta leaves pet ether extract; GLCF: G. gummi-gutta leaves chloroform extract; GLEA: G. gummi-gutta leaves ethyl acetate extract; GLAC: G. gummi-gutta leaves acetone extract; GLET: G. gummi-gutta leaves ethanol extract.

IV. DISCUSSION

The low IC$_{50}$ value in ethyl acetate extract indicated the presence of chemical compounds in it which can inhibit alpha glucosidase. There was no literature regarding the evaluation of alpha glucosidase inhibitory activity on G. cambogia. So we gathered information on other species of Gracina. Ryu H W et al (2011) demonstrated alpha glucosidase inhibitory activity (IC$_{50}$ 3.2 µg/ml) of ethanol extract of the fruit case of Garcinia mangostena whose most abundant chemical constituents were xanthones. Research work carried out by Ngoupayo J et al (2008) isolated four new depsidones named brevipsidones A—D from Garcinia brevipedicellata which were alpha glucosidase inhibitors. Mahayasih et al (2017) evaluated alpha glucosidase inhibitory activity on active fractions of ethyl acetate and methanol extracts of Garcinia lateriflora Blume leaves. The study indicated that fraction 13 of ethyl acetate extract and fraction 10 of methanol extract showed the highest percent inhibition compared with the other fraction with IC$_{50}$ values 8.96 µg/ml and 18.52 µg/ml respectively. These reports were in line with our results which indicated significant alpha glucosidase inhibitory activity for ethyl acetate extract when compared with other extracts of G. cambogia. Anti-diabetic activity was determined in terms of inhibition of alpha-glucosidase. Inhibition of alpha-glucosidase was revealed delay in the degradation of carbohydrates. Therefore, alpha-glucosidase inhibitors have been used as therapeutic agents for the management of type 2 diabetes.[8]

In vitro alpha glucosidase inhibitory activity of fruit rind extract of Garcinia gummi-gutta

In various extracts of G. gummi-gutta fruit, ethanol (GFET) extract was found to be more active due to its low IC$_{50}$ value, followed by ethyl acetate and acetone extract which was 16.48±1.69 µg/ml, 19.03±0.93 µg/ml and 21.61±1.47 µg/ml respectively (Table 2). It was observed that the percentage inhibitory activity for alpha glucosidase enzyme was high for ethyl acetate (95.01±0.87 %) at its highest concentration (100 µg/ml). The percentage inhibitory activity of chloroform and pet ether extract were 88.60±3.90 % and 91.21 ± 2.67 % at 100 µg/ml. When compared to Acarbose, there was significant difference in IC$_{50}$ values of different extracts of G. cambogia. The IC$_{50}$ value of different extracts of G. gummi-gutta fruit and Acarbose was found in the order: AB<GFET<GFPEA<GFAC<GFCH<GFPE (Figure 5).[9,10]
Figure 5. The percentage inhibition α-glucosidase enzyme by different extracts of G. cambogia fruits. The average of % inhibition is presented. Data are mean±SEM (n=3). AB: Acarbose; GFPE: G. cambogia fruits pet ether extract; GFCF: G. cambogia fruits chloroform extract; GFEA: G. cambogia fruits ethyl acetate extract; GFAC: G. cambogia fruits acetone extract; GFET: G. cambogia fruits ethanol extract.

Figure 6. The values are mean of three replicates ± SEM. Statistics analyzed for ANOVA one-way regulated utilising Dunnett’s collective analogy test. * represents p<0.05, ** represents p<0.01, *** represents p<0.001 when compared to Acarbose. AB: Acarbose; GFPE: G. gummigutta fruits pet ether extract; GFCF: G. gummigutta fruits chloroform extract; GFEA: G. gummigutta fruits ethyl acetate extract; GFAC: G. gummigutta fruits acetone extract; GFET: G. gummigutta fruits ethanol extract.

In various extracts of G. gummigutta fruit, ethanol (GFET) extract was found to be more active due to its low IC_{50} value, followed by ethyl acetate and acetone extract which was 11.45±1.11 µg/ml, 18.88±0.42 µg/ml and 26.73±1.93 µg/ml respectively (Table 2). It was observed that the
percentage inhibitory activity for alpha amylase enzyme was high for ethanol (94.07±0.57 %) at its highest concentration (100 µg/ml). The percentage inhibitory activity of chloroform and pet ether extract were 92.58±2.45 % and 85.61 ± 0.98 % at 100 µg/ml (Figure.7). When compared to Acarbose, different extracts of G. gummi-gutta demonstrated no significant difference in IC₅₀ values, except for GFPE (p<0.001). [17]. The IC₅₀ value of different extracts of G. gummi-gutta fruit and Acarbose was found in the order: AB<GFET<GFEA<GFAC<GFCH<GFPE (Figure.7,8).

Figure 7. The percentage inhibition α-amylase enzyme by different extracts of G. gummi-gutta fruits. The average of % inhibition is presented. Data are mean±SEM (n=3). AB: Acarbose; GFPE: G. gummi-gutta fruits pet ether extract; GFCF: G. gummi-gutta fruits chloroform extract; GFEA: G. gummi-gutta fruits ethyl acetate extract; GFAC: G. gummi-gutta fruits acetone extract; GFET: G. gummi-gutta fruits ethanol extract.

Fig 8: The values are mean of three replicates ± SEM. Data were analyzed by one-way ANOVA regulated utilising Dunnett’s collective analogy test. * represents p<0.05, ** represents p<0.01, *** represents p<0.001 when compared to Acarbose. AB: Acarbose; GFPE: G. gummi-gutta fruits pet ether extract; GFCF: G. gummi-gutta fruits chloroform extract; GFEA: G. gummi-gutta fruits ethyl acetate extract; GFAC: G. gummi-gutta fruits acetone extract; GFET: G. gummi-gutta fruits ethanol extract.

The α-amylase and α-glucosidase inhibitors play a significant role in regular blood sugar in clinics. Cumulative studies have indicated that some of Garcinia plants e.g. G. cambogia [4], G. xanthochymus [5], G. kola [6], and G. mangostana [7, 8], G. pedunculata [9], G. prainiana [10] contain plenty of biflavonoid and phenolic compounds. These compounds have been found to

inhibit the enzyme activity of α-amylase and α-glucosidase to perform the anti-diabetic effect. Thus, our results illustrated that G. gummi-gutta extracts had significant potential to manage blood sugar[16,17,18].

V. CONCLUSION

The outcome of the current evaluation expresses the fact that, of the five different extracts of Garcinia gummi-gutta leaves, ethylacetate fraction has shown better activity of IC50 values 9.81±1.17 μg/ml for alpha amylase and 11.45±1.11 μg/ml for alpha amylase inhibition when compared to Aarbose 4.50±0.70 μg/ml standard and among the fruit rind extract ethanol extract 18.54 ± 1.36 μg/ml and 16.48±1.69 μg/ml was shown better activity when compared with the standard drug acarbose8.867±0.41. Studies have shown that fruit rind extract contains hydroxyxeric acid and leaves with flavanoid may be the factor effective α-amylase and α-glucosidase inhibitors, which may be helpful to reduce the postprandial glucose levels. Hence, further studies may throw light on the antidiabetic potential of G. gummi-gutta fruit rind alongwith leafy extract especially for management of type 2 diabetes.

REFERENCES


