Research Article

Coccinia grandis Extract Exerts Antihyperglycemic Effect through its Antioxidant, α-Amylase and α-Glucosidase Inhibitory Activities: *An in vitro* Study

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ABSTRACT

An increasing demand for herbal medicines has shifted the attention from synthetic to natural antioxidants and antidiabetic agents. In this study, the edible plant *Coccinia grandis* fruits (CGF) were assessed for radical scavenging and antioxidant potentials based on NO (nitric oxide) radical scavenging activity, $ABTS^{\cdot}$ [(2,2'-Azino-bis(3-ethylbenzothiazoline- 6-sulfonic acid) diammonium salt] and FRAP- Ferric reducing antioxidant power. *In vitro* α-amylase and α-glucosidase inhibitory activities were evaluated by using porcine pancreatic α-amylase and yeast α-glucosidase, respectively. The results from free radical scavenging and antioxidant tests clearly indicated the concentration dependent free radical scavenging and antioxidant activities. The extract exhibited the best inhibitory activities against α-amylase and α-glucosidase with IC_{50} (half maximal inhibitory concentration) values of 117.64± 4.54 µg/mL and 81.6 ± 3.64 µg/mL, respectively. These data suggest that the possible mechanisms underlying antihyperglycemic activity of *C. grandis* extract, presumably by its radical scavenging/antioxidant activities and through inhibition of α- amylase and α-glucosidase. This study also provides scientific evidence to corroborate the efficacy of this plant as an alternative antidiabetic agent.

Key words: Diabetes mellitus, *Coccinia grandis*, Antioxidant, α-glucosidase, α-amylase.

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INTRODUCTION

The total predicted increase in numbers of people with diabetes from 2012 to 2030 is about 180 million (371.33 million to 551.87 million, respectively), an astonishing increase of 48% from 2012 at an annual growth of 2.7%, which is twice the annual growth of the total world adult population. Forty-two percent of the anticipated absolute global increase of 180 million people with diabetes is projected to occur in India and China alone. According to International Diabetes Federation (IDF), nearly 5 million people died due to diabetes and its related complications, in 2012.[1] Therefore, new concepts in the management of diabetes have aroused a curiosity among researchers as well as among the patients throughout the world.

In countries such as India and China, use of herbal medicines is a very common practice from ancient time, and herbal medicines are considered to be much safer and less expensive therapeutic strategies for the treatment of various diseases. A proper scientific investigation of traditional herbal remedies, in particular, can provide valuable leads for the development of alternative drugs and strategies for the management of diabetes.[2] Role of herbs in the management and control of diabetes has emerged fast over the years with the discovery

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Kingdom: Plantae
Order : Cucurbitales
Family : Cucurbitaceae
Genus : *Coccinia*
Species: *C. grandis* (L) J. Voigt

Figure 1: (A) Mature unripe whole fruits. (B) Small slices, air dried under shade and (C) Pulverized to fine powder of *Coccinia grandis*.[26]

of hypoglycemic effect of Bitter Melon (*Mormodica charantia*).[3,4]

The search for new pharmacologically active agents obtained by screening natural sources, such as medicinal plants or their extracts can lead to potent and specific inhibitors for amylase and α-glucosidase.[5] The use of α-glucosidase inhibitors, such as acarbose that can also inhibit pancreatic α-amylase revealed that the complications of DM, such as onset of renal, retinal and neurological changes and the development of ischemic diseases are prevented or delayed.[6] Long-term day-to-day management of diabetes, with acarbose is well tolerated and can improve glycaemic control as monotherapy, as well as in combination therapy.[7] Natural inhibitors of carbohydrate degrading enzymes (α-amylase and α-glucosidase) especially from plant sources offer an attractive strategy for the control of postprandial hyperglycemia.

Coccinia grandis (L.) Voigt, the ivy gourd, also known as baby watermelon. In traditional medicine, fruits have been used to treat leprosy, fever, asthma, bronchitis, and jaundice. The fruit possesses mast cell-stabilizing, antianaphylactic, and antihistaminic potential.[8] In Bangladesh, the roots are used to treat osteoarthritis and joint pain. A paste made of leaves is applied to the skin to treat scabies.[9] Scientific investigations have supported the efficacy of leaf extracts in amelioration of diabetic conditions.[10] The juice of the roots and leaves is used to treat diabetes, and the aqueous and ethanolic extracts of the plant exhibit hypoglycemic action.[11] The leaves of *C. grandis* have been shown to stimulate insulin secretion in diabetic rats.[12]

However, the scientific record on the antioxidant and antidiabetic efficacy of unripe mature fruits of *C. grandis* is very poor and scarce. Hence, the present study was aimed to evaluate the antioxidant effect and α-amylase and α-glucosidase inhibitory activities of its fruits *in vitro*, which has not been reported despite its traditional use in diabetes.

MATERIALS AND METHODS

Chemicals and reagents

Porcine pancreatic α-amylase was obtained from HiMedia Laboratories Mumbai, India. Acarbose was obtained from Bayer Pharmaceuticals Pvt. Ltd. (Mumbai, India). Yeast α-glucosidase, *p*-nitrophenyl α-D-glucopyranoside, ABTS-2,2'-Azino-bis(3-Ethylbenzthiazoline-6-Sulfonate) as sulfonic acid, Trolox [(+/-)–6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid] and DNSA (dinitrosalicylic acid) were obtained from Sigma-Aldrich (Bangalore, Karnataka, India). All other chemicals and solvents were of analytical reagent grade. Water was purified by Milli Q Water Purification System, Millipore, USA.

Plant materials and extraction

Coccinia grandis (L.) Voigt mature unripe whole fruits were collected from the Kancheepuram District, Tamil Nadu State, India in the month of December 2019. The pharmacognostic authentication was done by Siddha Central Research Institute, Central Council for Research in Siddha, Department of AYUSH, Ministry of Health and Family Welfare, Government of India, Chennai-600 106. The fruits were cut into small slices, air dried under shade, pulverized to fine powder (shown in Figure 1) using a laboratory scale cutting mill and extracted with ethanol at 35°C for 24 hrs. The extract was filtered, centrifuged and the residue was subjected to subsequent extraction with the same solvent and stored at -20°C.

Nitric oxide (NO) radical scavenging activity

Nitric oxide (NO) generated from sodium nitroprusside was measured by the Griess reaction.[13] Sodium nitroprusside (5 μM) in phosphate-buffered saline was mixed with 3 mL of different concentrations (12.5-250 μg/mL) of CGF extract and the mixture was incubated at 25°C for 150 min. The samples were then allowed to react with the Griess reagent (1% sulfanilamide and 0.1% naphthyl ethylenediamine dihydrochloride in 2% H_3PO_4). The absorbance of the chromophore during the diazotization of nitrite with sulfanilamide and subsequent coupling with naphthyl ethylenediamine was measured at 546 nm. A similar procedure was repeated with respective solvent instead of the extract, which served as the control; L-ascorbic acid (12.5- 250 μg/mL) was used as the positive control. All the tests were performed in triplicate. The percentage of scavenging activity was measured using the formula:

% Inhibition = (Absorbance of Control- Absorbance of Test)/ Absorbance of Control × 100

Antioxidant assay using ABTS [(2,2'-Azino-bis(3-ethylbenzothiazoline- 6-sulfonic acid) diammonium salt]

In this method, the radical scavenging capacity was measured by using $ABTS^{\bullet+}$ solution radical cation. The assay was performed according to the method described by Thaipong *et al*., (2006) and Gan *et al*., (2010).[14,15] The stock solution of $ABTS^{\bullet+}$ was prepared by mixing 7.4 mM ABTS solution and 2.6 mM potassium per sulfate solution in the ratio of 1:1 and allowed to react for 12 h at room temperature in the dark. The $ABTS^{\bullet+}$ working solution was prepared by diluting the stock solution (3 mL stock solution in 100 mL volumetric flask, diluting it to the mark with methanol) to get the absorbance of 1.1±0.05 unit at 734 nm using a UV–visible spectrophotometer. A series of standard were prepared in the range of 0−250 μg/mL. Standard solutions (150 μL) and sample extract (150 μL) were placed in different test tubes then ABTS working solution (2850 μL) was added to each test tube. These tubes were kept in the dark for 30 min. After that their absorbance was taken at 734 nm. The % inhibition of both standard and samples were calculated and the concentration of ABTS content in the extract was reported as mg of trolox equivalent (TE)/g extract.

Antioxidant assay using ferric reducing antioxidant power (FRAP)

For FRAP assay, Fresh FRAP reagent was prepared by mixing 300 mM acetate buffer (100 mL), 10 mM TPTZ (2,4,6-tri(2-pyridyl)−s-triazine) solution (10 mL) and 20 mM $FeCl_3.6H_2O$ (10 mL) solution and kept warmed at 37°C until used in experiment. 300 mM acetate buffer pH 3.6 was prepared by dissolving sodium acetate trihydrate (3.1 g) in distilled water (500 mL) then glacial acetic acid (16 mL) was added and made up to the mark of 1 L with distilled water and checked for its pH. 10 mM TPTZ solution was prepared in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ was prepared in distilled water. Trolox was used as the standard and a calibration curve in the range of 0–250 μg/mL was prepared.[14,15] Standard solution (150 μL) and sample extract (150 μL) were allowed to react with FRAP solution (2850 μL) in different test tubes for 30 min in the dark. Reading of the colored solution (ferrous tripyridyltriazine complex) of standard and sample was taken at 593 nm. The concentration of FRAP content in the extract was reported as mg trolox equivalent (TE)/g extract.

Porcine pancreatic amylase inhibition assay

Chromogenic 3,5-dinitrosalicylic acid (DNSA) assay was employed to assess the α-amylase activity as reported earlier.[16] CGF Extract (25−200 μg/mL) was incubated with 50 μg/mL of porcine pancreatic α-amylase at 37°C for 10 min.[17] One percent starch was used as a substrate. α-amylase without extract was used as control. Acarbose was used as a reference standard. Reducing sugar was estimated using DNSA assay at A 540 nm and the inhibitory activity was calculated by using the formula:

$$\% \text{ Inhibition} = (A540_{Control} - A540_{Test})/ A540_{Control} \times 100$$

In vitro α-glucosidase inhibitory assay

α-glucosidase inhibitory activity was determined according to Kumar *et al*., (2010) and Gowri *et al*., (2007) with slight modifications.[18,19] In brief, 50 μL of test sample (25-250 μg/mL in phosphate buffer, pH 6.8) was reconstituted in 100 μL of 100 mM phosphate buffer, pH 6.8 and incubated with 50 μL yeast α-glucosidase (0.25 U/mL in phosphate buffer) for 15 min at 37°C before 50 μL of substrate (5 mM, *p*-nitrophenyl α-D-glucopyranoside, in phosphate buffer) was added and then incubated for 15 min at 37°C. The reaction was stopped by adding 1 mL of Na_2CO_3 (0.1 M). Release of *p*-nitrophenol was measured at 405 nm by spectrophotometer (T60U, PG Instruments Limited, United Kingdom). Individual blank for test sample was prepared by replacing substrate with 50 μL of the buffer. The control sample was prepared in similar manner but using 50 μL of buffer in place of test sample. Acarbose was used as a reference standard in all the experiments. All the tests were run in triplicate. The percentage of enzyme inhibition was calculated by using the formula:

$$\% \text{ Inhibition} = (A405_{Control} - A405_{Test})/ A405_{Control} \times 100$$

The IC_{50} (half maximal inhibitory concentration) values were determined by regression analysis of the data for at least five concentrations of sample.

Data analysis

All the results were expressed as mean ± standard deviation. Linear regression analysis was used to calculate the IC_{50} values. Data were analyzed using one-way analysis of variance (ANOVA) followed by the Dunnett post hoc test using GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA, USA). Differences were considered to be statistically different when p value was < 0.05.

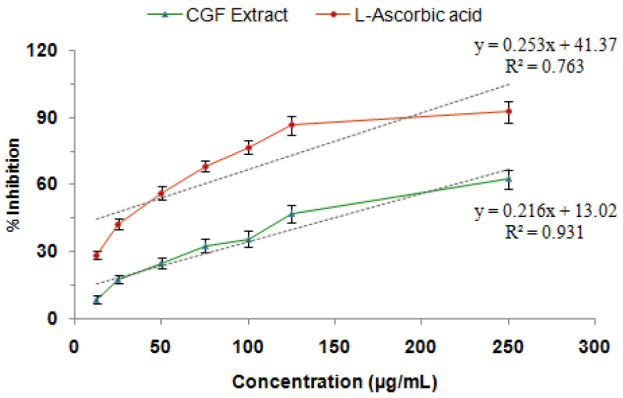

Figure 2: Nitric oxide (NO) scavenging activity of extract of CGF at different concentrations. Ascorbic acid was used as a reference antioxidant. Values are expressed as mean ± standard deviation (*n* = 3).

RESULTS

Nitric oxide (NO) scavenging activity

Results related to the NO radical scavenging activity of CGF extracts and positive control showed significant NO radical scavenging activity in a concentration-dependent manner (Figure 2). In this assay, the ethanol extract caused a potential inhibition with IC_{50} value of 0.15 mg/mL. The IC_{50} value of positive control ascorbic acid was found to be 0.04 mg/mL.

Antioxidant assays

The antioxidant ability of medicinal plants is associated with its medicinal values. In this study, the antioxidant activity of CGF was measured using two different assays, namely FRAP and ABTS. Ferric reducing antioxidant power (FRAP) assay depends on the reduction of ferric ion into ferrous ion.[20] ABTS assay depends on the antioxidant compound ability to scavenge ABTS radical. By this assay we can measure antioxidant capacity of lipophilic and hydrophilic compounds in the same sample. Both the assays are very simple, inexpensive and usually employed methods for the determination of antioxidant activity and can give reproducible results. In the present study, the antioxidant activity of CGF was found to be 53.67± 2.24 mg trolox equivalent (TE)/g extract using ABTS method and that of FRAP was found to be 48.25± 1.87 mg trolox equivalent (TE)/g extract (Table 1).

Porcine pancreatic amylase inhibition assay

The CGF extract was found to show maximum inhibition up to 62.21 ± 3.88% against porcine pancreatic a-amylase at the concentration of 250 µg/mL (Figure 3). The IC_{50} values of CGF and acarbose (positive control) were found to be 117.64± 4.54 µg/mL and 63.82± 2.17 µg/mL, respectively.

Table 1: Antioxidant activities of ethanol extract of CGF.

Method	Antioxidant capacity (mg TE/g)
ABTS	53.67± 2.24
FRAP	48.25± 1.87

Each value is expressed as mean ± SD from minimum of three independent experiments.
FRAP- Ferric reducing antioxidant power; ABTS- [(2,2′-Azino-bis(3-ethylbenzothiazoline- 6-sulfonic acid) diammonium salt]; TE- trolox equivalent

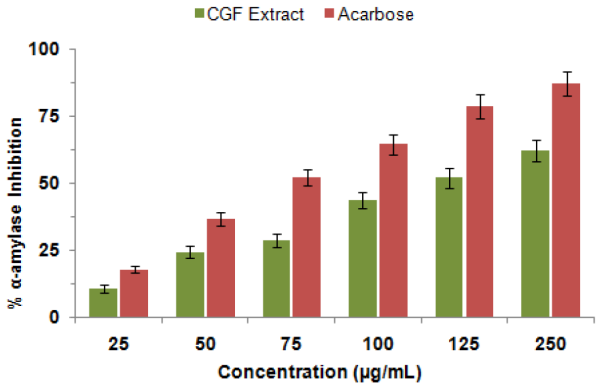

Figure 3: Inhibitory effect of CGF extract on pancreatic amylase activity
Values are expressed as mean ± standard deviation (*n* = 3).
Acarbose was used as positive control

In vitro α-Glucosidase inhibition assay

The CGF extract showed inhibitory activity against yeast α-glucosidase with the maximum inhibition of 67.64 ± 2.77 % at the concentration of 250 µg/mL, compared to 87.34 ± 4.64 % of acarbose at the same concentration. The IC_{50} of CGF extract was found to be 81.6 ± 3.64 µg/mL, whereas that of acarbose was 44.5 ± 2.34 µg/mL (Figure 4).

DISCUSSION

Diets biased towards plant-based components are known to provide a high amount of antioxidant phytochemicals, which offer protection against reactive oxygen species (ROS)- induced cellular damage.[21] Oxidation of biomolecules such as DNA, lipids and proteins by ROS play an important role in diabetes, cardiovascular disease, cancer, immune and inflammatory disorders and many other diseases related to the aging process.[22] This study has focused on the free radical scavenging, antioxidant capacities of CGF.

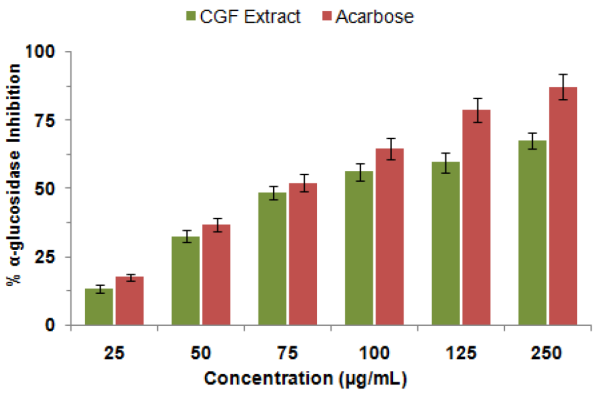

Figure 4: Inhibitory effect of CGF extract on α-glucosidase activity Values are expressed as mean ± standard deviation (*n* = 3). Acarbose was used as positive control

In the present study, ability of CGF to scavenge NO was analysed. The reactive oxygen species NO has been implicated in diabetes, inflammation, cancer, and other pathological conditions.[23] NO is a very unstable species under aerobic conditions.[24] The plant products have the property to counteract the effect of NO formation and, in turn, may be of considerable interest in relation to preventing the ill effects of excessive NO generation in the human body. Several studies have evaluated the relationship between the antioxidant activity of plant products and their phenolic content. Substances that are able to perform these reactions can be considered as antioxidants and radical scavengers.[25]

The trolox equivalent antioxidant capacity (TEAC) of CGF extract were measured using the ABTS and FRAP methods. The antioxidant activity of the extract was found to be 53.67±2.24 trolox equivalent (TE)/g for the ABTS method and 48.25± 1.87 mg trolox equivalent (TE)/g for the FRAP method. Based on NO radical scavenging and antioxidant activities (ABTS and FRAP) results, it can be inferred that the *C. grandis* has promising antioxidant capacity and novel drug candidate for diabetes mellitus. The radical scavenging and antioxidant activities of the CGF extract might be attributed to the presence of phenolic compounds and flavonoids.[26]

Potent inhibitors of mammalian α-amylase found in some vegetables and herbs have been known as effective antidiabetic treatment for diabetes. Therefore, this research investigated α-amylase inhibitory activity of CGF extract, since this enzyme is known as one of the key enzyme in human digestive system to degrade starch to monosaccharide's and cause the rise in blood glucose.[27] It is noteworthy that the selected plant with significant inhibitory activity (IC_{50} value 117.64 µg/mL, Figure 3) has been consistently used in traditional medicinal systems as antidiabetic treatments or consumed together with starch-based products as salads or incorporated into porridge.[28,29]

Uncontrolled hyperglycemia in diabetic patients is associated with profound complications, such as increased risk of coronary heart disease, peripheral vascular disease, and cerebrovascular disease.[30] The reduction of postprandial hyperglycemia has been approached by suppression of carbohydrate absorption from gastrointestinal tract.[31] Several plant extracts have been reported to exert antidiabetic property through α-glucosidase inhibition.[32-34] It was found in the present study that CGF extract also inhibited α-glucosidase *in vitro* with the IC_{50} of 81.6 µg/ml, whereas the IC_{50} of acarbose, an antidiabetic drug known to inhibit intestinal α-glucosidase, was found to be 44.5 µg/ml (Figure 4). Thus, from the results it can be inferred that the α-amylase and α-glucosidase inhibitory activities might contribute to the anti-hyperglycemic effect of the extract. The result also suggests that the extract may be clinically useful for the control of postprandial hyperglycemia.

CONCLUSION

From the aforementioned results, it can be concluded that mature unripe fruits of *Coccinia grandis* extract exerts antihyperglycemic activity via its free radical scavenging, antioxidant activities and inhibition of the enzymes, such as α-amylase and α-glucosidase. Further studies are required to isolate the active components from the extract and *in vivo* oral rodent efficacy studies in a disease model.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

ABBREVIATIONS

ABTS: [(2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt]; **CGF:** - *Coccinia grandis* fruits; **FRAP:** Ferric reducing antioxidant power; **IC_{50}:** half maximal inhibitory concentration.

SUMMARY

This study reports the antidiabetic efficacy of mature unripe fruits of an indigenous medicinal plant *Coccinia grandis*. The data suggest that the possible mechanisms underlying antihyperglycemic activity of *C. grandis* extract, presumably by its radical scavenging / antioxidant activities and through inhibition of α- amylase and α-glucosidase. Present findings provide experimental evidence that the fruits of *C. grandis* have potential antidiabetic activity which might be used as a functional food and safe remedy for the management and treatment of diabetes and associated complications. This study also revealed that the plant can be a promising source for development of natural novel antidiabetic drugs.

Authors' contributions

PM performed the experiments, analyzed/interpreted data and wrote the manuscript.

SM contributed to the concept, designed experiments, analyzed/interpreted data and finalized the manuscript.

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