CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.2605447

**Available online at: http://www.iajps.com** ***Research Article***

# ANTI-DIABETIC AND ANTIOXIDANT POTENTIAL OF *COCCINIA GRANDIS* LEAF EXTRACTS

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**Article Received:** January 2019 **Accepted:** February 2019 **Published:** March 2019

**Abstract:**

*The aim of the present study was to evaluate the anti-diabetic and antioxidant potential of Coccinia grandis leaf extract. Anti-diabetic activities were evaluated via α-amylase, α-glucosidase, Protein tyrosine phosphatase (PTP 1B), and dipeptidyl peptidase-IV (DPP-IV) enzyme inhibitory assays. In addition, scavenging assay using2,2-diphenyl-1-picrylhydrazyl (DPPH), peroxynitrite ($ONOO^-$), and total ROS was used to evaluate antioxidant potential. The MeOH extract of C. grandis exhibited the most potent inhibitory activities against α-amylase, α-glucosidase, PTP 1B and DPP-IV with the corresponding $IC_{50}$ values of 8.95 ± 0.30, 4.95 ± 0.02, 1.95 ± 0.03 and 19.81 ± 0.10 µg/mL, respectively, while the MeOH extract of C. grandis showed significant antioxidant effects in DPPH, $ONOO^-$and total ROSscavenging assay with the corresponding $IC_{50}$ values of 9.71 ± 0.16, 3.95 ± 0.21and 3.43 ± 0.13 µg/mL, respectively. These results support the traditional use of C. grandis in diabetic patients, especially for the control of postprandial plasma glucose level. However, further studies on identification of the active phytochemicals acting as enzyme inhibitors and in vivo carbohydrate-digesting enzyme inhibition are required to verify its potential clinical use in diabetes mellitus.*

**Keywords**: *C. grandis*; α-amylase; protein tyrosine phosphatase 1B; dipeptidyl peptidase-IV (DPP-IV); α-glucosidase.

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*Please cite this article in press Howlader Saurav et al.,* ***Anti-Diabetic And Antioxidant Potential Of Coccinia Grandis Leaf Extracts.,*** *Indo Am. J. P. Sci, 2019; 06(03).*

## INTRODUCTION:

Diabetes mellitus is an intensifying metabolic disorder associated with carbohydrate, fat and protein metabolism when the body does not produce adequate insulin or does not respond to the actual produced insulin, contributing to an increase of regarding blood glucose levels (hyperglycemia) and also triggering considerable and irreparable harm to body systems, for example blood vessels and nerves (Matsui *et al*., 2007). It is probably the escalating globally health issues at present leading to micro vascular (retinopathy, neuropathy and also nephropathy) and macro vascular (heart attack, stroke and peripheral vascular diseases) complications (Umar *et al*., 2010). The amount of diabetics increases in accordance with WHO approximation. It is envisioned that there are about 366 million people will tend to be diabetic throughout the world by the year 2030 (Wild *et al*., 2004). In Asia, the prevalence of diabetes is raising as an alarming rate and anticipated to increase 2-3 folds by 2030 (Shaw *et al*., 2010).

Hyperglycemia, a condition characterized by an abnormal postprandial enhance in the blood glucose level, has been linked to the onset of type 2 diabetes and also regarding oxidative dysfunction and failure of various organs, in particular the eyes, kidneys, nerves, heart, and blood vessels, possesses been shown to be likewise linked to hypertension (Haffner, 1998). Hyperglycemia-induced metabolic dysfunction may be caused by reactive oxygen species (ROS) produced in the mitochondrial electron transport chain (Brownlee, 2005). ROS such as the superoxide anion radical ($O_2^-$) and hydroxyl radicals ($OH^-$) are physiological metabolites formed as a result of respiration in aerobic organisms but their excessive levels have been linked to the onset of diseases such as cancer, stroke and diabetes (Niedowicz and Daleke, 2005). Therefore, the search for the discovery of antioxidant and antidiabetic agents from plant sources is an important strategy required to combat the widespread nature of this condition. This is because the present synthetic drugs have many drawbacks ranging from limited efficacy and several side effects such as hypoglycemia, weight gain and chronic tissue damage (Kane*et al*., 2005).

*C. grandis* L. Voigt. commonly known as “Ivy gourd” is a tropical plant belonging to the family Cucurbitaceae. It has been found in many countries in Asia and Africa. The roots, stems, leaves and whole plant of *C. grandis* are used in the treatment of jaundice, bronchitis, skin eruptions, burns, insect bites, fever, indigestion, nausea, eye infections, allergy, syphilis, gonorrhoea, etc. (Kirthikar and Basu, 1987; Umamaheswari and Chatterjee, 2007). The leaves of this species are widely used in folk medicine for reducing the amount of sugar in urine of patients suffering from diabetes mellitus. Literature suggests the use of this plant in the treatment of diabetes (Venkateswaran and Pari, 2003). The objective of the present study was to investigate the anti-diabetic and antioxidant activity of the different fractions of the methanolic extract of the leaves of *C. grandis* using *in vitro* models. Our data suggest that *C. grandis* may perhaps represent a source of new way to prevention or treatment associated with diabetes and oxidative damage-associated diseases.

## MATERIALS AND METHODS:

### Chemicals and Reagents

Yeast α-glucosidase, porcine pancreatic α-amylase, acarbose, p-nitrophenyl phosphate (pNPP), p-nitrophenyl α-D glucopyranoside (pNPG), 2,2-diphenyl-1-picrylhydrazyl (DPPH), L-ascorbic acid, L-penicillamine (L-2-amino-3-mercapto-3-methylbutanoic acid), diethylenetriaminepentaacetic acid (DTPA), and phenylmethylsulfonylfluoride (PMSF), DPPIV from porcine kidney, Gly-pro-p-nitroanilide were purchased from Sigma (St. Louis, MO, USA). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). PTP1B (human recombinant) was purchased from Biomol International LP (Plymouth Meeting, PA, USA). High quality dihydrorhodamine 123 (DHR 123) and 20,70-dichlorodihydrofluorescein diacetate (DCFH-DA) were purchased from Molecular Probes (Eugene, OR, USA), and $ONOO^-$ was purchased from Cayman Chemicals Co. (Ann Arbor, MI, USA). All chemicals and solvents used in the assays were of reagent grade, and were purchased from commercial sources.

### Plant Material

The plant sample of *C. grandis* leaves were collected in September, 2015 from Sylhet hill track, Bangladesh. The plant was identified by Bangladesh National Herbarium, Dhaka, where a voucher specimen has been deposited. Plants were then washed properly to remove dirty materials and air-dried for several days with occasional sun drying. These were then ground with a hammer grinder for better grinding. The dried leaves were ground into a coarse powder. Then, the dried powder was preserved in an airtight container against the re-absorption of moisture, oxidation, excessive heat or humidity, growth of moulds and bacteria and infestation by insects and rodents.

**Extraction and Fractionation of *C. grandis***

Dried powder of *C. Grandis* was refluxed with MeOH (3 × 3 L) for 3 h, and each filtrate was concentrateduntil dry in vacuo at $40^0$ C, resulting in MeOH extract(175.0 g). This extract was suspended in distilled $H_2O$ and then successively partitioned with *n*-hexane, $CH_2Cl_2$, EtOAc, and *n*-BuOH, to yield the *n*-hexane (14.9 g), $CH_2Cl_2$(19.8 g), EtOAc (51.5 g), and *n*-BuOH (39.6 g) fractions, respectively, as well as an $H_2O$ residue (52.5 g).

**Assay for DPPH radical scavenging activity**

The DPPH radical scavenging activity was evaluated using the method of Blois1958 with a slight modification. Test samples and DPPH were dissolved in MeOH. Each test sample (160 µL) at various concentrations was added to 40 µL of DPPH solution ($1.5 \times 10^{-4}$ M). After samples were gently mixed and left to stand at room temperature for 30 min, the optical density of each reactant was measured at 520 nm using a micro plate spectrophotometer (Molecular Devices, Sunnyvale, CA, USA). L-Ascorbic acid was used as the positive control.

**Assay for $ONOO^-$ scavenging activity**

The $ONOO^-$ scavenging activity was assessed by the modified method of Kooy *et al*., 1994, which involved monitoring highly fluorescent rhodamine 123 that was rapidly produced from non-fluorescent DHR 123 in the presence of $ONOO^-$. In brief, the rhodamine buffer (pH 7.4) consisted of 50 mM sodium phosphate dibasic, 50 mM sodium phosphate monobasic, 90 mM sodium chloride, 5.0 mM potassium chloride, and 100 µM DTPA. The final DHR 123 concentration was 5.0 µM. The assay buffer was prepared prior to use and placed on ice. The background and final fluorescent intensities were measured 5 min after treatment with and without the addition of authentic $ONOO^-$ (10 µM) dissolved in 0.3 N sodium hydroxide. The fluorescence intensity of the oxidized DHR 123 was evaluated using a fluorescence microplate reader (Bio-Tek Instruments Inc., FL 9 800, Winooski, UT, USA) at excitation and emission wavelengths of 480 and 530 nm, respectively. The values of the $ONOO^-$ scavenging activity were calculated as the final fluorescence intensity minus the background fluorescence, via detection of DHR 123 oxidation. L-Penicillamine was used as the positive control.

**Assay for inhibition of total ROS generation**

The generation of ROS was assessed using the ROS-sensitive fluorescence indicator DCFH-DA (LeBel and Bondy,1990). Male Wistar rats weighing 150-200 g were sacrificed by decapitation and the kidneys were quickly removed and rinsed in iced cold-buffer [100 mM Tris,1 mM EDTA, 0.2 mM PMSF, 1 µM pepstatin, 2 µM leupeptin, 80 mg/L trypsin inhibitor, 20 mM β-glycerophosphate, 20 mM NaF, 2 mM sodium orthovanadate (pH7.4)]. The tissues were immediately frozen in liquid nitrogen and stored at $-80^0$ C. 10 µL of each test sample (f.c.25 µg/mL) was added to 190 µL of kidney post mitochondrial fraction in a 50 mM potassium phosphate buffer. Then, the mixtures were loaded with 50 µL of DCFH-DA (12.5 mM) in a potassium phosphate buffer and shaken for 5 min. Finally, the fluorescence of 2′,7′-dichlorodihydrofluorescein (DCF), the oxidation product of DCFH-DA was measured on a microplate fluorescence spectrophotometer (Bio-Tek Instruments, Inc., Winooski, VT, USA) for 30 min at an excitation wavelength of 485 nm and an emission wavelength of 530 nm. Trolox was used as the positive control.

**Assay for α-Amylase inhibitory assay**

The α-amylase inhibitory assay was performed according to the method described by Kim *et al*., 2004 with slight modification. 10 µL α-amylase solution (1 unit $mL^{-1}$, distilled water (DW)) was premixed with 1 µL of sample solution at different concentrations (in 10% DMSO). Following incubation for 15 min, 180 µL of 1% starch solution in 20 mmol $L^{-1}$ sodium phosphate buffer (pH 6.9) was added to start the reaction. The reaction was carried out at 37.5 ◦ C for 5 min and terminated by addition of 500 µL of the DNS reagent (1% 3,5-dinitrosalicylic acid, 12% Na-K tartrate in 0.4 mol $L^{-1}$ NaOH). The reaction mixture was placed in a water bath at boiling point for 15 min and then cooled down to room temperature. α-Amylase activity was determined at 540 nm by using a spectrophotometer (Model U-3210, Hitachi Co., Tokyo, Japan). The $IC_{50}$ value was defined as the concentration of inhibitor required to inhibit 50% of the α-amylase inhibitory activity. For all tests, the inhibition assay was performed in triplicate.

**Assay for α-Glucosidase inhibitory activity**

The enzyme inhibition studies were carried out spectrophotometrically in a 96-well microplate reader using a procedure reported by Li *et al*., 2005. A total 60 µl reaction mixture containing 20 µl of 100 mM phosphate buffer (pH 6.8), 20 µl of 2.5 mM pNPG in the buffer, and 20 µl of sample (in a test concentration ranging from 0.5 to 200 µg/mL for the extract and fractions) dissolved in 10% DMSO, were added to each well, followed by 20 µl of 10 mM phosphate buffer (pH 6.8) containing 0.2 U/ml α-glucosidase to the mixture of treatment terminated wells. The plate was incubated at 37°C for 15 min, and then 80 µl of 0.2 mM sodium carbonate solution was added to stop

the reaction. Right after that, absorbance was recorded at 405 nm using VERSA max (Molecular Devices, Sunnyvale, CA, USA) microplate reader. Control contained the same reaction mixture except the same volume of phosphate buffer was added instead of sample solution. Acarbose was dissolved in 10% DMSO and used as a positive control. The inhibition percentage (%) was calculated as: [(Ac – As)/Ac] ×100 %, where, Ac is the absorbance of the control, and As, the absorbance of the sample.

**Assay for PTP 1B inhibitory activity**

The inhibitory activity of the plant extracts against human recombinant PTP1B was evaluated using pNPP as substrate Cui *et al*., 2006. To each 96 well (final volume 110 µl) were added 2 mM pNPP and PTP1B in a buffer containing 50 mM citrate (pH 6.0), 0.1 mM NaCl, 1 mM EDTA, and 1 mM DTT with or without sample. The plate was preincubated at 37°C for 10 min, and then 50 µl of pNPP in buffer was added. Following incubation at 37°C for 30 min, the reaction was terminated with the addition of 10 M NaOH. The amount of *p*nitrophenyl produced after enzymetic dephosphorylation was estimated by measuring the absorbance at 405 nm using VERSA max (Molecular Devices, Sunnyvale, CA, USA) microplate reader. The nonenzymatic hydrolysis of 2 mM PNPP was corrected by measuring the increase in absorbance at 405 nm obtained in absence of PTP1B enzyme. The inhibition percentage (%) was calculated as: [(Ac – As)/Ac] × 100 %, where, Ac is the absorbance of the control, and As, the absorbance of the sample. Ursolic acid was used as positive control.

**Assay for DPP-IV inhibitory activity**

The assay was performed as per Kojima *et al*., 1980. In brief, the assay was performed in 96 micro well plates. A pre-incubation volume 250µl contained 100mM Tris HCl buffer pH 8.4, 7.5µl of DPP IV enzyme (0.2U/ml) and various concentration of test material/reference inhibitor. This mixture was incubated at 37°C for 30 mins, followed by addition of 10µl of 1.4 mM Gly-pro-p-nitroanilide (substrate). The reaction mixture was incubated for 30 mins at 37°C and absorbance was measured at 410nm. Diprotein-A (Ile-Pro-Ile) was used as reference inhibitor.

**Statistical analysis**

Each result is expressed as the mean ± SEM of triplicates. Statistical significance was analyzed by one-way ANOVA and Student's t test (Systat; Evaston, IL, USA) and values were considered significant at $p< 0.05$.

## RESULTS:

**Antioxidant activities of the MeOH extract and its solvent soluble fractions from *C. grandis***

To find out potential antioxidant activities, we successively partitioned the crude extract with several solvents using a bioassay-guided fractionation strategy. The MeOH extract of *C. grandis* was dissolved in $H_2O$ and successively partitioned with *n*-hexane, $CH_2Cl_2$, EtOAc, and *n*-BuOH to obtain the respective solvent-soluble fractions and water residue. We then evaluated the total ROS as well as DPPH and $ONOO^-$ scavenging activities of the different solvent-soluble fractions. The DPPH radical scavenging activity was determined based on the formation of the DPPH-H non-radical form in the presence of hydrogen-donating antioxidants in the extract/or fractions that can be detected at 517 nm. The DPPH radical scavenging activity of the MeOH extract and its solvent-soluble fractions were tested at different concentrations using L-Ascorbicacid as a positive standard. As shown in Table 1, the highest DPPH scavenging activity was observed in the $CH_2Cl_2$ fraction with an $IC_{50}$value of 2.42 ± 0.03µg/mL, followed by the *n*-hexane fraction with an $IC_{50}$ value of 7.13 ± 0.09µg/mL compared to L-ascorbic acid ($IC_{50}$ = 2.81 ± 0.05µg/mL). $ONOO^-$ and ROS scavengers play important roles in various pharmacological effects and physiological conditions. Unlike DPPH radicals, $ONOO^-$ and ROS are generated in the body by cellular metabolism. $ONOO^-$ and ROS scavengers observed in vitro will at least, in part, ameliorate several oxidative stress-related diseases, including diabetes, aging, AD, and inflammation. As shown in Table 1, both $CH_2Cl_2$ and *n*-hexane fractions exhibited significant total ROS-scavenging activities with $IC_{50}$values of 0.45 ± 0.00 and 1.56 ± 0.08µg/mL, respectively. In addition, the value of the $CH_2Cl_2$ fraction was approximately eight fold less than trolox ($IC_{50}$ = 3.23 ± 0.06µg/mL). In accordance with DPPH and total ROS scavenging abilities, the highest $ONOO^-$ scavenging ability was also observed in the $CH_2Cl_2$ fraction with an $IC_{50}$value of 0.71 ± 0.03 µg/mL compared to L-penicilliamine ($IC_{50}$ = 1.30 ± 0.07 µg/mL).

Table 1. Antioxidant activities of the MeOH extract and its solvent soluble fractions from *C. grandis*

| Test Samples | $IC_{50}$ values (µg/mL)[a] ± SEM | | |
|---|---|---|---|
| | DPPH | $ONOO^-$ | Total ROS |
| MeOH | 9.71 ± 0.16 | 3.95 ± 0.21 | 3.43 ± 0.13 |
| *n*-hexane | 7.13 ± 0.09 | 1.29 ± 0.17 | 1.56 ± 0.08 |
| $CH_2Cl_2$ | 2.42 ± 0.03 | 0.71 ± 0.03 | 0.45 ± 0.00 |
| EtOAc | 36.15 ± 0.77 | 8.46 ± 0.32 | 6.98 ± 0.67 |
| *n*-BuOH | 40.13 ± 0.16 | 20.36 ± 0.04 | -60.17 ± 2.77 |
| $H_2O$ | NA | 49.81 ± 0.07 | NA |
| L-Ascorbic acid[b] | 2.81 ± 0.05 | | |
| L-Penicillamine[c] | | 1.30 ± 0.07 | |
| Trolox[d] | | | 3.23 ± 0.06 |

[a] $IC_{50}$ are expressed as to mean ± SEM of triplicate experiments

[b c d] Used as a positive control

**α-Amylase inhibitory activities of the MeOH extract as well as its different solvent soluble fractions of *C. grandis***

The methanolic extract of *C. grandis* against α-Amylase inhibitory was evaluated. Methanolic extract of *C. grandis* exhibited potent α-amylase inhibitory activity with $IC_{50}$ values of 8.95 ± 0.30 in comparison with the positive control of acarbose with respective $IC_{50}$ values of 188.23 ± 10.01 µg/mL. $CH_2Cl_2$ fraction exhibited the highest inhibitory activity with an $IC_{50}$ value of 1.75 ± 0.01 µg/mL whereas the *n*-hexane, EtOAc, *n*-BuOH fractions also showed significance $IC_{50}$ values of 3.56 ± 0.06, 18.23 ± 1.03, 40.14 ± 2.01µg/mL, respectively.

Table 2. α-Amylase inhibitory activity of different solvent-soluble fractions of *C. grandis*

| Test Samples | $IC_{50}$ values (µg/mL) [a] |
|---|---|
| MeOH extract | 8.95 ± 0.30 |
| *n*-hexane | 3.56 ± 0.06 |
| $CH_2Cl_2$ | 1.75 ± 0.01 |
| EtOAc | 18.23 ± 1.03 |
| *n*-BuOH | 40.14 ± 2.01 |
| $H_2O$ | >200 |
| Acarbose[b] | 188.23 ± 10.01 |

[a] $IC_{50}$ are expressed as to mean ± SEM of triplicate experiments
[b] Used as positive control in α-Amylase inhibitory assay

**α-Glucosidase inhibitory activities of the MeOH extract as well as its different solvent soluble fractions of *C. grandis***

As shown in Table 3, all the fractions exhibited significance α-glucosidase inhibitory activity in a concentration dependent manner with $IC_{50}$ values

ranging from 4.95 to 166.71 µg/mL. Among the tested fractions, the $CH_2Cl_2$ fraction displayed the highest inhibitory activity with an $IC_{50}$ value of 4.95 ± 0.03µg/mL compared to the positive control acarbose with an $IC_{50}$ value of 188.73 ± 11.05 µg/mL whether $CH_2Cl_2$ fraction showed more potential inhibitory activity than the positive control. In addition, the *n*-hexane, EtOAc, *n*-BuOH, $H_2O$ fractions also exhibited considerable inhibitory potential with $IC_{50}$ values of 2.56 ± 0.08, 18.46 ± 0.64, 60.18 ± 2.01, and 166.71 ± 5.28 µg/mL, respectively.

Table 3. α-Glucosidase inhibitory activity of different solvent-soluble fractions of *C. grandis*

| Test Samples | $IC_{50}$ values (µg/mL) [a] |
|---|---|
| MeOH extract | 4.95 ± 0.03 |
| *n*-hexane | 2.56 ± 0.08 |
| $CH_2Cl_2$ | 1.15 ± 0.01 |
| EtOAc | 18.46 ± 0.64 |
| *n*-BuOH | 60.18 ± 2.01 |
| $H_2O$ | 166.71 ± 4.28 |
| Acarbose[b] | 188.73 ± 11.05 |

[a]$IC_{50}$ are expressed as to mean ± SEM of triplicate experiments
[b] Used as positive control in α-Glucosidase inhibitory assay

**Protein Tyrosine Phosphatase (PTP 1B) inhibitory activities of the MeOH extract as well as its different solvent soluble fractions of *C. grandis***

The inhibitory potential of *C. grandis* against PTP 1B inhibitory were evaluated and the results are presented in the Table 4. Methanolic extract of *C. grandis* exhibited potent PTP 1B inhibitory activity with $IC_{50}$ values of 1.95 ± 0.03in comparison with the positive control of ursolic acid with respective $IC_{50}$ values of 1.23 ± 0.09 µg/mL.Among the tested fractions, the $CH_2Cl_2$ fraction displayed the highest inhibitory activity with an $IC_{50}$ value of 1.25 ± 0.01 µg/mL compared to the positive control ursolic acid with an $IC_{50}$ value of 1.23 ± 0.09µg/mL. $CH_2Cl_2$ fraction showed potential inhibitory activity whereas the *n*-hexane, EtOAc, *n*-BuOH, $H_2O$ fractions also showed considerable $IC_{50}$ values of 1.56 ± 0.08, 28.46 ± 1.03, 60.40 ± 2.01, and 196.71 ± 3.28 µg/mL, respectively.

Table 4. Protein tyrosine phosphatase 1B (PTP 1B) inhibitory activity of different solvent-soluble fractions of *C. grandis*

| Test Samples | $IC_{50}$ values (µg/mL) [a] |
|---|---|
| MeOH extract | 1.95 ± 0.03 |
| *n*-hexane | 1.56 ± 0.08 |
| $CH_2Cl_2$ | 1.25 ± 0.01 |
| EtOAc | 28.46 ± 1.03 |
| *n*-BuOH | 60.40 ± 2.01 |
| $H_2O$ | 196.71 ± 3.28 |
| Ursolic acid[b] | 1.23 ± 0.09 |

[a]$IC_{50}$ are expressed as to mean ± SEM of triplicate experiments
[b] Used as positive control in PTP 1B inhibitory assay

**Dipeptidyl Peptidase IV (DPP-IV) inhibitory activities of the MeOH extract as well as its different solvent soluble fractions of *C. grandis***

Since *C. grandis* exhibited the highest DPP IV inhibitory activity, it was selected for further investigation. The MeOH extract of *C. grandis* was

successively partitioned in order to obtain different solvent soluble fractions and their inhibitory potential against DPP IV enzymes was evaluated. As shown in Table 5, all the fractions showed DPP IV inhibitory activity in a concentration dependent manner with $IC_{50}$ values ranging from 7.25 to 96.71 µg/mL. Among the tested fractions, the $CH_2Cl_2$ fraction displayed the highest inhibitory activity with an $IC_{50}$ value of 7.25 ± 0.01 µg/mL compared to the positive control vildagliptin with an $IC_{50}$ value of 19.71 ± 0.20 µg/mL whether $CH_2Cl_2$ fraction showed more potential inhibitory activity than the positive control. In addition, the *n*-hexane, EtOAc, *n*-BuOH, $H_2O$ fractions also exhibited considerable inhibitory potential with 50% inhibition concentration ($IC_{50}$) values of 15.01 ± 0.04, 28.60 ± 1.03, 52.40 ± 3.01, and 96.71 ± 6.28 µg/mL, respectively.

Table 5. Dipeptidyl Peptidase IV (DPP-IV) inhibitory activity of different solvent-soluble fractions of *C. grandis*

| Test Samples | $IC_{50}$ values (µg/mL) [a] |
|---|---|
| MeOH | 19.81 ± 0.30 |
| *n*-hexane | 15.01 ± 0.04 |
| $CH_2Cl_2$ | 7.25 ± 0.01 |
| EtOAc | 28.60 ± 1.03 |
| *n*-BuOH | 52.40 ± 3.01 |
| $H_2O$ | 96.71 ± 6.28 |
| Vildagliptin[b] | 19.71 ± 0.20 |

[a]$IC_{50}$ are expressed as to mean ± SEM of triplicate experiments
[b] Used as positive control in DPP-IV inhibitory assay

## DISCUSSION:

Despite regarding improvements throughout biomedical science as well as the introduction regarding new treatment approaches, diabetes remains a major reason of new onset blindness, end stage renal diseases, along with cardiovascular diseases; all of these bring about the surplus morbidity and mortality throughout people who have diabetes (Lee *et al*., 2009). Based on the present perception of the pathophysiology regarding DM, multiple pharmacological and non pharmacological interventions have been developed within the last few decades to enhance glycemic control as well as slow diseases progression. Due to the fact plant therapies may provide numerous health benefits along with reduced toxicity, researchers tend to be constantly looking for effective drugs from natural sources intended for various ailments for instance diabetes. Even though there have been several current endeavors devoted to obtaining effective anti-diabetic agents coming from natural sources, research emphasizing natural plants with regard to efficient anti-diabetic agents is still in its infancy. As part of the continuous search for effective anti-diabetic agents coming from natural sources, we determined MeOH extracts from *C. grandis* and evaluated their antioxidant and anti-diabetic inhibitory potential employing DPPH, $ONOO^-$, total ROS, α- Amylase, α-Glucosidase, PTP 1B and DPP-IV inhibitory assay. *Coccinia grandis* belongs to the family Cucurbitaceae is a rapidly growing, perennial climber or trailing vine. *C.grandis* (L) Voigt plays a major role in the medicinal properties. The plant parts of *C. grandis* such as roots, leaves and fruits are used for numerous medicinal purposes like wound healing, ulcers, jaundice, diabetes and antipyretic. The leaf possesses hypoglycemic, antihyperglycemic, antioxidant properties and is also used to treat infective hepatitis (Ajay 2009; Gunjan *et al*., 2010;Mandal *et al*., 2009; Rajalakshmi *et al*., 1984). Bhattacharya *et al.*, 2010 evaluated the aqueous extract of leaves of *C. grandis* for antibacterial activity against *Shigella flexneri* NICED, *Bacillus subtilis*, *Escherichia coli*, *Salmonella choleraesuis*, *Shigella dysenteries*, and *Shigella flexneri*. Aqueous extract of *C.grandis* showed more significant antibacterial activity in comparison to ethanol extract. Moideen*et al*., 2011 evaluated Ethanol extract of root of*C. grandis*contain

flavonoids which are responsible forantioxidant activity. Methanol extracts of the fruit of *C. grandis* possess the potent antioxidant activity. The methanolextract of *C. grandis* contains glycoside and flavonoid. Theantioxidant activity of *C. grandis* is due to the reducingpower ability, hydrogen peroxide scavenging potential (Deshpande*et al.,* 2011; Mongkolsilp *et al.,* 2004). Ethanol and methanolextract shows the antioxidant activity (Ashwini *et al.,* 2012).*C. grandis*stem extract containing solvent petroleum,chloroform and ethyl acetate shows antioxidant activity. Ethylacetate possess potent antioxidant activity than petroleum (Deshpande *et al.,* 2011) *C. grandis* methanol extract and leaf powder contain the antioxidant principle (Mujumder *et al.,* 2008). Mallick *et al*., 2007 evaluated combined extracts of *Musa paradisiaca* and *Coccinia indica* aqueous extract of leaf for anti-diabetic activity in streptozotocin induced diabetes rats. The ethanolic extract of the aerial part decreases blood glucose levels and lipid parameters in streptozotocin induced diabetic rats at 100 or 200 mg/kg. Chronic administration of fruit extract 200 mg/kg for 14 days reduces the blood glucose level in alloxan induced diabetic rat (Gunjan *et al*., 2010). The aqueous extract of *Coccinia indica* reduced the blood glucose level; also reduced the cholesterol, protein and urea with prolonged treatment. *C. grandis* stimulated gluconeogenesis, or inhibited glycogenolysis in the diabetic rat liver. Treatment with Coccinia extract increases the total protein, SGPT, SGOT (Doss *et al.,* 2008). The *Coccinia indica* leaves extract exerts hypoglycemic activity on blood glucose and cholesterol, TG, LDL, VLDL level in alloxan induced diabetic rats (Manjula *et al.,* 2007). The hypoglycemic activity of *C. grandis* fruit evaluated by using alloxan induced diabetic rat. Ethanolic extract shows the decreased blood glucose level. Pectin from fruit reduces the blood glucose by decreasing the absorption of glucose from the intestine and increasing liver glycogen and decreasing glycogen phosporylase (Pekamwar*et al.,* 2013). Combined methanolic extract of leaves of *Coccinia indica* and *Salvadoraoleoides* shows the hypoglycemic activity (SaklaniI*et al.,* 2012). Alcoholic extract of *C. grandis* leaves (Eliza Jose, 2010) and stem have the capacity to lower the blood glucose level in normal fasted rats (Doss *et al.,* 2008). Ethyl acetate extract and petroleum ether extract of Coccinia contains triterpines, alkaloid, flavonoid, β-carotene which is responsible for the hypoglycemic activity (Pekamwar*et al.,* 2013).Our data revealed that the MeOH extract as well as different solvent soluble fractions of *C. grandis* possess promising antidiabetic and antioxidant potential which are attributed to its potent inhibitory activity against α-amylase, α-glucosiadse, PTP 1B, DPP-IV and antioxidant scavenging activity. Hyperglycaemia raises the generation of free radicals and also reduces the tissue antioxidative capability in diabetes. These types of imbalances bring about tissue oxidative stress. The tissue antioxidative potential needs to be elevated as a way to overcome oxidative damage. Our data suggested that methanolic extract of *C. grandis* showed potent scavenging activity on various in vitro antioxidant assays. Among the tested fractions, $CH_2Cl_2$ and *n*-hexane fractions showed potential activity compared to other polar fractions. As shown in Table 2, $CH_2Cl_2$ fraction exhibited potent α-amylase and α-glucosidase inhibitor with $IC_{50}$ value of 1.75± 0.01 µg/mL and 1.15 ± 0.01 µg/mL, respectively. The positive control of Acarbose, which is a well-known α-amylase and α-glucosidase inhibitor that is in clinical utilize, acquired an $IC_{50}$ value of 188.23 ± 10.01 µg/mL and 188.73 ± 11.05 µg/mL. Conversely, $CH_2Cl_2$ and *n*-hexanefractions displayed strong PTP 1B inhibitory activity with respected $IC_{50}$ values of 1.25± 0.01 and 1.56± 0.08 µg/mL, respectively when compared to the positive control Ursolic acid with $IC_{50}$ value of 1.23 ± 0.09 µg/mL. For DPP-IV inhibitory assay, $CH_2Cl_2$ fraction showed potent inhibitory activity followed *n*-hexane fraction with respected $IC_{50}$ values of 7.25± 0.01 and 15.01± 0.04 µg/mL, respectively. Results of the present study showed that the methanolic extract of *C. grandis* has marked anti-diabetic effects with a potential inhibitory activity. The antioxidant and anti-diabetic effect of different solvent soluble fractions of methanol leaves extract of *C. grandis* was evaluated for the first time in various in vitro experimental test models.

## CONCLUSION:

Taking into consideration the relevance and severity associated with diabetes, primarily cardiovascular diseases, nephropathies, retinopathies, and neuropathies, any kind of new therapeutic innovation can be associated with fascination to prevent deleterious consequences connected with hyperglycemia. In the present study, the MeOH extract as well as different solvent soluble fractions from *C. grandis* revealed encouraging inhibitory potential against α-amylase, α-glucosiadse, PTP 1B, DPP-IV and antioxidant scavenging assay. Considered together all these results illustrate the promising anti-diabetic potential of *C. grandis* that could be used for the treatment of diabetes and oxidative stress associated diseases. Nevertheless, the isolation of pure secondary metabolites from the plant will help us to further understand the mechanism of these activities and in the identification of primary compounds of clinical utility.

**Conflict of Interest**
The authors declare no conflicts of interest.

**Acknowledgements**
The authors acknowledge the Department of Pharmacy, Daffodil International University, Dhaka, Bangladesh to provide laboratory facilities and National Herbarium, Dhaka, Bangladesh for the identification of the plant.

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