

Comparative Studies On *Coccinia Grandis* Raw And Ripe Fruits For Its Phytochemical, *In Vitro* Antioxidant, Anti Diabetic And Hyperlipidemic Activity

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ABSTRACT

Purpose: To compare qualitative phytochemical contents, antioxidant, antidiabetic and hyperlipidemic activity of raw and ripe fruits of *Coccinia grandis* **Methods:** Raw and ripe fruit of *Coccinia grandis* were extracted with Soxhlet apparatus. The extract was screened for primary and secondary metabolites. High-performance liquid chromatography (HPLC), was used to find out the number of active compounds present in the extract. **Results:** The qualitative phytochemical analysis showed the presence of saponins, flavanoids, carbohydrates, proteins, alkaloids etc in both red and green extract. HPLC analysis showed that the red extract possessed more amounts of phytochemicals than green extract. The amount of protein was found to be higher in unripe fruit. The amount of carbohydrate was found to be higher in ripe than in green (9.8 mg/mL and 6.2mg/mL respectively). The results of the study showed that ripe fruit of *Coccinia* has maximum inhibition against α -amylase inhibition (63.87%) at a concentration of 160 μ g/ml. Our experimental studies showed that HMG-CoA reductase (62.59%), DPPH scavenging (59.97%) and ABTS scavenging (51.3%) activity at a higher concentration of ripe fruit extract used for the assays. **Conclusion:** This result implies that the fruit extracts has the efficacy to maintain and reduce postprandial hyperglycemia, hypercholesterolemia and reactive oxygen species (ROS) production through its biological activities. We can suggest the inclusion of ripe fruit is more favored because of the higher antioxidant activity of ripe is found to be more active and can be included in our diet or in the form of any formulations. Further, in vivo studies and clinical trials must be done to validate this scientific claim.

Keywords: *Coccinia grandis*, HPLC, DPPH, α -amylase, HMG-CoA.

INTRODUCTION

Coccinia grandis (*Coccinia indica*), the ivy gourd, also referred to as baby watermelon, little gourd, gentleman's toes or even gherkin is actually an exotic vine belonging to the family Cucurbitaceae, in the genus *Coccinia* Wight & Arn. Other Common Names of Ivy includes Kowai fruit, Small Gourd, Scarlet-Fruited Gourd, Scarlet Gourd, Kovakka, little gourd, tonde Kai, *Cephalandra indica*, and Tindora. It is actually native to northern and eastern Africa, Arabia to tropical south and southeast Asia. Ivy gourd is referred to as Telakucha in Bangladesh, Gourde Écarlate De L'Inde Tindola in French and Gol Kankri in Nepali. Ovoid to ellipsoid berry shaped vegetable is in fact a storeroom of several health promoting

Nutrients, vitamins and Minerals. Previous study reported the hypoglycemic activity of *Coccinia indica* wight Arn [1,2]. The alcoholic extract of *Coccinia indica* was found to be more active in reducing blood glucose level, this extract was subjected to further fractionation. A review reported on India medical herbs *Amaranthus paniculatus*, *Aerva lantha*, *Coccinia indica*, *Cassia auriculata* and *Coriandrum sativum* indicating that plants could be source of dietary antioxidant supplies [3,4]. Earlier studies analysed the antioxidant activity of medicinal plant namely *Gymnema sylvestre* (Asclepiadaceae), *Coccinia indica* (Cucurbitaceae), *Catharanthus roseus* (white), *C. roseus* (purple) (Apocynaceae), *Momordica charantia* var. *charantia* and *M.*

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charantia var. *muricata* (Cucurbitaceae) which are used to treat diabetes mellitus in India [5,6]. The extracts were found to have different level of antioxidant activity in the system tested. The hepatoprotective and antioxidant activity of *Coccinia grandis* root extracts against paracetamol induced hepatic oxidative stress in Wistar albino rats was reported in earlier studies [7]. It was concluded from the result that ethanolic extract of *Coccinia grandis* possess hepatoprotective and antioxidant activity against paracetamol induced hepatotoxicity in wistar albino rats. Based on the pharamaceutical activities reported in earlier studies are conduced and used in ayurvedic medicine [8]. Earlier studies investigated the phytochemical constituents, antioxidant and anti-inflammatory activities to justify the use of the plants in folklovic medicine [9]. Phytochemical screening of *Coccinia grandis* fruit in different organic solvent revealed the presence of alkaloid, carbohydrates, tannins, resins, proteins, and diterpenes. The result obtained in this study showed that fruits of *Coccinia grandis* have antioxidant and anti-inflammatory activity. Earlier studies reveal that ethanolic leaf extract of this plant has potential effect against several bacterial strains compare to root [10]. The present review is the pool information that highlights the botany, chemical constituents, pharmacological activities and recent research in *C. cordifolia*. Our study has also revealed that ethanolic leaf extract of this plant has potential effect against several bacterial strains compare to root. *Coccinia grandis* has been used in traditional medicine as a household remedy for various diseases. The whole plant of *Coccinia grandis* having pharmacological activities like analgesic, antipyretic, anti-inflammatory, antimicrobial, antiulcer, antidiabetic, antioxidant, hypoglycemic, hepatoprotective, antimalarial, antidyslipidemic, anticancer, mutagenic. In the present study we carried out the comparative study on the raw and ripe fruits of *Coccinia grandis* for its phytochemical, invitro antioxidant, antidiabetic and hyperlipedemic activity

2.MATERIALS AND METHODS

2.1 Collection of plant Materials

The fruit of *Coccinia grandis* was collected from local market of Therthally, Kannur district. It was identified

by taxonomist Dr. Krishna raj R, Department of Botany, Baselius College, Kottayam, Kerala. One kilogram of raw (CGG) and ripe fruits (CGR) of *C. grandis* were shade dried during the month of March 2025 and it was powdered in a grinder.

2.2 Method of Extraction

The extraction was carried out using a soxhlet extractor. 53.83g of CGR and 53.16 g of CGG powder samples was taken separately in a starch free cotton bag. 50% ethanol in water (hydroalcoholic) was used for extraction purpose. The extraction was carried out until the solvent becomes colourless. The extract was kept in a temperature-controlled evaporator below 50°C and the yield of each extract was noted.

2.3 Qualitative Phytochemical Analysis

1) Test for saponin:

The test solution was mixed with water and shaken and observed for the formation of froth, which is stable for 15 minutes for a positive result.

2) Test for Alkaloids:

Test solution was treated with few drops of Hager's reagent. Formation of yellow precipitate would show a positive result for the presence of alkaloids.

3) Test for Flavonoids:

Test solution when treated with few drops of FeCl_3 solution would result in the formation of blackish red colour indicating the presence of flavonoids.

4) Test for Tannin:

Sample was mixed with 5% FeCl_3 solution. Blue-black precipitate indicates the presence of tannins.

5) Test for proteins:

Test solution was treated with 10% NaOH solution and 2 drops of 0.1% CuSO_4 solution. Formation of pink/violet colour observed.

6) Test for free amino acid:



Test solution when boiled with 0.2% solution of Ninhydrin, would result in the formation of purple color suggesting the presence of free amino acids.

7) Test for carbohydrate:

Test solution was mixed with few drops of Benedict's reagent and boiled in water bath, observed for the formation of reddish brown precipitate to show a positive result for the presence of carbohydrate.

8) Test for phenol:

FeCl₃ was used to test the presence of phenols. The presence of phenols was indicated by the appearance of green colouration when the reagent was added to extract. (Janarthanan and Senthil, 2013)

3 Quantitative Phytochemical Analysis

3.1 Estimation carbohydrate

Carbohydrates are dehydrated with concentrated H₂SO₄ to form "Furfural", which condenses with anthrone to form a green color complex which can be measured by using colorimetrically at 620nm (or) by using a red filter. Anthrone react with dextrins, monosaccharides, disaccharides, polysaccharides, starch, gums and glycosides [11].

3.2 Estimation of Protein

According to the Beer-Lambert Law, the absorption of the sample is directly proportional to the concentration of the species – in this case peptide bonds. Hence absorption spectroscopy using a spectrophotometer is a quantitative method which can be used to determine the concentration of total protein, following the Biuret test. Measure the absorbance of standard and sample against reagent [12].

3.3 Estimation Total Flavanoid Content

The extract was evaporated to dryness to obtain the crude extract. The principle involved in Aluminium chloride (AlCl₃) colorimetric method is that AlCl₃ forms acid stable complexes with the C-4 keto groups and either the C-3 or C-5 hydroxyl group of flavones and flavonols. Total flavonoid content was calculated

from a calibration curve, and the result was expressed as mg rutin equivalent per g dry weight [13].

3.4 Estimation and Identification of flavonoids by HPLC

The estimation of flavanoids was carried out using Shimadzu lab solutions. The solvent system used was methanol- water system and 10 microlitre of sample was injected and detector used was at 370 and 1280 nm. The column used was of 10m and run for 40 minutes and the chromatogram was taken and the analysis was carried out and this was compared with the standard flavanoids like quercetin, apigenin, myrcetin, luteolin and kaemferol and the amount was determined.

1. Determination of Antioxidant activity

4.1 2,2'-Azino-Bis 3-ethylbenzothiazoline-6-sulfonic acid assay (ABTS)

For the ABTS Assay, the ABTS radical solution was made by mixing 05 mL of a 07 mM ABTS solution with 88 µL of a 140 mM potassium persulfate solution. The resulting mixture was mixed with water at a ratio of 1:44. 100µL/ml of the fruit extracts solution was combined with 100µL of the ABTS reagent for each fruit extract in various concentration (10–100 µg/mL), and the mixture was allowed to react [14].

$$\% \text{ of scavenging} = \frac{\text{Absorbance of Ascorbic acid} - \text{Absorbance of extract}}{\text{Absorbance of Ascorbic acid}} \times 100$$

Absorbance of Ascorbic acid

This formula measures the ability of the test sample to scavenge ABTS free radicals, which is indicated by a reduction in absorbance.

4.2 2,2-Diphenyl-1-picryl hydrazyl assay (DPPH)

In the DPPH assay, the DPPH free radical transforms into a stable, non-radical form by reacting with electrons or hydrogen atoms. The sample extract is prepared as a stock solution at 100 µg/mL (10 mg/100 mL). The final concentrations of 10, 20, 40, 60, 80, and 100 µg/mL are serially diluted to 1 mL, 2 mL, 4 mL, 6 mL, 8mL, and 10 mL solvents. The same method is used to make ascorbic acid a standard. Each



test tube combines one millilitre of a 0.3 mM DPPH solution with 2.5 milliliters of the sample or standard. Using methanol as the blank, absorbance is measured at 517 nm using a UV spectrophotometer following a 15-minute incubation period at 37°C. This process calculates the antioxidant activity of the sample using the following formula:

$$\% \text{ of scavenging} = \frac{\text{Absorbance of Ascorbic acid} - \text{Absorbance of extract}}{\text{Absorbance of Ascorbic acid}} \times 100$$

This calculation provides the percentage of DPPH radicals scavenging activity by the sample or standard, indicating its antioxidant potential [14].

5. Determination of *In vitro* Antidiabetic Activity

5.1 α -Amylase Inhibition Study:

The α -amylase inhibitory activity of the fruit extracts was evaluated using a slightly modified standard procedure [15]. Different concentrations of the test sample (10, 20, 40, 80, 160, and 320 $\mu\text{g/mL}$), and a control (without the test sample or standard) were mixed with a solution of α -amylase (100 μL , 0.1mg/mL). The reaction mixtures were pre-incubated at 37°C for 15 minutes. Subsequently, 100 μL of starch solution was added to initiate the enzymatic reaction, and the incubation was continued at 37°C for 60 minutes. Each tube was filled with 100 μL of iodine reagent and 10 μL of 1 M HCl to stop the reaction. At 565 nm, the final solution's absorbance was measured.

The α -amylase inhibitory activity was calculated using the formula:

$$\% \text{ inhibition} = \frac{\text{OD of Control} - \text{OD of test}}{\text{OD of Control}} \times 100$$

In this method, the optical density (OD) of control refers to the absorbance measured for the control sample (lacking the test sample or standard). At the same time, the OD of the test denotes the absorbance of the sample at different concentrations. The α -amylase inhibitory potential of fruit extracts are determined by comparing the absorbance values of the test samples with those of the control [15].

6. Determination of Anti-Hyperlipidemic activity

6.1 β -Hydroxy β -methylglutaryl-CoA (HMG-CoA inhibitory assay):

The spectrophotometric approach evaluated the test sample's (CVC) HMG-CoA reductase inhibitory assay activity. A reaction mixture including 60 μL of NADPH (400 μM), HMG CoA Substrate (400 μM), and potassium phosphate buffer (100 mM, pH7.4) containing KCl (120 mM), EDTA (1 mM), and DTT (5 mM) was mixed with extract samples of various concentrations (10-100 $\mu\text{g/mL}$). After 10 minutes of incubation at 37°C, the absorbance at 340 nm was measured for the reaction mixture. Atorvastatin was used as a positive control.

$$\% \text{ inhibition} = \frac{\text{OD of Control} - \text{OD of test}}{\text{OD of Control}} \times 100$$

This formula compares the optical density of the test sample to that of the control to quantify the test sample's inhibitory effect on HMG-CoA reductase [16].

7. RESULTS

7.1 Qualitative Analysis

The extract of both green and red *Coccinia grandis* was taken in 50 % hydroalcohol and the extractive value was found to be 29.243% for red and 34.05755% for green respectively. The qualitative phyto chemical analysis showed the presence of saponins, flavanoids, carbohydrates, proteins, alkaloids etc in both red and green extract. It was found that the red extract possessed more amounts of phytochemicals than green extract (Table 1).



Table 1: Shows the presence of phytochemicals present in CGR and CGG

Phytoconstituents	Coccinia Extract (CGR)	Coccinia Extract (CGG)
Carbohydrate	++	+
Flavanoids	++	+
Proteins	+++	+++
Tannins	-	-
Saponin	+	++
Alkaloids	++	++

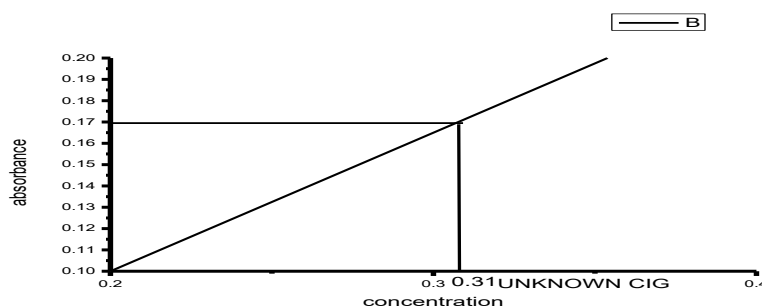
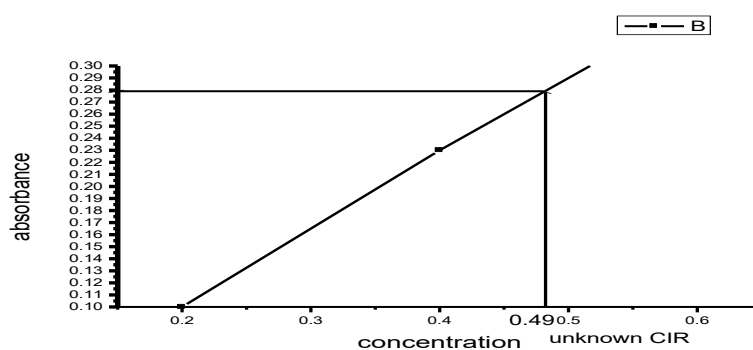
7.2 Quantitative Analysis

As a part of the study, we calculated quantitatively the amount of protein and it was found that the amount of protein was found to be 1.42g/dL in green and 0.789g/dL in red extract respectively. The amount of protein was found to be higher in CGG (Table 2). The amount of carbohydrate was compared with standard glucose and the amount was found to be higher in CGR than in CGG (9.8 mg/mL and 6.2mg/mL

respectively). The amount was found to be highest in red extract than in green one (Fig 1 & 2).

Table :2 shows the amount of protein present in raw and ripe *Coccinia grandis*

Samples	Concentration (g/dL)
CGG	1.42
CGR	0.789

Fig.1: Showed the amount of carbohydrate in green *Coccinia grandis* (CGG)**Fig 2:** Showed the amount of carbohydrate in red *Coccini grandis* (CGR)

The total flavanoids was quantitatively estimated and it was found that the amount was found to be 0.18µg/100µg in red extract and 0.12µg/100µg in green extract (Fig 3). To identify the flavanoids, HPLC was carried out and the amount of major active

principal present was tabulated in Table 3. HPLC analysis has identified that the presence of quercetin (1.030687779 mg/L) in CGG and the presence of Myricetin, Kaemferol and Apigenin was found in CGR and the amount found to be 49.5528mg/L, 0.2563mg/L, 0.3040 mg/L respectively.

Fig.3: Showed the Estimation of total flavanoid content of red and green *Coccinia grandis*

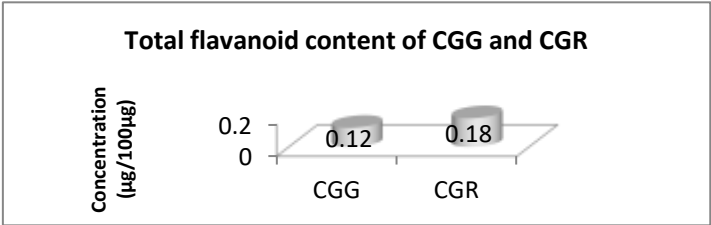


Table 3: Showing the quantity of flavonoids identified by HPLC

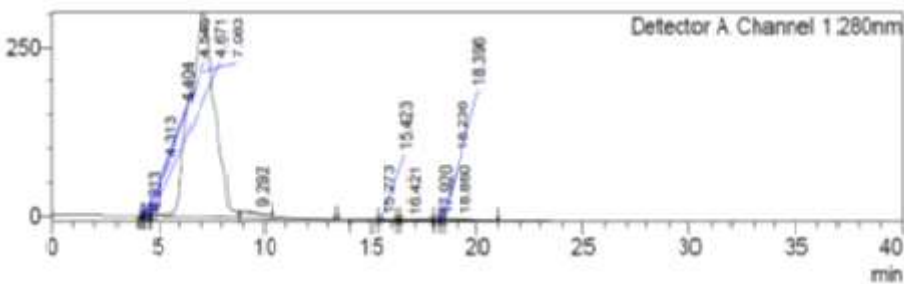
Flavoniods	CGR (mg/ml)	CGG (mg/ml)
Myricetin	49.5527731	BDL*
Quercetin	BDL*	1.030687779
Kaempferol	0.256294964	BDL*
Apigenin	0.303948494	BDL*

*BDL: Below the Detection Limit

Table 4: Showing the retention time of the standard flavanoids using HPLC

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	3.099	19668	1191	0.000		V	
2	3.544	1827	183	0.000		V	
3	3.840	5008	202	0.000		V	
4	4.602	1210	77	0.000		V	
5	5.827	967652	44151	17.144	mg/L	V	Myricetin
6	7.120	57140	1669	0.000		V	
7	8.135	1103	161	0.000		V	
8	8.804	1117220	37965	15.414	mg/L	V	Quercetin
9	10.505	1015399	29856	15.168	mg/L	V	Luteolin
10	12.895	1135	105	0.000		V	
11	13.895	1071968	26220	15.346	mg/L	V	Kaempferol
12	15.318	634647	13201	15.218	mg/L	V	Apigenin
Total		4893976	154981				

Figure 4: Chromatogram showing the retention time of the flavanoids identified from green *C. grandis* (CGG)



7.3 Determination of *In vitro* antioxidant activity (ABTS and DPPH Assay)

The antioxidant activity by ABTS inhibition assay of the extracts showed that in CGR as the concentration increases the absorbance also increases up to 500 μ g and in the case of CGG and CGR. But CGR is showing the higher IC₅₀ in 500 μ g/ml when compared with CGG extract (Fig 5). In DPPH scavenging study,

the % inhibition increase upto 160 μ g/ml and further increase of concentration decreases the antioxidant activity and it is behaving as prooxidant which may be due to the synergistic effect of the extract. Whereas the concentration increases the % inhibition was increased in CGR (Fig 6). The higher antioxidant activity of CGR is may be due to the presence myricetin and other compounds. Whereas the activity of CGG is may be due to the presence quercetin.

Fig 5: Showing the ABTS scavenging activity of CGG and CGR extracts

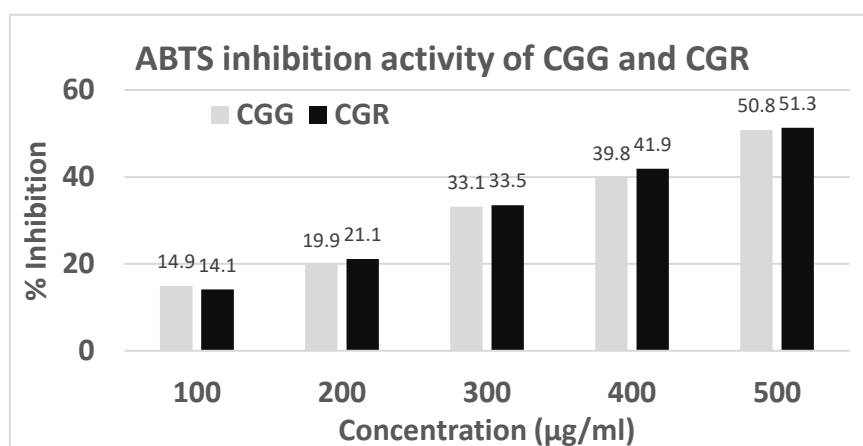
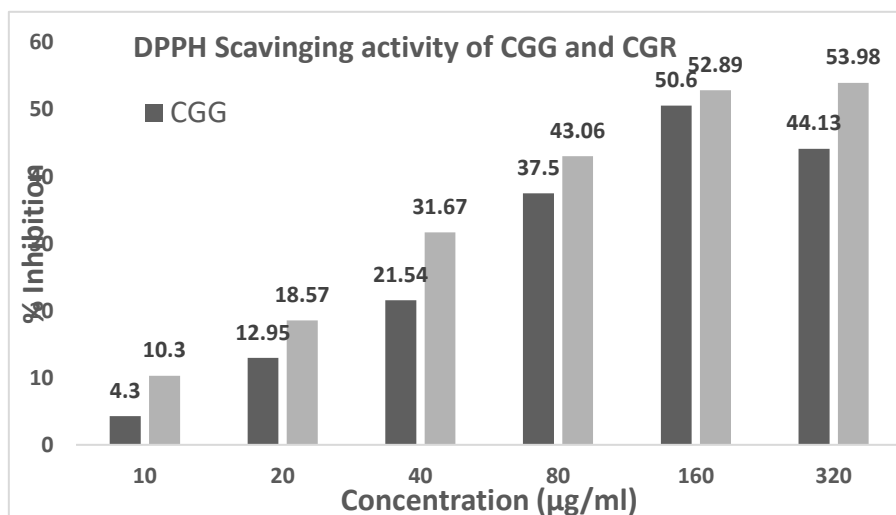


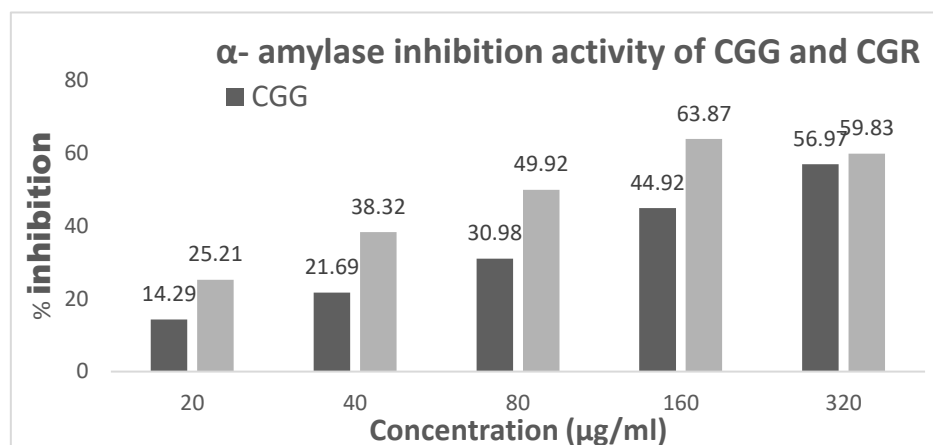
Fig 6: Showing the DPPH scavenging activity of CGG and CGR extracts



7.4 Determination of *In vitro* antidiabetic and antihyperlipidemic activity

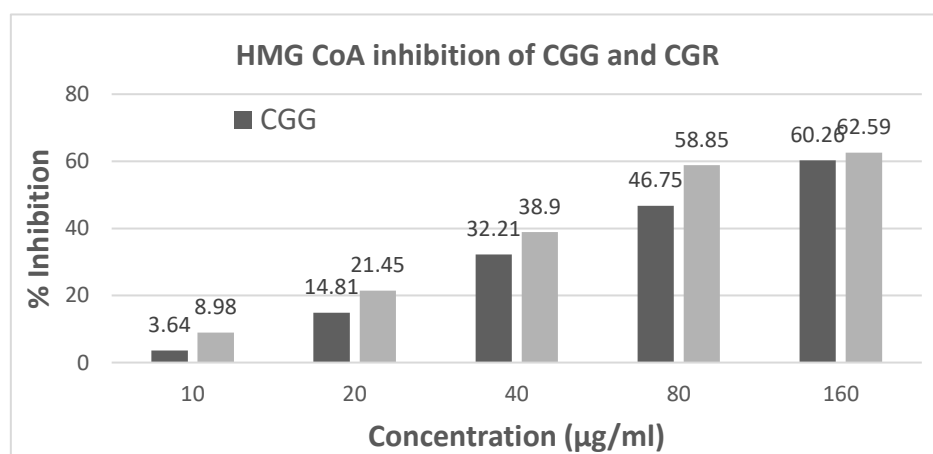
Green fruit of *Coccinia* achieved a maximum α -amylase inhibition of 63.87% at a concentration of 160 μ g/ml, whereas CGG showed 56.97% at a

concentration of 320 μ g/ml (Fig 7). This substantial inhibition indicates that both *Coccinia* extracts effectively impedes the enzymatic breakdown of complex carbohydrates into polysaccharides, oligosaccharides, disaccharides and monosaccharides.

Fig 7: Showing the α - amylase inhibition activity of CGG and CGR extracts

Regarding Antihyperlipidemic activity, Coccinia extracts showed inhibition of 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase,

achieving a maximum inhibition of 62.59% at a concentration of 120µg/ml by CR, whereas CGG showed 60.26% at a concentration of 120µg/ml (Fig 8).

Fig 8: Showing the HMG CoA inhibition activity of CGG and CGR extracts

8 DISCUSSION

Type 2 Diabetes Mellitus (T2DM) is the most common form of diabetes and frequently remains undiagnosed or inadequately managed. More than 90% of individuals with T2DM exhibit dyslipidemia, which contributes significantly to the progression of atherosclerotic cardiovascular disease, hypertension, and end-stage renal disease [17]. Persistent hyperglycemia in T2DM promotes the glycation of extracellular proteins such as collagen, elastin, and laminin, leading to the formation of Advanced Glycation End Products (AGEs). These AGEs accumulate in tissues including vascular walls, basement membranes, and the eye lens, thereby contributing to diabetic complications such as

nephropathy, cataracts, microangiopathy, and atherosclerosis.

Currently available oral antidiabetic therapies include biguanides, sulfonylureas, meglitinides, thiazolidinediones (TZDs), DPP-4 inhibitors, SGLT2 inhibitors, and α -glucosidase inhibitors [18]. Although these drugs are effective in glycemic control, prolonged use may result in adverse effects including gastrointestinal disturbances, dizziness, cobalamin deficiency, edema, elevated liver enzymes, respiratory infections, headaches, and injection-site reactions. Hence, there is a growing need for affordable and safer therapeutic alternatives capable of addressing the multifactorial complications associated with diabetes.



Oxidative stress plays a crucial role in the progression of diabetes and its associated complications. Antioxidants neutralize free radicals and thereby reduce oxidative damage, making them important in the prevention and management of diabetic complications [19]. In the present study, *Coccinia* exhibited remarkable antioxidant activity, demonstrating a strong ability to scavenge free radicals and minimize oxidative stress. Its effectiveness against a broad range of reactive oxygen species indicates its therapeutic potential in preventing oxidative damage associated with chronic metabolic disorders such as diabetes. By protecting cellular components from oxidative injury, *Coccinia* may contribute to improved metabolic health and reduced disease progression.

The findings of this in vitro investigation further revealed that *Coccinia* possesses promising antidiabetic and antihyperlipidemic properties. Enzymes such as α -amylase and α -glucosidase are involved in carbohydrate digestion and are key contributors to postprandial hyperglycemia [20]. The fruit extracts of *Coccinia* demonstrated significant inhibitory activity against α -amylase, suggesting their potential to delay carbohydrate digestion and glucose absorption. This mechanism may help attenuate postprandial blood glucose elevations, thereby supporting glycemic regulation in diabetic conditions.

Diabetic dyslipidemia is commonly characterized by elevated triglyceride levels, decreased HDL cholesterol, and increased small dense LDL particles. These lipid abnormalities are primarily associated with insulin resistance-induced free fatty acid mobilization [21]. HMG-CoA reductase is the key regulatory enzyme in cholesterol biosynthesis, catalyzing the conversion of HMG-CoA to mevalonate, a precursor of cholesterol [22]. Inhibition of this enzyme suppresses hepatic cholesterol synthesis, resulting in reduced circulating cholesterol levels [23,24]. The observed antihyperlipidemic activity of *Coccinia* may therefore contribute to the prevention of cardiovascular complications associated with diabetes.

Recent studies have emphasized the involvement of free radicals in the pathogenesis of diabetes and its complications [25]. The bitter components present in

Coccinia may stimulate TAS2R receptors, leading to the secretion of glucagon-like peptide-1 (GLP-1), which plays an important role in lowering blood glucose levels. In addition, polyphenolic compounds in medicinal plants are known to activate bitter taste receptors within the gastrointestinal tract [26]. These receptors establish an important relationship between nutrient sensing and metabolic regulation. Based on the phytochemical investigations and in vitro findings of the present study, *Coccinia* may act synergistically as a potent antidiabetic agent in both Non-Insulin Dependent Diabetes Mellitus (NIDDM) and Insulin Dependent Diabetes Mellitus (IDDM) by improving insulin regulation and reducing the risk of diabetic complications.

9 CONCLUSION

The results of the present study indicate that the antioxidant potential of both raw and ripe fruits of *Coccinia grandis* may be associated with the presence of diverse phytochemical constituents occurring in varying concentrations. The antioxidant properties of flavonoids and related phytochemicals identified in the fruit have been widely recognized. Comparatively, the ripe fruit exhibited greater antioxidant activity, suggesting that it may be more beneficial for dietary incorporation or for use in herbal supplement formulations.

In addition, the in vitro investigations demonstrated promising antidiabetic and antihyperlipidemic activities of *Coccinia grandis*. Nevertheless, these experimental findings do not conclusively establish its therapeutic efficacy against Type 2 Diabetes Mellitus (T2DM) and associated lipid abnormalities. Therefore, further in vivo investigations and controlled clinical studies are required to validate these findings and to comprehensively evaluate the safety, efficacy, and clinical applicability of *Coccinia grandis* as a potential therapeutic agent.

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Conflict of interest: No conflict of interest is associated with this work.



Contribution of authors: We declare that this work was done by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. All authors read and approved the final manuscript.

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