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A Study On Quantitative Estimation Of Secondary Metabolites And In Vitro Dipeptidyl Peptidase IV (DPP-IV) Inhibitory Activity Of Coccinia Grandis Fruit Extract.

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Abstract

Nature provides an abundant source of medicines that can be used to treat diabetes. A range secondary metabolites, including alkaloids, terpenoids and flavonoids have shown notable anti-diabetic effects. These natural compounds can increase insulin secretion, boost insulin sensitivity, and lower blood glucose levels. Coccinia grandis (ivy gourd) have demonstrated promising potential in the field of medicine due to their rich phytochemical composition. Various cultural settings have traditionally utilized these herbs for their purported therapeutic properties, particularly in the field of diabetes management.

Objective: The present study objective is to investigate the antidiabetic potential of Hydroalcoholic extract Coccinia grandis fruit through in vitro assays with DPP-IV enzyme inhibition and quantitative estimation with standard producers.

Materials and Methods: The current research investigated DPP-IV inhibitory mechanism of C.Grandis fruit extract by utilizing the spectrophotometric approach and Sitagliptin serves as a positive control. The efficacy of inhibition has been evaluated by percent inhibition.

Results: The findings indicated that the extract effectively suppressed DPP-IV activity, comparable to sitagliptin but at a concentration that was ten times greater. The extract exhibited IC50 value of 88.22µg/mL, whereas the IC50 value of sitagliptin was 44.68µg/ml. The total flavonoid, phenolic, and alkaloid content in the fruit of coccinia grandis was measured to be 3.15 mg/100mg, 2.24mg/100mg, and 2.14 mg/100 respectively.

Conclusion: the results indicate that the Hydroalcoholic extract of coccinia grandis has higher flavonoid content compared to alkaloids and phenols which may have a potential DPP-IV inhibitory effect that can serve potential anti-diabetic agent.

Keywords: Antidiabetic, DPP-IV, Coccinia grandis, Inhibitory activity.

Introduction

A chronic metabolic disease called diabetes mellitus is typified by abnormally elevated blood glucose levels. It has become a major global health concern. Given the rising incidence of diabetes and its concomitant problems, there is an emerging need to investigate natural cures and alternative medicines as potential means of managing the condition. Plant sources are generally regarded as non-toxic, with fewer adverse effects compared to synthetic sources.

The scientific community has shown considerable interest in investigating plant-based extracts that may possess antidiabetic effects in this particular setting. Many secondary metabolites have pharmacological properties, making them valuable in medicine. Compounds such as flavonoids, alkaloids, and terpenoids have been used in traditional and modern medicine for their anti-inflammatory, antioxidant, antimicrobial, and anticancer activities, and significant anti-diabetic effects. Secondary metabolites from plants, such as polyphenols and flavonoids, have been sources of therapeutic agents for antidiabetic activity. They act by improving insulin secretion, and insulin sensitivity, and reducing the absorption of glucose ((1).

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Researchers have discovered the effectiveness of several medicinal plants in managing diabetes (2). The underlying mechanism in most of these cases remains unknown despite the desired outcome being achieved. In order to assess the effectiveness of drugs in treating diabetes, in vitro tests can be employed as an initial means of screening. These tests allow for the evaluation of a large number of potential therapeutic candidates. They could potentially offer valuable insights into the mechanism by which therapeutic agents work (3).

Conventional pharmacological treatments for diabetes target various aspects of glucose metabolism. Metformin reduces hepatic glucose production, sulfonylureas stimulate pancreatic insulin secretion, and thiazolidinediones enhance insulin sensitivity in peripheral tissues (4). Alpha-glucosidase and alpha-amylase inhibitors, such as acarbose and voglibose, delay carbohydrate digestion and absorption, thereby moderating postprandial blood glucose levels (van de Laar 2008). DPP-IV inhibitors, such as sitagliptin, saxagliptin, vildagliptin, linagliptin, and alogliptin, are often prescribed medications for the treatment of diabetes (5).

Researchers have an equal interest in antidiabetic drugs that target the incretin pathway in particular. The intestinal mucosa produces endogenous hormones called incretins. These primarily consist of glucagon-like peptide 1 (GLP-1) and insulin-tropic polypeptide (GIP). They decrease blood glucose levels and respond to glucose by increasing insulin secretion (Thakare et al., 2017). 60% of the insulin released by the body comes from the incretin pathway, which is mediated by GLP-1 and GIP (6). Therefore, 2 primary methods to GLP-1 therapies for diabetes management include prolonging the duration of GLP-1 circulation in the liver by inhibiting the enzyme DPP-IV which belongs to a serine protease family and has the ability to break down the incretins GLP-1 and GIP-1 very quickly (7). DPP-IV inhibitors primarily function by impeding the rapid breakdown of incretins, such as GLP-1, thereby ensuring a sustained impact on insulin stimulation (8).

The development of diabetic-friendly foods for the prevention as well as treatment of type 2 diabetes mellitus (T2DM) is a significant opportunity presented by the food and natural health product businesses. While these conventional drugs are effective, they can cause side effects like nasopharyngitis and pancreatitis with DPP-IV inhibitors (9). Interest in natural products for the treatment of diabetes is increasing because of their efficacy and lower side effects, with phytochemicals offering promising avenues for new treatments due to their diverse mechanism of action (10). Although the effectiveness of natural DPP-IV inhibitors (eg, berberine from Berberis aristata and plamitine from Coscinium fenestratum, as well as flavonoids in most fruits and vegetables) that augment incretin hormone activity may not be comparable to those synthetic ones (11).

The fruits of Coccina grandis (ivy gourd) possess pharmacological characteristics due to their substantial phytochemical makeup. Herbs in Different Cultural Contexts: Several cultures have used these herbs traditionally for their so-called medicinal purposes, especially related to diabetes. Coccinia grandis (Indian ivy gourd / kowai) C. grandis has a pharmaceutical application as an "India replacement for insulin," and it is valuable in diabetes (12). This plant basially contains several secondary metabolites such as flavonoids (quercetin, polyphenols), Xyloglucan polymers, glycosides and alkaloids along with cryptoxanthins; carotenoid - Carotene-, terpenes- Taraxerol]) and saponuns. A systematic approach for glucose metabolism in controlling diabetes by C. grandis fruit and leaf extract studies have been reviewed while emphasizing the multiple mode of actions paradigm through animal or cell models (13).

This study is ahead to find out some clue for antidiabetic property of Hydroalcoholic extract Coccinia grandis fruit by in vitro tests such as DPP-IV enzyme inhibition as well quantitative estimation for secondary metabolites. With this we will know how it works and if could also use as a drug to treat diabetes.

Material and methods

Plant Material

Fruit of coccinia grandis for the present study was taken from market area.. A botanist, who holds the position of Assistant Professor in the Department of Botany at S.V. University, Tirupati, verified the plant material. The verified specimen was allocated a voucher number 0579 for future reference.

The fruits were first dried in the shade, then crushed into coarse powder and passed through a 40-mesh sieve to obtain a fine powder. The fine powder was then sealed into an airtight container. The extraction was carried out with a dried material powder (100g) soaked in 60% ethanol hydro-alcoholic solution. Plant material exaction requirement chemicals were solute by the solvent, so 7days maceration was performed. After 7 days dissolution maceration, the mixture was filtered to separate liquid extract and solid residue. The solvent was evaporated from the filtered liquid to get a concentrated extract of Coccinia grandis (14).

Quantitative estimation:

Determination of total flavonoid content

Determination of flavonoids Hydroalcoholic extracts were determined by Aluminium chloride method in the fruit of Coccinia grandis (15). The

150µL of 5% $NaNO_2$ solution was mixed with the test sample, which contained 1mg/mL of extract. After that, the mixture has been then incubated for 5 minutes at room temperature. After adding 150µL of 10% aluminium chloride to the mixture, it was allowed to sit undisturbed for 60 minutes. The next step was to add 2 millilitres of sodium hydroxide (1M) solution to the mixture. To make it up to 5 millilitres in volume, distilled water was added. After a good mixing, the ingredients were set aside for another fifteen minutes to relax. The measurement of absorbance relative to the blank was conducted at a wavelength of 510nm using a spectrophotometer. The identical standard procedure was employed to analyze the standard quercetin solution at various concentrations ranging from 5, 10, 20, 30, 40, and 50ug/ml, and the calibration line was established. The amount of flavonoids was measured and expressed as milligrammes of quercetin equivalents (mg/100mg).

Determination of total Alkaloid content:

Estimation of alkaloid content in the HACG fruit was done by spectrophotometric method. An alkaloid and bromocresol green (BCG) react to produce a yellow chemical, which is the basis of this method. (16).

Briefly, 1mg/ml hydroalcoholic extracts of Coccinia grandis fruit were dissolved in 2N HCl double in distilled water and filtered. After transferring 1 mL of the solution to a separatory funnel, it was washed three times with 10 mL of chloroform. Using 0.1N NaOH, the pH of this mixture was brought to a neutral level. Then, 5 millilitres of BCG solution and 5 millilitres of phosphate buffer have been then added to the mixture. The solution was vigorously shaken while adding 1, 2, 3, and 4mL of chloroform, and the resultant product was then separated. A 10 mL volumetric flask was used to hold the collected materials. The required volume was then achieved by diluting them with chloroform. At 470 nm, the complex's absorbance in chloroform was measured in comparison to a blank sample made in the same way.

The total Alkaloid content has been quantified and reported in milligrams of Atropine equivalents (mg/100mg).

Determination of total Phenolic content:

The folin-Ciocalteu (17) approach has been utilized the total phenolic content (TPC). A 1 mL aliquot of Coccinia grandis or a standard Gallic acid solution (5, 10, 20, 30, 40, or 50 µg/ml) has been then added to a 25 mL volumetric flask that held 10ml of distilled water already. A blank reagent devoid of any substance was made using distilled water. The mixture was stirred after 1 milliliter of Folin-Ciocalteu phenol reagent was added. A volume of 10 mL of a 7.5% Na2CO3 solution was added to the mixture after 5mins. After that, the volume was raised to the appropriate level. Using a UV-visible (Systronics) at 760 nm, the absorbance against the reagent blank was measured following a 30- to 45-minute incubation period at room temperature.

The hydroalcoholic extracts of Coccinia grandis fruit were tested for total phenolic component content in milligrammes of gallic acid equivalent (mg/100mg). An equation developed from a typical gallic acid graph was used to produce this measurement. The samples were analyzed in duplicate.

DPP-IV enzyme activity measurement

The evaluation of this assay was conducted utilizing a DPP-IV inhibitor screening kit (BML-SE434, Enzo Life Sciences, and USA with minimal alterations. The" test samples were diluted to various concentrations ranging from 6.25 to 100µg/mL after being dissolved in the assay buffer included in the kit. Using the assay buffer, the DPP-IV enzyme was diluted at a volume-to-volume ratio of 1:4. The initial activity wells were prepared by adding 50µL of substrate, 10µL of diluted DPP-IV, and 40µL of assay buffer. The assay buffer (30µL), sitagliptin (positive control), 10µL of DPP-IV, 50µL of the substrate, and 10µL of test samples were all placed in the inhibitory activity wells. The plate was incubated for 10 minutes at room temperature before adding the substrate and starting the reaction. Next, the plate was sealed with a ninety-six well cover sheet and foil and placed in an incubator at a temperature of 37 ◦C for a duration of 30 minutes. Following the incubation period, Stop the reaction by adding 25ul of 25% acetic acid and read the absorbance at 405nm with a microplate reader (18). The subsequent equation was employed to determine the inhibitory "activity:

% Inhibition = [(Absorbance control – Absorbance test sample) / Absorbance control] × 100

The IC 50 values were obtained by plotting the percent inhibition against the inhibitor concentration. Subsequently, these values were obtained by non-linear regression analysis utilizing the average inhibitory values.

Results

Quantitative Phytochemical Analysis of Hydroalcoholic extracts of Coccinia grandis fruit (HACG).

Total flavonoid content:

To estimate the TFC, a calibration curve using quercetin (a type of flavonoid) was prepared. The quercetin was used as a standard and measured for its absorbance at various concentrations (µg/ml). Y= 0.0188X + 0.0021, where X is the Quercetin equivalent (QE) and Y is the absorbance, was used to calculate the flavonoid content of the coccinia

grandis, depending on the quercetin absorbance at 420nm. The calibration curve has an R2 value of 0.9996.
The TFC in the fruit of coccinia grandis was measured to be 3.15 mg/100mg (Table 1).

Total alkaloid content:
The chemical was synthesized to have a peak absorption wavelength and a yellow color. At a pH of 4.7, the chloroform completely extracted this combination. Various concentartions of atropine were used to construct the calibration curve shown in Figure 2. The standard curve yields the following equation: y = 0.0036x + 0.0238, where x is the concentration in μg/mL and y is the absorbance. A value of 0.9919 is obtained as the r-value from the standard curve.
Table 1 shows that the total flavonoid content in coccinia grandis fruit was 2.14 mg/100 mg as determined by the standard plot (y = 0.0036x + 0.0238).

Total phenolic content:
The quantification of total phenols was performed using a standard calibration plot of gallic acid. The construction was carried out within the concentration range of 5-50 μg/ml, yielding a coefficient of determination (R2) of 0.9981 (Figure 3). According to the standard plot of gallic acid (y= 0.0189x+0.0478), the fruit of coccinia grandis was found to have a phenols content of 2.24mg/100mg (Table 1).

Effect of hydro-alcoholic extracts from C. Grandis fruits on DPP-IV enzyme inhibition.
The DPP-IV enzyme inhibitory activity of the hydroalcoholic extracts of HACG. The fruit was examined using in vitro assays. Sitagliptin served as the benchmark for comparison. The experiments were replicated 3times times, and each replication showed excellent agreement. The findings indicated that the extract effectively suppressed DPP-IV activity, comparable to sitagliptin but at a concentration that was ten times greater. The information on concentration and percentage of inhibition is presented in Table 2.
The determined IC50 value of the extract was 88.22 μg/mL while the IC50 value of sitagliptin was 44.68μg/mL shown in table 2.

Discussion and conclusion
Phytochemicals are specific chemical components found in plants that have unique effects on the human body's physiology (19). In addition to their physiological activity, these phytochemicals have medicinal properties. Alkaloids, flavonoids. Phenolic, terpenoids and essential oils are significant bioactive phytochemicals (20). It is critical to screen plant products to evaluate their safety and identify the kind and severity of any adverse effects generated by medicinal plants since people often take herbal remedies without medical supervision. The researchers investigated the nutrients and numerous phytochemicals that have the potential to be used in the production of pharmaceuticals and as dietary supplements. This is regarded as an essential first step.
The fruits of the Coccinia grandis plant were found to contain a number of compounds with potential medicinal uses. It contained alkaloids, glycosides (saponins), terpenes (sesquiterpene lactone type) steroids/sterols and or another satisfied group of compounds while phenolic/flavonoid saccharine has tannin. Studies show that phytochemicals in this plant have many positive aspects, such as relieving pain, resolving high fever (21), antimicrobial activity inhibiting and killing infectious organisms which cause infection or disease states like Diabetes,Cancer, Malaria and Asthma (22).
Some of these extracts contain phenolic compound, which has an antimicrobial action inhibiting the growth and changing the mechanisms associated with microorganisms proliferation (23). Tannins have been demonstrated to possess anti-diarrheal, antibacterial, antisecretolytic as well as antihelminthic effects(24) Flavonoids have been studied for their antidiabetic, antioxidative, anti-inflammatory immunoregulatory activities and may also reduce cancer development and viral infection; though. (25). These bioactive compounds have been discovered to offer diverse therapeutic qualities that could be advantageous in the clinical treatment of various illnesses.
The quantitative study confirmed the presence of alkaloids, phenols, and flavonoids in both the extracts of Coccinia grandis fruits in a significant amount. The study findings are shown in Table 1.
Current study aims the antidiabetic activity of Coccinia grandis fruit with concomitant in vitro biological assays. The results found that DPP-IV inhibitory activities were substantial of C. grandis One of the main therapeutic approaches in type II diabetes is based on inhibition of this enzyme because it can potentiate incretin activity leading to increased insulin secretion.
Coccinia grandis showed notable inhibitory effects on DPP-IV, an "enzyme responsible for degrading incretin hormones such as GLP-1 and GIP. These hormones play a crucial role in enhancing glucose-dependent insulin secretion and suppressing glucagon release, thereby" regulating blood glucose levels (7and 8). The determined IC50 value of the extract was 115.38μg/mL respectively while the IC50 value of sitagliptin was 44.68μg/mL.
In addition to the above information, potential applications related to DPP-IV inhibitory and

antidiabetic impact could be performed with plant extracts in clinical trial. Interest in natural DPP-IV inhibitors has increased because these agents have fewer adverse effects than synthetic inhibitors and are effective at lowering blood glucose levels (26). For example, compounds in Coccinia grandis may affect the incretin pathway to enhance insulin sensitivity and secretion (27).

Therefore, Coccinia grandis could be used as potent antidiabetic agents due to its diverse mechanisms of action. Inhibiting the DPP-IV enzyme provides a whole new approach to correct two critical abnormalities in type 2 diabetes, enhancing incretin activity and suppressing glucagon action.

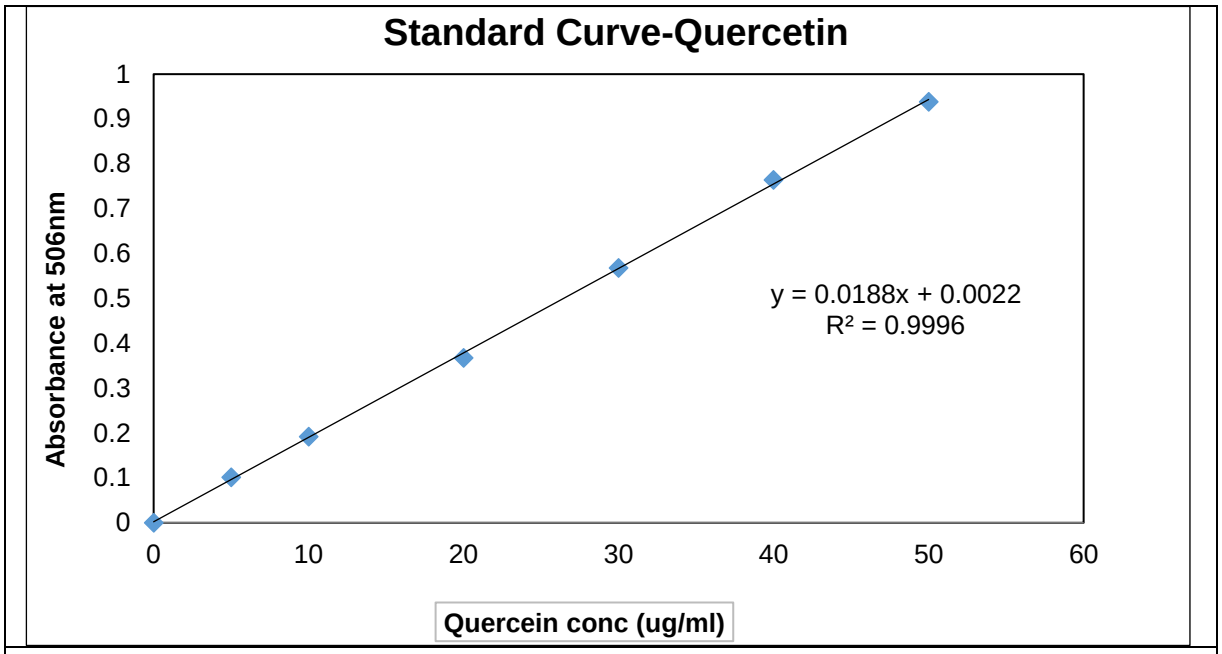

Figure 1. Calibration Curve of Quercetin for Flavonoid Determination

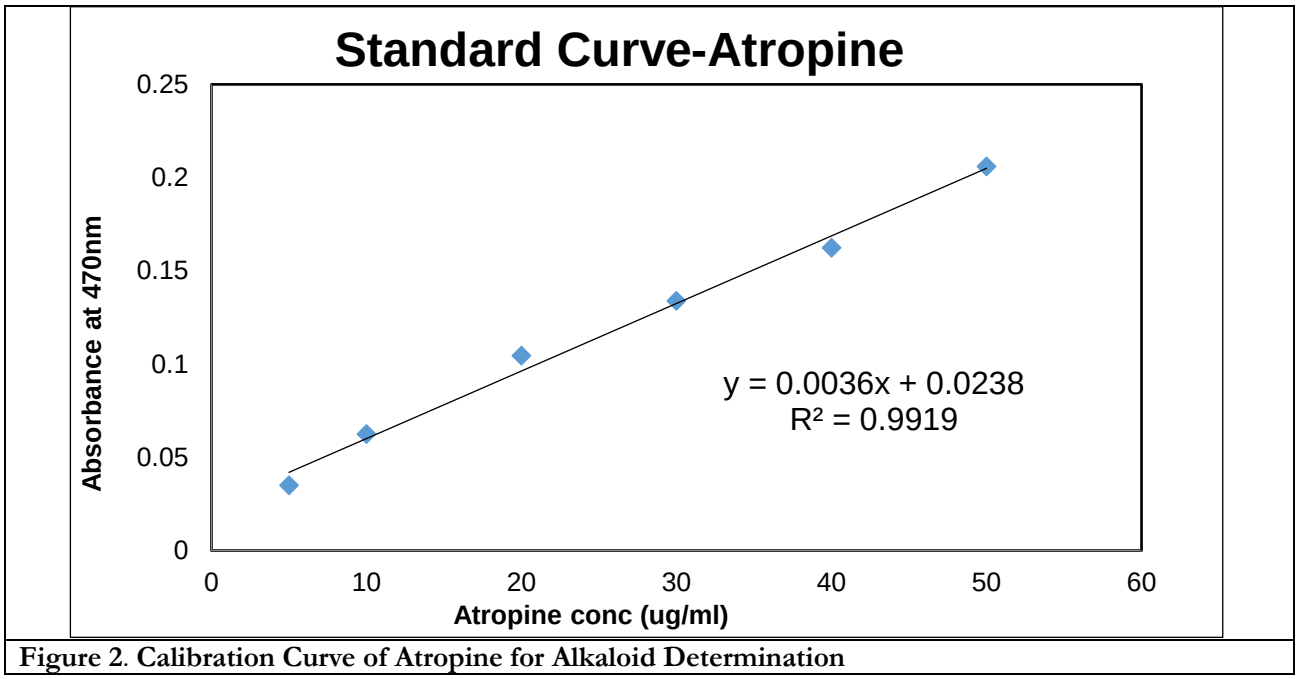

Figure 2. Calibration Curve of Atropine for Alkaloid Determination

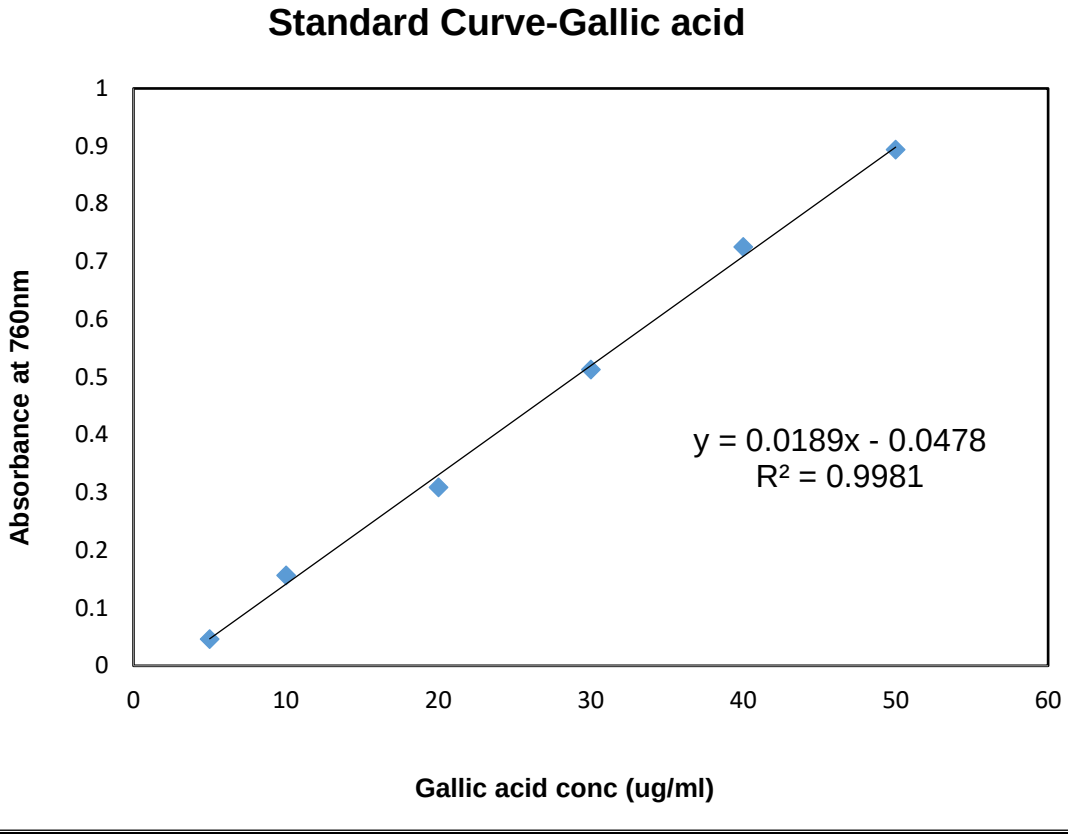

Figure 3. **Calibration Curve of Gallic acid for Phenol Determination**

Table 1. Total Flavonoid, Alkaloids and Phenolic contents in hydroalcholic extract of Coccinia grandis (HACG) fruit.

S.no.	Plant used	Plant used	Phytochemical constituents	Concentration
1	HACG	FRUIT	Flavonoids	3.15mg of quercetin/100gm
			Alkaloids	2.14 mg of atropine/100mg
			Phenols	2.24 mg of GAE/gm

Table 2: The Comparative results of in vitro DPP-IV enzyme inhibitory activity of different Test compounds.

Test compounds	% inhibition at Conc. (ug/ml)					IC 50 (ug/ml)
	6.25	12.5	25	50	100	
Sitagliptin	12.62± 0.02	24.02± 0.01	43.88± 0.01	68.54± 0.00	89.58± 0.01	44.68
HACG Fruit	1.57± 0.00	5.11± 0.01	15.06± 0.01	29.57± 0.01	54.54± 0.01	88.22
n=3; the values are given as mean± SD						

References

1. Hanhineva, K., Törrönen, R., Bondia-Pons, I., Pekkinen, J., Kolehmainen, M., Mykkänen, H., & Poutanen, K. (2010). Impact of dietary polyphenols on carbohydrate metabolism. International Journal of Molecular Sciences, 11(4), 1365-1402.
2. Stamp, N. (2003). Out of the quagmire of plant defense hypotheses. *The Quarterly Review of Biology, 78*(1), 23-55. https://doi.org/10.1086/367580
3. Roy, A., & Geetha, R. V. (2013). In vitro α-amylase and α-glucosidase inhibitory activities of the ethanolic extract of Dioscorea villosa

tubers. *International Journal of Pharma and Bio Sciences, 4*(4), 49-54.
4. Nathan, D. M., Buse, J. B., Davidson, M. B., Ferrannini, E., Holman, R. R., Sherwin, R., & Zinman, B. (2009). Medical management of hyperglycemia in type 2 diabetes: A consensus algorithm for the initiation and adjustment of therapy. *Diabetes Care, 32*(1), 193-203.
5. Schernthaner, G., & Schernthaner, G. H. (2020). The role of thiazolidinediones and sulfonylureas in the treatment of type 2 diabetes mellitus. *Drugs, 80*(17), 1863-1880. https://doi.org/10.1007/s40265-020-01408-w
6. Thakare, V., et al. (2017). Role of conventional oral antidiabetic drugs in management of type 2 diabetes mellitus. *International Journal of Research in Medical Sciences, 5*(3), 749-758.
7. Lin SR, Chang CH, Tsai MJ, Cheng H, Chen JC, Leong MK, Weng CF (2019) The perceptions of natural compounds against dipeptidyl peptidase 4 in diabetes: from in silico to in vivo. Therapeutic advances in chronic disease 10: e2040622319875305. https://doi. org/10.1177/2040622319875305
8. Ahrén B. DPP-4 inhibitors. Best Pract Res Clin Endocrinol Metab. 2007 Dec;21(4):517-33. doi: 10.1016/j.beem.2007.07.005. PMID: 18054733.
9. American Diabetes Association. (2023). Standards of Medical Care in Diabetes—2023. *Diabetes Care, 46*(Supplement 1), S1-S264. https://doi.org/10.2337/dc23-S001
10. Patel, D. K., Prasad, S. K., Kumar, R., & Hemalatha, S. (2012). An overview on antidiabetic medicinal plants having insulin mimetic property. Asian Pacific Journal of Tropical Biomedicine, 2(4), 320-330.
11. Zhang, L., Zhang, J., & Ling, S. (2019). Antidiabetic effects of palmatine by increasing insulin sensitivity via PTP1B and PI3K/Akt signal pathway in insulin-resistant hepatocytes. *European Journal of Pharmacology, 853*, 1-8.
12. Chopra, R., Chopra, I., Handa, K., & Kapur, L. (1958). *Indigenous Drugs of India* (2nd ed.). UN Dhar and Sons Pvt. Ltd.
13. Putra, I. M. W. A., Fakhrudin, N., Nurrochmad, A., & Wahyuono, S. (2022). Antidiabetic activity of *Coccinia grandis* (L.) Voigt: bioactive constituents, mechanisms of action, and synergistic effects. *Journal of Applied Pharmaceutical Science, 12*(01), 041-054. https://doi.org/10.7324/JAPS.2021.120103
14. Iqbal, E., Salim, K. A., & Lim, L. B. L. (2015). Phytochemical screening, total phenolics, and antioxidant activities of bark and leaf extracts of Goniothalamus velutinous (Airy Shaw) from Brunei Darussalam. Journal of King Saud University - Science, 27(3), 224-232.
15. Kariyone T, Hashimoto Y, Kimura M. Microbial studies of plant components. IX. Distribution of flavonoids in plants by paper chromatography. J Pharm Soc. 1953;73:253–6.
16. Shamsa F, Monsef H, Ghamooshi R, Verdian-rizi M. Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. Thai J of Pharm Sci. 2008;32:17–20.
17. Ali HFM, El-Ella FMA & Nasr NF (2010) Screening of chemical analysis, antioxidant antimicrobial and antitumor activities of essential oil of oleander (Nerium oleander) flower. International journal of biological Chemistry 4(4): 190–202.
18. González-Montoya, M., Hernández-Ledesma, B., Mora-Escobedo, R., & Martínez Villaluenga, C. (2018). Bioactive peptides from germinated soybean with anti-diabetic potential by inhibition of dipeptidyl peptidase-IV, α-amylase, and α-glucosidase enzymes. *International Journal of Molecular Sciences, 19*(10), 2883. https://doi.org/10.3390/ijms19102883.
19. Vimala, A., Thamizharasi, T., Sathish, S. S., Palani, R., & Vijayakanth, P. (2013). Phytochemical studies on selective medicinal plants. *International Journal of Research in Engineering and Biosciences, 1*(1), 57-62
20. Anwar, F., Jamil, A., Iqbal, S., & Sheikh, M. A. (2006). Antioxidant activity of various plant extracts under ambient and accelerated storage of sunflower oil. Grasas y Aceites: International Journal of Fats and Oils, 57(3), 189-197.
21. Aggarwal, A. S., Suralkar, U. R., Chaudhari, S. G., Deshpande, S. V., Garud, A. A., & Talele, S. G. (2011). Analgesic and antipyretic activity of methanolic extract of Coccinia grandis L. leaves in experimental animals. *Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2*(1), 175-182.
22. Bhattacharya, B., Lalee, A., Mal, D. K., & Samanta, A. (2011). In vivo and in vitro anticancer activity of Coccinia grandis (L.) Voigt (Family: Cucurbitaceae) on Swiss albino mice. *Journal of Pharmacy Research, 4*(1), 567-569.
23. Udayakumar, R., Kasthurirengan, S., Vasudevan, A., Mariashibu, T. S., Rayan, J. J., Choi, C. W., Ganapathi, A., & Kim, S. C. (2010). Antioxidant effect of dietary supplement Withania somnifera L. reduces blood glucose levels in alloxan-induced diabetic rats. Plant Foods for Human Nutrition, 65(2), 91–98.
24. Singh, G., Passari, A. K., Leo, V. V., Mishra, V. K., Subbarayan, S., Singh, B. P., et al. (2016). Evaluation of phenolic content variability along with antioxidant, antimicrobial, and cytotoxic potential of selected traditional medicinal plants from India. *Frontiers in Plant Science, 7*, 407

25. Raj, J. B., & Kalaivani, R. (2016). Comparative in-vitro evaluation of antihelmintic property of leaves and rhizome of Costus pictus D. Don against albendazole. *National Journal of Physiology, Pharmacy and Pharmacology, 6*(5), 438-441
26. Liu, H., Wang, J., Liu, M., Zhao, X., & Pang, L. (2013). Inhibitory effects of palmatine from *Mahonia bealei* (Fort.) Carr. on alpha-glucosidase: Kinetics and mechanism. *Journal of Functional Foods, 5*(4), 1671-1678.
27. Kumar, S., Kumar, V., & Prakash, O. (2011). Antidiabetic and hypolipidemic activities of *Dillenia indica* extract in diabetic rats. *Journal of Ethnopharmacology, 135*(1), 34-42.