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Evaluation of A-Amylase and *A*-Glucosidase Inhibitory Effects of Bioactive Compounds of *Coccinia grandis* (L.) Voigt Leaves

[1]*A. Doss and* [2]*M. Vijayasanthi*

[1]Department of Microbiology, Kamaraj College, Thoothukudi, Tamil Nadu State, South India
[2]Department of Microbiology, Ayya Nadar Janaki Ammal College (Autonomous),
Sivakasi, Tamil Nadu State, South India

Abstract: The present study was conducted to determine the inhibitory potential of bioactive phytochemicals against key enzymes related to type 2 diabetes. The inhibitory potential of bioactive compounds (flavonoids, tannins, anthocyanins, total phenols) against the enzymes was determined by using *A*-amylase and *A*-glucosidase inhibition assays. The bioactive phytochemicals showed significant inhibitory activity on the amylase and glucosidase, thus suggesting that the phytochemicals might be useful in the treatment to limit dietary fat and glucose absorption and the accumulation of fat in adipose tissue.

Key words: Bioactive Compounds · Flavonoids · Tannins · Inhibitors

INTRODUCTION

Diabetes mellitus is a non-communicable disease considered to be one of the three leading causes of death world-wide [1]. Currently, there are over 150 million diabetics worldwide and this is likely to increase to 300 million or more by the year 2025. According to the World Health Organization (WHO) reports, India already leads the world with the largest number of diabetic subjects (nearly 40 million) and it is predicted that this number would reach almost 80 million by the year 2030. This would represent approximately 20 per cent of the total diabetic population of the world. The most prevalent form both in the global and Indian scenario is the noninsulin dependent diabetes mellitus (NIDDM type 2) which is associated with elevated postprandial hyperglycemia. The prevalence and morbidity associated with type 2 diabetes mellitus continues to increase throughout the world. The secondary complications in diabetes mostly from micro and macro vascular changes [2].

The therapeutic advance for treating diabetes is to decrease postprandial hyperglycemia. This can be achieved through the inhibition of carbohydrate hydrolyzing enzymes such as alpha glucosidase and alpha amylase [3]. A-amylase and A-glucosidase are the well-known enzymes playing a key role in the management of hyperglycemia-linked Type 2 diabetes [4]. Higher plants, animals and microorganisms are found to produce a large number of different protein inhibitors of alpha amylases and alpha glucosidases in order to regulate the activity of these enzymes. Some of these enzyme inhibitors act by directly blocking the active centre of the enzyme at various local sites. *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, gonorrhea etc. The leaves of the plant possess antidiabetic, anti-inflammatory, antipyretic, analgesic, antispasmodic, antimicrobial, cathartic and expectorant activities [5,6]. The leaves contain Triterpenoids, alkaloids and tannins [7]. In the present study the A-glucosidase and A-amylase inhibitory activity of the some crude bioactive compounds from *Coccinia grandis* has been evaluated by *in vitro* assays.

MATERIALS AND METHODS

Collection of Plants: The leaves of *C. grandis* were collected from Coimbatore district, Tamilnadu, India and authenticated by Dr. S.P. Anand, Assistant Professor,

Corresponding Author: Dr. A. Doss, Assistant Professor, Department of Microbiology, Kamaraj College, Thoothukudi, India.

PG and Research Department of Botany, National College, Tiruchirappalli. A voucher specimen is being maintained in the Department of Microbiology, Kamaraj College, Tuticorin, Tamilnadu, South India.

Total Phenols: The phenolic compounds in crude extracts of *C. grandis* were extracted with 10 ml of 80% aqueous ethanol in the dark at room temperature on a magnetic stirrer for 2h. Then the contents were centrifuged at 13,000x g for 10 min and the supernatant was recovered and used for the analysis of phenolic compounds according to the method described by Mazza *et al*. [8]. Briefly, the method consists of mixing 10 ml of extract with 240 ml of a solution of 2% HCL in 75% ethanol in a 96 well-flat bottom micro titration plate. The absorbance of the solution was monitored at 280 nm with a spectrophotometer using (+)-catechin (0-200µg/ml for total phenols).

Extraction of Flavonoids: The hot water extract was prepared by boiling 200 g of fresh leaves with 1 ltr. of distilled water for 3h. The final volume was reduced to 200 ml. The water extract was centrifuged and the supernatant was obtained. Excess of ethanol was added to the supernatant to precipitate the high molecular weight polysaccharides fraction (500 mg), which was filtered and concentrated in vacuum and extracted with ethyl acetate. The ethyl acetate soluble fraction (flavonoids) and the aqueous fraction (Proanthocyanidin) were obtained [9].

Extraction of Tannins: Sample of the powdered leaf (3 g) was boiled in 5 ml of distilled water for 3 min on a hot plate. The mixture was filtered while hot and the resulting filtrate was used to carry out ferric chloride test [10]. Sample of the filtrate (1.0 g) was transformed into a beaker and 10ml of distilled water was added. This was boiled for 5 min. Two drops of 5% ferric chloride ($FeCl_2$) was then added. Production of greenish precipitate indicated the presence of tannins.

Analysis of Hypoglycemic Activity

α-Amylase Inhibition Assay: The A-amylase inhibition activity of various bio active compounds extracted from *C.grandis* was determined according to the assay described by Worthington [11]. 500 µl of each bioactive compounds (25-125 µg/ml) and 500 µl of 0.02 M sodium phosphate buffer (pH 6.9) containing A-amylase solution (1 unit liberates 1.0 mg of maltose from starch in 3 min at pH 6.9 and temperature 25°C) were taken and incubated at 25°C for 10 min. After the pre-incubation, 500 µl of 1 % starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube at regular time intervals. After the incubation at 25°C for 30 min the reaction was stopped with 0.1 ml of dinitrosalicylic acid reagent. The test tubes were then incubated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was then diluted with the addition of 15 ml of distilled water and the absorbance was measured at 540 nm. The readings were compared with the control, which contain buffer instead of sample extract. The results were calculated by using the following equation.

A-Amylase inhibition activity (%) = Acontrol-Aextract / Aontrol x 100

α-Glucosidase Inhibition Assay: The A-glucosidase inhibition activity of various bioactive compounds of the present investigation was analyzed according to the method of Worthington [12]. 50 µl of each bioactive compounds (25-125 µg/ml) and 100 µl of 0.1 M phosphate buffer (pH 6.9) containing A-glucosidase solution (1 unit /ml) were taken in a 96 well flat-bottom micro titration plate and incubated at 25°C for 10 min. After the pre-incubation, 50 µl of 5 mM p-nitrophenyl-A-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added to each well at regular time intervals and the reaction mixture was incubated at 25°C for 5 min. After the incubation period the absorbance readings were recorded at 405 nm and compared to a control that had 50 µl of buffer solution in place of the extract. The results were calculated according to the following equation and expressed as percentage of A-glucosidase inhibition

A-Glucosidase inhibition activity (%) = Acontrol-Aextract / Acontrol x 100

RESULTS AND DISCUSSION

Diabetes mellitus (DM) is a common metabolic disease characterized by elevated blood glucose levels, resulting from absent or inadequate pancreatic insulin secretion, with or without current impairment of insulin action. Reports from the World Health Organization (WHO) indicate that diabetes mellitus is one of the major killers of our time [13], with people in Southeast Asia and the Western Pacific being most at risk [14]. DM has been identified by Indian Council of Medical Research (ICMR) as one of the refractory diseases for which satisfactory treatment is not available in modern allopathic system of medicine and suitable herbal preparations are to be

Table 1: Bioactive compounds contents of *Coccinia grandis* (L.) Voigt

Bioactive compounds	Yield (mg / 100g of sample)
Total phenols	1560
Tannins	43
Flavonoids	268
Proanthocyanidin	54

investigated [15]. The leaves of *C.grandis* of the present study were found to contain appreciable levels of various bioactive compounds such as total Phenolics (1560 mg/100 g of sample), tannins (43 mg/100 g of dry sample), flavonoids (268 mg/100 g of dry sample) and proanthocyanidin (54 mg/100 g of dry sample) (Table 1).

The inhibitory activity of bioactive compounds of *C. grandis* on A amylase and alpha glucosidase was investigated in this study and the results are shown in Table 2 and 3. The A-amylase enzyme inhibition activity of various bioactive compounds such as total Phenolics (17.83-38.7%), tannins (11.9-34.73%), flavonoids (17.96-44.8%) and proanthocyanidin (8.13-24.93%) extracted from the leaves of *C. grandis*. Among the various bioactive compounds, the flavonoids was found to exhibit maximum level of A-amylase inhibition activity (44.8%), whereas, the moderate A-amylase inhibition activity was detected in proanthocyanidin (24.93%) (Table 2). The A-glucosidase enzyme inhibition activity of various bioactive compounds such as total Phenolics (38.1-49.06%), tannins (38.96-50.86%), flavonoids (45.0-76.16 %) and anthocyanins (18.0-35.26 %) extracted from the leaves of *C.grandis*. Among the various bioactive compounds, the flavonoids were found to exhibit maximum level of A-glucosidase inhibition activity (76.16%), whereas, the moderate A-amylase inhibition activity was detected in anthocyanins (Table 3).

A-amylase and A-glucosidase are the well-known enzymes playing a key role in the management of hyperglycemia-linked Type 2 diabetes [4]. Comparison of A-amylase and A-glucosidase inhibition activity in the present drugs indicated that the *C.grandis* leaves have a lower level of A-amylase inhibition activity when compared to A-glucosidase inhibition activity. It is a well-known fact that the dietary management of hyperglycemia-linked Type 2 diabetes can be targeted through whole foods that have a high A-glucosidase and moderate level of A-amylase inhibition [16,17]. Because, excessive A-amylase inhibition leads to accumulation of undigested starch in the intestine and consequent stomach distension and discomfort.

Table 2: The percentage inhibition of A-galactosidase by bioactive compounds of *Coccinia grandis* (L.) Voigt

	Percentage of Inhibition				
	Concentrations (µg/ml)				
Bioactive compounds	25	50	75	100	125
Total Phenols	38.1 ± 0.26	40.9 ± 0.36	43.8 ± 0.52	45.23 ± 0.32	49.06 ± 0.40
Tannins	38.96 ± 0.55	39.86 ± 0.32	42.96 ± 0.45	47.83 ± 0.20	50.86 ± 0.61
Flavonoids	45.0 ± 0.32	52.13 ± 0.41	59.16 ± 0.20	65.4 ± 0.45	76.16 ± 0.20
Proanthocyanidin	18.0 ± 0.40	22.23 ± 0.32	29.0 ± 0.71	30.96 ± 0.45	35.26 ± 0.37

Values are expressed as mean ± SD (n = 3)

Table 3: The percentage inhibition of A-amylase by bioactive compounds of *Coccinia grandis* (L.) Voigt

	Percentage of Inhibition				
	Concentrations (µg/ml)				
Bioactive compounds	25	50	75	100	125
Total Phenols	17.83 ± 0.20	20.96 ± 0.35	26.0 ± 0.15	30.86 ± 0.61	38.7 ± 0.51
Tannins	11.9 ± 0.45	18.86 ± 0.41	26.9 ± 0.45	31.86 ± 0.32	34.73 ± 0.64
Flavonoids	17.96 ± 0.45	29.06 ± 0.40	31.93 ± 0.40	38.26 ± 0.30	44.8 ± 0.43
Proanthocyanidin	8.13 ± 0.41	12.93 ± 0.50	18.03 ± 0.75	21.06 ± 0.20	24.93 ± 0.70

Values are expressed as mean ± SD (n = 3)

The recent pharmacological studies showed the screening of A-amylase and A-glucosidase inhibition from natural sources especially medicinal plants is increasing. Inhibition of these enzymes has been recently developed from medicinal plants. There are many natural resources with the A-glucosidase inhibitory activity and some of them are more specific for sucrose inhibition rather than maltase inhibition. Inhibition of A-amylase, maltase and sucrose by a polyphenolic extract of green tea has been reported [18, 19]. Our present research suggest that the presence of polyphenolic compounds of *C.grandis* may have a potentially important role in managing diabetes via the inhibition of A-amylase and A-glucosidase enzyme activities.

The presence of potent A-glucosidase inhibitory activity therefore, appears more important in controlling the release of glucose from disaccharides in the gut than A-amylase inhibition. However, moderate A-amylase inhibition with potent A-glucosidase inhibitory activity may offer better therapeutic strategy that could slow down the availability of dietary carbohydrate substrate for glucose production in gut. Results of the present study showed a higher level of A-glucosidase inhibition activity and moderate level of A-amylase inhibition activity, which is more desirable for the dietary management of hyperglycemic linked type 2 diabetes.

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