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In-vitro Anti-diabetic and Antioxidant Efficacy of Methanolic Extract of *Canthium coromandelicum* Leaves

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Authors' contributions

This work was carried out in collaboration between both authors. Author SS designed the study and performed statistical analysis author STS wrote the protocol, performed the statistical analysis and managed the analyses of the study. Both authors read and approved the final manuscript.

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ABSTRACT

Oxidative stress plays a major role in diabetic complications. The study aim was to investigate the *in-vitro* antidiabetic and antioxidant activities of methanolic extract of *Canthium coromandelicum* leaves. The plant material was extracted with methanol and the methanolic extract was screened for *in-vitro* antioxidant activity using 1, 1-diphenyl-2-picryl hydrazyl (DPPH) assay. The efficiency of the antidiabetic activity of the plant extract was evaluated against α-amylase and α-glucosidase digestive enzymes. The study revealed that the *C. coromandelicum* extract exhibited significant α-amylase and α-glucosidase inhibitory activities with an IC_{50} value of 31.52 ± 0.42 and 41.49 ± 0.28 µg/mL respectively and compared with standard acarbose drug. The extract efficiently scavenging DPPH radical with IC_{50} values of 65.46 ± 0.50 µg/ml. Therefore, the extract could be a promising therapeutic in management of diabetic complications.

Keywords: In-vitro anti-diabetic; antioxidant; Canthium coromandelicum; DPPH; α- amylase.

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1. INTRODUCTION

Oxidative stress caused by the imbalance between free radicals and cellular oxidants scavengers in favour of free radicals has been implicated in the aetiology of insulin resistance and diabetic complications [1]. ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) and DPPH assay were widely used to monitor the antioxidant potential of the extract. These methods accommodated for Single Electron Transfer (SET) and Hydrogen Atom Transfer (HAT) activities [2,3]. Diabetes mellitus is the most common metabolic disorder caused by the insufficient secretion of insulin by the pancreas or the inability of the insulin produced to control blood glucose [4,5]. Pancreatic *α*- Amylase and *α*-Glucosidase inhibitors offer an effective way to lower the levels of postprandial hyperglycemia via delayed breakdown of carbohydrates [6,7].

Canthium coromandelicum (Rubiaceae family) is a bushy thorny herb, found throughout the western ghats and coast of the coromandel region of India. *Canthium* herbal medicine is used for the treatment of diabetes among major tribal groups in South India [8,9]. However, medicinal folklores usage still lacks scientific validation. In this current work, the *in vitro* antioxidant activity and *α*-Glucosidase, α-Amylase inhibitory potentials of *C. coromandelicum* leaves extract was evaluated. This is the first report on the *in vitro* anti-diabetic potential of methanolic extract of *C. coromandelicum* leaves to the best of our knowledge.

2. MATERIALS AND METHODS

2.1 Plant Materials

Leaves of *C. coromandelicum* were collected from Thanjavur, Tamil Nadu.

The leaves were identified by Botanical Survey of India, Coimbatore and the voucher samples are kept in the BSI herbarium for reference (BSI/SRC/5/23/2011-12/Tech-542). A voucher specimen has been deposited in the herbarium of the department.

2.2 Preparation of Extract

The leaves were washed thoroughly with tap water and in distilled water and then dried at room temperature. The dried leaves were ground to a fine powder in a mechanic grinder. About 20 gm of powdered plant material extracted with 200 ml of methanol, filtered through Whatmann No.1 filter paper and the solvent was removed by evaporating in a water bath, which gave rise to a solid mass of the extract.

2.3 DPPH Scavenging Activity

DPPH scavenging activity of the extract was determined by the method of Jain and Agarwal, [10]. Various concentrations of *C. coromandelicun* extract (10 µl, 20 µl, 30 µl, 40 µl & 50 µl)) of test solution and 50 µl of DPPH (0.659 mM) solution was incubated at 25°C for 20 and the absorbance was read at 510 nm using shimadzu UV 1800 spectrophotometer, same procedure used for control without sample. Ascorbic acid served as positive control. The % inhibition was calculated according to the following equation % Inhibition = $(A_0 - At) / A_0 \times 100$. Where A_0 was the absorbance of the control (blank, without extract) and at was the absorbance in the presence of the extract.

2.3.1 *In vitro* antidiabetic activity

Percentage inhibition of the enzymes α-amylase and α-glucosidase were carried out by previously optimized procedure [11].

2.3.2 *In vitro* α -amylase inhibitory assay

A starch solution (1% w/v) was prepared by stirring 1 g starch in 100 ml of 20 mM of phosphate buffer (pH 6.9) containing 6.7mM of sodium chloride. The enzyme solution was prepared by mixing 27.5 mg of porcine pancreatic amylase α amylase (PPA) in 100 ml of 20 mM of phosphate buffer (PBS, pH 6.9) containing 6.7 mM of sodium chloride. To 100 µl of (10, 20, 30, 40, 50 µg/ml) *C. coromaldelicum* extract, 200 µl porcine pancreatic amylase was added and the mixture was incubated at 37°C for 20 min. To the reaction mixture 100 µl (1%) starch solution was added and incubated at 37ºC for 10 min. The reaction was stopped by adding 200 µl DNSA (1 g of 3,5 di nitro salicylic acid, 30 g of sodium-potassium tartarate and 20 ml of 2N sodium hydroxide was added and made up to a final volume of 100 ml with distilled water) and kept it in a boiling water bath for 5 minutes. The reaction mixture diluted with 2.2 ml of water and absorbance was read at 540 nm. For each concentration, blank tubes were prepared by replacing the enzyme solution with 200 µL in distilled water. Control, representing 100%

enzyme activity was prepared in a similar manner, without extract. The experiments were repeated thrice using the same protocol [12].

2.3.3 *In vitro* α-Glucosidase inhibition assay

The inhibition of α-glucosidase activity was determined using the modified published method [13]. One mg of α-glucosidase was dissolved in 100 ml of phosphate buffer (pH 6.8). To 100 µl of (10, 20, 30, 40, 50 µg/ml) *C. coromaldelicum* extract, 200 µl α-glucosidase were added and the mixture was incubated at 37ºC for 20 min. To the reaction mixture 100 µl 3mM *p*-nitrophenyl – D glucopyranoside (p-NPG) was added and incubated at 37ºC for 10 min. The reaction was terminated by the addition of 2 ml Na_2CO_3 0.1 M and the α-glucosidase activity was determined spectrophotometrically at 405 nm on spectrophotometer UV-VIS (Shimadzu UV-1800) by measuring the quantity of *p*-nitrophenol released from p-NPG.

Acarbose was used as a positive control of α-amylase and α-glucosidase inhibitor. The concentration of the extract required inhibiting 50% of α-amylase and α-glucosidase activity under the assay conditions was defined as the IC_{50} value. The concentration of the *C. coromaldelicum* extract required to scavenge 50% of the radicals (IC_{50}) was calculated by using the percentage scavenging activities at five different concentrations of the extract. Percentage inhibition (I %) was calculated by I % = (Ac-As)/Ac X 100, where Ac is the absorbance of the control and as is the absorbance of the sample.

2.4 Data Analysis

The values are represented as Mean ± SEM of triplicates. Microsoft office excel was used to calculate IC_{50} value.

3. RESULTS

3.1 *In vitro* Antioxidant Activity

3.1.1 DPPH scavenging activity

The inhibition concentration at 50% inhibition (IC_{50}) was the parameter used to compare the radical scavenging activity. DPPH radical scavenging activity in a concentration-dependent manner (Table 1) and the IC_{50} were found to be 65.46 ± 0.50 µg/ml. However, the activity was less when compared with the standard, ascorbic acid (IC_{50} value 25.89 ± 1.62 µg/ml). A lower IC_{50} meant better radical scavenging activity. *C. coromandelicum* extract showed a good scavenging activity on DPPH radical.

3.1.2 *In vitro* antidiabetic activity

The percentage inhibition of α-amylase and α-glucosidase exhibited by the methanol extract of *C. coromandelicum* are depicted in the table. IC_{50} values are compared with standard drug acarbose and listed in Table 2.

The percentage inhibition of α-amylase and α-glucosidase exhibited by the methanol extract of PHF are depicted in Fig. 1 and Fig. 2.

4. DISCUSSION

Free radicals causing many diseases to human [14]. Antioxidants derived from natural sources, scavenge the free radicals and protect cells from damage [14]. Antioxidant drugs derived from natural sources prevent and treat oxidative stress-related diseases [15,16]. Synthetic antioxidant drugs have more side effects. So, people prefer natural sources, which have fewer side effects [17]. Only few methods have been used for the determination *in vitro* and *in vivo* antioxidant activity [18-20]. To study the antioxidant activity of *C. coromandelicum* plant extract, the ability to scavenge the stable free radical DPPH was evaluated. DPPH is one of the stable free radicals, mostly used for evaluating scavenging activity of antioxidant standards, showing absorbance at 517 nm [21], which decreases in the presence of free radical scavengers. By accepting hydrogen from a corresponding donor, the colour of DPPH solution changed from purple colour to yellow [22-25]. This DPPH scavenging method widely used for evaluating *in vitro* antioxidant activity of plant extracts [26,27]. A lot of studies have been reported by using DPPH assay [28-30]. Fig. 1 illustrated the DPPH radical scavenging activity of *C. coromandelicum* extract showed a scavenging effect, which increases with the increasing concentration of the sample, which showed a dose-dependent manner. At 100 µg/mL concentration, methanolic extract of *C. coromandelicum* showed the DPPH scavenging activity of 68.20 ± 0.68%. This result showed that *C. coromandelicum* leaves containing radical scavenging compounds with proton-donating ability. IC_{50} was also calculated. A lower IC_{50} value means greater antioxidant activity.

Indeed, the methanol fraction showed the highest DPPH radical inhibition value (IC_{50}=65.46 ± 0.50 µg/mL). The previous study supported the current study [31].

In modern medicine, there is no medication to treat diabetes without side effects [32]. Medicinal plants are safer, cost-effective when compared to commercial antidiabetic drugs. α-Amylase and α-Glucosidase assays were used to evaluate antidiabetic activity in the present research. α-Glucosidase acting as a key enzyme for carbohydrate digestion [33], can significantly decrease the postprandial hyperglycemia [34]. Pingale et al. (2017) study revealed significant antidiabetic activity of the methanol extract *Canthium parviflorum Lam.* in a dose-dependent manner, the study was quite interesting since the antidiabetic activity of the leaf extract was comparable with metformin [35].

C. coromandelicum extract showed inhibitory effects on both enzymes tested as presented in Table 2. The result revealed that the tested extract inhibited α-Amylase and α-Glucosidase activity concentration-dependently (10–50 mg/mL). In the present study, *C. coromandelicum* inhibits α-amylase 70.20 ± 0.64% and acarbose inhibits 72.72±0.04% (Fig. 1). *C. coromandelicum* inhibits glucosidase 59.12% ± 0.42 compared with standard drug acarbose which inhibits 80.72 ± 0.03% (Fig. 2). This

Table 1. DPPH radical scavenging activity of *C.coromandelicum* extract at different concentrations

Concentration (µg/ml)	% Inhibition	
	***C. coromandelicum* extract**	**Ascorbic acid (Standard)**
20	23.64 ± 0.25	38.20 ± 0.23
40	35.32 ± 0.75	49.90 ±0.24
60	48.28 ± 0.12	60.21 ± 0.16
80	59.34 ± 0.70	72.98 ± 0.90
100	68.20 ± 0.68	89.78 ± 0.46
IC_{50}	65.46 ± 0.50	25.89 ± 1.62

Table 2. IC_{50} values of standard drug acarbose and methanol extract of *C. coromandelicum*

Sample	IC_{50}(µg/mL)	
	α-amylase	**α-glucosidase**
Acarbose	15.95±0.12	13.54±0.20
C. coromandelicum extract	31.52±0.42	41.49±0.28

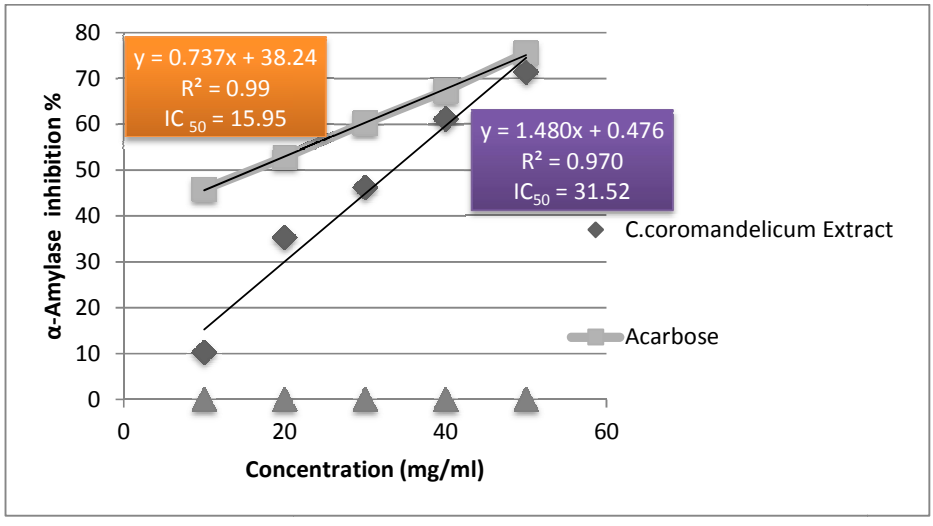

Fig. 1. The α-Amylase inhibitory activity of *C. coromandelicum* extract and standard drug Acarbose

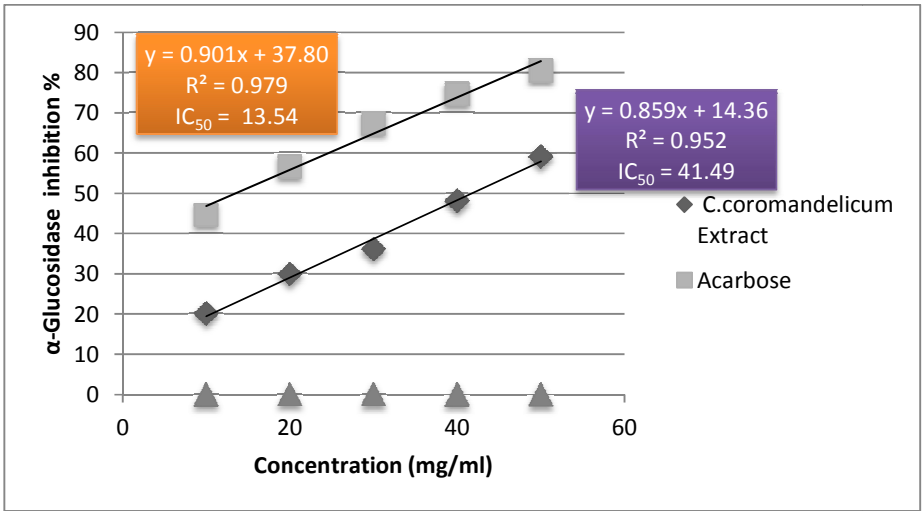

Fig. 2. The α-Glucosidase inhibitory activity of *C. coromandelicum* extract and standard drug Acarbose

indicates that the methanolic extract of *C. coromandelicum* is very potent α-amylase and α-glucosidase inhibitor in comparison with acarbose. The inhibitory effect observed for methanolic extract of *C. coromandelicum* may be associated with the presence of other phytoconstituents [36,37]. These phyto-constituents, have fewer side effects and are less expensive compared to synthetic drugs [38] and they perform several other biological activities [39]. All these activities were based on phytoconstituents such as tannins, terpenoids, steroids, glycosides, flavonoids and phenolic compounds present in the *C. coromandelicum* extract [40]. Therefore, the extract could be a beneficial drug in the treatment of diabetic complications.

5. CONCLUSION

The methanol extract of the *C. coromandelicum* leaves had the best antioxidant effect against the DPPH. The inhibitory effect of *C. coromandelicum* extract on carbohydrate hydrolysing enzymes α-amylase and α-glucosidase were evaluated. These enzyme inhibitors antagonize the activity of these enzymes and delaying the digestion of carbohydrate which prevents the sudden rise in blood glucose level, especially after a meal. Therefore, inhibition of these two enzymes is an attractive approach for the management of diabetes. As a result, we found that the *C. coromandelicum* extract has inhibitory activity against α-amylase and α-glucosidase and this therapeutic approach for the management of type 2 diabetes mellitus.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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