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Research Article

IN VITRO ASSESSMENT OF ETHANOLIC EXTRACT OF *CUCUMIS MADERASPATANUS* LEAVES AS AN α-AMYLASE AND α-GLUCOSIDASE INHIBITOR

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ABSTRACT

Objective: The current study was planned to study the phytochemicals, Antioxidant, and inhibitor activity of alpha-glucosidase and alpha-amylase from leaf extract of *Cucumis maderaspatanus.*

Methods: Extract was extracted using a soxhlet apparatus using solvents, such as n-hexane, chloroform, ethyl acetate, ethanol, and aqueous. The extracts were evaporated using a rotary evaporator. The phytochemicals were measured qualitatively and quantitatively and the antioxidant activity was done and the IC50 value was calculated.

Result: The qualitative analysis of the ethnolic leaf extract of *C. maderaspatanus* shows the presence of carbohydrates, terpenoids, phenol compounds, tannins, saponins, flavonoids, alkaloids, anthocyanins, betacyanins, quinones, glycosides, sterol, and coumarins. Quantitative analysis of ethanolic leaf extract of *C. maderaspatanus* showed that the presence of phenols (98.63±0.03), flavonoids (80.35±0.78), saponin (16.56±0.04), alkaloids (12.56±0.05), and total antioxidant (130.18±2.45). The IC50 value of various parameters such as DPPH (IC50=265), Nitric oxide (IC50=213), Hydrogen peroxide (IC50=355), Hydroxyl (IC50=290), Superoxide (IC50=145), alpha-amylase activity (IC50=48), and alpha-glucosidase activity (IC50=76).

Conclusion: From the study, it was concluded that the ethanolic leaf extracts of *C. maderaspatanus* have good antioxidant capacity, alpha-amylase, and alpha-glucosidase inhibitor activity due to phytochemicals present in it.

Keywords: *Cucumis maderaspatanus*, EECM- Ethanolic leaf Extracts of *Cucumis maderaspatanus* and DPPH-2,2-diphenyl-1-picrylhydrazyl.

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INTRODUCTION

Herbal medicines have played a significant role in curing various diseases and have contributed to the development of synthetic drugs with fewer side effects [1]. Diabetes mellitus is a chronic metabolic disorder that disrupts the metabolism of carbohydrates, proteins, fats, electrolytes, and water. When blood sugar levels rise, this condition is referred to as hyperglycemia. Hyperglycemia occurs when the blood sugar level exceeds the normal range due to insulin produced by the beta cells in the pancreas. Key enzymes, such as alpha-amylase and alpha-glucosidase are crucial for carbohydrate metabolism, as they facilitate the digestion of carbohydrates. Alpha-amylase breaks down long-chain carbohydrates, while alpha-glucosidase converts starch and disaccharides into glucose. These digestive enzymes are essential for carbohydrate digestion and absorption in the intestines. Alpha-glucosidase acts in the small intestine to convert complex carbs into glucose. Inhibiting the action of these enzymes may assist minimize carbohydrate digestion [2]. Thus, natural inhibitors of alpha-amylase and glucosidase generated from dietary plants can serve as effective therapeutic medicines for treating hyperglycemia with low adverse effects. Animal studies have shown that alpha-amylase inhibitors can lower high glucose levels by converting starch into simple sugars, which is particularly important for individuals with diabetes, as low insulin levels hinder the rapid clearance of glucose from the bloodstream [3]. This study will look at the phytochemicals, antioxidant characteristics, and inhibitory effects of alpha-glucosidase and alpha-amylase in *Cucumis maderaspatanus.* Leaf extract. The leaves are recognized for their effectiveness in treating hyperglycemia with minimal adverse effects. This plant, belonging to the Cucurbitaceae family, is commonly known as gourds [4] and is utilized in traditional medicine in India [5]. The fruit of *C. maderaspatanus* is also consumed in various dishes throughout South India [6,7].

METHODS

Collection of leaves from *C. maderaspatanus*

The leaves of *C. maderaspatanus*, known as musumusukkai in Tamil, were sourced from a medicinal plant vendor and verified for authenticity.

Extraction of leaves from *C. maderaspatanus*

To prepare the extract, the leaves of *C. maderaspatanus* were shade-dried and ground using a mechanical grinder. The resulting powder was extracted with n-hexane, chloroform, ethyl acetate, ethanol, and water through a Soxhlet apparatus, concentrated, and then lyophilized until a dry powder was achieved. This extract was stored in a glass bottle for the duration of the experiment.

Preliminary phytochemical screening

Phytochemical analysis was conducted on the powdered samples following standard procedures to identify the various constituents, as outlined by SofowaraA [8], Trease [9], and Harborne [10].

Sample preparation

For the preparation, 100 mg of n-hexane, ethyl acetate, ethanol, and aqueous leaf extracts of *C. maderaspatanus* were weighed, dissolved, and diluted to 100 mL in separate standard flasks (resulting in a concentration of 1 mg/mL). This extract was utilized for further studies.

Test for carbohydrates

To test for carbohydrates, combine 2 mL of the leaf extract with 1 mL of Molisch's reagent and a few drops of strong sulfuric acid. The appearance of purple color indicates the presence of carbohydrates.

Test for terpenoids
To detect terpenoids, take 0.5 mL of the leaf extract, add 2 mL of chloroform, and carefully introduce strong sulfuric acid; a red-brown color at the interface indicates terpenoids.

Test for triterpenoids
To test for triterpenoids, combine 1.5 mL of the leaf extract with 1 mL of Liebermann-Burchard Reagent (acetic anhydride and concentrated sulfuric acid). A blue or green hue suggests the presence of triterpenoids.

Test for phenols
For phenols, mix 1 mL of leaf extract with 2 mL of distilled water, and then add a few drops of 10% ferric chloride. The appearance of a green color indicates the existence of phenols.

Tannin test
To test for tannins, combine 1 mL of the leaf extract with 2 mL of 5% ferric chloride; if a greenish-black tint develops, tannins are present.

Saponin test
For saponins, add 2 mL of leaf extract to 2 mL of purified water. Shake the ingredients in a graduated cylinder for 15 min. Saponins can be found when a 1 cm layer of foam forms.

Flavonoid test
To test for flavonoids, mix 5 mL of weak ammonia solution with a portion of the aqueous leaf extract. Then, cautiously add pure sulfuric acid. A yellow color suggests the presence of flavonoids.

Alkaloid test
To detect alkaloids, mix 2 mL of the leaf extract with 2 mL of strong hydrochloric acid, and then add a few drops of Mayer's reagent. The presence of alkaloids is shown by the green hue.

Anthocyanin and betacyanin test
To test for anthocyanins and betacyanins, combine 2 mL of the leaf extract with 1 mL of 2N sodium hydroxide and heat at 100°C for 5 min. Betacyanins can be identified by their yellow tint.

Quinone test
For quinones, combine 1 mL of the leaf extract with 1 mL of strong sulfuric acid. A red color suggests the presence of quinones.

Glycoside test
To test for glycosides, mix 3 mL of chloroform with 2 mL of the leaf extract and add 10% ammonia solution. The formation of a pink color indicates the presence of glycosides.

Cardiac glycoside test
For cardiac glycosides, take 0.5 mL of the leaf extract and add 2 mL of glacial acetic acid along with a few drops of 5% ferric chloride. Layer this with 1 mL of concentrated sulfuric acid. A brown ring at the interface indicates the presence of cardiac glycosides.

Steroid and phytosteroid test
To test for steroids and phytosteroids, mix 2 mL of the leaf extract with 5 mL of chloroform and filter it. Then, add 2 mL of acetic anhydride and 2 mL of sulfuric acid to the filtrate. A color change from violet to blue or green and this indicates the presence of steroids.

Test for phlobatannins
Take 1.0 mL of the leaf extract and add a few drops of 10% ammonia solution. If a pink color appears, it indicates the presence of phlobatannins.

Anthraquinones
Take 1.0 mL of the leaf extract and mix in a few drops of 2% HCl. The appearance of a red precipitate suggests the presence of anthraquinones.

Coumarins
To check for Coumarins, take 1 mL of the leaf extract and add 1 mL of 10% sodium hydroxide. A yellow color formation indicates the presence of coumarins.

Proteins and amino acids
Take 2.0 mL of the leaf extract, add a few drops of 0.2% Ninhydrin, and heat the mixture. The formation of a purple color indicates the presence of amino acids and proteins.

Quantitative analysis
The quantitative phytochemical analysis was performed using extracts of ethyl acetate, ethanol, and water.

Estimation of total phenols
A 100µL sample of the extract was combined with 2 mL of 2% Na_2CO_3. After allowing the mixture to sit for 2 min, 100µL of Folin-Ciocalteu reagent was added. The tubes were then left at room temperature for 30 min, and the absorbance was measured at 743 nm against a blank. The same procedure was applied to a standard solution of gallic acid. The results were expressed in mg/g of the sample. The total phenol content in the leaf extracts of *C. maderaspatanus* was determined following the method outlined by Yu *et al.* [11].

Estimation of total flavonoids
To 1 mL of the extract, 3 mL of methanol, 0.2 mL of 1 M potassium acetate, 0.2 mL of 10% aluminum chloride, and 5.6 mL of distilled water were added. This mixture was allowed to sit at room temperature for 30 min. The absorbance was then measured at 415 nm using a UV spectrophotometer. The same method was used for the standard quercetin. The flavonoid content in the leaf extracts of *C. maderaspatanus* was expressed in mg/g of the sample, as determined by the method of Chang *et al.* [12].

Determination of saponins
A 10 mg sample of the extract was dissolved in 5 mL of 80% aqueous methanol, and 50 µL of this solution was placed in each test tube. To each tube, 0.25 mL of vanillin reagent (8% in 99.9% ethanol) was added. The tubes were then placed in an ice-cold water bath, and 2.5 mL of 72% sulfuric acid was carefully added to the sides of the test tubes. The contents were mixed and allowed to sit for 3 min before being heated at 60°C for 10 min. The developed color was measured using a spectrophotometer against a reagent blank. Saponin was used as the standard, and the same procedure was followed for the standard as well. The saponin content in the leaf extracts of *C. maderaspatanus* was determined using the method.

Estimation of alkaloids
The alkaloid content in the leaf extract of *C. maderaspatanus* was assessed using the method outlined by Harborne *et al.* [10]. A sample of 5 g was placed in a 250 mL beaker, and 200 mL of 20% acetic acid in ethanol was added. The mixture was covered and allowed to sit for 4 h. Afterward, it was filtered, and the extract was concentrated in a water bath to one-quarter of its original volume. Concentrated ammonium hydroxide was then added drop by drop until precipitation occurred completely. The solution was left to settle, and the precipitate was collected through filtration. The weight of the precipitate was expressed in mg/g of the sample, using Isoquinoline alkaloid as a standard.

Antioxidant assay
Determination of DPPH radical scavenging activity
For the DPPH radical scavenging activity, 0.2 mL of plant extract samples at various concentrations (100, 200, 400, 800, and 1000 µg/mL) were placed in separate test tubes. Next, 0.8 mL of TrisHCl buffer (100 mM, pH 7.4) was added, followed by 1.0 mL of DPPH (500 µM in ethanol) solution. The mixture was incubated at room temperature for 30 min, and the absorbance was measured at 517 nm. BHT served as the standard. The percentage of DPPH scavenging activity was calculated, and the IC50 value was determined. A decrease in the absorbance of the

reaction mixture indicates a stronger DPPH radical scavenging activity. The DPPH radical scavenging activity of *C. maderaspatanus* leaf extract was evaluated using the method described by Koleva *et al.* [14].

Calculation

$$\% \text{ Inhibition} = (\text{Control} - \text{Test})/\text{Control} \times 100$$

Assessing reducing power

The reducing power of *C. maderaspatanus* leaf extract was evaluated using the method described by Oyaizu [15]. Different test tubes were prepared with 1 mL of *C. maderaspatanus* leaf extract at concentrations of 10, 20, 50, 75, and 100 µg/mL. To each tube, 1.0 mL of 200 µM potassium phosphate buffer was added, while a separate tube containing only 1.0 mL of potassium buffer served as a blank. Next, 2.5 mL of potassium ferric cyanide was introduced to all test tubes. The mixtures were then incubated at 50°C for 20 min before being centrifuged at 3000 rpm for 10 min. After that, 2.5 mL of 10% trichloroacetic acid was added to the solutions, which were then centrifuged at 3000 g for 10 min. From the supernatant, 2.5 mL was transferred to new test tubes, followed by the addition of 2.5 mL distilled water and 0.5 mL of 0.1% FeCl3. The absorbance was measured at 700 nm, with BHT serving as the standard.

Evaluating nitric oxide scavenging potential

Nitric oxide is produced from the reaction of sodium nitroprusside in an aqueous solution at physiological pH, where it interacts with oxygen to form nitrite ions, which can be quantified using the Griess reaction. For this assessment, a reaction mixture was prepared containing 3.0 mL of 10 mM sodium nitroprusside in phosphate-buffered saline along with *C. maderaspatanus* leaf extract at concentrations of 50, 100, 200, 400, and 800 µg/mL. This mixture was incubated at 25°C for 150 min. After incubation, 0.5 mL aliquots were taken and mixed with 0.5 mL of Griess reagent. The resulting chromophore was measured at 546 nm, with BHT used as a control. The nitric oxide radical scavenging activity was determined following the method outlined by Green *et al.* [16].

Calculation

$$\% \text{ Inhibition} = (\text{Control} - \text{Test})/\text{Control} \times 100$$

Evaluation of superoxide anion radical scavenging ability

The superoxide radical scavenging activity of *C. maderaspatanus* leaf extract was assessed using the method described by Liu *et al.* [17]. In this process, superoxide anion radicals were generated in a PMS-NADH system through the oxidation of NADH, and their activity was evaluated by measuring the reduction of NBT to blue formazan. To a 1.0 mL solution of 156 µM NBT, 1.0 mL of 468 µM NADH and 0.5 mL of various concentrations of the extract (50, 100, 250, 500, and 750 µg/mL) were added. Following this, 100 µL of PMS was introduced, and the mixture was incubated at room temperature for 5 min. The absorbance was then recorded at 560 nm, with BHT serving as the standard.

Calculation

$$\% \text{ Inhibition} = (\text{Control} - \text{Test})/\text{Control} \times 100$$

Assessment of hydrogen peroxide radical scavenging activity

The hydrogen peroxide scavenging potential of *C. maderaspatanus* leaf extract was evaluated using the method outlined by Ruch *et al.* [18]. A 4 mM solution of H_2O_2 was prepared in phosphate buffer (pH 7.4). Different concentrations of the extract (100, 200, 400, 800, and 1000 µg/mL) were added to 0.6 mL of the H_2O_2 solution. After 10 min, the absorbance of H_2O_2 was measured at 230 nm against a blank solution containing phosphate buffer without H_2O_2. The results were compared with BHT, and the percentage of H_2O_2 scavenged by the extract was calculated at 230 nm.

Calculation

$$\% \text{ Inhibition} = (\text{Control} - \text{Test})/\text{Control} \times 100$$

Assessment of hydroxyl radical scavenging activity

The assay involved the sequential addition of 0.1 mL of EDTA, 0.01 mL of FeCl3, 0.1 mL of H_2O_2, 0.36 mL of deoxyribose, and 1.0 mL of the extract (at concentrations of 50, 100, 250, 500, and 1000 µg/mL) dissolved in distilled water, along with 0.33 mL of phosphate buffer (50 mM, pH 7.9) and 0.1 mL of ascorbic acid. This mixture was incubated at 37°C for 1 h. After incubation, 1.0 mL of the mixture was combined with 1.0 mL of 10% TCA and 1.0 mL of 0.5% TBA to produce a pink chromogen, which was then measured at 532 nm. The hydroxyl radical scavenging activity of the extract was expressed as the percentage inhibition of deoxyribose degradation, calculated using a specific formula. BHT served as the standard reference. The hydroxyl radical scavenging activity of *C. maderaspatanus* leaf extract was evaluated following the method by Halliwell *et al*., [19].

Calculation

$$\% \text{ Inhibition} = (\text{Control} - \text{Test})/\text{Control} \times 100$$

Evaluation of total antioxidant capacity

The total antioxidant capacity of *C. maderaspatanus.* leaf extract was evaluated using the method reported by Preito *et al*., [20]. This assay relied on the extract reducing molybdenum (VI) to molybdenum (V), which resulted in the production of a green phosphate Mo (V) complex in an acidic environment. The sample solution (100 µg/mL) was combined with a reagent solution of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The tubes were sealed and placed in a boiling water bath at 95°C for 60–90 min. After cooling to room temperature, the absorbance at 695 nm was measured against a blank with a spectrophotometer. The results were reported as BHT equivalents.

Calculation

$$\% \text{ Inhibition} = (\text{Control} - \text{Test})/\text{Control} \times 100$$

Determination of alpha-amylase inhibitory activity

The assay mixture consisted of 200 µL of sodium phosphate buffer, 20 µL of enzyme, and 20 µl of *C. maderaspatanus* leaf extract at concentrations of 20, 40, 60, 80, and 100 µg/mL. This mixture was incubated for 10 min at room temperature, after which 200 µL of starch was added to each tube. The reaction was halted by adding 400 µL of DNS reagent, followed by 5-min incubation in a boiling water bath. The mixture was then cooled, diluted with 15 mL of distilled water, and the absorbance was measured at 540 nm. Control samples were prepared without the extracts. The percentage of inhibition was calculated using the following formula:

Calculation

$$\% \text{ Inhibition} = (\text{Control} - \text{Test})/\text{Control} \times 100$$

Determination of alpha-glucosidase inhibitory activity

The inhibitory effect of *C. maderaspatanus* leaf extract on alpha-glucosidase was assessed using the method described by Bachhawat *et al.* The reaction mixture included 50 µL of phosphate buffer, 10 µL of alpha-glucosidase, and 20 µL of the leaf extract at concentrations of 20, 40, 60, 80, and 100 µg/mL, which were pre-incubated at 37°C for 15 min. Following this, 20 µL of p-nitrophenyl-α-D-Glucopyranoside (PNPG) was added as a substrate and incubated at 37°C for an additional 30 min. The reaction was terminated by adding 50 µL of sodium carbonate. The resulting yellow color was measured at 405 nm. Each experiment included appropriate blanks, and acarbose at various concentrations (20–100 µg/mL) was used as a standard. Control samples were prepared without the extracts, and the results were expressed as a percentage of inhibition.

Calculation

$$\% \text{ Inhibition} = (\text{Control} - \text{Test})/\text{Control} \times 100$$

Statistical analysis
The IC50 values and corresponding graphs were calculated using Graph Pad Prism Software 6.0. The results of this study are presented as mean±SD based on six parallel measurements.

RESULTS AND DISCUSSION

The results and discussion reveal that Table 1 highlights the presence of various phytochemicals, including phenols, flavonoids, alkaloids, carbohydrates, glycosides, saponins, sterols, tannins, proteins, and amino acids across different extracts such as n-hexane, chloroform, ethyl acetate, ethanol, and aqueous extracts. The n-hexane extract of *Cucumis maderaspatanas* demonstrated the presence of phenols and tannins, while the chloroform extract contained phytoconstituents, such as sterols. The ethyl acetate extract revealed the presence of phenols, flavonoids, carbohydrates, proteins, and amino acids. The ethanol extract showed a rich variety of phytochemicals, including phenols, flavonoids, alkaloids, carbohydrates, glycosides, saponins, sterols, tannins, and proteins and amino acids. Similarly, the aqueous extract also contained phenols, flavonoids, alkaloids, carbohydrates, glycosides, saponins, tannins, and proteins and amino acids. Among all the extracts, the ethanolic extract exhibited the highest diversity of phytoconstituents, making it the preferred choice for further analyses, such as quantitative phytochemical analysis, antioxidant assays, and the determination of alpha-amylase and alpha-glucosidase inhibitory activities.

Quantitative phytochemical analysis
The quantitative phytochemical analysis is presented in Table 2, which details the total phenol content expressed as mg/g for various extracts of *C. maderaspatanas*. The ethyl extract has a value of 3.9±0.04, the ethanol extract shows 98.62±0.03, and the aqueous extract is at 79.2±0.27. In addition, Table 2 provides insights into the total flavonoid content, measured as mg/g for different solvent extracts of *C. maderaspatanas*. The ethyl extract records 7.1±0.08, the ethanol extract is at 80.35±0.78, and the aqueous extract shows 76.97±0.05. Furthermore, the table highlights the saponin content, also expressed in mg/g for the various solvent extracts. The ethyl extract shows 7.42±0.05, the ethanol extract is at 16.56±0.04, and the aqueous extract has a value of 19.11±0.14. Finally, the quantitative analysis of alkaloid content, equivalent to Isoquinoline alkaloid, is also illustrated in Table 2, with the ethyl extract at 7.1±0.08, the ethanol extract at 80.35±0.78, and the aqueous extract at 76.97±0.05.

Antioxidant assay
The overall antioxidant capacity is detailed in Table 2. The antioxidant capacity equivalent of BHT was measured in mg/g of extract. The Ethyl acetate extract showed a value of 110±1.68, while the Ethanolic extract reached 130.18±2.45, and the aqueous extract recorded 115.36±0.63.

DPPH assay
Table 2 presents the total antioxidant capacity. The antioxidant capacity equivalent of BHT was expressed in mg/g of extract. The Ethyl acetate extract had a value of 110±1.68, the Ethanolic extract was at 130.18±2.45, and the aqueous extract measured 115.36±0.63.

Regarding DPPH radical scavenging activity, the ethanolic extract exhibited a maximum scavenging activity of around 85.5% at a concentration of 1000 µg/mL from *C. maderaspatanas*, with an IC50 value of 265.

Reducing activity
The reducing activity of the samples was found to be dependent on their concentration, as illustrated in Fig. 1. Generally, the ethanolic extracts from dried samples exhibited a higher reducing power, measuring at 0.994±1.3.

Nitric oxide scavenging assay
For the nitric oxide scavenging assay, the activity reached 80.43% at an ethanolic extract concentration of 800µg. The percentage inhibition corresponds to an IC50 value of 213, while BHT shows a value of 170.

Table 1: Qualitative phytochemical analysis of *Cucumis maderaspatanus*

S. No.	Plant constituents	n-hexane	C	EA	E	A
1	Carbohydrates	-	-	+	+	+
		-	-	+	+	+
		-	-	+	+	+
2	Terpenoids	-	-	+	+	+
		-	-	+	+	+
		-	-	+	+	+
3	Phenolics Compounds	+	-	+	+	+
		+	-	+	+	+
4	Tannins	+	-	+	+	+
		+	-	+	+	+
5	Saponins	-	-	-	+	+
		-	-	-	+	+
6	Flavonoids	-	-	+	+	+
		-	-	+	+	+
7	Alkaloids	-	-	-	+	+
		-	-	-	+	+
		-	-	-	+	+
8	Anthocyanins and Betacyanins	-	-	-	+	+
		-	-	-	+	+
		-	-	-	+	+
9	Quinones	-	-	+	+	+
		-	-	+	+	+
10	Glycosides and Cardiac glycosides	-	-	-	+	+
		-	-	-	+	+
		-	-	-	+	+
11	Sterols	-	+	-	+	-
		-	+	-	+	-
12	Coumarins	-	-	+	+	+
		-	-	+	+	+
13	Proteins and Amino acids	-	-	+	+	+
		-	-	+	+	+

+: Present, -:Absent, P: Petroleum ether, C: Chloroform, E: Ethanol, A: Aqueous extracts

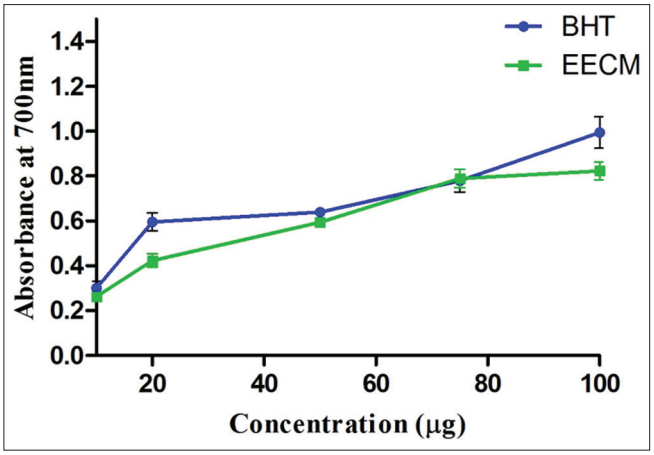

Fig. 1: Reducing activity

Superoxide anion radical scavenging assay
For superoxide anion radical scavenging, the ethanolic extract showed a maximum activity of around 80.15% at 1000 µg/mL, with an IC50 value of 145.

Hydrogen peroxide radical scavenging assay
When examining hydrogen peroxide radicals, the ethanolic extract demonstrated a maximum scavenging activity of about 85.43% at a concentration of 1000 µg/mL, with an IC50 value of 340.

Table 2: Quantitative phytochemical analysis of *Cucumis maderaspatanus*

S. No	Plant constituents	EA	E	A
1	Phenol (mg/g)	3.9±0.04	98.62±0.03	79.2±0.27
2	Flavonoid (mg/g)	7.1±0.08	80.35±0.78	76.97±0.05
3	Saponin (µg/mL)	7.42±0.05	16.56±0.04	19.11±0.14
4	Alkaloids (µg/mL)	4.32±0.06	12.56±0.05	5.34±0.03
5	Total Antioxidant (g of extract)	110±1.68	130.18±2.45	115.36±0.63

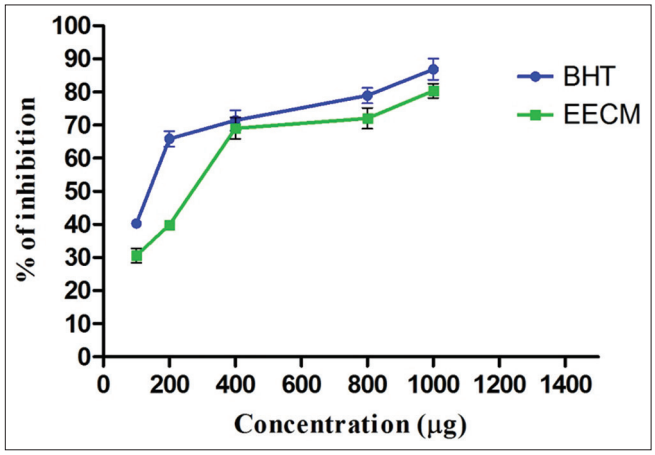

Fig. 2: DPPH scavanging activity

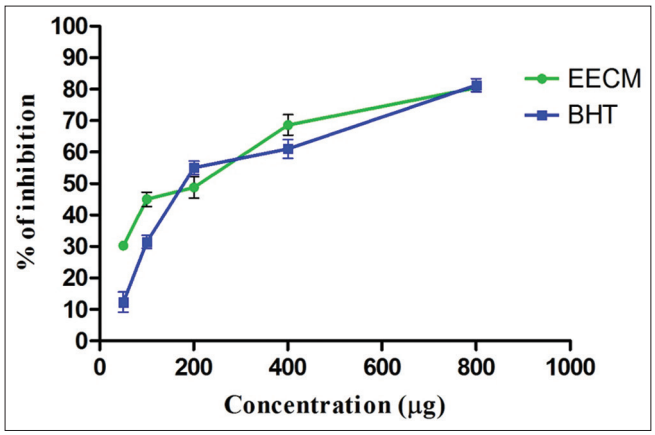

Fig. 3: Nitric oxide scavenging assay

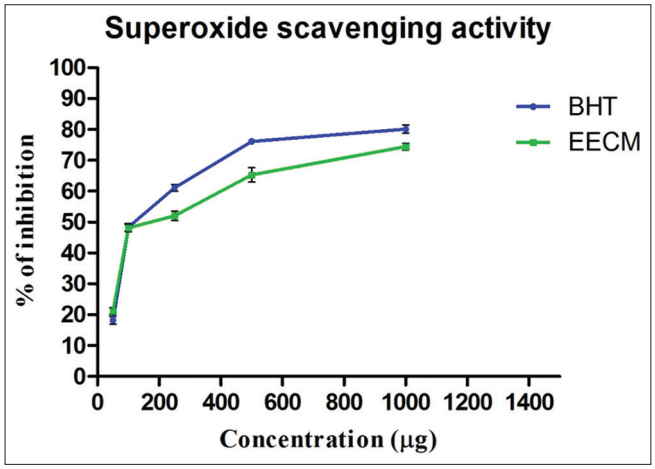

Fig. 4: Superoxide anion radical scavenging assay

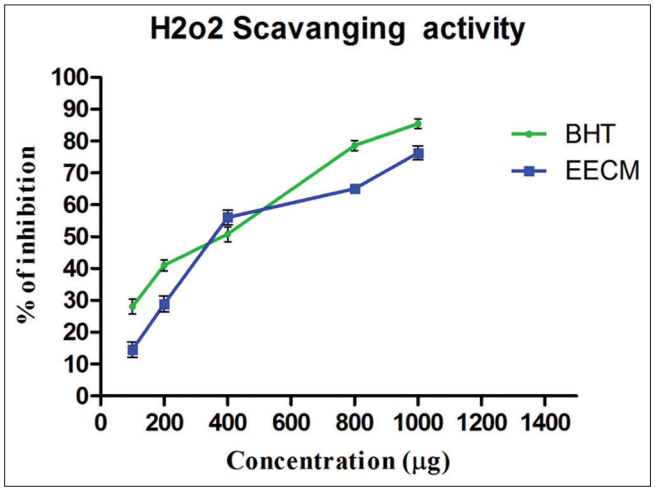

Fig. 5: Hydrogen peroxide radical scavenging assay

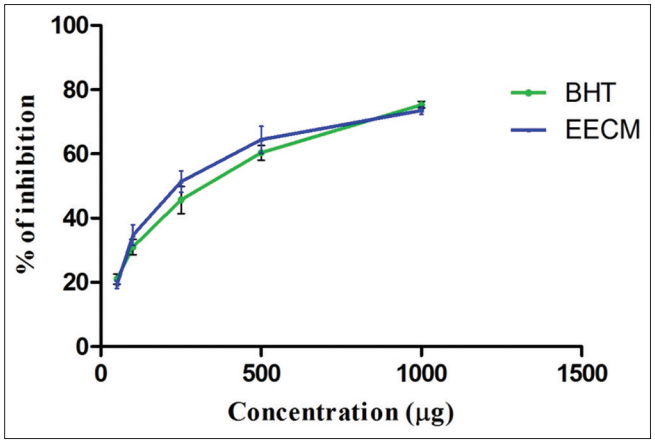

Fig. 6: Hydroxyl scavenging activity

Hydroxyl scavenging activity

The IC50 for hydroxyl scavenging activity is 290, while BHT has 230. The maximum scavenging activity is approximately 75.358 at 1000 µg/mL.

Table 3 and Figs. 1-6 shows the IC50 value calculated for all the models.

Alpha amylase activity

Regarding alpha-amylase activity, as shown in Table 4 and Fig. 7, the ethanolic extract of *C. maderaspatanus* exhibited a maximum activity of about 82.24 at a concentration of 100 µg/mL, with an IC50 value of 48.

Alpha-glucosidase activity

Table 4 and Fig. 8 show the ethanolic extract of *C. maderaspatanus.* has a maximal alpha-glucosidase activity of 68.18 at 100 µg/mL, with an IC50 of 76.

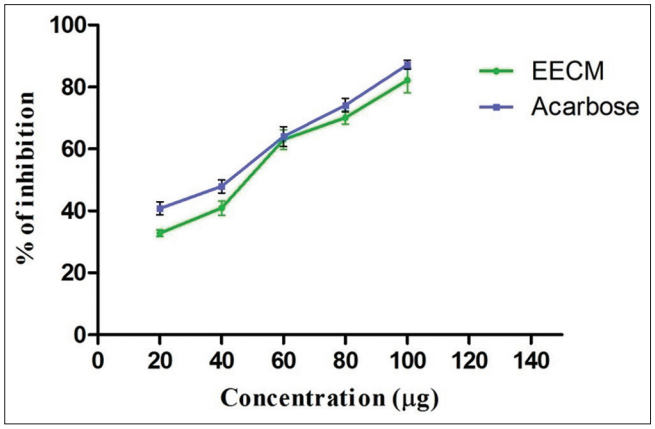

Fig. 7: Determination of alpha-amylase inhibitory activity

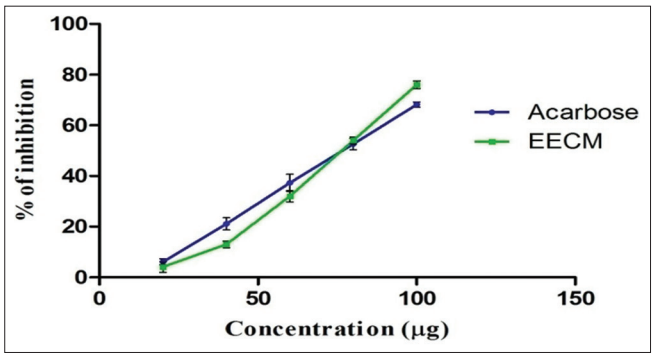

Fig. 8: Determination of alpha - glucosidase inhibitory activity

Table 3: IC_{50} value of *Cucumis maderaspatanus* leaf extracts

S. No	Free radical scavenging method	Ic50 value	
		Ethanol	BHT Standard
1	DPPH	265	135
2	Nitric oxide	213	170
3	Hydrogen peroxide	340	355
4	Hydroxyl	290	230
5	Superoxide	145	120
6	Amylase	48	44
7	Beta-glucosidase	76	77

Table 4: Alpha-amylase and alpha-glucosidase activity

S. No.	Assay	Ic50 value	
		Ethanol	Acarbose Standard
1	Amylase	48	44
2	Alpha-glucosidase	76	77

DISCUSSION

Phenolic compounds found in plants are significant antioxidants, serving as primary defenders against oxidative stress. Their antioxidant properties stem from their ability to donate hydrogen atoms to free radicals, which helps neutralize these harmful molecules. The structural characteristics of these compounds enable them to effectively scavenge free radicals. Polyphenols and flavonoids exhibit a range of beneficial activities, including antioxidative, antidiabetic, anticarcinogenic, antimicrobial, antiallergic, antimutagenic, and anti-inflammatory effects. Research by Shimada et al. and Arbaayah and Umi [27] highlighted that mushroom extracts possess reducing power due to their ability to donate hydrogen ions, which stabilize molecules by accepting these ions. The capacity for reduction is often a strong indicator of a compound's antioxidant potential. The presence of phenols and flavonoids in methanolic extracts of Artocarpus heterophyllus was found to significantly inhibit alpha-amylase and alpha-glucosidase. Nair *et al*. [2] reported that phenols exhibit superior antidiabetic properties. The interaction between phenols, flavonoids, and tannins with the hydroxyl group at the catalytic site of alpha-amylase is crucial for their inhibitory effects, with hydroxylation of flavonoids enhancing glycosidase inhibition.

CONCLUSION

The findings indicate that the ethanolic leaf extracts of *C. maderaspatanus.* possess the superior antioxidant capacity and notable alpha-amylase and alpha-glucosidase inhibitory activities, primarily due to phytochemicals, such as phenols, flavonoids, and tannins. More research is needed to isolate and characterize these phyto active compounds to validate their antidiabetic effects through additional *in vivo* and *in vitro* studies.

CONFLICTS OF INTEREST

There are no conflicts of interest. We herewith submit the manuscript entitled as *in vitro* assessment of ethanolic extract of *C. maderaspatanus* leaves as an α-amylase and α-glucosidase inhibitor for the research paper in the journal of *Asian Journal of Pharmaceutical and Clinical Research*. A review of *C. maderaspatanus* collected and a manuscript written by Dr. K. Nadhiya, P. Mahalakshmi done the analysis, such as phytochemical, antioxidant, and inhibitory activity of alpha-amylase and alpha-glucosidase analysis for this methodology instructed by Dr. K. Nadhiya and Calculations and statistical analysis done by. K. Nadhiya and Dr. P. Kalaivani. Analyzed data checking and approved by Dr. K. Nadhiya and Dr. P. Kalaivani. No funding for research paper.

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