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Original Article

Anti-Diabetic and Free Radical Scavenging Activity of Phytochemicals from *Caesalpinia bonducella*

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Abstract

Diabetes mellitus is a metabolic disease resulted with high blood glucose levels due to oxidative stress that leads to many complications. Natural compounds derived from herbal plants are alternative source that increasing globally in the process of diabetes drug development. The present study investigates the potential of plant natural compounds for its therapeutic capability to treat diabetic mellitus. Phytochemicals were extracted and purified from seeds of the plant *Caesalpinia bonducella*. The functional group in *Caesalpinia bonducella*was confirmed by UV-VIS and FTIR spectroscopic technique. The seed extract's in vitro antioxidant properties in various concentration (0.25 to 1 g/mL) were carried out against the DPPH and H_2O_2 onion radical scavenging assay. In vitro studies of anti-diabetic activity were also evaluated by inhibiting key enzymes that are involved in glucose metabolism such as alpha amylase. The seed extract was found to inhibit the enzyme responsible for glucose metabolism and so maximum inhibition was observed in 0.25 mg/mL and the compound was found to be highly reactive against free radicals. In the DPPH assay, the plant seed extract has a maximum inhibitory activity of 92.04% at high concentration. Additionally, for the H_2O_2 scavenging test, the inhibition of the plant seed extract at 0.75 g/mL concentration was 88.3%. This suggests that the plant's phytocompounds may offer therapeutic benefits for the treatment of diabetes and conditions linked to oxidative stress. In vivo investigations of the study's findings are further necessary to validate the phytocomplex's efficacy and open the door to the creation of cutting-edge treatments for antioxidants and diabetes.

Keywords: *Anti-diabetic activity, Caesalpinia bonducella, enzyme inhibition, free-radical scavenging action*.

Introduction

Diabetes mellitus (DM) a complex disorder affecting the overall metabolism resulting in abnormal lipid profile, impaired insulin, impaired secretion of antioxidant enzymes and a rise in reactive oxygen species (ROS) due to an intensification of oxidative stress (Seuring *et al*., 2015). Oxidative stress when stimulated by the production of free radicals during hyperglycaemia, it then contributes to the development and progression of diabetes (Shamran & Jaffat, 2020). Diabetes with ROS generation is a main factor for dysregulation of endothelial cell functions with impairments in vasodilation and angiogenic properties (Kolluru *et al*., 2012). In patients with increasing levels of glucose together with dyslipidaemia may develops microangiopathies with oxidative stress (Fadheel, 2018).

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Hyperglycaemia may promote ROS through mitochondrial electron transport chain and the enzyme superoxide leads to development of diabetes-related vascular damage (Du *et al*., 2000). On the other hand, increased ROS speedup atherosclerotic inflammation by increasing the formation of oxidised LDL, increasing insulin resistance, and decreasing endothelial nitric oxide synthase (eNOS) (Soppert *et al*., 2020). In addition, mitochondrial dysfunction was a state of pre-existence before diabetes mellitus where increased ROS destructs pancreatic β-cells and declines peripheral tissue insulin sensitivity (Yuan *et al*., 2019). Antioxidant defense system is affected functionally by slow progression of DM where excess ROS-induced oxidative stress mediates protein storage modifications with accumulated useless proteins rather than degradation (Masschelin *et al*., 2020). Reduced synthesis of the antioxidants glutathione peroxidase, catalase, and superoxide dismutase (SOD) could be the cause of the rise in ROS levels (Bhatti *et al*., 2022). Therefore, research studies addressed the possible participation of natural products as antioxidants, in ameliorating the diabetic condition and preventing the development of complications (Rahimi-Madiseh *et al*., 2016; Pasupuleti *et al.,* 2020).

The enzyme pancreatic alpha amylase catalyses the first stage of the breakdown of carbohydrates, which entails turning starch into maltose and then glucose. When this dietary starch is hydrolyzed, postprandial hyperglycemias (PPHG) are raised (Ponnusamy *et al*., 2011). Inhibition of this enzyme causes significant reduction in the post prandial increased concentration of blood glucose and therefore considered as an important approach in blood glucose management (Poovitha & Parani, 2016). An Indian herb belonging to the Caesalpiniaceae family is called *Caesalpinia bonducella*. Although it is widespread in the Andaman and Nicobar Islands, India, and Sri Lanka, it is found worldwide (Konan *et al*., 2014; Shukla *et al*., 2010). However, to synchronize the effect of ROS in DM, we investigated the effect of *C. bonducella* seed extracts for its composition by qualitative preliminary phytochemical screening and FTIR. Determination of α-amylase inhibitory potential and antioxidant activity of *C. bonducella* seed extracts were also explored.

Materials and methods

Preparation of plant material

Caesalpinia bonducella (L.) fresh seed was gathered from Lalgudi area in Tiruchirappalli, Tamilnadu and got authenticated by John Britto Rapinat Herbarium, Department of Botany, St Josph's College, Tiruchirappalli. After three sterile distilled water washes, the seeds were chopped into small pieces, shade dried for two weeks, and then ground into a coarse powder that was sealed in an airtight receptacle. The material was then extracted using the soxhlet apparatus, which exploits the polarity of solvents. First, the plant material was dissolved in ethanol, which was extracted using that solvent. Next, the remaining material was extracted using water, which has a different polarity in a 1:4 ratio. This method eases the withdrawal of active constituents in the plants (Abubakar *et al*., 2020).

Phytochemical studies

Using aqueous plant seed extract, a preliminary qualitative analysis was performed to verify the presence of several phytoconstituents. Test for alkaloids: use 1 mL of Dragendorff's reagent to 2 mL of extract. The presence of alkaloids was revealed by the formation of an orange-red precipitate (Kancheria *et al*., 2019). Test for flavonoids: a little amount of the test material was dissolved in the same amount of 95% ethanol, along with three to five drops of concentrated hydrochloric acid. The development of a cherry red hue suggested the presence of flavonoids (Pant *et al*., 2017; Shaw *et al.,* 2021). Test for glycoside and quinone: To 2 mL of extract, added around 0.5 mL of glacial acetic acid and two to three drops of ferric chloride. One millilitre of concentrated H_2SO_4 was added to this mixture. When deep blue color started to develops, it indicated the presence of glycosides (Kancheria *et al*., 2019). To 3 mL of chloroform, approximately 3 mL of plant seed extract was added. Approximately 5 percent potassium hydroxide was added after the layer separated. The presence of quinones was revealed by the appearance of red colour (Maria *et al*., 2018). Test for tannins:2 millilitres of 5% FeCl3 were added to one millilitre of plant extract, and the creation of a dark blue or greenish-black colour indicated the presence of tannins (Kancheria *et al*., 2019). Test for coumarins: To the plant extract, about 3 mL of 10% NaOH was added. Positive results were reported in the

formation of yellow color (Le thi *et al*., 2021). Test for saponins: An aqueous plant extract (5 mL) was mixed vigorously with two drops of olive oil. The resulted frothing confirmed the presence of saponins (Gul *et al*., 2017). Test for steroids and terpenoids: A 0.5g powdered plant extract was mixed with 10 mL of chloroform. To create a layer underneath, it was filtered and combined with one millilitre of strong sulphuric acid and one millilitre of acetic anhydride. Steroids were indicated by the formation of green color (Auwal *et al*., 2014). The presence of terpenoids is shown by the formation of a reddish-brown color after 0.1 g of the plant extract is mixed with 0.4 ml of chloroform and concentrated sulphuric acid (Dubale *et al.,* 2023).

UV-Visible and FTIR analysis

Structural clarification of biomolecules present in plant extract was determined with spectroscopic technique of FTIR and UV-Visible spectroscopy. The infra-red spectrum produced through the measured amount of electromagnetic absorption and depending on the spectrum, organic molecular structure was identified from the plant seed extract (Altemimi *et al*., 2017). The plant material extracted through Soxhlet apparatus was used for the analysis (Akhtar *et al*., 2022).

DPPH scavenging assay

The technique for scavenging free radicals using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) in methanol is known as the DPPH scavenging assay. Antioxidants caused the colour of DPPH to change from purple to yellow. Approximately 2.4 mL of a solution containing 0.1 mM DPPH in methanol was produced and combined with 1.6 mL of plant seed extract in methanol at several doses ranging from 0.25 to 1g/mL. After 30 minutes, the absorbance was measured spectrophotometrically at 517 nm. As a control, ascorbic acid was employed. The following formula was used to determine the percentage (%) of DPPH radical scavenging activity: % DPPH radical scavenging activity= $\{(A_0 - A_1)/A_0\} \times 100$, where A_0 represents the absorbance control and A_1 represents the absorbance standard. To get the IC_{50}, the percentage of inhibition was plotted versus the concentration (Baliyan *et al*., 2022).

H_2O_2 scavenging assay

For hydrogen peroxide scavenging assay, 0.6 mL of H_2O_2 solution (4 mM) and 0.1M phosphate buffer were added (pH 7.4). This solution was used for make up to 0.4 mL of plant seed extracts (0.25–1g/mL). After mixing the reaction mixture, it was incubated for ten minutes. Afterwards, ascorbic acid was used as the positive control for measuring the absorbance at 230 nm. Scavenging ability was calculated using the following equation: H2O2 scavenging effect % = $A_\circ - A_1/A_\circ \times 100$ where A_o is the absorbance control and A_1 is the absorbance standards (Baliyan *et al*., 2022)

Determination of alpha-amylase enzyme inhibition

Boiling 100 mL of distilled water with 0.1 g of potato starch produced the enzyme substrate. 100 millilitres of distilled water were mixed with 27.5 mg of alpha-amylase to create the enzyme solution. 200 µL of 3,5-dinitro-salicylic acid (DNSA) colour reagent (96 mM DNSA, 2 M sodium potassium tartrate tetrahydrate, and 2 M NaOH) was added to the colorimetric reagent. The plant seed extracts were then combined with starch, which reacts with alpha-amylase at 25°C. In this case, the positive control was acarbose. By converting 3,5-dinitro salicylic acid to 3-amino-5-nitro salicylic acid, which was measured at 540 nm, the amount of maltose generated was ascertained. The inhibition (*I*%) of alpha-amylase was: [Ac−(As−Ab)/Ac] ×100 where Ac denotes Absorbance of the negative control (uninhibited reaction), As denotes Absorbance of the sample (inhibited reaction), and Ab denotes Absorbance of the sample blank (enzyme omitted).

Results and Discussion

Phytochemical analysis

Phytochemicals present in the *Caesalpinia bonducella* plant seed extract was analysed qualitatively which yield the results as shown in Table 1.

Table 1: Preliminary Phytochemical Analysis of plant seeds extract of *Caesalpinia bonducella*

S.NO	Phytochemicals	Aqueous extract
1.	Alkaloids	++
2.	Flavonoids	++
3.	Glycosides	++
4.	Quinone	+++
5.	Tannins	++
6.	Coumarins	++
7.	Saponins	++
8.	Steroids	+++

*(+) positive; (-) negative

Saponin proved to have cytotoxic effects in cancer cells but no normal cells, have free radical scavenging activity through upregulation of antioxidant related genes including NrF2 (Khan *et al*., 2022). Plant steroid's structure is related to animal steroids and exhibited cardiotonic and anti-microbial activity (Dey *et al.,* 2020). Through its cardiotonic action,it involves controlling cellular proliferation and intracellular Ca2+ homeostasis (Orellana *et al*., 2016). Because of their anti-inflammatory and antioxidant properties, phytochemical tannins are essential for neuroprotection in the event of problems from diabetes (Omar *et al*., 2022). Because of their anti-inflammatory properties, flavonoids may have an additional effect to the endogenous scavenging compounds during injury, increasing the activity of the endogenous antioxidants. During injury, more reactive oxygen species (ROS) will be formed, which leads to the depletion of the endogenous scavenging compounds (Mawlood *et al*., 2022). *Caesalpinia bonducella*extract with the presence of saponins, steroids, tannins and flavonoids have highlighted its potential on cancer cells through cardiotonic, antimicrobial effects, neuroprotective qualities and antioxidant benefits. The results indicates new path for therapeutic applications while validating the plant's traditional medicinal usage.

UV-VIS Analysis

The qualitative UV/VIS profile of plant seed extract of *Caesalpinia bonducella* was analysed using the wavelength of 200 nm to 1100nm. The spectrum showed the peaks at 218.05, 585.10, 675.75, 762.80, 860.05, 989.50 and 1036.80nm with the absorption 4.0000, 0.6608, 0.6432, 0.6315, 0.5880, 0.5678 and 0.5748 as shown in Table 2.

Table 2: UV-VIS analysis of *Caesalpinia bonducella* plant seed extract

S.NO	Wave length	Absorbance
1.	218.05	4.0000
2.	585.10	0.6608
3.	675.75	0.6432
4.	762.80	0.6315
5.	860.05	0.5880
6.	989.50	0.5678
7.	1,036.80	0.5748

Absorption bands observed that related to UV-Vis spectrum of *Caesalpinia bonducella* plant seed extract was displayed in Figure 1. Absorption spectrum of *Caesalpinia bonducella* plant seed extract was almost transparent in the wavelength region of 200 to 1100nm. However, the appearance of peak in the region from 200 to 400 nm showed the presence of unsaturated and heteroatomic groups. The spectrum also confirms the presence of organic chromophores within the plant extract. Two absorption maxima in the ranges of 230–285 nm and 300–350 nm made up the flavonoids' spectra. Key details about the makeup of flavonoids can be gleaned from the exact location and relative strengths of these spectra (Zou *et al*., 2022). Due to the difficulty in identifying the absorption peaks with any ingredient, this spectrum is limited. Thus, FTIR was also utilized to enable the proper constituent identification.

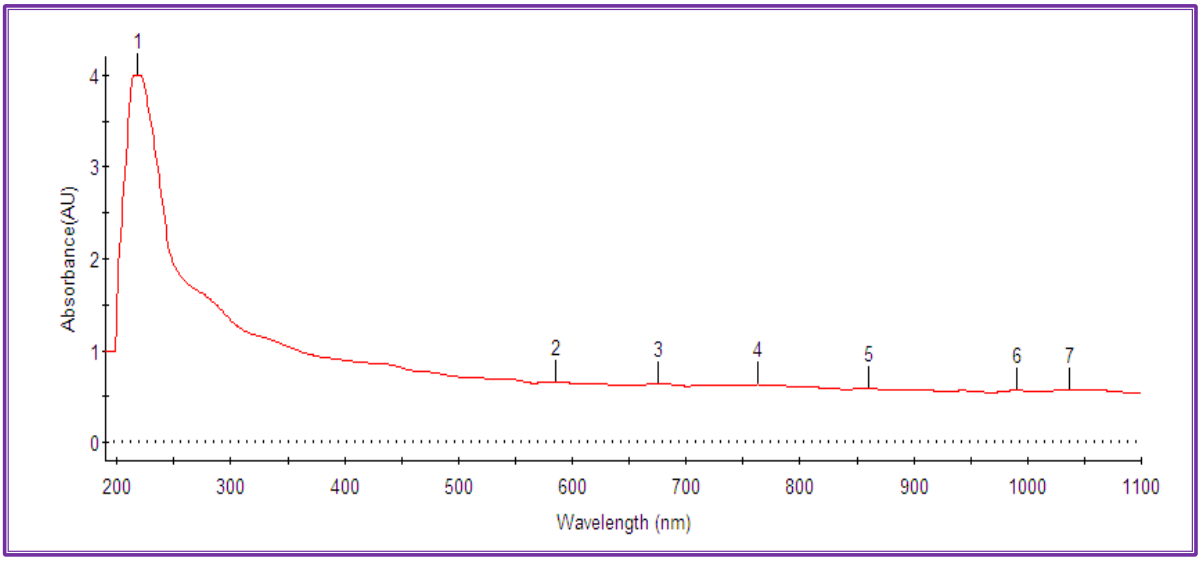

Figure 1: UV-Visible spectrum ofplant seed extract of*Caesalpinia bonducella*

FTIR Analysis

The FTIR spectrum was utilised to determine the functional groups present in the plant seed extract. Based on the peak's ratio of the plant seed extract of *Caesalpinia bonducella*, FTIR analysis verified the existence of alcohol, phenol, alkanes, aldehyde, aromatic compound, secondary alcohol, aromatic amines, and halogen compound. As seen in Figure 2, the spectrum with many absorption bands indicated the existence of active functional groups in the sample extract.

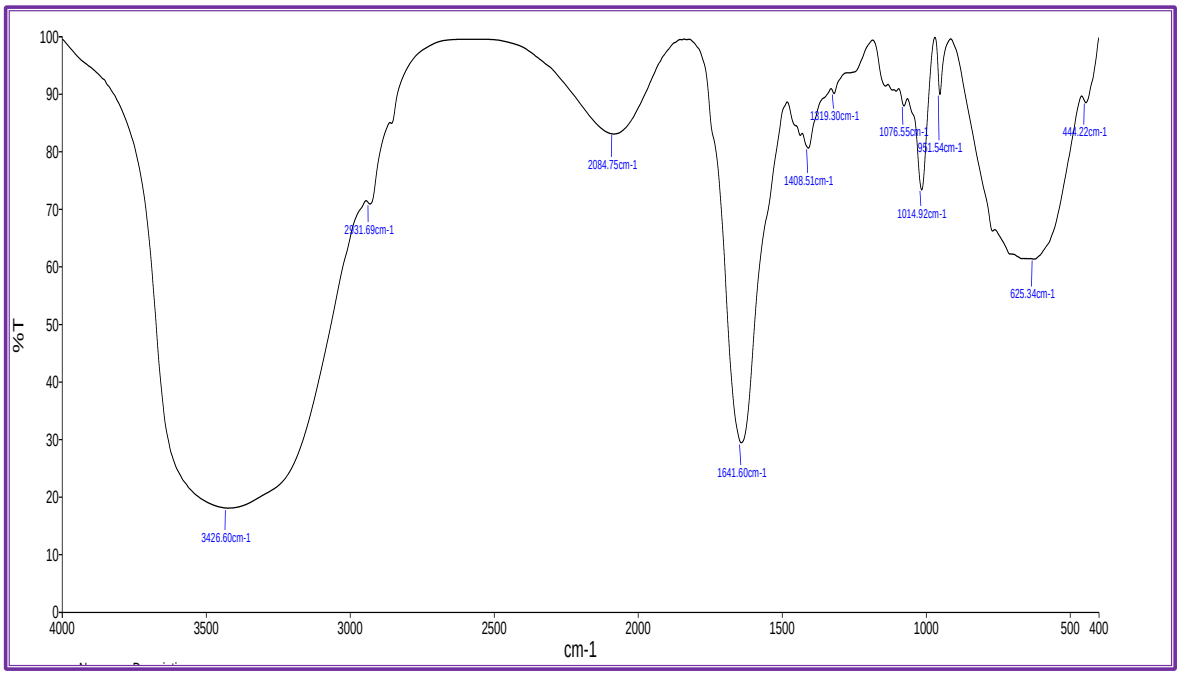

Figure 2: FTIR spectrum of plant seed extract of *Caesalpinia bonducella*

Both the 3426 and 1014 cm^{-1} periods have high intensity peaks, and the 1319 and 1014 cm^{-1} periods have some decreasing intensity peaks. Alcohol and phenol were proven to be present by the band at 3426 cm^{-1}, which correlated to a hydrogen-bonded O-H stretch. The peak at 1641 cm^{-1} was associated with an amide-related C=O stretch. The N=O bend at the peak at 1319 cm^{-1} was recognised as nitro groups. According to Table 3, the peak at 1014 cm^{-1} suggested C-O stretch vibrations to esters groups, while the faint band at 1076 cm^{-1} indicated C-O stretch vibrations

demonstrated the existence of ethers. This spectroscopic analysis reveals the chemical makeup together with functional groups responsible for the plant's pharmacological characteristics.

Table 3: Phytocomponents identified in plant seed extract of *Caesalpinia bonducella* by FTIR

Sl.No.	Wavenumber (cm^{-1})	Functional group	Identified phytocompounds
1.	3426	Hydrogen-bonded O-HStretch	Phenols and alcohols
2.	1641	C=O Stretch	Amides
3.	1319	N=O Bend	Nitro groups
4.	1076	C-O Stretch	Ethers
5.	1014	C-O Stretch	Esters

DPPH Assay

The antioxidant activity of *Caesalpinia bonducella* plant seed extract was determined by DPPH assay where the available DPPH was reacted with proton donors such as phenols in the sample extract. The free radical scavenging activity by means of IC50 values for various concentration of given plant seed extracts was shown in Table 4. *C. bonducella* plant seed extract exhibited the highest (92.04%) activity at high concentration of 1g/mL, actively pursued by 0.75g/mL concentration (79.53%). This plant seed extract concentration showed to contain low phenolic content whereas plant seed concentration of 0.25 and 0.50g/mL showed comparatively weaker scavenging activity (< 50%). The extracts demonstrated dose-dependent suppression of DPPH free radical scavenging activity, with a good potential of approximately 92% at 1g/mL concentration. The results are in accordance with the results observed in *C. tetragonoloba* seed extract where the extract concentration of 0.5 mg/ml enhanced the antioxidant activity (Joshi *et al.,* 2023)

Table 4: IC_{50} values of plant seed extract of *Caesalpinia bonducella*by DPPH assay

S.No	Concentration of plant extract (g/mL)	DPPH assay
1	**0.25**	37.50±0.18
2	**0.50**	40.83±2.41
3	**0.75**	79.53± 0.64
4	**1.00**	92.04±2.66

Such antioxidant activity of the *Caesalpinia bonducella* plant seed extract suggest that the extracts are an excellent source of antioxidants that occurred naturally along with the presence of alkaloids, sterols, phenolic compounds (flavonoids) and glycosides. These phytochemicals are excellent free radical scavengers considered as an important herb for prevention of cardiovascular disease and diabetes. Baliyan *et al.* (2023) showed that the DPPH radical scavenging activity of *Ficus religiosa* was 43.41% and Tupe *et al.* (2013) reported IC50 value as 96.01% for *Caesalpinia bonducella.*

Hydrogen peroxide scavenging Assay

Results display the plant seed extract's (*Caesalpinia bonducella*) capacity to scavenge H2O2 as shown in Table 5. The percentage of inhibition was observed from 50 to 82.5% for various concentrations of extract such as 0.25, 0.50, 0.75 and 1g/mL respectively. It was found that the inhibition percentage of the plant seed extract at 0.75 g/mL concentration was 88.3% when compared to high concentration of plant seed extract (1g/mL showed 82.5%). The results are in line with the reported study where tested extract of *(Ocimum basilicum* L.) exhibited high scavenging action against hydrogen peroxide (Nadeem *et al*., 2022).

Table 5: IC_{50} values of *Caesalpinia bonducella*seed extract by H_2O_2 assay

S.No	Concentration of plant extract (g/mL)	H_2O_2 assay
1	**0.25**	50 ± 3.5
2	**0.50**	72 ± 2.9
3	**0.75**	88.3 ± 3.8
4	**1.00**	82.5 ± 5.3

Hydrogen peroxide produced during physiological oxidative stress condition can converted into hydroxyl radicals and cause deleterious effects to cells by easily penetrated into the cell membranes. Anti-oxidants on the other hand, donates electron to hydrogen peroxide to neutralize it (Najeh *et al*., 2022)

Inhibitory action of α-Amylase

The inhibitors of α-amylase usually bind to 1, 4- α glycosidic linkages of polysaccharide and inhibits the further breakdown of polysaccharide into simpler forms (Wickramaratne *et al*., 2016). Assay performed therefore demonstrated the significant inhibitory potential of *Caesalpinia bonducella*plant seed extract with value of 85% for high concentration (1 g/mL). The IC_{50} value for 0.75g/mL plant seed extract concentration was 80.83%, followed by 79.16% and 53.33% for 0.50 and 0.5g/mL concentration of plant seed extract as shown in Table 6.

Table 6: α-amylase inhibitory activity of *Caesalpinia bonducella*seed extract

Concentration of plant extract (g/mL)	α-amylase inhibitory activity(%)
0.25	53.33 ± 0.25
0.50	79.16 ± 2.34
0.75	80.83 ± 4.58
1.00	85 ± 7.54

One of the main therapeutic approach in diabetic management is to decrease blood glucose level by diet which is achieved by blunting of carbohydrate diet digestion through inhibition of digestive enzymes such as alpha-amylase and alpha-glucosidase. It was reported that several natural sources act as amylase inhibitors (Ogunyemi *et al.,* 2022). One such natural plant source, *Caesalpinia bonducella*seed extract showed significant α-amylase inhibitory effects when compared to acarbose as the plant phtoconstituents involves terpenes, flavonoids and alkaloids. Such starch blockers that is plant-based, may provide a prospective therapeutic approach for the diabetic management (P *et al*., 2011). Pancreatic α-amylase, is the calcium-based metalloenzyme catalyst for polysaccharide molecules like amylose, amylopectin, glycogen, and other maltodextrins to hydrolyze their α-1,4 glycosidic linkages. Anti-hyperglycemic drug with amylase inhibiting action is frequently associated with substantial gastrointestinal side effects (Kashtoh & Baek, 2023). Similar results were observed in *Acacia nilotica* seed extracts that demonstrated notable inhibitory effects on enzymes. In particular, the seed methanolic extract has shown ability to inhibit α-amylase, which can help control blood sugar levels by delaying the digestion and absorption of carbohydrates (Ojo *et al*., 2024). It was reported that several species of plants within the same family revealed the presence of terpenoids, glycosides, alkaloids, and tannins when checked for phytochemical analysis. This may be due to the highest concentration of all the chemical classes listed above that shown to exhibit the most promising antidiabetic effect (Jaber, 2023).*Caesalpinia bonducella* as a natural starch blocker, is a plant-based therapeutic approach for diabetes management. The presence of phytochemicals in the extract supported its efficacy in α-amylase inhibition, by slowing down glucose absorption.

Conclusion

This research investigated the effectiveness of using Caesalpinia bonducella plant seed extract, for treating diabetes. Analysis of the extract revealed the presence of phytochemicals like saponins, steroids, tannins and flavonoids which have pharmacological properties such as cytotoxic, anti-microbial, anti-inflammatory and neuroprotective effects. Spectroscopic techniques like UV-VIS and FTIR confirmed the existence of these compounds in the extract. The DPPH assay showed that the antioxidant activity was dose dependent with a 92.04% scavenging activity at the concentration (1 g/mL). The hydrogen peroxide scavenging test displayed inhibition rates at concentrations reaching a peak of 88.3% at 0.75 g/Ml. Moreover the α-amylase assay demonstrated notable inhibitory effects. These findings highlight Caesalpinia bonducellas potential as a source of antioxidants and bioactive

compounds that could be beneficial for preventing and managing diseases in individuals, with diabetes.

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Conflicts of interest

Authors did not disclose any conflicts of interest.

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