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Original article

In silico studies and evaluation of *in vitro* antidiabetic activity of berberine from ethanol seed extract of *Coscinium fenestratum* (Gaertn.) Colebr

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ABSTRACT

Background and objective: Tree turmeric or *Coscinium fenestratum* (Gaertn.) Colebr is a recent focus climber tree for its various potent therapeutic activities. The present study focused on establishing the potent antidiabetic activity of ethanol seed extract of *Coscinium fenestratum* (CF) after confirmation through *in silico* molecular docking study.

Methods: The Soxhlet method was used for the extract preparation using ethanol solvent. Further, phytochemical screening and TLC were performed to detect the compound responsible for the antidiabetic activity. The accessible structure from the plant extract was used in an *in-silico* docking investigation based on that prediction. For the diabetes docking investigation, the alpha-amylase (HPA) protein with PDB ID: 4X9Y was chosen. After that, alpha-amylase and alpha-glucosidase inhibition assay techniques were also used to test the in vitro antidiabetic activity.

Results: Berberine was detected and identified by TLC with an Rf of 0.37 and was found to be responsible for the antidiabetic activity. AutoDock4.2 was employed for *in silico* study, and the binding energy of berberine was −7.84, demonstrating the significant antidiabetic properties of the ethanol extract from CF seeds.

Conclusion: The ethanol seed extract of CF may have an anti-diabetic effect because of the presence of berberine in the extract as one of the phytochemicals. However, additional research is required to ascertain the potential synergistic or antagonistic effects amongst various CF seed extract constituents.

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1. Introduction

Natural remedies have effectively treated and prevented many diseases with fewer adverse effects. Although there are quite a few synthetic medicines available on the market for the treatment of disorders, most customers are dissatisfied with these drugs owing to their negative effects on their health. As a result, people have gravitated toward natural herbal treatments in recent decades, even though the onset of action is slow yet efficient in curing ailments at the root level. This is because natural herbal remedies can cure ailments at the system level. One of the most critical and widespread chronic health disorders listed among them is diabetes. Recent research has shown that sudden shifts in lifestyle are one of the primary factors contributing to the development of non-communicable diseases like diabetes (Moksha and Rebecca, 2015). India currently has the second-highest number of diabetics globally, with 66.84 million, behind China, which had 96.28 million in 2016 (Pushpanathan et al., 2016). However, recent data suggested that India will become the first country with a high number of diabetics due to fast food consumption and an uncontrolled lifestyle (Ghazanfar et al., 2016). According to current epidemiological estimates, the prevalence of diabetes in Saudi Arabia is an alarming 18%. (Alotaibi et al., 2017). Hence, we felt it necessary to determine safe and effective treatment modalities that can manage diabetes and its complications, and one of the alternate options is natural plants. Herbals are prescribed long ago for their potent medicinal effectiveness and even WHO has also suggested wide applications of herbals in many health conditions, especially for diabetes treatment (Moradi et al., 2018). Natural plant drugs play an immense role in controlling diabetes with their potent bioactive compounds, such as metformin, from *Galega officinalis,* used to control glucose levels (Vivó-Barrachina et al., 2022). Further, Turmeric, Ginkgo, Stevia, Gymnema, and many other plant-based chemicals are used in lowering glucose uptake through its transporters by triggering insulin release (Salehi et al., 2019).

Oflate, *Coscinium fenestratum* (Gaertn.) Colebr. (CF) is generally recalled as 'tree turmeric' (F: Menispermaceae). The wood of CF is present in the conventional staining of cloths with a yellowish-brown cylindrical stem and yellowish on the inside. Recently, the CF plant species has not been cultivated. However, most of its populations have been heavily exploited in their natural habitats (Naincy and Vasudeva, 2022). In India, the main source of CF trees is in the Western Ghats region in crevices of rocks and trees as a climbing plant (Danapur et al., 2020). The stem produces a yellow dye that can be used either by itself or in conjunction with other coloring agents, such as turmeric. The roots contain many alkaloidal bioactive components viz. berlambine, dihydroberlambine, 12, 13-dihydro-8-oxo berberine, tetrahydroberberine, oxyberberine and noroxy hydrastinine (Das et al., 2018). Stem contents berberine especially. Leaves and fruits also contain flavonoids, saponin, and alkaloids (Tripathi et al., 2022). Leaves contain ecdysterone, a little percentage of berberine, and aporphine alkaloids (Ashalatha and Gopinath, 2019). Seeds contain ceryl alcohol, hentriacontane, palmitic acid, sitosterol, and saponin with some resinous material (Kashyap et al., 2016). All the parts of the CF plant were reported for many therapeutic activities. Traditionally, the CF plant showed versatile therapeutic efficacies viz. antidiabetic, anti-inflammatory, asthma, and antiseptic activities (Krishnamoorthy et al., 2019) whereas, fruits showed anthelmintic and antioxidant activity (Das et al., 2018) but no reports for the CF seeds though the seed contains some effective bioactive chemicals. Hence, this is the first time reported potent antidiabetic activity for the ethanol CF seed extract with predicted *in silico* docking study.

2. Materials and methods

2.1. Selection of seed and extraction

The seeds of CF were procured from the Indian Institute of Horticultural Research, Bangalore. They were identified with the help of scientists from the same Institute. The seed sample was preserved as an herbarium (No: KCP/2021-2022-CF seed-514/COG) for future reference (Fig. 1).

The hard seeds broke the coats to get inside solid yellow parts. Approximately 100 seeds were broken, and all the inner material was collected for the different extraction processes. About 50 g of oven-dried (50 °C for 30 min) powder sample was extracted using 250 ml of ethanol solvent by Soxhlet apparatus for 6 hrs. The oven temperature for the extraction was 40 degrees Celsius. After extraction, the solution containing extract was filtered using Whatman filter paper (No: 41, ashless) and dried using a rotary flash evaporator at 45 degrees C for 30 min.

2.2. Phytochemical screening

As per standard literature, various chemical tests were performed for phytochemical screening. All the tests were carried out to know the presence of a group of phytochemicals in CF seed extract (Das et al., 2022).

2.3. Identification and separation of constituents

The seed extract was further run with TLC using precoated silica gel and a mixture of mobile phases (n-Hexane: chloroform: methanol; 5:4:2) and identified the presence of the desired compound in the ethanol CF extract.

2.4. In silico docking study

2.4.1. Preparation of target

The protein was obtained from RCSB PDB a protein data bank. A human pancreatic alpha-amylase (HPA) protein was selected for the docking studies of Diabetes. It was obtained in pdb format, and the protein was converted to target by removal of the water molecules, energy minimization, addition of charges such as Kollmann and Gasteigercharges, and the protein's missing atoms were added. For energy minimization, MOE2018software was used and for molecular docking studies, AutoDock4.2 was used.

2.4.2. Preparation of ligand

Berberine was obtained from the Chem draw 3D, and saved in sdf format. Openbabelsoftware was used to convert ligands from sdf to pdb format. Later using AutoDock4.2, the aromaticity criterion and several torsions were set and saved in pdbqt format.

2.5. In-Vitro anti-diabetic activity

2.5.1. Alpha-amylase inhibition assay

The procedure developed by prior researchers is followed for doing the alpha-amylase inhibition experiment (Pant et al., 2013; Ramachandran et al., 2013) with modification. Separately, 1.5 ml of each of the ethanolic extracts of CF seed of various strengths and the standard acarbose (100–400 g/ml) were combined with 1.5 ml of the -amylase enzyme (1%), 1.5 ml of sodium acetate buffer, and 1.5 ml of the enzyme (pH-7.2). 2 ml of a 1% starch solution was added after the mixture had been incubated at room temperature for 20 min. The combination above is incubated at 37 °C for

Fig. 1. CF seeds and powder.

30 min. The mixture is then given 1.5 ml of the 3,5-dinitro salicylic acid reagent. Five minutes are spent with the mixture in a bath of hot water. With a UV–Visible spectrophotometer, the absorbance is measured at 540 nm. The average was calculated after three copies of each experiment were performed.

Finally, the percentage inhibition of α-amylase inhibition was calculated as:

$$\% \text{ inhibition} = (A0 - A1/A0) \times 100$$

where A0 = The absorbance of control.

A1 = The absorbance of the test or standard.

2.5.2. Alpha-glucosidase inhibition assay

The inhibitory action was assessed by combining different concentrations of the sample, ethanolic extracts of CF seed (100–500 mg/ml), with 1 ml of starch solution (2% w/v maltose) in 0.2 M tris buffer (pH 8). For 10 min, the reaction mixture was incubated at 37 °C. One ml of alpha-glucosidase enzyme (1 U/ml) was added to the reaction to start it, and it was then incubated at 35 °C for 40 min. After that, 2 ml of 6 N HCl was added to end the reaction. At 540 nm, a spectrophotometer was used to quantify the color's intensity based on the earlier method of Vennila and Pavithra, 2015 with modification.

The percentage inhibition was calculated as:

$$\text{Percent inhibition} = \frac{Absorbance\ \ of\ \ control - Absorbance\ \ of\ \ smaple}{Absorbance\ \ of\ \ control} \times 100.$$

Further, IC_{50} values were calculated for all the assay methods. The amount of inhibitor needed to inhibit a substance's action by 50% under test conditions is known as the inhibitory concentration (IC_{50}) value. Plots of percent inhibition versus log inhibitor concentration were used to determine the IC_{50} values, which were then derived from the mean inhibitory values.

2.6. Statistical analysis

The values are expressed as the mean ± standard error of the mean. The result is also expressed as an IC_{50} value. The IC_{50} value was calculated using logarithmic regression analysis.

3. Results

3.1. Extract yield

The extract was allowed to dry. The dried extract was weighed. The % yield of each plant extract was calculated. The % of yield obtained was 10.24 for alcoholic extract. The section was preserved in the refrigerator till further use.

3.2. Chemical test and TLC identification

Various chemical tests were performed, revealing the presence of various phytoconstituents in the ethanol CF seed extract depicted in Table 1. After that, TLC was performed using an n-hexane, chloroform, and methanol solvent mixture at a ratio of 5:4:2 and compared with standard berberine. Under UV light, it was detected as berberine was present in the extract (Rf: 0.37) (Fig. 2), and due to the presence of the bioactive compound, it gives many therapeutic activities. Among them, in the present study, anti-diabetic activity was performed.

3.3. Docking studies

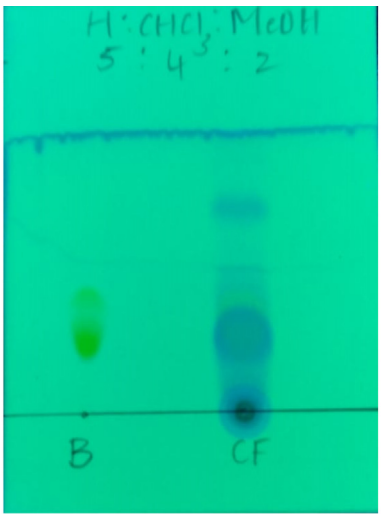
In the present study, we are performing docking studies for berberine which was docked against the proteins of type II Diabetes. Berberine is docked with the major target protein (Human pancreatic amylase) with PDB ID: 4X9Y. The binding energy of berberine was found to be −7.84. In addition, hydrogen bonds were formed between the target molecule and the ligand. For berberine, there are five hydrogen bonds and three alkyl bonds (Fig. 3).

3.4. ADMET studies

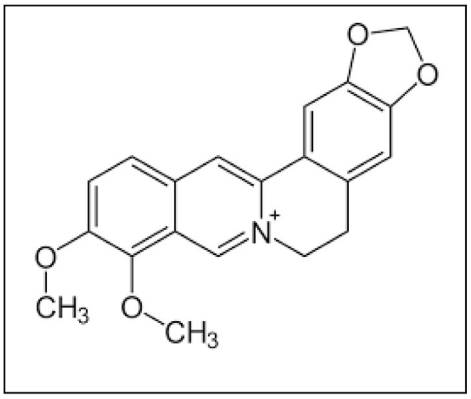
ADMET Studies were conducted for berberine using pkSCM. For developing and discovering new drugs, absorption, distribution, metabolism, elimination, and toxicity play a significant role. The

Table 1
Various phytochemical tests for CF ethanol seed extract.

Chemical tests	Phytoconstituents
Alkaloids	Present
Saponins	Present
Phytosterol	Present
Triterpenoids	Present
Tannins	Present
Proteins	Present
Carbohydrate	Absent



TLC of CF ethanol seed extract



Berberine structure

Fig. 2. TLC of Berberine in the CF ethanol seed extract.

SMILES format of ligands is used to predict their properties. We evaluated water solubility, intestinal absorption, CNS permeability, LD50, hepatotoxicity, etc. (Tables 2 and 3).

4. *In vitro* antidiabetic activity

a) Alpha-Amylase inhibition assay.

Percentage inhibition of Alpha-amylase by ethanol seed extract of CF was performed; results are shown in Fig. 4. Inhibition activity was recorded as 19.46 %, 38.19 %, 52.09 %, and 61.22 % in 100, 200, 300, and 400 μg/ml concentrations. The same activity was recorded higher for the standard Acarbose at the same concentrations (36.11 %, 52.10 %, 64.28 %, and 76.2 %, respectively at the same concentrations).

From Fig. 4, the result of IC_{50} values was calculated for the standard and the extract and found to be 1.96 and 3.02, respectively. Interestingly, dose-dependent activity was recorded for both the extract and the standard.

b) Alpha-Glucosidase Inhibition Assay.

Percentage inhibition of alpha-glucosidase by ethanol seed extract of CF was performed; results are shown in Fig. 5. The inhibitory action was assessed by combining different concentrations of the sample (100–400 μg/ml) and resulted in a similar alpha-amylase inhibition activity. It was reported that extract at a 400 μg/ml concentration resulted in 54.22 percent inhibition, whereas the standard gave 57.38 percent. Dose-dependent inhibition was recorded for both standard and extract. Further, from the Fig. 5, the IC_{50} values were calculated for the standard and the extract and found to be 3.50 and 3.78, respectively.

5. Discussion

Diabetes-related microvascular complications include retinopathy, nephropathy, and neuropathy. At the same time, the term "macrovascular complications" refers to an increase in the number of events connected to atherosclerosis, such as "myocardial infarction" and "stroke" (Pothireddy et al., 2014). One of the most effective treatments for diabetes is to reduce the amount of carbohydrates absorbed after eating. To be absorbed in the duodenum and upper jejunum, complex starches, oligosaccharides, and disaccharides must first be broken down into monosaccharides by alpha-amylase and alpha-glucosidases (Mohamed et al., 2012). Most natural products work by virtue of inhibiting these enzymes required for breaking down polysaccharides into monosaccharides, thereby preventing postprandial hyperglycemia. Antidiabetic effects are present in plants because of flavonoids, terpenoids, saponins, carotenoids, alkaloids, and glycosides (Afrisham et al., 2015). Specifically, individual bioactive substances such as palmatine, berberine, honokiol, amorfrutins, trigonelline, gymnemic acids, gurmarin, and phlorizin demonstrated antidiabetic potential (Jacob and Narendhirakannan, 2019).

In the current investigation, the extraction process was carried out using ethanol as the solvent because it was readily available and less hazardous than methanol. Because of its high dielectric constant (24.55), ethanol can extract the maximum amount of bioactive components from the plant, including polyphenols, tannins, flavonoids, terpenoids, and alkaloids (Azmir et al., 2013). For this reason, ethanol was chosen as the solvent for preparing the extract in the current investigation. In addition, several chemical analyses carried out on the CF seed extract confirmed the existence of alkaloids as one of the phytochemicals present in the sample. TLC and specific chemical testing confirmed the presence of berberine, the primary active ingredient in CF seed extract. Berberine was shown to be among the alkaloids. Earlier studies supported the idea that berberine plays a significant role in preventing diabetes through multiple mechanisms. These mechanisms include increased insulin utilization, modulation of gut microbiota without systemic anti-infective activity (Han et al., 2011), and stimulation of glucose uptake markedly. Earlier studies also supported the idea that berberine plays a significant role in preventing diabetes. As a result, berberine was investigated for its potential to combat diabetes in vitro.

The chemical structure of berberine and other isoquinoline alkaloids is very different from that of other hypoglycemic medications commonly used, such as sulphonylureas, biguanides, thiazolidinediones, or acarbose. Berberine and similar isoquinoline alkaloids are known to have anti-diabetic properties. It's possible that the improved glucose metabolism that berberine brings about is due to the stimulation of glycolysis, which is linked to the sup-

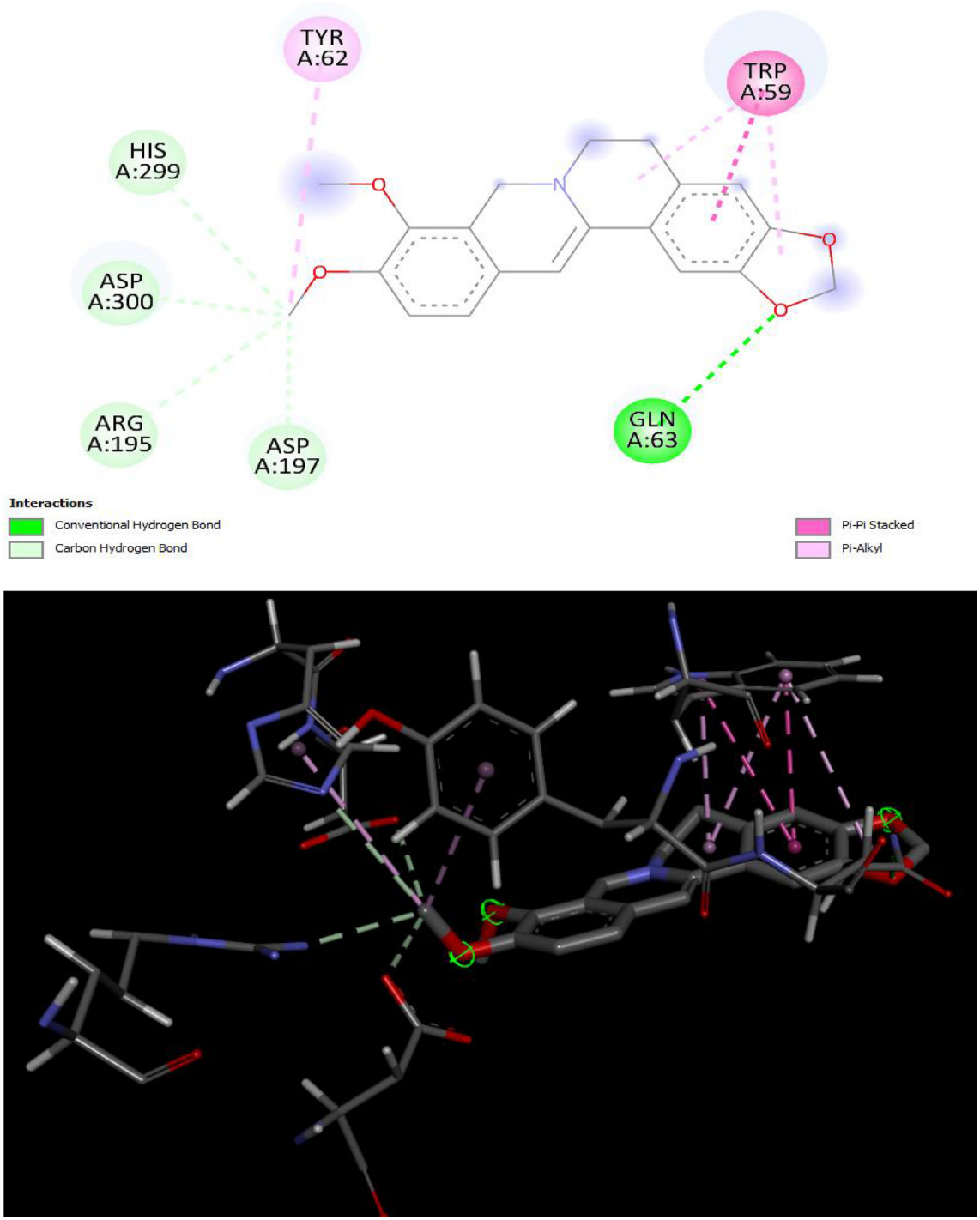


Fig. 3. 2D and 3D structure of binding of berberine with the PDB ID: 4X9Y protein.

pression of oxidation in the mitochondria (Yin et al., 2008). In addition, berberine has the potential to inhibit alpha-glucosidase. Disaccharidase activities were inhibited, and the amount of glucose transported through the intestinal epithelium was reduced (Kong et al., 2004). Based on the previous research and its effectiveness against diabetes, a further prediction was carried out by an *in silico* docking study. The results of this study revealed a strong binding affinity with the protein that caused Alzheimer's disease. They showed that the compound inhibited enzyme activity with a relatively low level of toxicity. In conclusion, for confirmation, in vitro alpha-amylase and alpha-glucosidase activities were carried out. The results showed that there was a significant inhibition of the enzyme activity in comparison to the standard. A report that was very similar to this was previously shown by prior researchers (Ramana Murty Kadali et al., 2017; Paun et al., 2020; Van et al., 2022).

The enzyme alpha-amylase catalyzes the process by which the 1,4-glucosidic linkages in the starch are broken down into glucose. Degradation of starch in the diet is linked to increased postprandial hyperglycemia. It does this by inhibiting the amylase in the diges-

Table 2
ADMET study of Berberine.

Property	Model name	Berberine
Absorption	Water solubility	−3.973
Absorption	$CaCO_2$ permeability	1.734
Absorption	Intestinal absorption(human)	97.147
Absorption	Skin Permeability	−2.576
Absorption	P-glycoproteinsubstrate	Yes
Absorption	P-glycoprotein Iinhibitor	No
Absorption	P-glycoprotein IIinhibitor	Yes
Distribution	VDss (human)	0.58
Distribution	Fraction unbound(human)	0.262
Distribution	BBB permeability	0.198
Distribution	CNS permeability	−1.543
Metabolism	CYP2D6 substrate	No
Metabolism	CYP3A4 substrate	Yes
Metabolism	CYP1A2 inhibitor	Yes
Metabolism	CYP2C19 inhibitor	No
Metabolism	CYP2C9 inhibitor	No
Metabolism	CYP2D6 inhibitor	Yes
Metabolism	CYP3A4 inhibitor	Yes
Excretion	Total Clearance	1.27
Excretion	Renal OCT2substrate	No
Toxicity	AMES toxicity	Yes
Toxicity	Max. tolerated dose(human)	0.144
Toxicity	hERG I inhibitor	No
Toxicity	hERG II inhibitor	No
Toxicity	Oral Rat AcuteToxicity (LD50)	2.571
Toxicity	Oral Rat ChronicToxicity (LOAEL)	1.89
Toxicity	Hepatotoxicity	Yes
Toxicity	Skin Sensitisation	No
Toxicity	*T.Pyriformis* toxicity	0.354
Toxicity	Minnow toxicity	−0.277

Table 3
Nature of binding affinity for berberine with human pancreas protein.

Descriptor	Berberine
Molecular Weight	336.367
LogP	3.0963
#Rotatable Bonds	2
#Acceptors	4
#Donors	0
Surface Area	144.867

tive tract, which slows down the rate at which starch is broken down in the stomach and reduces postprandial hyperglycemia (Kajaria et al., 2013). Two alpha-amylase inhibitors, acarbose, and miglitol are responsible for reducing postprandial glucose levels. They do this by blocking alpha-amylase activity in the small intestine, which delays the absorption of carbohydrates and slows the release of glucose from starches (Kalra, 2014). The inhibitory effect was strengthened by increasing the concentration of ethanolic extract and acarbose from 100 to 400 g/ml. The experiment's findings revealed that conventional acarbose possessed much higher percent inhibition values when compared to ethanol extract. The alpha-glucosidase enzyme, inhibited by the CF seed extract, likewise produced a very similar result to the one described above. The overall activity may be caused by the presence of berberine in the seed extract, which is one of the active ingredients that can be found alongside other active principles.

6. Conclusion

The anti-diabetic effect of the ethanol CF seed extract and its potential bioactive constituent were tested for the first time in vitro. Phytochemical analysis indicated that the CF seed extract contains berberine, one of the most important bioactive substances. At first, the docking of berberine was accomplished with the help of AutoDock4.2, and the toxicity tests were carried out with the assistance of the pkCSM tool. The score of berberine for its ability to bind was found to be −7.84. The number of hydrogen bonds discovered to exist is five, and they were discovered to be created by amino acids such as GLN63, ARG195, ASP197, HIS299, and ASP300. According to the results of docking studies and ADMET experiments conducted on berberine, it was found to have superior absorption and much less toxicity. Following this, in vitro alpha-amylase and alpha-glucosidase enzyme inhibitory activities by the CF extract demonstrated that the CF seed is a potent antidiabetic agent. Additionally, it was established that berberine, one of the abundantly available bioactive components, has promising antidiabetic potential.

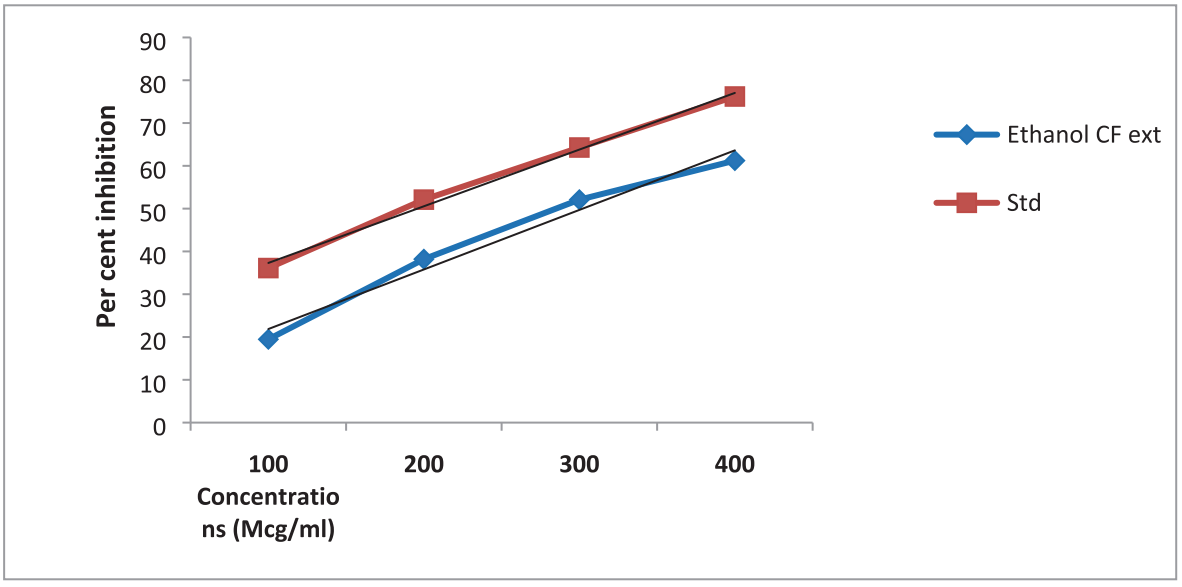


Fig. 4. *In vitro* α-amylase inhibitory activity of ethanol CF seed extract.

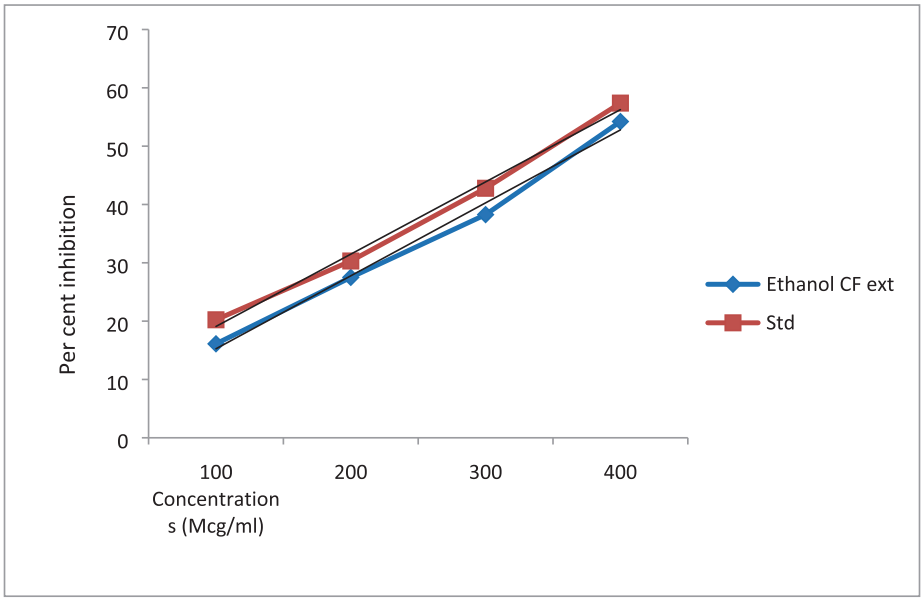


Fig. 5. *In vitro* alpha-glucosidase inhibitory activity of ethanol CF seed extract.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Afrisham, R., Aberomand, M., Ghaffari, M., Siahpoosh, A., Jamalan, M., 2015. Inhibitory effect of *Heracleum persicum* and *Ziziphus jujuba* on activity of alpha-amylase. J. Bot. 2015, 824683.

Alotaibi, A., Perry, L., Gholizadeh, L., Ganmi, A.A., 2017. Incidence and prevalence rates of diabetes mellitus in Saudi Arabia: An overview. J. Epidemiol. Global Health. 7 (4), 211–218.

Ashalatha, Gopinath, S.M., 2019. Phytochemical profiling of Coscinium fenestratum (Gaertn.) Colebr Cultivar, by Liquid chromatography-Mass spectrometry. Int. J. Curr. Microbiol. App. Sci. 8 (1), 3194–3201.

Azmir, J., Zaidul, I.S.M., Rahman, M.M., Sharif, K.M., Mohamed, A., Sahena, F., Omar, A.K.M., 2013. Techniques for extraction of bioactive compounds from plant materials: Areview. J. Food Eng. 117 (4), 426–436.

Danapur, V., Haleshi, C., Sringeswara, A.N., 2020. Endangered Medicinal Plant *Coscinium fenestratum* (Gaertn.) Colebr - A Review. Pharmacogn J. 12 (5), 1077–1085.

Das, K., Dang, R., Sivaraman, G., Rajasekharan, P.E., 2008. Phytochemical screening for various secondary metabolites, antioxidant and anthelmintic activity of Coscinium fenestratum fruit pulp- A new bio source for the novel drug discovery. Turkish. J. Pharmaceut. Sci. 5 (2), 156–165.

Das, K., Syed Mohammed, B.A., Khan, S.M., Amrutha, S., Alamri, A.S., Alhomrani, M., et al., 2022. Phytochemical investigation and evaluation of *in vitro* anti-inflammatory activity of *Euphorbia hirta* ethanol leaf and root extracts: A comparative study. J. King Saud Univ. Sci. 34, (7) 102261.

Ghazanfar, H., Rizvi, S.W., Khurram, A., Orooj, F., Qaiser, I., 2016. Impact of insulin pump on quality of life of diabetic patients. Indian J. Endocrinol. Metab. 20 (4), 506–511.

Han, J., Lin, H., Huang, W., 2011. Modulating gut microbiota as an anti-diabetic mechanism of berberine. Med. Sci. Monit. 17 (7), RA164-7.

Jacob, B., Narendhirakannan, R., 2019. Role of medicinal plants in the management of diabetes mellitus: A review. 3Biotech. 9, 4. https://doi.org/10.1007/s13205-018-1528-0.

Kajaria, D., Ranjana, T.J., Tripathi, Y.B., Tiwari, S., 2013. In-vitro α amylase andglycosidase inhibitory effect of ethanolic extract of antiasthmatic drug - Shirishadi. J. Adv. Pharm. Technol. Res. 4 (4), 206–209.

Kalra, S., 2014. Alpha glucosidase inhibitors. J. Pak. Med. Assoc. 64 (4), 474–476.

Kashyap, S., Kapoor, N., Kale, R.D., 2016. *Coscinium fenestratum*: Callus and suspension cell culture of the endangered medicinal plant using vermicompost extract and coelomic fluid as plant tissue culture media. Am. J. Plant Sci. 7, 899–906.

Kong, W., Wei, J., Abidi, P., Lin, M., Inaba, S., Li, C., Wang, Y., Wang, Z., Si, S., Pan, H., Wang, S., Wu, J., Wang, Y., Li, Z., Liu, J., Jiang, J.D., 2004. Berberine is a novel cholesterol-lowering drug working through a unique mechanism distinct from statins. Nat. Med. 10 (12), 1344–1351. https://doi.org/10.1038/nm1135.

Krishnamoorthy, K., Gangadharan, G., Senguttuvan, J., Subramaniyam, P., Ali, M.A., Hemaid, F.A., et al., 2019. The potential of antioxidant activity of methanolic extract of *Coscinium fenestratum* (Goetgh.) Colebr (Menispermaceae). Saudi J. Biol. Sci.. 26 (5), 1037–1042.

Mohamed, E.A., Siddiqui, M.J., Ang, L.F., Sadikun, A., Chan, S.H., Tan, S.C., Asmawi, M. Z., Yam, M.F., 2012. Potent α-glucosidase and a-amylase inhibitory activities of standardized 50% ethanolic extracts and sinensetin from *Orthosiphon stamineus* Benth as anti-diabetic mechanism. BMC Complement Altern. Med. 12, 176. https://doi.org/10.1186/1472-6882-12-176.

Moksha, J., Rebecca, J., 2015. A clinical study of sensory and pain threshold values of upper limb nerves in diabetes mellitus patients. Int. J. Diabetes Dev. Ctries. 35 (3), S393–S397.

Moradi, B., Abbaszadeh, S., Shahsavari, S., Alizadeh, M., Beyranvand, F., 2018. The most useful medicinal herbs to treat diabetes. Biomedi. Res. Therapy. 5 (8), 2538–2551.

Naincy, S., Vasudeva, R., 2022. Endangered Liana of the Western Ghats, *Coscinium fenestratum* (Gaertn.) Colebr: An Overview. Indian. J. Ecol. 49 (4), 1247–1251.

Pant, G., Sai, K., Babasaheb, S., Reddy, R., Sibi, G., 2013. In vitro α-amylase and α-glucosidase inhibitor activity of abutilon indicum leaves. Asian J. Pharm. Clin. Res. 6 (5), 22–24.

Paun, G., Neagu, E., Albu, C., Savin, S., Radu, G.L., 2020. In Vitro Evaluation of Antidiabetic and Anti-Inflammatory Activities of Polyphenolic-Rich Extracts from *Anchusa officinalis* and *Melilotus officinalis*. ACS Omega 5, 13014–13022.

Pothireddy, S., Chukka, S., Shankaraiah, P., 2014. Evaluation of antidiabetic, antidyslipidemic & hepatoprotective activity of *Homalium zeylanicum* in alloxan induced diabetic rats. Int. J. Res. Dev. Pharm Life Sci. 3 (3), 1004–1010.

Pushpanathan, P., Srikanth, P., Seshadri, K.G., Selvarajan, S., Pitani, R.S., Kumar, T.D., et al., 2016. Gut microbiota in Type 2 diabetes individuals and correlation with monocyte chemoattractant protein1 and interferon gamma from patients attending a tertiary care centre in Chennai, India. Indian J. Endocrinol. Metab. 20 (4), 523–530.

Ramachandran, S., Rajasekaran, A., Adhirajan, N., 2013. In Vivo and In Vitro Antidiabetic Activity of Terminalia paniculata Bark: An Evaluation of Possible Phytoconstituents and Mechanisms for Blood Glucose Control in Diabetes. ISRNPharmacol. 2013, 484675.

Ramana Murty Kadali, S.L.D.V., Das, M.C., Vijayaraghavan, R., Shanmukha, I., 2017. *In vitro* evaluation of antidiabetic activity of aqueous and ethanolic leaves extracts of *Chloroxylon swietenia*. Nat. J. Physiol. Pharmacy Pharmacol. 7 (5), 486–490.

Salehi, B., Ata, A., Kumar, N.V.A., Sharopov, F., Ramírez-Alarcón, K., Ruiz-Ortega, A., et al., 2019. Antidiabetic Potential of Medicinal Plants and Their Active Components. Biomolecules. 9, 551. https://doi.org/10.3390/biom9100551.

Tripathi, D., Misra, A., Chaudhary, M.K., Srivastava, S., 2022. Evaluation of *Coscinium fenestratum* (Goetgh.) Colebr. stem extracts for urolithiasis and quantification of bioactive alkaloids to validate the traditional claims. Nat. Prod. Res. 19, 1–6. https://doi.org/10.1080/14786419.2022.2134863.

Van, L.V., Pham, E.C., Nguyen, C.V., Duong, N.T.N., Thi, T.V.L., Truong, T.N., 2022. *In vitro* and *in vivo* antidiabetic activity, isolation of flavonoids, and *in silico* molecular docking of stem extract of *Merremia tridentata* (L.). Biomed. Pharmacother. 146, 112611.

Vennila, V., Pavithra, V., 2015. In-vitro alpha amylase and alpha glucosidase inhibitory activity of various solvent extracts of *Hybanthus enneaspermus* Linn. World J. Pharm. Pharm. Sci. 4 (4), 1425–1437.

Vivó-Barrachina, L., Rojas-Chacón, M.J., Navarro-Salazar, R., Belda-Sanchis, V., Pérez-Murillo, J., Peiró-Puig, A., et al., 2022. The Role of Natural Products on Diabetes Mellitus Treatment: A Systematic Review of Randomized Controlled Trials. Pharmaceutics. 14 (1), 101. https://doi.org/10.3390/pharmaceutics14010101.

Yin, J., Gao, Z., Liu, D., Liu, Z., Ye, J., 2008. Berberine improves glucose metabolism through induction of glycolysis. Am. J. Physiol. Endocrinol. Metab. 294 (1), E148–E156. https://doi.org/10.1152/ajpendo.00211.2007.