

RESEARCH ARTICLE

Evaluation of antidiabetic potential of extracts of *Cryptolepis dubia* (Burm.f.) M. R. Almeida

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Abstract

Diabetes mellitus is a group of metabolic illnesses marked by chronic hyperglycemia, which can harm a variety of organs, including the kidneys, heart, nerves, eyes, and blood vessels. Despite the availability of synthetic hypoglycemic medicines, the negative side effects need alternative therapy, particularly those derived from natural sources. This study looks into the antidiabetic characteristics of *Cryptolepis dubia* (Burm.f.) M.R. Almeida, a plant that has traditionally been used for medicinal purposes. Extracts from *Cryptolepis dubia* stems and leaves were examined for their inhibitory effects on α -amylase and α -glucosidase enzymes, as well as glucose uptake in yeast and HepG2 cells. The ethanolic leaf extract inhibited α -amylase at the highest rate (83.20%), whereas the aqueous stem extract inhibited α -glucosidase significantly (81.50%). The glucose uptake studies revealed that both stem and leaf extracts significantly increased glucose absorption, comparable to the standard medication Metformin. In addition, the cell viability assay revealed that the extracts were non-toxic. These findings indicate that *C. dubia* extracts have strong anti-diabetic activities, underlining their potential as alternative therapeutic agents for diabetes control.

Keywords: *Cryptolepis dubia*, Antidiabetic, HepG2 cells, Apocynaceae, Glucose uptake.

Introduction

A collection of metabolic diseases known as diabetes mellitus share hyperglycemia as a common symptom. The kidneys, heart, nerves, eyes, and blood vessels are all harmed by chronic hyperglycemia. It is brought on by an inherited, acquired, or inefficient pancreatic insulin production insufficiency, as well as by inadequate insulin that is generated. It is caused by either insufficient insulin hormone secretion, insufficient insulin uptake by target cells, or a combination of these. Changes in lifestyle, medical diagnosis, and treatment are necessary for this disease. Within the next 25 years, it is expected to rank

among the primary debilitators and murderers in the world (Li *et al.* 2004). Up until today, diabetes management has been a worldwide issue for which there is no proven cure. While several synthetic medications have been produced for patients, no one has ever been known to have fully recovered from diabetes. The current generation of oral hypoglycemic medications has unfavourable side effects. Alternative therapy is therefore necessary, and it is imperative that we move towards various indigenous plant and herbal compositions (Satyanarayana *et al.* 2006). Certain medicinal plants have been used empirically as antidiabetic and antihyperlipidemic treatments and have recently been reported to be helpful in the treatment of diabetes worldwide. Diabetes and its complications remained a serious medical issue even after pharmaceutical companies introduced effective anti-diabetic medications. The capacity of these plants to stimulate insulin production, prevent intestinal glucose absorption, or support metabolites involved in insulin-dependent activities is thought to be the source of their antihyperglycemic actions (Khan and Kesarkar 2021, Satyanarayana *et al.* 2006). There are about 400 plant species with hypoglycemic activity that have been documented in the literature; yet, the presence of certain compounds makes it appealing to search for novel antidiabetic medications derived from natural plants, which show several, safe ways to treat diabetes mellitus. Glycosides, alkaloids, terpenoids, flavonoids, cardenoids, etc. are found

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How to cite this article: Khan, S.Y., Shinde, R.D (2025). Evaluation of antidiabetic potential of extracts of *Cryptolepis dubia* (Burm.f.) M. R. Almeida. *J. Indian bot. Soc.*,105(3):231-237 Doi: 10.61289/jibs2025.01.20.301

Source of support: Nil

Conflict of interest: None.



Figure 1: (L) - Flowering in *Cryptolepis dubia*; (R) - Leaf and flower buds of *Cryptolepis dubia* (Photograph taken by author Saif Khan and his student Shubham Patkar from The Conservation Education Centre (CEC) of Bombay Natural History Society (BNHS), Goregaon under guidance of staff member Ms. Kiran Thumma.

in most plants and are often linked to antidiabetic effects. One such plant *Cryptolepis dubia* (Burm.f.) M.R.Almeida from family Apocynaceae is generally known as Jambu Patra, Krishna Sariva in Sanskrit, Karanta in Hindi, and Wax leaved climber in English. This perennial creeper with milky juice can be found throughout the country, from Western Kashmir to Assam, reaching elevations of 1200 m in the Himalayas and as far south as Kerala (Ragunathan and Mitra 1999). Global distribution ranges from northern Pakistan, Nepal, and Bhutan to India, Sri Lanka, and Myanmar (Bhakuni 1969). The herb is used in traditional medicine for anti-ulcerative, anti-inflammatory, anti-diarrheal, antibacterial, cough, blood purifier, breastfeeding, bone fracture, and rickets treatment. *Cryptolepis dubia* includes phytocomponents such as cryptosin, sarverogenin, isosarverogenin glycosides, a novel nicotinoyl glucoside, cryptolepain, and burchanin (Meher *et al.* 2020). It also has antioxidant, hepatoprotective, analgesic, antiinflammatory, chondroprotective, immunomodulatory, anticancer, insecticidal and cardiotoxic effects (Venkateshwara *et al.* 1989, Purushothaman *et al.* 1988, Sunil *et al.* 1980, Pande *et al.* 2006, Khare and Shah 1983, Padmalochana *et al.* 2013, Hanprasertpong *et al.* 2014). This study aimed to investigate the anti-diabetic properties of *Cryptolepis dubia*, this marks a crucial milestone in assessing the quality of the crude drug for future development.

Materials and Methods

Materials used

All the chemicals like Starch powder, Iodine solution, Potassium Phosphate buffer (pH 6.5 - 6.8), Porcine pancreatic alpha amylase enzyme (EC 3.2.1.1), Iodine reagent, alpha glucosidase enzyme (EC 3.2.1.20), Sodium carbonate, p-NPG (p-nitrophenyl glucanopyranoside), Dextrose powder, Bovine serum albumin, Glutamine, Phosphate buffered saline (1x) and MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) were all of analytical grade and purchased from Sisco Research Laboratories (SRL) Pvt. Ltd., Mumbai. Standard drugs like Metformin and Acarbose along with Dry Yeast powder were purchased from local pharmacy. Materials for cell culture like DMEM (Dulbecco's Modified Eagle Medium), FBS (Fetal Bovine Serum) and Antibiotic solution (100x - Penicillin Streptomycin solution) were purchased from Hi - Media and finally the Glucose Oxidase Assay Kit (GAGO20) was purchased from Merck - Sigma Aldrich.

Collection and Identification of Plant Material

The various plant parts, such as roots, stems, leaves, flowers, and fruits, were taken from The Conservation Education Centre (CEC) of Bombay Natural History Society (BNHS), Goregaon, Mumbai which is situated abutting to biodiverse forest of Sanjay Gandhi National Park as seen in Figure 1. Plant parts were collected often from June to October in the years 2021 to 2023. Fresh specimens were compared to previously collected herbariums from various regions of the country for authentication at Blatter Herbarium, St. Xavier's College, Mumbai, Maharashtra. The specimen matches with the Blatter herbarium specimen No. NI - 979 of N. A. Irani. The plant's GPS location (19°09'47.4"N 72°53'33.3"E) was also recorded for future reference. Plant specimens were deposited and stored at Blatter Herbarium, St. Xavier's College, Mumbai, Maharashtra. (BLAT).

Preparation of Extract

We were not able to collect the required amount of roots, flowers and fruits in order to proceed with pharmacological work, so we decided to work with the stem and leaf material. A total of 50 gms of ground-up stem and leaves plant were extracted one after the other using solvents that progressively increased in polarity i.e ethanol and water. Plant stem and leaf material extraction was carried out using a Soxhlet equipment for 48 hours at 31 degrees Celsius. To get a concentrated extract and lower the volume to 50 millilitres, the solvent was eliminated using a rotary evaporator unit. Using Whatman No. 1 filter paper, extracts were filtered. The pre-weighed screw-capped bottle containing the concentrated extract was refrigerated at 40°C. The extracts were carefully labelled as CDLAE (*Cryptolepis dubia* leaf aqueous extract), CDLEE (*Cryptolepis*

dubia leaf ethanolic extract), CDSAE (*Cryptolepis dubia* stem aqueous extract) and CDSEE (*Cryptolepis dubia* stem ethanolic extract).

Alpha Amylase Inhibitory Assay

Pheiffer *et al.* (2018) found that α -amylase had inhibitory action. To summarize, 20 μ L of diluted extract samples (100-1000 μ g/mL) and duplicate 5 μ L aliquots of 10 mg α -amylase in 100 mL phosphate buffer were reacted in separate wells of a 96-well plate and incubated at 37°C for 10 minutes. There were duplicate control wells that used buffer instead of the enzyme. After adding 20 μ L of a 2% starch solution to each reaction well, incubate for 30 minutes at 37 °C before adding 75 μ L of iodine reagent (Odeyemi and Dewar 2019). Separate wells containing acarbose were used instead of extracts. Each well's absorbance at 580 nm was measured with an ELISA plate reader (Spectra Max ID3). The % inhibition was then calculated using the equation below:

$$\% \text{ inhibition} = ([A - B]/A) \times 100,$$

where A represents the absorbance of the control reaction mixture (containing buffer instead of the enzyme), and B represents the absorbance of the enzyme-containing reaction mixture.

Alpha Glucosidase Assay

The α -glucosidase enzyme was suppressed according to (Sun *et al.* 2014). To begin, duplicate aliquots of extract fractions (100-1000 μ g/mL), α -glucosidase enzyme (30 μ L, 50 μ g/mL), and potassium phosphate buffer (80 μ L, 70 mM, pH 6.8) were added to 96-well plates. The wells were then incubated for 5 minutes at 37 degrees Celsius. There were controls with buffer instead of enzyme and no substrate. After that, each well received a 20 μ L aliquot of 10 mM PNPG and was incubated for 20 minutes at 37°C. A 100 μ L solution of 100 mM Na₂CO₃ (Sigma, Johannesburg, South Africa) was added to each well, and the absorbance at 405 nm was measured using an ELISA plate reader (Spectra Max ID3) (Odeyemi and Dewar 2019). Separate wells containing acarbose, rather than extracts, were also created and used as standards. The % inhibition was then calculated using the equation below:

$$\% \text{ inhibition} = ([A - B]/A) \times 100,$$

where A represents the absorbance of the control reaction mixture (containing buffer instead of the enzyme), and B represents the absorbance of the enzyme-containing reaction mixture.

Glucose Uptake in Yeast Cells

To create clear supernatant fluids, a 15% (v/v) suspension of commercial baker's yeast was prepared in distilled

water following centrifugation (5500 rpm, 5 min). Mix one milliliter of glucose solution (5, 10, and 25 mM) with different concentrations of plant extracts (100 - 1000 μ g/mL) and incubate for 10 minutes at 37°C. To start the process, 100 μ L of yeast suspension was added. The mixture was vortexed and incubated at 37°C for an additional 60 minutes. After 60 minutes, the tubes were centrifuged at 4000 rpm for 5 minutes to measure the amount of glucose in the supernatant (Cirillo, 1962). Metronidazole was the prescription drug. The % inhibition was then calculated using the equation below:

$$\% \text{ inhibition} = ([A - B]/A) \times 100,$$

where A represents the absorbance of the control reaction mixture (containing buffer instead of the enzyme), and B represents the absorbance of the enzyme-containing reaction mixture.

Cell culture

The National Centre for Cell Sciences (NCCS), located in Pune, India, provided the HepG2 cell line. The cell line as seen in Figure 2 was cultured in Dulbecco's Modified Eagle Medium (DMEM), which was enhanced with 10% foetal bovine serum (FBS), 100 U/ml pen-strep antibiotic solution, and 2 mM glutamine. T25 cm² cell culture flasks with a 5% CO₂ environment were used to cultivate the cells at 37°C. HepG2 cells were cultivated then incubated for 48 hours. The cells at 90 - 100% confluency were split and then seeded 5000 cells/mL in 96 well plates.

Cell viability Assay

The MTT test was employed to assess the alterations in cell viability when various plant extract fractions were present. In 96-well plates, 5000 cells/mL of the cells were planted. Subsequently, varying quantities (100 -1000 μ g/mL) of the samples were added, and the cells were cultured for an additional 24 hours. Following treatment, the plates were incubated for 24 and 48 hours so that the MTT test could be used to undertake cytotoxic analysis. Yellow tetrazole 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was produced at a concentration of 5 mg/ml. Ten millilitres of MTT were then added to each well, and the mixture was allowed to incubate for four hours. Following the formation of purple formazone crystals, 100 litres of dimethyl sulfoxide (DMSO) were used to dissolve them. These crystals were detected in a multiwell ELISA plate reader (Spectra max ID3) at 570 nm. The percentage of viability was applied to the optical density measurement (Ashokkumar *et al.* 2014).

Glucose Uptake in HepG2 cells

HepG2 cells were cultivated in culture plates at a density of 5000 cells/mL and allowed to adhere and grow for 24 hours

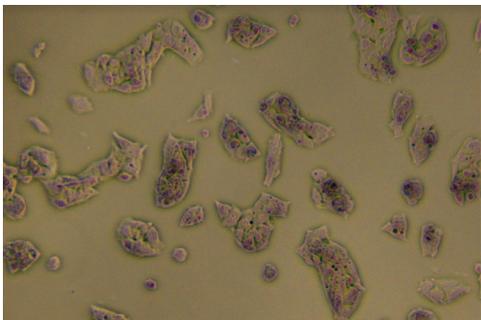


Figure 2: Culture of HepG2 cells growing in T25 flask (Picture taken at 60% confluency)

at 37°C in an incubator with 5% CO₂ added for humidity. Prior to the experiment, cells were preincubated plant extracts at varying doses (10 µL, 100 – 1000 µg/mL) for 48 hours at 37°C. After that, the wasted culture medium was aspirated and replaced with 25 µL of the incubation medium (DMEM), which was then incubated for three hours at 37 °C after being diluted with 10 mM glucose, 0.1% bovine serum albumin (BSA), and phosphate-buffered saline (PBS). Subsequently, 10 microliters of the incubation media were extracted from each well and placed into a fresh 96-well plate. Following the manufacturer’s instructions, the glucose assay kit (Sigma GAGO20 – Product Code Number 1003591187) was used to measure the amount of glucose in the medium. Next, an ELISA plate reader (Spectra max ID3) was used to determine the absorbance at 540 nm. By deducting the cell-containing wells from the cell-free wells, the amount of glucose utilised by the cells was determined. The cell viability assay, which was previously mentioned, was also used to determine the cell viability. Metformin was utilised as the positive control, and untreated cells with just the incubation buffer and no test sample were employed as the negative control.

Statistical analysis

All of the following assays were performed in triplicate, and the O.D values were then calculated to determine the mean and standard deviation. The O.D mean ± SD was entered into the aforementioned formulas and the percent inhibition was calculated. The data was subsequently analysed using one-way ANOVA. The statistical significance level was set at 5% (p < 0.05) based on the previous literature review of plant that suggests its medicinal potential in multiple ethnobotanical papers. Except for the glucose uptake assay in HepG2 cells assay, where p values were set at the 1% level of significance (p < 0.01). Graphs were made using Microsoft Excel 2016.

Results And Discussion

Alpha amylase assay and Alpha Glucosidase Assay

A diet high in carbohydrates leads to a sudden spike in blood glucose levels because the complex carbohydrates

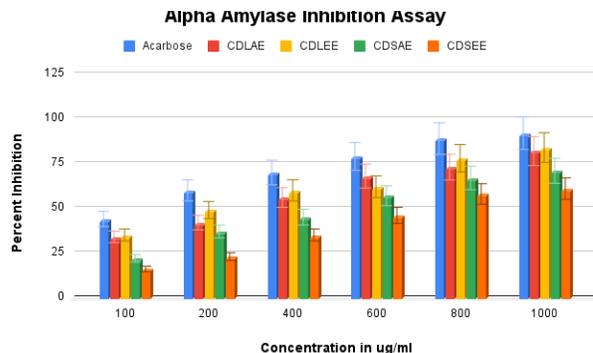


Figure 3 : Inhibitory effects of standard Acarbose and various plant extracts on alpha amylase activity

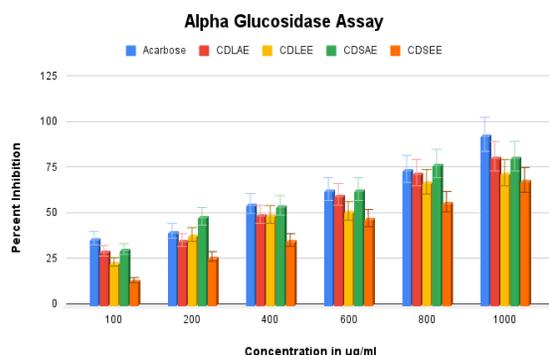


Figure 4 : Inhibitory effects of standard Acarbose and various plant extracts on alpha glucosidase activity

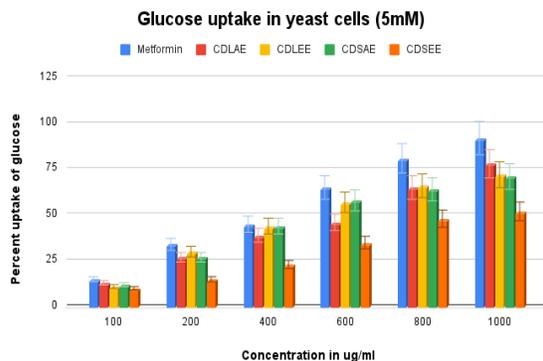


Figure 5: Percent uptake of 5 mM of glucose uptake in yeast cells

in food are quickly broken down and absorbed in the intestine by the enzymes α-amylase and α-glucosidase, which convert the carbohydrates into simple sugars that can be absorbed (monosaccharides) as shown in Figures 3 and 4. α-amylase and α-glucosidase, two enzymes that hydrolyze saccharides, have proven to be effective oral hypoglycemic medications for managing hyperglycemia,

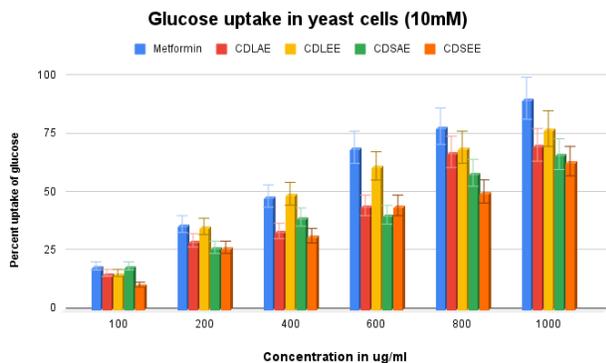


Figure 6: Percent uptake of 10 mM of glucose uptake in yeast cells

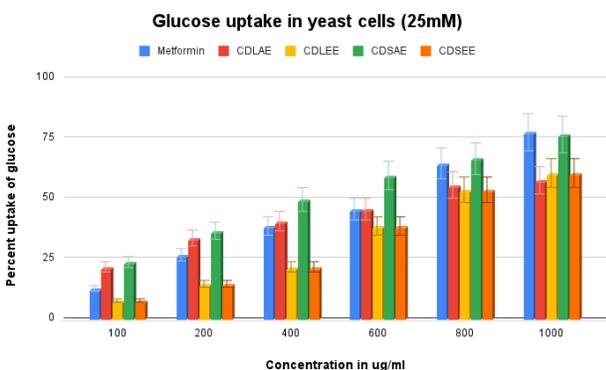


Figure 7: Percent uptake of 25 mM of glucose uptake in yeast cells

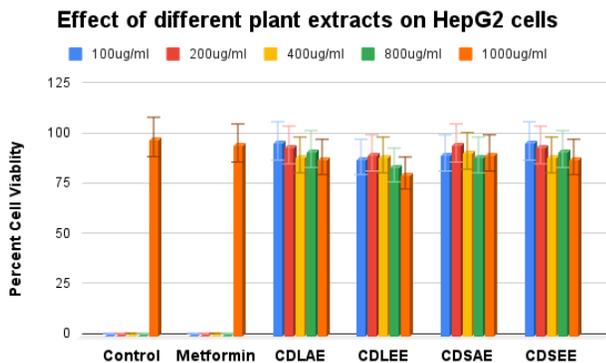


Figure 8: Cytotoxicity of aqueous and ethanolic extract of *C. dubia* leaf and stem at different concentrations on HepG2 cells

particularly in individuals with type II diabetes mellitus. These inhibitors reduce the rate of glucose absorption and hence the postprandial plasma glucose rise by delaying and lengthening the overall duration of carbohydrate digestion. In addition to traditional antidiabetic medications, synthetic hypoglycemia medications such as acarbose, miglitol, and voglibose are prescribed; nevertheless, these inhibitors have been linked to gastrointestinal adverse effects, including diarrhoea, flatulence, and discomfort in the abdomen. This

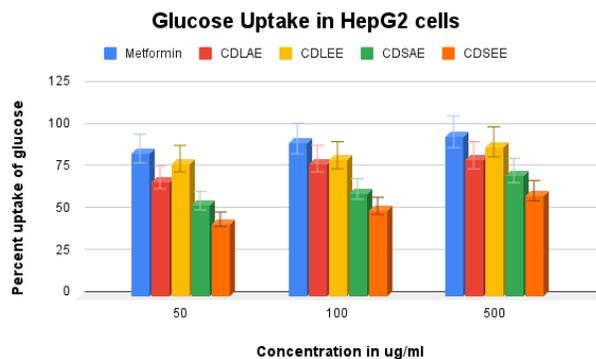


Figure 9: Effects of aqueous and ethanolic extract of *C.dubia* stem and leaf at different concentrations on glucose uptake in HepG2 cells. Metformin was utilized as positive control; level of significance for this assay is set at $p < 0.01$

has led to an increasing interest in finding novel, potent α amylase and α -glucosidase inhibitors from plants that have negligible or no negative effects (Kwon *et al.* 2006, Gin and Rigalleau 2000, Bhandari *et al.* 2008, Ahmed and Kumar 2016). Extracts of *Cryptolepis dubia* effectively inhibit alpha amylase and α -glucosidase enzymes. At doses of 100, 200, 400, 600, 800, and 1000 μ g/ml, the extracts reduced the activity of alpha amylase and alpha glucosidase, as measured by percentage inhibition assays. In the alpha-amylase assay, CDLEE demonstrated significantly greater inhibitory activity than standard drug Acarbose, achieving 83.20% inhibition ($p = 0.01$). CDLAE also exhibited significant inhibition compared to Acarbose, with 81.34% inhibition ($p = 0.03$). Similarly, CDLAE and CDSAE significantly inhibited alpha-glucosidase enzyme activity compared to Acarbose, showing 81.50% and 81.04% inhibition, respectively ($p = 0.02$ and $p = 0.04$), as above values are less than set p value of 0.05, this shows that results were significant ; moreover ethanolic extracts performed poorly in comparison to aqueous extracts. In the alpha amylase and alpha glucosidase assays, the IC50 values of Acarbose were 116.27 μ g/ml and 138.88 μ g/ml, respectively. In the alpha amylase assay, CDLEE had a superior IC50 value of 147.05 μ g/ml than acarbose, but in the alpha glucosidase assay, CDSAE had a higher IC50 value of 166.67 μ g/ml. Leaf and stem extracts had significant anti-diabetic efficacy, possibly due to inhibition of intestinal α -amylase and α -glucosidase.

Glucose Uptake in Yeast Assay

The process by which glucose is transported across the membrane of yeast cells has drawn interest as an *in vitro* technique for determining the hypoglycemic impact of different substances and therapeutic herbs. An indicator of the amount of glucose taken up by the yeast cells is the amount of glucose that is still in the medium after a given amount of time. According to recent research on the transport of glycosides and non-metabolizable sugars, stereospecific membrane carriers are thought to be involved

in the transport of sugars across yeast cell membranes. Yeast cells (*Saccharomyces cerevisiae*) are known to have highly sophisticated glucose transport, and it is generally accepted that glucose is transported in yeast by a facilitated diffusion process (Pitchaipillai and Ponniah 2016). Figures 5, 6, and 7 depict the rate at which glucose is transferred over the cell membrane in the yeast cell system. The yeast cells demonstrated dose-dependent glucose absorption. The best results were seen when treated with the CDLEE. The proportion of glucose uptake by the yeast cells rose with glucose concentrations (5, 10, and 25 mM). The CDLAE and CDLEE samples showed superior activity at 5mM and 10mM glucose concentrations, indicating 70.32% - 77.54% glucose absorption compared to the standard drug Metformin ($p = 0.04$ and $p = 0.03$, respectively). At 25 mM glucose, the IC₅₀ value of Acarbose was determined to be 217.33 ug/ml, while CDSAE was determined to be 249.08 ug/ml. Overall, we conclude that both the leaf and the stem of a plant have a high capacity to absorb glucose from the environment. This can be related to the fact that if a plant is used as an antidiabetic treatment, it may boost the body's GLUT transporters' ability to absorb glucose from blood tissue.

Glucose Uptake in HepG2 cells

Cell viability was assessed at various sample concentrations (100-1000 µg/mL). The cell viability was initially assessed to determine the cytotoxicity of all four plant extracts. This was used to determine whether the plant extracts were harmful to the cells, and it was also used to confirm the correct dosage for the glucose utilization assay in HepG2 cells. The MTT-based experiment revealed no harmful effects at concentrations ranging from 100 to 1000 ug/ml of plant extracts, with cell viability percentages ranging from 84.03% to 98.54%. The glucose uptake in HepG2 cells treated with the four plant extracts at varied doses (50, 100, and 500 µg/mL) is shown in Figure 9. CDLEE significantly increased glucose consumption in HepG2 cells ($p = 0.005$) at 500 µg/mL and 100 µg/mL compared to the standard drug Metformin. However, both CDLEE and CDLAE demonstrated a concentration-dependent increase in glucose absorption, which was substantial when compared to normal Metformin. CDLEE at 500 ug/ml showed 89.38% glucose utilization at 500 µg/mL compared to metformin (95.34%), the positive control. The toxicity assay demonstrated that all four plant extracts were non-toxic to HepG2 cells at all concentrations tested, as illustrated in Figure 8. Cell death was reported at a rate of less than 6%. The low proportion of cell death observed with plant extracts and metformin indicates a considerable reduction in glucose use in the cells.

Conclusion

The current study looked at the antidiabetic properties of *Cryptolepis dubia* extracts, specifically their ability to inhibit key enzymes involved in carbohydrate metabolism and their effect on glucose uptake in yeast and HepG2 cells. The

ethanolic leaf extract (CDLEE) had the best inhibitory action against alpha amylase, with an inhibition rate of 83.20% at a dosage of 1000 µg/mL. Similarly, the aqueous leaf extract (CDLAE) inhibited alpha amylase by 81.34% at the same quantity. Furthermore, both the aqueous leaf (CDLAE) and stem extracts (CDSAE) showed strong inhibitory efficacy against alpha glucosidase. CDLEE demonstrated a dose-dependent increase in glucose absorption in yeast cells, showing its potential to improve glucose utilization. CDLEE significantly enhanced glucose absorption in HepG2 cells at all tested doses, with the strongest impact seen at 250 µg/mL. The phytochemical examination of the extracts revealed the presence of bioactive components such as flavonoids, tannins, and saponins, which are most likely responsible for the observed antidiabetic benefits. Overall, the findings indicate that *Cryptolepis dubia* extracts, particularly the ethanolic leaf extract, have promise antidiabetic activities. These extracts effectively block key enzymes involved in carbohydrate digestion and boost glucose absorption in both yeast and liver cells, suggesting their potential as natural medicinal agents for treating diabetes. Furthermore, CDLEE's capacity to greatly increase glucose absorption at low concentrations implies a potent mode of action that should be further investigated in the creation of new antidiabetic medicines. The discovery of bioactive chemicals in these extracts offers up possibilities for future study to isolate, define, and understand the molecular mechanisms behind their actions. Furthermore, assessing the safety and efficacy of these extracts in in vivo models and clinical trials would be critical for verifying their therapeutic potential and prospective incorporation into diabetes care strategies. This study adds to the growing body of evidence that supports the use of traditional medicinal plants in modern healthcare, emphasizing the relevance of researching plant-based chemicals for novel medication discoveries.

Acknowledgments

The authors thank Mr. Alok Gude, Incharge Head Department of Botany, and Dr. Priya Sunderrajan, Professor, Department of Life Science and Biochemistry and Director of CAIUS Research Laboratory, St. Xavier's College Autonomous Mumbai, for providing the essential research facilities. We also thank Dr. Vishwas Sarangdhar, Consultant Biotechnologist and Co-Investigator CAIUS Research Laboratory, St. Xavier's College Autonomous Mumbai, and Dr. Manoj Kumar Bhat, DBT Distinguished Scientist, National Centre of Cell Science, Pune, for teaching and guiding us through the animal tissue culture assays and techniques.

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