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EVALUATION OF ANTI DIABETIC ACTIVITY OF CRATEVA RELIGIOSA BARK EXTRACT

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Abstract

Diabetes mellitus remains a significant global health challenge, with increasing prevalence and complex treatment demands, particularly for Type 2 diabetes. This study investigates the in vitro anti-diabetic potential of Crateva religiosa bark extract, a medicinal plant traditionally used in Ayurveda and Unani systems. Methanolic extract of the bark was evaluated for its α-amylase inhibitory activity and hydrogen peroxide (H_2O_2) scavenging capacity to elucidate mechanisms relevant to glycemic control and oxidative stress mitigation. Results demonstrated a dose-dependent inhibition of α-amylase, indicating effective suppression of starch hydrolysis and potential attenuation of postprandial hyperglycemia. Remarkably, the extract exhibited superior antioxidant activity, with a 95.38% H_2O_2 scavenging rate, outperforming standard antioxidants. These dual actions—enzyme inhibition and potent free radical neutralization-are likely attributable to the presence of bioactive phytochemicals such as flavonoids, tannins, alkaloids, and saponins. The findings underscore the therapeutic promise of C. religiosa as a multi-targeted natural agent for Type 2 diabetes management, addressing both metabolic dysregulation and oxidative stress, key contributors to disease progression. Future in vivo investigations and clinical evaluations are essential to validate efficacy, safety, and mechanistic pathways, paving the way for integration into complementary diabetes care paradigms.

Keywords: Crateva religiosa, Type 2 diabetes mellitus, α-Amylase inhibition, Antioxidant activity, Hydrogen peroxide scavenging.

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Introduction

Diabetes mellitus, including types 1 and 2, has heavily burdened public health over the past few decades due to its high mortality rate and challenging treatment regimen [1,2]. In 2021, prevalence of diabetes among adults aged 20–79 worldwide was 10.5%, affecting more than 536 million people. By 2045, the number is expected to increase to 12.2%, affecting 783 million people [3, 4].

Crateva religiosa, also known as the sacred garlic pear or temple plant belonging to family Capparidaceae, is native to tropical Asia and several South Pacific islands [5]. It is also grown in parts of the African continent for its fruit. *Crateva religiosa* is used in Ayurvedic medicine for its diuretic properties, which promote urine flow and assist in flushing out toxins. Compounds in the plant are thought to help dissolve kidney stones and reduce the recurrence of stone formation. Additionally, it can provide relief from symptoms like burning sensations and discomfort during urination. The plant has been used in Ayurveda and Unani medicine systems. Different parts of the plant, such as the leaves, stem bark, and root bark, are used to treat ailments like kidney stones, hypertension, malaria, diabetes, respiratory disorders, pain, and inflammation. *Crateva religiosa* contains alkaloids, glycosides, saponins, terpenoids, phenols, and volatile oils. Research indicates that *Crateva religiosa* exhibits analgesic, anti-inflammatory, antimicrobial, antioxidant, hepatoprotective, antifungal, and antiarthritic properties [6]. The primary aim of this study was to evaluate the anti-diabetic activity of *Crateva religiosa* bark extract and understand its potential mechanisms of action in managing diabetes mellitus, particularly Type 2 diabetes.

Materials and Methods

Extraction of *Crateva religiosa* bark

The bark from mature *C. religiosa* trees was collected. The powdered bark was soaked in methanol for 72 hours to extract the bioactive compounds. It was filtered to separate the liquid extract from the solid residue and concentrated to evaporate the solvent. The final extract was stored in a dark, airtight container to preserve its properties [7].

α-amylase enzyme inhibitory assay

A volume of 0.5 ml of α-amylase enzyme was mixed with 0.2 ml of *C. religiosa* bark extract at various concentrations (100, 200, 300, 400, 500 µg/ml). The final reaction volume was adjusted to 1 ml with phosphate buffer (pH 6.8), if needed. The reaction mixture was incubated at 37°C for 10 minutes. Then, 0.5 ml of soluble starch (1%) was added to the reaction mixture, and it was incubated for an additional 30 minutes. To stop the reaction, 1.0 ml of DNS reagent was added. The mixture was heated in a boiling water bath for 5 minutes and then cooled to room temperature. The absorbance was measured at 540 nm using a spectrophotometer [8].

$$Inhibition\ (\%) = \frac{\left(\begin{matrix}Absorbance\ of \\ sample\end{matrix} - \begin{matrix}Absorbance\ of \\ blank\end{matrix}\right)}{\begin{matrix}absorbance\ of \\ control\end{matrix}} \times 100 \quad \text{Equation 1}$$

Free Radical Scavenging Activity: Hydrogen Peroxide (H_2O_2) Scavenging Assay

This assay measures the ability of a sample to scavenge hydrogen peroxide, a reactive oxygen species (ROS) that can cause oxidative damage in biological systems. A 40 mM H_2O_2 solution was prepared in 50 mM phosphate buffer (pH 7.4) and stored in an amber bottle to minimize light-induced degradation. Test samples were dissolved in distilled water or methanol at various concentrations (e.g., 10, 20, 50, 100, 200 µg/mL). A standard antioxidant solution (Ascorbic acid or BHT) was also prepared. A volume of 0.6 mL of the H_2O_2 solution was mixed with 0.4 mL of the test sample or standard. For the control, 0.6 mL of H_2O_2 was mixed with 0.4 mL of buffer without the sample. The mixtures were incubated for 10 minutes at room temperature in the dark. The absorbance was measured at 230 nm using a UV-visible spectrophotometer [9]. The percentage inhibition of hydrogen peroxide is calculated using:

$$\text{Scavenging activity}(\%) = \left(\frac{A_{517}\ \text{of control} - A_{517}\ \text{of sample}}{A_{517}\ \text{of control}}\right) \times 100 \quad \text{Equation 2}$$

Results and Discussion

α-amylase enzyme inhibitory assay

The results are presented in Table 1 and demonstrate a clear dose-dependent inhibition of α-amylase activity by *C. religiosa* bark extract. The optical density (OD) at 540 nm decreased progressively with increasing extract concentration, from 0.643 at 100 µg/mL to 0.354 at 500 µg/mL, compared to 0.897 in the absence of inhibitor. This reduction indicates that the extract significantly inhibits α-amylase, an enzyme responsible for starch breakdown, thus potentially reducing postprandial hyperglycemia-a critical aspect in managing Type 2 diabetes.

Table 01. Alpha- Amylase inhibition activity of C. religiosa bark extract

TEST	ALPHA-AMYLASE ENZYME 0.5 ML	BUFFER pH 6.8 (ML)	0.2 ML INHIBITOR OF DIFFERENT CONCENTRATIONS (UG/ML)	STARCH 1% (ML)	DNS (ML)	OD 540 NM
Blank (no starch no t inhibitor)	0.5	1.0	-	-	1.0	0.00
Without inhibitor (add enzyme, buffer substrate, DNS, no extract)	0.5	1.0	-	1.0	1.0	0.897
With inhibitor (enzyme,	0.5	1.0	100	1.0	1.0	0.643

buffer, extract)	0.5	1.0	200	1.0	1.0	0.530
	0.5	1.0	300	1.0	1.0	0.477
	0.5	1.0	400	1.0	1.0	0.464
	0.5	1.0	500	1.0	1.0	0.354

*Presence of an inhibitor gives less absorbance (0.354); Absence of an inhibitor gives more absorbance (0.897)

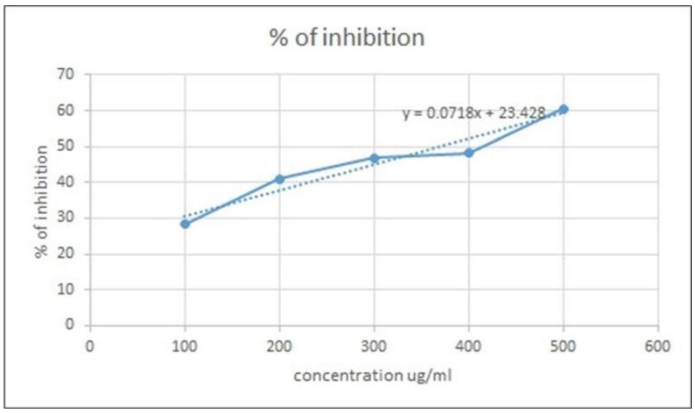

Fig.1. Percentage of Inhibition

Hydrogen Peroxide (H_2O_2) Scavenging Assay

Furthermore, the hydrogen peroxide (H_2O_2) scavenging assay revealed that the *C. religiosa* extract exhibited remarkable antioxidant activity, with a scavenging efficiency of 95.38%, substantially higher than that of the standard antioxidant (51.49%). This superior free radical quenching ability underscores the extract's capacity to mitigate oxidative stress, a major contributor to β-cell dysfunction and insulin resistance in diabetic pathology.

These findings support the antidiabetic potential of *C. religiosa* bark extract, attributed to both α-amylase inhibition and potent antioxidant activity. The presence of bioactive phytoconstituents such as flavonoids, tannins, and phenolic compounds may underlie these effects. These dual mechanisms—enzyme inhibition and oxidative stress attenuation—suggest a multi-targeted approach in diabetes management, making the extract a promising candidate for further in vivo evaluation and therapeutic development.

Conclusion

This study explores the anti-diabetic potential of *Crateva religiosa* bark extract, focusing on its phytochemical constituents and *in vitro* anti-diabetic activity. The study highlights the role of C. religiosa in traditional medicine and modern pharmacology, emphasizing its phytochemicals such as alkaloids, flavonoids, saponins, and tannins, which exhibit hypoglycemic, antioxidant, and anti-inflammatory properties.

The findings of this study demonstrate that *Crateva religiosa* bark extract exhibits significant in vitro α-amylase inhibitory activity and exceptional hydrogen peroxide scavenging capacity, indicating strong antidiabetic and antioxidant potential. The dose-dependent inhibition of α-amylase suggests that the extract may effectively modulate carbohydrate metabolism, thereby reducing postprandial glucose levels. Additionally, the extract's superior ability to neutralize reactive oxygen species reinforces its role in alleviating oxidative stress, a key factor in the progression of diabetes and its complications. These results highlight the therapeutic promise of *Crateva religiosa* as a natural, multi-targeted agent in the management of Type 2 diabetes mellitus. Further in vivo studies and clinical trials are warranted to validate its efficacy, safety, and pharmacological profile for integration into complementary diabetes care strategies.

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Conflicts of interest

The authors declare no conflicts of interest.

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Informed Consent and Ethical Considerations

Not Applicable

Author Contribution

All authors are contributed equally.

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