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Inhibitory Effect Of Rubus Ulmifolius Leaf Extract On Alpha-Amylase Activity: Phytochemical Profiling And In Vitro Evaluation For Diabetes Management

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Abstract
Diabetes mellitus is a chronic metabolic disorder with increasing global prevalence. Controlling postprandial hyperglycemia by inhibiting digestive enzymes such as alpha-amylase is a validated therapeutic approach. Natural inhibitors from plants are valued for their safety and multifunctional bioactivity. This study aimed to evaluate the alpha-amylase inhibitory potential of an aqueous extract from Rubus ulmifolius leaves, known in traditional medicine for antidiabetic properties. Extraction by successive hexane followed by methanol/acetone reflux yielded a rich polyphenolic profile including flavonoids and tannins. The extract inhibited alpha-amylase activity in vitro with an IC50 of 1.80 mg/mL. Comparative analysis with the standard inhibitor acarbose and a detailed phytochemical assay corroborate the potential of R. ulmifolius as a complementary antidiabetic agent. Further in vivo and mechanistic studies are recommended.

Keywords
Diabetes, Alpha-amylase inhibition, Rubus ulmifolius, Polyphenols, Flavonoids, Natural inhibitors, Traditional medicine

INTRODUCTION

Diabetes mellitus, characterized by chronic hyperglycemia due to insulin dysfunction, affects over 400 million people worldwide and remains a leading cause of morbidity and mortality **(World Health Organization [WHO], 2016)**. Elevated postprandial glucose contributes significantly to diabetes complications such as cardiovascular disease and neuropathy. Alpha-amylase, a critical enzyme for starch hydrolysis in the digestive tract, represents a key target for therapeutic intervention **(Lebovitz, 1997).**

Commercial alpha-amylase inhibitors like acarbose effectively delay carbohydrate digestion and glucose absorption but cause side effects including gastrointestinal discomfort **(Inzucchi, 2002; He, 1998).** This has incentivized the search for safer natural inhibitors derived from medicinal plants with traditionally recognized antidiabetic effects.

Rubus ulmifolius Schott, a wild blackberry native to Mediterranean regions, is used ethnomedicinally to treat diabetes and inflammatory conditions **(Ali et al., 2017; Tabarki et al., 2017).** Previous phytochemical studies reveal its leaves are rich in flavonoids, tannins, and phenolic acids, compounds associated with antioxidant and enzyme inhibitory activities **(Chaban et al., 2014; Martini et al., 2009)**. However, detailed assessments of its alpha-amylase inhibitory potential remain limited.

This study investigates the phytochemical composition and in vitro inhibitory activity of *R. ulmifolius* leaf extract on alpha-amylase to scientifically validate its traditional use and explore its potential as an adjunct natural therapy for glycemic control.

MATERIALS AND METHODS

Plant Material Collection and Authentication

Fresh *Rubus ulmifolius* leaves were collected from Tlemcen, Algeria, during spring 2023. Botanical identification was confirmed by experts at the Department of Botany, University of Abou Bekr Belkaid.

Leaves were washed, shade dried, and ground to a fine powder using a mechanical grinder, stored in airtight containers until extraction.

Extraction Procedure

Sequential extraction was performed to fractionate the bioactive compounds based on polarity, following protocols adapted from **Thalapaneni et al. (2008)**. Approximately 200 g of dried leaf powder was first refluxed with hexane (boiling point 69°C) for 6 hours to remove lipophilic substances. Post filtration, the marc was refluxed with a methanol/acetone mixture (1:1 v/v) for 8 hours to extract polar phenolic compounds. The combined extracts were concentrated under reduced pressure at 40°C using a rotary evaporator. Yield was calculated as the dry extract mass over initial plant mass × 100.

Phytochemical Characterization

Total Polyphenols

Determined by the Folin-Ciocalteu method **(Boizot & Charpentier, 2006).** Briefly, 0.5 mL of extract (1 mg/mL) was mixed with 2.5 mL Folin-Ciocalteu reagent (diluted 1:10), incubated for 5 min, then 2 mL of 7.5% sodium carbonate solution was added. After 30 min incubation at room temperature, absorbance at 765 nm was recorded. Gallic acid was used to generate the calibration curve (0–500 mg/L).

Flavonoid Content

Quantified by the aluminum chloride colorimetric assay **(Dewanto et al., 2002).** Extract aliquots (0.5 mL) were mixed with 0.1 mL of 10% aluminum chloride, 0.1 mL potassium acetate (1 M), and 4.3 mL of distilled water. After 30 min at room temperature, absorbance was measured at 415 nm. Catechin was used as the standard.

Condensed Tannins

Measured using the vanillin-HCl assay as described by **Sun et al. (1998).** Extract (0.5 mL) was treated with 3 mL of vanillin (4% in methanol) and 1.5 mL concentrated HCl, incubated for 15 min at room temperature. Absorbance was read at 500 nm. Catechin was the reference standard.

Alpha-Amylase Inhibition Assay

Porcine pancreatic alpha-amylase (EC 3.2.1.1) enzyme powder (Sigma-Aldrich, MW ~13 kDa, specific activity 13 U/mg) was dissolved in phosphate buffer (pH 6.9, 0.02 M, with 6 mM NaCl) to prepare a 3.9 U/mL enzyme solution **(Bernfeld, 1955; Thalapaneni et al., 2008).**

- Various extract concentrations (0.2, 0.4, 0.8, 1.2, 1.8 mg/mL) were preincubated with enzyme for 10 min at 37°C.
- Soluble starch (1% w/v in buffer) was added as substrate, and incubated again for 15 min.
- The release of reducing sugars was quantified by addition of 3,5-dinitrosalicylic acid (DNSA) reagent, followed by boiling for 5 min.
- Following cooling on ice, absorbance was measured at 540 nm.

Acarbose (Larimel® tablets) diluted in buffer served as positive control with known IC50 ~0.038 mg/mL**.** Negative controls contained enzyme and substrate without inhibitor. Percent inhibition was calculated as:

$$\%\text{Inhibition} = \left(1 - \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{control}} - A_{\text{blank}}}\right) \times 100$$

Each test was done in triplicate.

Statistical Analysis

Data were expressed as mean ± standard deviation from at least three independent experiments. IC50 values were calculated using nonlinear regression curve fitting with GraphPad Prism 9. Differences between means were assessed by ANOVA with significance set at $p < 0.05$.

RESULTS

Extraction Yield and Phytochemical Profile

Methanol/acetone extraction resulted in a dry yield of 26.74%. Quantitative phytochemical results are presented in Table 1.

Table 1: Quantitative phytochemical compound from Methanol/acetone extraction of *Rubus ulmifolius* leaves

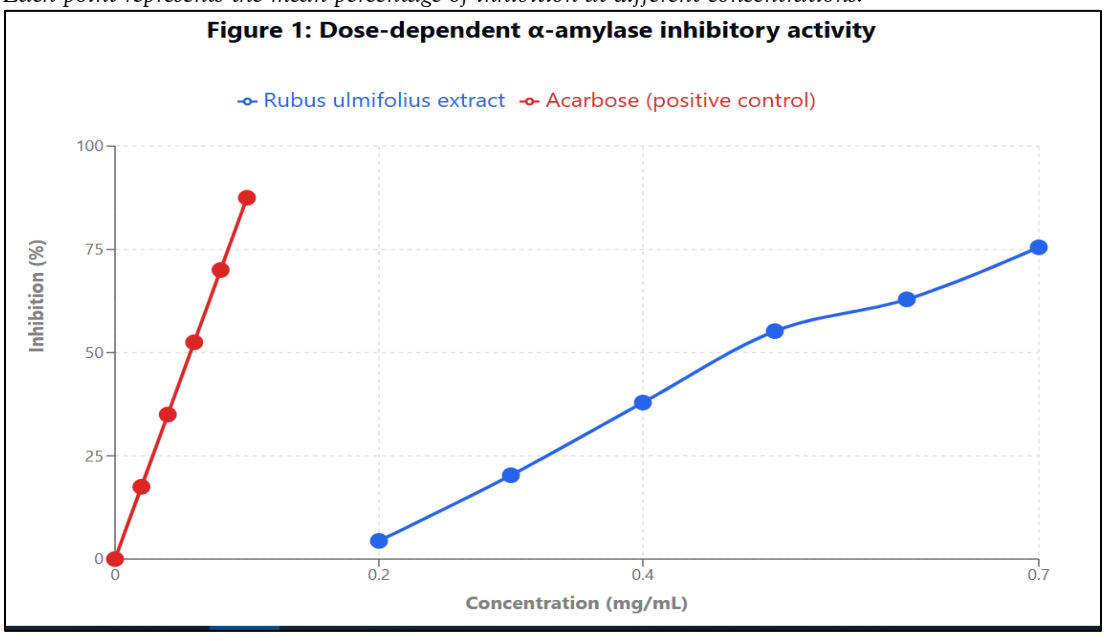
Parameter	Content (mg/g dry extract)	Standard Used
Total Polyphenols	59.11 ± 2.68	Gallic acid (GAE)
Flavonoids	19.04 ± 0.33	Catechin (CE)
Condensed Tannins	11.11 ± 0.32	Catechin (CE)

Values represent the mean ± standard error of the mean (SEM)

Alpha-Amylase Inhibitory Activity

The extract exhibited dose-dependent alpha-amylase inhibition **(Figure 1).** The calculated IC50 was 1.80 ± 0.07 mg/mL, significantly higher than acarbose's IC50 of 0.038 ± 0.003 mg/mL (Table 2), indicating moderate inhibitory potency.

Each point represents the mean percentage of inhibition at different concentrations.



Each point represents the mean percentage of inhibition at different concentrations.

Figure 1. Dose-dependent inhibition of α-amylase activity by *Rubus ulmifolius* extract and acarbose (positive control).

The extract showed an IC_{50} of 1.80 ± 0.07 mg/mL, while acarbose exhibited an IC_{50} of 0.056 ± 0.003 mg/mL, indicating moderate inhibitory potency of the plant extract.

Table 2: IC50 of the *Rubus ulmifolius* Extract

Sample	IC50 (mg/mL)
***Rubus ulmifolius* extract**	1.80 ± 0.07
Acarbose (positive control)	0.056 ± 0.003

Values represent the mean ± standard error of the mean (SEM)

Despite lower potency, the extract's inhibition is notable given its natural origin and complex phytochemical matrix.

DISCUSSION

The results show that Methanol/acetone extraction of *Rubus ulmifolius* leaves contains a significant amount of total polyphenols. These findings confirm a rich phenolic composition consistent with literature reports for *Rubus* species **(Martini et al., 2009; Tabarki et al., 2017).**

This study demonstrates that *Rubus ulmifolius* leaf extract effectively inhibits alpha-amylase activity in vitro, supporting its ethnomedicinal antidiabetic use. Inhibition of this key starch-digesting enzyme slows glucose release, thereby limiting postprandial blood sugar spikes, a critical aspect of type 2 diabetes management **(Akhtar et al., 2017).**

The high total polyphenol and flavonoid content plays a major role in this inhibitory mechanism. These compounds directly interact with alpha-amylase either by binding to its active site, preventing substrate attachment, or through allosteric modulation that changes the enzyme's conformation to reduce its activity **(Tundis et al., 2010).** Among these polyphenols, flavonoids such as quercetin have well-documented alpha-amylase inhibitory actions, via competitive or non-competitive binding depending on the study **(Chaban et al., 2014; Martini et al., 2009).**

Compared to acarbose, a clinically used alpha-amylase inhibitor, the extract shows moderate activity but potentially fewer side effects, especially fewer gastrointestinal disturbances. This can be explained by the extract's natural complex mixture, whose synergistic effects seem to attenuate adverse reactions **(Gray & Flatt, 1997).**

Moreover, these polyphenols provide significant antioxidant benefits. They reduce oxidative stress by scavenging free radicals, a process that worsens diabetic complications such as neuropathy and nephropathy **(Marles & Farnsworth, 1995).** Thus, *Rubus ulmifolius* leaf extract combines hypoglycemic and antioxidant effects, two complementary properties in diabetes management.

However, limitations must be noted. The crude extract used likely dilutes the concentrations of active compounds by the presence of other constituents. This heterogeneity can affect the reproducibility and potency of the observed effects. Therefore, isolation and characterization of the bioactive molecules responsible for enzymatic inhibition are necessary for clearer insight into their precise mode of action.

Further studies are also indispensable, including in vivo testing on diabetic animal models to confirm therapeutic efficacy and safety before moving to clinical trials. Recent research on related *Rubus* species highlights the pharmacological richness of these plants, justifying further investigation **(Ali et al., 2017; Sebnem Selen Isbilir & Yagar, 2016).**

CONCLUSION

The methanol/acetone leaf extract of *Rubus ulmifolius* contains substantial polyphenols, flavonoids, and tannins that confer moderate in vitro alpha-amylase inhibitory activity. These findings scientifically rationalize traditional uses against diabetes and suggest potential as a nutraceutical supplement targeting postprandial hyperglycemia. Further biochemical characterization and in vivo assessments are crucial next steps.

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