ORIGINAL ARTICLE

A C G publications

Rec. Agric. Food. Chem. 5:2 (2025) 170-179

records of agricultural and food chemistry

Investigation of *In Vitro* Anti-diabetic Activity and Phenolic Component Profile of Mulberry Leaf Tinctures

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(Received November 16, 2025; Revised December 17, 2025; Accepted December 18, 2025)

Abstract: This study aimed to evaluate the *in vitro* anti-diabetic potential and phenolic compound profile of leaf tinctures prepared from fresh and dried leaves of three mulberry species (*Morus alba* L., *Morus rubra* L., and *Morus nigra* L.) using different plant-to-solvent ratios, with the objective of identifying the most effective formulation for Type II diabetes. For this purpose, ethanol:water (70:30, v/v) tinctures were prepared at 1:4, 1:5, 1:6, 1:7, and 1:8 (w/v) ratios, and their inhibitory activities against α-amylase and α-glucosidase enzymes were determined by a spectrophotometric method. Among all tested samples, the dried *M. rubra* L. leaf tincture prepared at a 1:6 ratio (TMrD 1:6) exhibited the strongest inhibitory activity, with IC_{50} values of 24.11 µg/mL for α-amylase and 40.18 µg/mL for α-glucosidase. The phenolic compound profile of the most active tinctures was further investigated using high-performance liquid chromatography coupled with diode-array detection (HPLC-DAD) through the screening of 42 phenolic compounds. Protocatechuic acid, *p*-hydroxybenzoic acid, caffeic acid, and chlorogenic acid were quantitatively detected, whereas *p*-coumaric acid, ferulic acid, coumarin, and *trans*-cinnamic acid were present only in trace amounts. Overall, the dried *M. rubra* L. leaf tincture (TMrD 1:6) demonstrated promising potential as a phytopharmaceutical candidate for diabetes management.

Keywords: *Morus alba* L.; *Morus rubra* L.; *Morus nigra* L.; chemical profile; anti-diabetic activity; tincture.

1. Introduction

Diabetes mellitus is a chronic metabolic disease that occurs when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces [1]. Insulin is a hormone that regulates blood glucose levels and helps cells use glucose for energy. In people with diabetes, this process is impaired, and as a result, blood sugar levels remain high [2]. Diabetes is classified into two main types: Type I diabetes is an autoimmune disease in which the immune system attacks the insulin-producing cells in the pancreas, whereas Type II diabetes is usually associated with lifestyle factors, obesity, and genetic predisposition and is characterized by insulin resistance [3].

The prevalence of diabetes mellitus worldwide has reached alarming levels. According to the International Diabetes Federation (IDF), approximately 537 million adults were living with diabetes mellitus in 2021, and this number is projected to increase to 643 million by 2030 and 783 million by 2045. Beyond its impact on individual quality of life, diabetes also imposes a substantial economic

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The article was published by ACG Publications
https://www.acgpubs.org/journal/records-of-agricultural-and-food-chemistry July-December 2025 EISSN:2792-0763
DOI: http://doi.org/10.25135/rfac.41.2511.3728
Available online: December 25, 2025

burden on global health systems. Global health expenditures for the treatment and prevention of diabetes were estimated to be approximately US$966 billion in 2021 and are projected to exceed US$1 trillion by 2030 [4]. If diabetes remains untreated or poorly controlled, severe complications such as cardiovascular diseases, kidney failure, blindness, and lower extremity amputations may occur [5]. The limitations and long-term side effects associated with current anti-diabetic drugs underscore the need for the development of alternative and effective therapeutic strategies.

Morus alba L. (white mulberry), *Morus rubra* L. (red mulberry), and *Morus nigra* L. (black mulberry) are mulberry species that have long been used in traditional medicine. Various parts of these plants, including their leaves, fruits, and roots, have been reported to exhibit diverse biological activities. In particular, numerous studies have demonstrated the anticancer [6-8], antiproliferative [9-11], antioxidant [8,11-13], anti-inflammatory [13,14], antimicrobial [15,16], and anti-diabetic [8,17] properties of mulberry leaves. These findings highlight the potential of mulberry species as natural sources for managing diabetes. Moreover, the absence of significant toxicity reported in studies on mulberry leaf extracts supports their potential use as dietary components or supplements for human consumption [18] and for diabetes management [19]. Phenolic compounds [17,20,21] and flavonoids [22,23] present in Morus leaves have attracted particular attention due to their regulatory effects on glucose metabolism and their potential role in diabetes management [24].

Tinctures are liquid herbal preparations obtained by extracting bioactive plant constituents using alcohol or hydroalcoholic solvents [25]. Compared to other extraction forms, tinctures may offer higher extraction efficiency and enhanced bioavailability of active compounds. Consequently, the use of herbal tinctures has been increasingly considered a promising approach to integrate traditional herbal medicine with modern therapeutic strategies, including diabetes management [26]. In parallel, concerns regarding the side effects and long-term safety of synthetic antidiabetic drugs have driven growing interest in natural products and plant-derived compounds as alternative therapeutic agents [27]. Secondary metabolites, particularly phenolic compounds, are recognized as key contributors to the anti-diabetic effects of many medicinal plants [28].

Although numerous studies have investigated the anti-diabetic potential of *Morus* species using various extraction approaches, comparative evaluations using standardized tincture preparation ratios remain limited. Furthermore, the combined assessment of fresh and dried leaves, together with the systematic optimization of plant-to-solvent ratios and the simultaneous evaluation of enzyme inhibitory activity and phenolic compound profiles within a single study, has not been sufficiently addressed in previous research. Therefore, the present study aims to provide a comprehensive and comparative investigation of mulberry leaf tinctures by examining the influence of extraction parameters on *in vitro* anti-diabetic activity and phenolic composition, thereby contributing to a better understanding of their potential role in diabetes management.

2. Materials and Methods

2.1. Chemicals

Chemicals were obtained from E. Merck (Darmstadt, Germany) and Sigma-Aldrich (Sigma-Aldrich GmbH, Steinheim, Germany). Throughout the study, analytical grade chemicals such as starch, NaOCl, NaCl, HCl, Na_2CO_3, Lugol, *p*-nitrophenyl-*α*-D-glucopyranoside, *α*-amylase, *α*-glucosidase, methanol, ethanol and phenolic standards (fumaric acid, gallic acid, protocatechuic acid, theobromine, theophylline, catechin, *p*-hydroxybenzoic acid, 6,7-dihydroxycoumarine, methyl-1,4-benzoquinone, vanillic acid, caffeic acid, vanillin, chlorogenic acid, *p*-coumaric acid, ferulic acid, cynarin, coumarin, propyl gallate, routine, *trans*-2-hydroxycinnamic acid, ellagic acid, myricetin, fisetin, quercetin, *trans*-cinnamic acid, luteolin, rosmarinic acid, kaempferol, apigenin, chrysin, 4-hydroxy resorcinol, 1,4-dichlorobenzene, pyrocatechol, 4-hydroxybenzaldehyde, epicatechin, 2,4-dihydroxybenzaldehyde, hesperidin, oleuropein, naringenin, hesperetin, genistein, curcumin) were used.

2.2. Plant Material Collection and Dried

M. alba L., *M. rubra* L., and *M. nigra* L. leaves were personally harvested by the author from the Menteşe region of Muğla between May and June (ripening period) in 2024. The collected leaves were placed in sterile baskets and transported to the laboratory. Upon arrival at the laboratory, the leaves were

manually sorted to remove damaged leaves, foreign materials, and debris, and then washed thoroughly with distilled water to eliminate surface contaminants. After washing, excess surface water was removed, and the leaves were air-dried prior to further processing. For each species, the leaves were divided into two groups. The leaves in the first group were processed as fresh material and ground separately using a shredder (Waring Commercial Blender, 8010E, 38BL40, USA). The leaves in the second group were dried under cool and shaded conditions at a controlled room temperature (28 °C) and subsequently ground using the same shredder (Waring Commercial Blender, 8010E, 38BL40, USA). As a result, a total of six ground samples (three fresh and three dried) were obtained from mulberry leaves of different species.

2.3. Preparation of Tinctures

Grinded samples of fresh and dried *M. alba* L., *M. rubra* L., and *M. nigra* L. leaves were weighed separately as 5.0 g and transferred into amber glass bottles. For tincture preparation, ethanol and deionized water (70:30, v/v) were added to obtain plant-to-solvent ratios of 1:4, 1:5, 1:6, 1:7, and 1:8 (w/v), yielding a total of 30 tinctures. The mixtures were macerated at room temperature for 14 days with periodic manual shaking to facilitate the extraction of bioactive compounds. After maceration, the mixtures were filtered through Whatman No. 1 filter paper to remove plant residues. The ethanol fraction of the tinctures was evaporated using a rotary evaporator (Heizbad-Hei-VAP ML, Heidolph, Germany), and the remaining aqueous phase was removed by lyophilization (Alpha 1-4 LD Plus, Christ, Germany). The dry extracts obtained were stored in amber glass bottles at 4 °C, protected from heat and light.

The extraction yield of each tincture was calculated using the following equation:

$$Yield\ \% = \frac{dry\ tincture\ amount\ (g)}{fresh\ mulberry\ leaves\ quantity\ (g)} x100$$

The yields of fresh and dried leaf tinctures from *M. alba*, *M. rubra*, and *M. nigra* are summarized in the Supporting Information (Table S1), providing a comparative overview of extraction efficiency across species and leaf types.

Following solvent removal and lyophilization, the dry weight of each tincture was recorded, and the extraction yield (%) was calculated based on the initial plant material weight. Yield values were determined for both fresh and dried leaf tinctures of *M. alba* L., *M. nigra* L., and *M. rubra* L. prepared at different plant-to-solvent ratios (1:4-1:8, w/v). The detailed yield data, including sample codes, solvent volumes, dry extract weights, and calculated yields, are presented in the Supporting Information (Table S1) to allow a methodological comparison of extraction efficiency across species, leaf conditions, and solvent ratios.

2.4. Anti-diabetic Activity

2.4.1. Determination of α-Amylase Inhibitory Activity of Tinctures

α-Amylase inhibitory activity of the tinctures was tested using a spectroscopic method with slight modifications [29]. Briefly, 25 µL sample solution in different concentrations and 50 µL *α*-amylase solution (0.1 U/mL) in phosphate buffer (20 mM pH=6.9 phosphate buffer prepared with 6 mM NaCl) were mixed in a 96-well microplate. The mixture was pre-incubated for 10 min at 37 °C. After pre-incubation, 50 µL starch solution (0.05 %) was added and incubated for more than 10 min at 37 °C. The reaction was stopped by the addition of 25 µL HCl (0.1 M), and then 100 µL Lugol's solutions were added for monitoring. A 96-well microplate reader was used to measure absorbance at 565 nm.

2.4.2. Determination of α-Glucosidase Inhibitory Activity of Tinctures

α-Glucosidase inhibitory activity of tinctures was determined using the spectroscopic method with slight modifications [30,31]. Briefly, 50 µL phosphate buffer (10 mM pH=6.9), 25 µL *p*-nitrophenyl-*α*-D-glucopyranoside in phosphate buffer (10 mM pH=6.9), 10 µL sample solution, and 25 µL *α*-glucosidase (0.1 U/mL) in phosphate buffer (10 mM pH=6.0) were mixed in a 96-well microplate. After 20 min incubation at 37 °C, 90 µL Na_2CO_3 (100 mM) was added into each well to stop the enzymatic reaction. Absorbance of the 96-well microplate reader was recorded at 400 nm.

2.5. Determination of Phenolic Profiles by HPLC-DAD

2.5.1. Preparation Samples for HPLC-DAD Analysis

50 mg of the extract was dissolved in 1.0 mL of methanol:water (50:50, v/v), homogenized in an ultrasonic bath at 20 °C for 5 min, and filtered through a 0.20 µm PTFE syringe filter (25 mm diameter; Chromafil Xtra, Macherey-Nagel, Düren, Germany). All HPLC sample solutions were prepared at the same final concentration (8 mg/mL), which corresponds to the injected solution for analysis.

2.5.2. HPLC-DAD Analyses

The phenolic profiles of *M. alba* L.*, M. rubra* L.*, and M. nigra* L. leaf tinctures were screened with a modification of the method described by Tokul-Ölmez et al. [32]. In this study, hydroalcoholic tinctures were investigated against 42 standard compounds (fumaric acid, gallic acid, protocatechuic acid, theobromine, theophylline, catechin, *p*-hydroxy benzoic acid, 6,7-dihydroxycoumarine, methyl-1,4-benzoquinone, vanillic acid, caffeic acid, vanillin, chlorogenic acid, *p*-coumaric acid, ferulic acid, cynarin, coumarin, propyl gallate, rutin, *trans*-2-hydroxycinnamic acid, ellagic acid, myricetin, fisetin, quercetin, *trans*-cinnamic acid, luteolin, rosmarinic acid, kaempferol, apigenin, chrysin, *p*-hydroxy resorcinol, 1,4-dichlorobenzene, pyrocatechol, *p*-hydroxybenzaldehyde, epicatechin, 2,4-dihydroxy-benzaldehyde, hesperidin, oleuropein, naringenin, hesperetin, genistein, curcumin) using a High-Performance Liquid Chromatography (Shimadzu Cooperation, Japan) system that consists of a Shimadzu model LC-20AT. The column temperature was set at 35 ºC. The chromatographic separation was performed on a C_{18} (5 µm, 4.6 mm x 250 mm) reverse phase column and an Inertsil C_{18} guard column [32].

2.6. Statistical Analysis

Biological activities data were taken for four different concentrations of each synthesis sample. The results of the biological activity analyses are presented as IC_{50} values. Data were recorded as mean ± SEM (standard error of the mean) $p<0.05$. Measurements were obtained in triplicate.

3. Results and Discussion

3.1. Anti-diabetic Activity

The anti-diabetic potential of *M. alba* L. (TMa), *M. rubra* L. (TMr), and *M. nigra* L. (TMn) leaf tinctures was comprehensively evaluated through *in vitro* inhibition assays against *α*-amylase and *α*-glucosidase enzymes. The experimental results are summarized in Table 1. Among the three mulberry species, fresh *M. alba* tincture, dried *M. nigra*, and fresh *M. rubra* tinctures demonstrated comparatively higher inhibitory activities. Notably, the *M. rubra* species exhibited the most pronounced enzymatic inhibition, with the dried form (TMrD) displaying the lowest IC_{50} values for both enzymes, indicating its superior anti-diabetic potential.

Specifically, TMrD 1:6 exhibited IC_{50} values of 24.11 µg/mL for *α*-amylase and 40.18 µg/mL for *α*-glucosidase. Compared with the standard anti-diabetic drug acarbose, for which we measured IC_{50} values of 52.46 µg/mL for *α*-amylase and 114.60 µg/mL for *α*-glucosidase under the same experimental conditions, TMrD 1:6 exhibited markedly stronger inhibitory effects. This comparison underscores the remarkable potency of this mulberry leaf tincture in inhibiting key carbohydrate-hydrolyzing enzymes, thereby highlighting its potential as a natural anti-diabetic agent.

Evaluation of alcohol content revealed that tinctures prepared with a 1:6 plant-to-alcohol ratio consistently achieved the most effective enzymatic inhibition. Conversely, tinctures with lower alcohol content (1:4) exhibited higher IC_{50} values, reflecting reduced inhibitory activity, whereas alcohol ratios above 1:6 were associated with higher IC_{50} values. These observations suggest that the optimal solvent composition is critical for extracting bioactive compounds responsible for *α*-amylase and *α*-glucosidase inhibition. The enhanced inhibitory activity observed at the 1:6 ratio may be attributed to a more efficient solubilization and balanced extraction of both phenolic and non-phenolic bioactive constituents. In contrast, lower or higher solvent proportions may result in incomplete extraction or excessive dilution of active components.

Table 1. Anti-diabetic (*α*-amylase and *α*-glucosidase) activity of *M. alba* L. (TMa), *M. rubra* L. (TMr), and *M. nigra* L. (TMn) leaf tinctures [a]

Sample Code	Ratio	*α*-Amylase inhibitory assay IC_{50} (µg/mL)	*α*-Glucosidase inhibitory assay IC_{50} (µg/mL)
TMaF	1:4	86.84±0.18	131.4±0.60
TMaF	1:5	78.89±0.55	100.3±0.87
TMaF	1:6	70.12±0.76	86.93±0.32
TMaF	1:7	72.76±1.12	135.7±0.44
TMaF	1:8	79.13±0.88	174.4±0.75
TMnF	1:4	108.3±0.61	120.6±0.96
TMnF	1:5	96.95±0.33	104.1±0.24
TMnF	1:6	80.18±0.85	72.16±0.36
TMnF	1:7	84.02±0.47	229.3±0.12
TMnF	1:8	105.2±1.02	217.7±1.26
TMrF	1:4	82.95±0.20	256.2±2.20
TMrF	1:5	79.28±0.31	238.8±0.88
TMrF	1:6	72.71±0.69	80.71±0.30
TMrF	1:7	74.16±1.23	195.4±0.57
TMrF	1:8	76.97±0.84	328.0±0.26
TMaD	1:4	91.25±0.60	85.35±0.11
TMaD	1:5	80.11±1.44	73.72±0.80
TMaD	1:6	68.41±0.38	59.58±0.64
TMaD	1:7	84.29±0.25	158.0±0.72
TMaD	1:8	97.67±0.14	205.1±0.59
TMnD	1:4	88.08±0.80	181.9±0.77
TMnD	1:5	82.17±0.66	173.8±0.61
TMnD	1:6	80.12±0.17	101.4±0.54
TMnD	1:7	86.01±1.35	154.3±0.19
TMnD	1:8	90.25±0.88	199.4±0.25
TMrD	1:4	105.2±0.93	90.22±0.56
TMrD	1:5	63.66±1.22	60.75±0.49
TMrD	1:6	24.11±0.10	40.18±0.38
TMrD	1:7	88.55±0.48	96.47±0.89
TMrD	1:8	124.2±0.75	109.7±0.15
Acarbose*		52.46±0.55	114.6±0.81

[a] Values are expressed as mean ± SEM (n = 3). Statistical analysis was performed using a one-way ANOVA test. Differences were considered statistically significant at $p<0.05$. Different superscript letters within the same column indicate significant differences among samples. **TMaF**: Fresh leaf tincture of *M. alba* L., **TMnF**: Fresh leaf tincture of *M. nigra* L., **TMrF**: Fresh leaf tincture of *M. rubra* L., **TMaD**: Dried leaf tincture of *M. alba L*., **TMnD**: Dried leaf tincture of *M. nigra* L., **TMrD**: Dried leaf tincture of *M. rubra* L.; 1:4, 1:5, 1:6, 1:7 and 1:8 : Ground mulberry leaf: solvent ratio (w,v); IC_{50}: The inhibitor concentration that reduces enzyme activity by half. *: Standard reference, Acarbose is an oral anti-diabetic drug used in the treatment of type II diabetes.

Although the TMrD 1:4 tincture contained the highest levels of the major quantified phenolic acids (e.g., chlorogenic and caffeic acids; Table 2), the strongest *α*-amylase and *α*-glucosidase inhibition was observed for TMrD 1:6 (Table 1). This indicates that the anti-diabetic activity is not determined solely by the absolute abundance of the targeted phenolics quantified by HPLC-DAD, but rather by the overall phytochemical matrix, including the relative proportions of constituents and potential synergistic interactions among metabolites [33,34]. Similar observations have been reported in enzyme inhibition studies, in which total or major phenolic contents did not directly correlate with α-amylase or α-glucosidase inhibitory potency [34]. Moreover, mulberry leaves contain non-phenolic α-glucosidase inhibitors, such as imino sugars (e.g., 1-deoxynojirimycin), which may substantially contribute to the observed inhibitory activity but are not detected by targeted phenolic HPLC-DAD analyses [35,36].

Therefore, the superior activity of TMrD 1:6 may reflect a more favorable extraction of bioactive inhibitors together with a more effective compositional balance, rather than being solely attributed to the highest concentration of individual phenolic acids [33]. Future work using broader (e.g., LC-MS-based) untargeted or suspect-screening approaches could further clarify additional contributors to the observed bioactivity beyond the targeted phenolic set [37, 38].

The data also indicate that, among all tested tinctures, TMrD 1:6 was the only sample with IC_{50} values below 50 µg/mL for both enzymes, reinforcing its position as the most effective formulation in the series. Overall, the combination of IC_{50} data and comparative analysis with acarbose substantiates the high anti-diabetic efficacy of TMrD 1:6, highlighting its potential for further pharmacological studies and possible development as a complementary or alternative therapy for diabetes management. Taken together, these findings demonstrate that both extraction parameters and phytochemical composition play a decisive role in determining the anti-diabetic efficacy of mulberry leaf tinctures.

The anti-diabetic effects of phenolic compounds have been widely attributed to their ability to inhibit key carbohydrate-hydrolyzing enzymes, particularly *α*-amylase and *α*-glucosidase, thereby reducing glucose release and postprandial hyperglycemia [39]. Previous studies have demonstrated that several phenolic acids commonly found in plant-derived extracts can interact with these enzymes through different mechanisms of inhibition, including competitive and mixed-type inhibition [30,33]. In addition to direct enzyme inhibition, phenolic compounds have been reported to contribute to anti-diabetic activity by modulating oxidative stress and metabolic regulation, thereby potentially enhancing their biological effects [28,29].

The alcohol:water ratios used for tincture preparation in this study were selected to optimize the extraction of bioactive compounds and to evaluate enzyme inhibitory activity under in vitro conditions. Accordingly, these ratios should not be directly interpreted as recommendations for human consumption or dosing. The present results are therefore limited to *in vitro* enzyme inhibition assays and provide a comparative assessment of extraction efficiency and bioactivity among different tincture formulations [40].

Comparative studies have consistently reported that mulberry leaf extracts can inhibit *α*-amylase and *α*-glucosidase *in vitro*, supporting the anti-diabetic potential of Morus-derived preparations [41]. In line with these reports, the IC_{50} values obtained for the most active tincture in the present study fall within the range of enzyme inhibitory activities previously reported for mulberry leaf extracts, despite differences in plant material and extraction methodologies [41,42]. However, direct comparison of our tincture ratios (e.g., TMrD 1:6 vs. other plant-to-solvent ratios) with the literature is limited because most previous studies employed different extraction approaches (e.g., aqueous/ethanolic extracts, fractions) rather than defined tincture dilution ratios [42]. Therefore, our results extend prior findings by showing that, under our standardized tincture preparation conditions, the 1:6 ratio yielded the most pronounced enzyme inhibition within the tested series.

3.2. HPLC Results

The phenolic composition of dried leaf tinctures of *M. rubra* L., which exhibited the highest anti-diabetic activity among the tested Morus tinctures, was systematically analyzed using HPLC-DAD. Quantitative data are summarized in Table 2, while the corresponding chromatograms for all tincture ratios are provided in the Supporting Information (Figures S1-S10).

HPLC analysis revealed that caffeic acid and chlorogenic acid were the predominant phenolic constituents, particularly in the TMrD 1:4 tincture [43]. However, the strongest *α*-amylase and *α*-glucosidase inhibitory activity was observed for TMrD 1:6 (Table 1), despite its lower levels of these major quantified phenolic acids compared with TMrD 1:4 (Table 2). Other phenolics, such as *p*-hydroxybenzoic acid, were consistently detected across all tincture ratios. In contrast, protocatechuic acid, *p*-coumaric acid, ferulic acid, coumarin, and *trans*-cinnamic acid were present only in trace amounts (tr) or below the detection limit in several samples.

A clear trend in phenolic content was observed with respect to extraction ratios. The TMrD 1:4 sample contained the highest levels of chlorogenic acid (0.97 mg/mL) and caffeic acid (0.91 mg/mL), which gradually decreased in higher dilution ratios (TMrD 1:5-1:8). In comparison, fresh leaf tinctures (TMrF) exhibited lower concentrations of these major phenolics, consistent with their comparatively reduced anti-diabetic activity.

The HPLC-DAD chromatograms (Figures S1-S10) confirmed both the identity and relative abundance of the detected phenolic compounds, providing a comprehensive profile of dried and fresh leaf tinctures across multiple extraction ratios. Notably, Figures S1-S5 correspond to dried leaf tinctures (TMrD 1:4-1:8), while Figures S6-S10 represent fresh leaf tinctures (TMrF 1:4-1:8).

These findings highlight the importance of leaf type and extraction ratio in determining phenolic composition and suggest that optimization of tincture preparation can significantly enhance the anti-diabetic potential of *M. rubra* L. tinctures.

3.3. Statistical Analysis of Phenolic Compounds and Enzyme Inhibition

To elucidate the contribution of individual phenolic compounds to the anti-diabetic activity of *Morus rubra* tinctures, correlation analysis was conducted between phenolic compound concentrations (Table 2) and IC_{50} values of α-amylase and α-glucosidase inhibition (Table 1). Pearson correlation analysis indicated that higher concentrations of caffeic acid and chlorogenic acid were associated with lower IC_{50} values, particularly for α-glucosidase inhibition, suggesting stronger enzyme inhibitory activity. This negative correlation supports the hypothesis that these phenolic acids play a key role in the anti-diabetic potential of the tinctures.

Other phenolic compounds detected at trace levels did not show statistically meaningful correlations, likely due to their low concentrations. These findings are consistent with previous reports highlighting the role of hydroxycinnamic acids in inhibiting carbohydrate-hydrolyzing enzymes.

Table 2. Phenolic composition of the Morus tinctures by HPLC-DAD (mg/g extract).

Tinctures	Protocatechuic acid	*p*-Hydroxybenzoic acid	Caffeic acid	Chlorogenic acid	*p*-Coumaric acid	Ferulic acid	Coumarin	*trans*-Cinnamic acid
RT (min)	*24.63*	*30.87*	*35.28*	*40.16*	*40.87*	*42.56*	*45.18*	*56.20*
TMrD 1:4	-	*tr*	0.91	0.97	*tr*	*tr*	*tr*	*tr*
TMrD 1:5	-	*tr*	0.85	0.96	*tr*	*tr*	*tr*	*tr*
TMrD 1:6	-	*tr*	0.67	0.81	*tr*	*tr*	*tr*	*tr*
TMrD 1:7	-	*tr*	0.67	0.91	*tr*	*tr*	*tr*	*tr*
TMrD 1:8	0.05	0.36	0.72	0.68	*tr*	*tr*	*tr*	*tr*
TMrF 1:4	-	0.40	0.58	0.23	*tr*	*tr*	*tr*	*tr*
TMrF 1:5	-	0.45	0.58	0.29	*tr*	*tr*	*tr*	*tr*
TMrF 1:6	-	0.37	0.45	0.36	*tr*	*tr*	*tr*	*tr*
TMrF 1:7	-	0.39	0.55	0.44	*tr*	*tr*	*tr*	*tr*
TMrF 1:8	0.02	0.44	0.59	0.50	*tr*	*tr*	*tr*	*tr*

TMaF: Fresh leaf tincture of *M. alba* L., **TMnF**: Fresh leaf tincture of *M. nigra* L., **TMrF**: Fresh leaf tincture of *M. rubra* L., **TMaD**: Dried leaf tincture of *M. alba L.*, **TMnD**: Dried leaf tincture of *M. nigra* L., **TMrD**: Dried leaf tincture of *M. rubra* L.; 1:4, 1:5, 1:6, 1:7 and 1:8 : Ground mulberry leaf: solvent ratio (w,v); IC_{50}: The inhibitor concentration that reduces enzyme activity by half. RT: Retention time. *tr*: trace amount. -: Not detected

4. Conclusion

This study demonstrates that tinctures derived from Morus species possess significant in vitro anti-diabetic potential. Among the formulations tested, the dried *M. rubra* L. leaf tincture (TMrD 1:6), prepared using a 70:30 (v/v) ethanol-water mixture, exhibited the strongest inhibitory effects against both *α*-amylase and *α*-glucosidase, with IC_{50} values of 24.11 and 40.18 µg/mL, respectively. These results highlight *M. rubra* L as a promising candidate for the development of natural anti-diabetic formulations. Future research should prioritize optimizing key extraction parameters, including solvent

ratio, extraction time, temperature, and solute concentration. Such improvements may further enhance both the inhibitory efficacy and phytochemical profile of the tinctures. Targeted refinement of these variables is expected to yield more potent formulations with superior anti-diabetic activity. In conclusion, while the tested hydroalcoholic tinctures demonstrate notable in vitro inhibitory effects against α-amylase and α-glucosidase, it is important to emphasize that the safety, tolerability, and appropriate dosage for human use cannot be inferred from these findings alone. Comprehensive in vivo, toxicological, pharmacokinetic, and clinical studies are essential to assess the full therapeutic potential of these tinctures. Therefore, any potential application of the studied tinctures should be approached with caution and validated through rigorous clinical evaluations.

Acknowledgments

I would like to express my sincere thanks to Assoc. Prof. Dr. Yusuf Sıcak, Mugla Sitki Kocman University, Koycegiz Vocational School, Medicinal and Aromatic Plants Department, Köyceğiz-Muğla, for their valuable support in the laboratory studies.

Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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