

Solvent-Dependent Enzyme-Inhibitory, Antioxidant, and Cytotoxic Effects of *Cinnamomum verum* Bark Extracts

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Abstract

Cinnamomum verum bark is widely used as a culinary spice and in traditional medicine and contains diverse phytochemicals with potential biological activities. This study evaluated the in vitro α -amylase and α -glucosidase inhibitory activities, phytochemical contents, antioxidant properties, and effects on HepG2 cell viability of *C. verum* bark extracts prepared using 100% methanol (CVB 100), 50% methanol (CVB 50), and distilled water (CVB DW). The methanolic extracts showed higher measured α -amylase and α -glucosidase inhibitory activities than the aqueous extract. Phytochemical analysis revealed numerical variation among the extracts, with CVB 50 exhibiting the highest measured total phenolic and tannin contents, whereas CVB DW showed the highest measured total flavonoid content. All three extracts exhibited concentration-dependent DPPH and ABTS radical scavenging activities. In the ABTS assay, the measured IC₅₀ values were 5.44 ± 0.04 , 6.11 ± 0.34 , and 8.58 ± 0.10 μ g/mL for CVB 100, CVB 50, and CVB DW, respectively, while FRAP measurements indicated relatively comparable reducing capacities among the extracts. The MTT assay showed a concentration-dependent reduction in HepG2 cell viability, with recorded viability values at 100 μ g/mL of 48.98%, 24.77%, and 23.78% for CVB 100, CVB 50, and CVB DW, respectively. Because one extract was prepared per solvent and measurements were performed in technical replicates, comparisons among extraction solvents are interpreted descriptively.

Overall, the findings demonstrate multiple measurable in vitro biological activities of *C. verum* bark extracts and support further investigation using independent extraction replicates, detailed phytochemical characterization, mechanistic studies, and appropriate biological validation.

Keywords: *Cinnamomum verum*; α -amylase; α -glucosidase; antioxidant activity; phenolic compounds; HepG2 cells.

Introduction

Cinnamon (*Cinnamomum verum* or *Cinnamomum zeylanicum*) is a widely used spice and medicinal plant that has attracted considerable scientific attention because of its rich content of phytochemicals with antioxidant, enzyme-inhibitory, antimicrobial, and antiproliferative properties (Zhang et al., 2023). The biological activity of cinnamon is mainly attributed to phenolic acids, flavonoids, tannins, and volatile compounds such as cinnamaldehyde and eugenol, which vary depending on the plant part, geographic origin, and extraction procedure. Among these factors, the choice of extraction solvent is especially important because it strongly influences the spectrum and quantity of metabolites recovered from plant tissues (Gilani and Najafpour, 2022). As a result, cinnamon extracts prepared with different solvents may differ markedly in chemical composition and biological efficacy (Yang et al., 2012).

Solvent-dependent extraction is a key issue in phytochemical research because plant metabolites differ in polarity, solubility, and stability (Dama et al., 2024). Polar solvents such as water and methanol are generally effective for extracting hydrophilic compounds, including many phenolics and flavonoids, whereas less polar solvents may be more suitable for compounds with intermediate or lipophilic character. Studies on cinnamon have shown that extraction conditions can alter the yield of total phenolics, tannins, flavonoids, and other bioactive constituents, which in turn affects the observed bioactivity (Weisany et al., 2023).

More recent work has also demonstrated that advanced solvent systems can improve the recovery of cinnamic acid, cinnamaldehyde, and related compounds from cinnamon residues, confirming that solvent selection can substantially affect extraction efficiency and chemical fingerprinting (Zhang et al., 2023). Therefore, comparing extracts prepared with different solvents is important for evaluating how solvent selection influences phytochemical recovery and the resulting biological activities (Khattak et al., 2025). Cinnamon extracts are particularly known for their antioxidant potential. Oxidative stress is implicated in the development of numerous diseases, including diabetes, inflammation, cardiovascular disorders, and cancer-related processes, and antioxidant-rich plant extracts are therefore frequently investigated as potential sources of bioactive compounds. Previous studies have demonstrated that phytochemical-rich plant extracts can exhibit measurable antioxidant activity in vitro (Murshed et al., 2024). In addition, phytochemical and biological investigations of *C. verum* bark have reported the presence of bioactive constituents together with antioxidant-related activities (Murshed et al., 2025). More recent work evaluating methanolic plant extracts has further demonstrated measurable phytochemical and antioxidant properties in vitro (Murshed et al., 2026). Collectively, these observations support continued investigation of the relationship between extraction conditions, phytochemical composition, and antioxidant activity.

Inhibition of carbohydrate-digesting enzymes, particularly α -amylase and α -glucosidase, represents an important strategy for modulating postprandial hyperglycemia (Rahu et al., 2025). Cinnamon-derived phytonutrients have also been investigated for their potential roles in metabolic regulation and glucose homeostasis through multiple cellular and molecular pathways (Tjandrawinata et al., 2026). Previous studies have reported enzyme-inhibitory activities for plant-derived phytochemicals, including inhibition of α -glucosidase, while the magnitude of these effects may vary according to phytochemical composition (Khan et al., 2019). Extraction conditions have also been shown to influence the yield and biological properties of cinnamon-derived preparations, including their antioxidant and antiproliferative activities (Kallel et al., 2019). Accordingly, evaluating enzyme inhibition together with phytochemical content and antioxidant activity within the same experimental framework may provide a more integrated assessment of the biological properties of *C. verum* extracts.

Cinnamon has also been investigated for cytotoxic and antiproliferative activities in cell-based experimental models (Khedkar et al., 2023). Previous studies have reported that cinnamon extracts and cinnamon-derived constituents can affect cancer-cell proliferation and survival through several cellular mechanisms (Kwon et al., 2010; Han et al., 2024). However, observations from in vitro cell-based assays should be interpreted cautiously and should not be considered direct evidence of therapeutic efficacy. In the present study, the HepG2 assay was therefore used as an in vitro assessment of the effects of the extracts on cell metabolic viability. When considered together with phytochemical, antioxidant, and enzyme-inhibitory measurements, this assay provides an additional biological endpoint for comparing the investigated extracts.

Despite the growing body of literature on cinnamon bioactivity, a clear gap remains in studies that directly compare the effects of extraction solvent on phytochemical yield, antioxidant capacity, enzyme inhibition, and HepG2 cytotoxicity within a single experimental design. Many reports have focused on a single biological endpoint or a single extraction method, making it difficult to compare how different extraction solvents affect the chemical composition and biological activities of cinnamon extracts.

Addressing this gap is important because the selection of solvent influences both the analytical profile and the functional properties of the extract (Gilani and Najafpour, 2022).

The present study aimed to evaluate how extraction solvent influences the phytochemical and biological properties of *C. verum* bark extracts prepared using 100% methanol, 50% methanol, and distilled water. Total phenolic, tannin, and flavonoid contents were determined, together with α -amylase and α -glucosidase inhibitory activities. Antioxidant activity was evaluated using DPPH, ABTS, and FRAP assays, while the effects of the extracts on HepG2 cell viability were assessed using the MTT assay. By

integrating these measurements, the study provides a comparative in vitro evaluation of the chemical and biological profiles of *C. verum* bark extracts obtained using different extraction solvents.

Materials and Methods

Plant Material and Preparation of Extracts

Dried bark of *Cinnamomum verum* J. Presl was obtained from a commercial herbal supplier in Riyadh, Saudi Arabia, and authenticated botanically. The bark samples were cleaned, air-dried at room temperature for 5 days, and ground into a fine powder using a laboratory mill. The powdered material was stored in airtight containers protected from light and moisture until extraction.

Three extraction solvents were used: absolute methanol (100%), 50% methanol in distilled water (v/v), and distilled water. Briefly, the powdered bark was extracted separately with each solvent by maceration at room temperature under continuous agitation. The extracts were filtered through Whatman No. 1 filter paper. Methanolic extracts were concentrated under reduced pressure using a rotary evaporator, whereas aqueous extracts were evaporated to dryness. The dried extracts were stored at 4°C in amber glass vials until further analysis. Stock solutions were freshly prepared before each experiment.

Chemicals and Reagents

Unless otherwise stated, all chemicals and reagents used in this study were of analytical grade and were purchased from Merck (Darmstadt, Germany). These included α -amylase, α -glucosidase, acarbose, soluble starch, p-nitrophenyl- α -D-glucopyranoside (pNPG), Folin–Ciocalteu reagent, gallic acid, quercetin, aluminum chloride, DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)], potassium persulfate, methanol, and other analytical-grade reagents used throughout the study.

For the cytotoxicity experiments, Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), penicillin–streptomycin solution, and HepG2 cells were obtained from Invitrogen (Carlsbad, CA, USA). MTT reagent and dimethyl sulfoxide (DMSO) were purchased from Merck (Darmstadt, Germany).

Determination of α -Amylase Inhibitory Activity

The inhibitory activity of *C. verum* extracts against α -amylase was evaluated using a colorimetric method based on starch hydrolysis. Different concentrations of the extracts were incubated with an α -amylase solution before the addition of the starch substrate. After incubation, the reaction was terminated, and absorbance was measured spectrophotometrically using a PerkinElmer UV–Visible

spectrophotometer (PerkinElmer Inc., USA). The percentage inhibition was calculated relative to the control group, and acarbose was used as a positive control.

Determination of α -Glucosidase Inhibitory Activity

α -Glucosidase inhibitory activity was determined using p-nitrophenyl- α -D-glucopyranoside (pNPG) as substrate.

Various concentrations of the extracts were incubated with an α -glucosidase solution, followed by the addition of pNPG. The released p-nitrophenol was quantified spectrophotometrically, and percentage inhibition was calculated relative to the untreated control. Acarbose served as the reference inhibitor.

Total Phenolic, Tannin, and Flavonoid Contents

Total phenolic content (TPC) was determined using the Folin–Ciocalteu method with gallic acid as the calibration standard and expressed as mg gallic acid equivalents (GAE)/g dry extract. Total tannin content was estimated using the Folin–Ciocalteu-based method and expressed as mg GAE/g dry extract. Total flavonoid content (TFC) was determined using the aluminum chloride colorimetric assay with quercetin as the reference standard and expressed as mg quercetin equivalents (QE)/g dry extract.

DPPH Radical Scavenging Assay

The antioxidant activity of the extracts was evaluated using the DPPH free-radical scavenging assay. Various extract concentrations were mixed with freshly prepared DPPH solution and incubated in the dark at room temperature. Absorbance was measured using a PerkinElmer UV–Visible spectrophotometer, and the percentage inhibition and IC₅₀ values were calculated.

ABTS Radical Scavenging Assay

ABTS radical cation scavenging activity was assessed using a pre-formed ABTS^{•+} solution generated by reaction with potassium persulfate. Extract solutions at different concentrations were incubated with the ABTS radical solution, and absorbance reduction was measured spectrophotometrically. The scavenging percentage and IC₅₀ values were subsequently calculated.

Ferric Reducing Antioxidant Power (FRAP) Assay

Ferric reducing antioxidant power was determined using freshly prepared FRAP reagent. Extract samples were mixed with FRAP reagent and incubated under controlled conditions. The formation of the Fe²⁺–TPTZ complex was measured spectrophotometrically. Antioxidant reducing power was calculated from an ascorbic acid calibration curve and expressed as mg ascorbic acid equivalents per gram of dry weight (mg AAE/g DW).

Cell Culture

Human hepatocellular carcinoma (HepG2) cells were obtained from Invitrogen (Carlsbad, CA, USA). Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS) and antibiotics consisting of 100 µg/mL streptomycin and 100 U/mL penicillin. Cell cultures were maintained at 37°C in a humidified atmosphere containing 5% CO₂ using a Thermo Scientific CO₂ incubator (Thermo Fisher Scientific, USA). The culture medium was replaced every 1–2 days, and cells were sub-cultured upon reaching approximately 90% confluence.

Cytotoxicity Assay (MTT)

The cytotoxic activity of *C. verum* extracts against HepG2 cells was evaluated using the MTT assay. Cells were seeded into 96-well plates at a density of 1×10^4 cells/well and incubated for 24 h at 37°C and 5% CO₂. The extracts were prepared as stock solutions and diluted to final concentrations ranging from 3.125 to 100 µg/mL. Following treatment for 48 h, 10 µL of MTT solution (0.5 mg/mL) was added to each well and incubated for an additional 3 h. Subsequently, 100 µL of DMSO was added to dissolve the resulting formazan crystals. Absorbance was measured at 570 nm using a BioTek microplate reader (BioTek Instruments, USA). Cell viability (%) was calculated relative to untreated control cells.

Statistical Analysis

All analytical measurements were performed in technical triplicate, and the results are presented as mean $\pm$ standard deviation (SD). One extract was prepared per solvent and analyzed in technical triplicate. Therefore, comparisons among the extraction solvents were interpreted descriptively, and no inferential statistical comparisons were performed among the solvent extracts.

Results

The α -amylase inhibitory activity of *C. verum* bark extracts showed numerical variation among the extracts prepared using the three extraction solvents. The 100% methanolic extract (CVB 100) exhibited the highest measured α -amylase inhibitory activity, followed by the 50% methanolic extract (CVB 50), whereas the distilled-water extract (CVB DW) showed the lowest measured activity. Because one extract was prepared per solvent and each extract was analyzed in technical triplicate, these results are interpreted descriptively and do not establish statistically significant differences in α -amylase inhibitory activity among the extraction solvents (Figure 1).

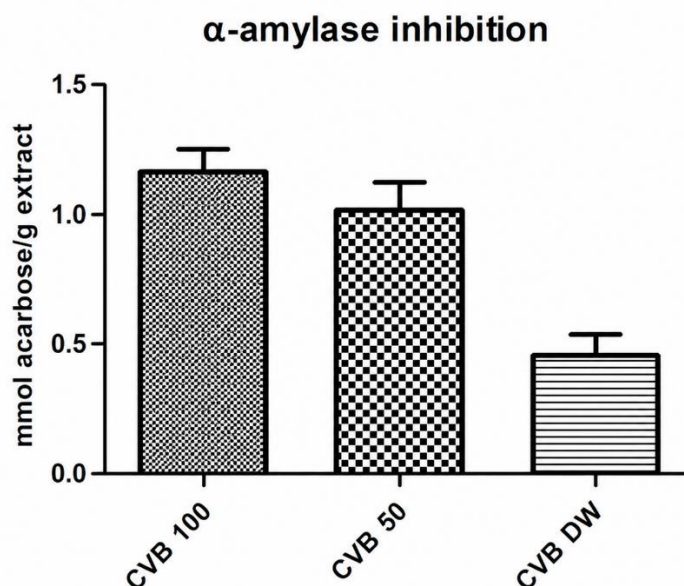


Figure 1. α -Amylase inhibitory activity of *Cinnamomum verum* bark extracts prepared using 100% methanol (CVB 100), 50% methanol (CVB 50), and distilled water (CVB DW), expressed as mmol acarbose equivalents per gram of extract. Values are presented as mean $\pm$ SD of three technical measurements performed on one extract per solvent.

The α -glucosidase inhibitory activity of *C. verum* bark extracts showed numerical variation among the three extraction solvents. The 50% methanolic extract (CVB 50) exhibited the highest measured α -glucosidase inhibitory activity, closely followed by the 100% methanolic extract (CVB 100), whereas the distilled-water extract (CVB DW) showed the lowest measured activity. Because one extract was prepared per solvent and each extract was analyzed in technical triplicate, these results are interpreted descriptively and do not establish statistically significant differences in α -glucosidase inhibitory activity among the extraction solvents (Figure 2).

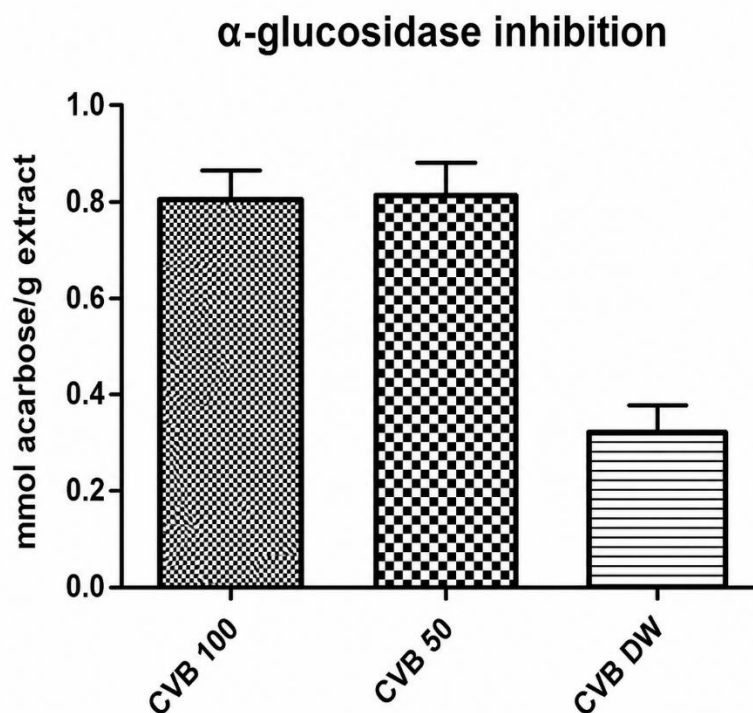
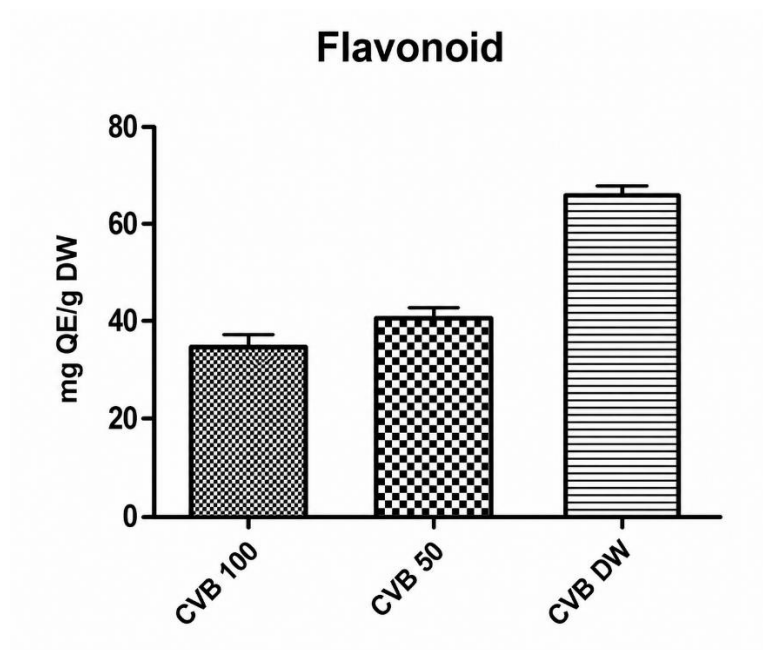


Figure 2. α -glucosidase inhibitory activity of *Cinnamomum verum* bark extracts prepared using 100% methanol (CVB 100), 50% methanol (CVB 50), and distilled water (CVB DW), expressed as mmol acarbose equivalents per gram of extract. Values are presented as mean $\pm$ SD of three technical measurements performed on one extract per solvent.

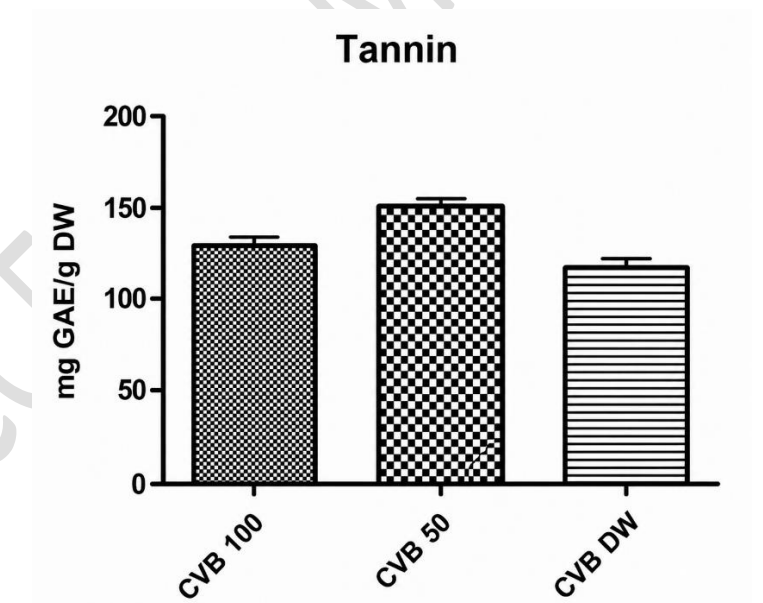
Phenolic, tannin, and flavonoid content

The total phenolic, tannin, and flavonoid contents of the *Cinnamomum verum* bark extracts obtained with different extraction solvents are presented in Table 1 and Figures 3–5. Numerical variations were observed among the extracts. The 50% methanol extract (CVB 50) exhibited the highest measured total phenolic content (185.54 ± 0.53 mg GAE/g DW) and total tannin content (148.29 ± 0.41 mg GAE/g DW), whereas the distilled water extract (CVB DW) showed the highest measured total flavonoid content (65.07 ± 0.60 mg QE/g DW). Because one extract was prepared per solvent and each extract was analyzed in technical triplicate, these results are presented descriptively and do not establish statistically significant differences in extraction efficiency among the solvents.



217

218 **Figure 3.** Total flavonoid content of *Cinnamomum verum* bark extracts prepared using 100% methanol
 219 (CVB 100), 50% methanol (CVB 50), and distilled water (CVB DW), expressed as mg quercetin
 220 equivalents per gram of dry weight (mg QE/g DW). Values are presented as mean $\pm$ SD of three
 221 technical measurements performed on one extract per solvent.



222

223 **Figure 4.** Total tannin content of *Cinnamomum verum* bark extracts prepared using 100% methanol
 224 (CVB 100), 50% methanol (CVB 50), and distilled water (CVB DW), expressed as mg gallic acid
 225 equivalents per gram of dry weight (mg GAE/g DW). Values are presented as mean $\pm$ SD of three
 226 technical measurements performed on one extract per solvent.

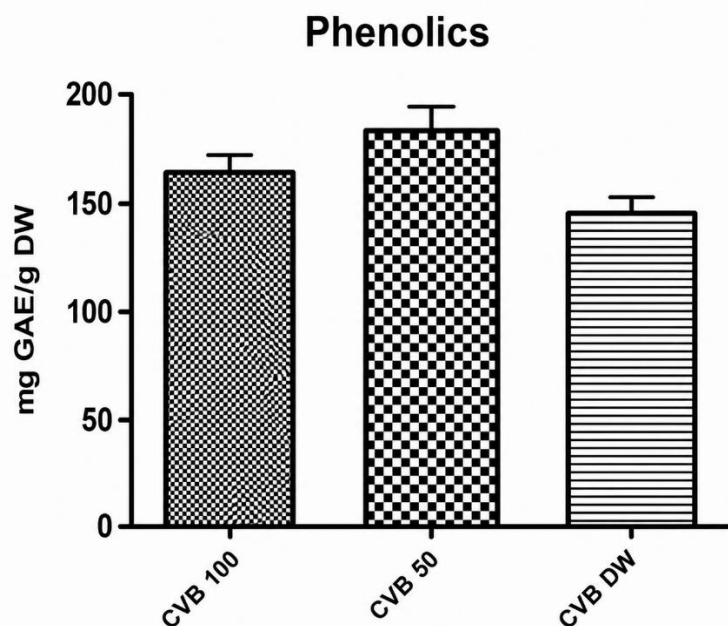


Figure 5. Total phenolic content of *Cinnamomum verum* bark extracts prepared using 100% methanol (CVB 100), 50% methanol (CVB 50), and distilled water (CVB DW), expressed as mg gallic acid equivalents per gram of dry weight (mg GAE/g DW). Values are presented as mean $\pm$ SD of three technical measurements performed on one extract per solvent.

Table 1. Total phenolic, tannin, and flavonoid contents of *Cinnamomum verum* bark extracts prepared using 100% methanol (CVB 100), 50% methanol (CVB 50), and distilled water (CVB DW).

Phytochemical assay	CVB 100 mean $\pm$ SD	CVB 50 mean $\pm$ SD	CVB DW mean $\pm$ SD
Total phenolic content (mg GAE/g DW)	160.27 $\pm$ 0.72	185.54 $\pm$ 0.53	145.62 $\pm$ 0.30
Total tannin content (mg GAE/g DW)	129.11 $\pm$ 0.54	148.29 $\pm$ 0.41	117.98 $\pm$ 0.25
Total flavonoid content (mg QE/g DW)	35.59 $\pm$ 0.46	39.68 $\pm$ 0.34	65.07 $\pm$ 0.60

Values are presented as mean $\pm$ SD of three technical measurements performed on one extract per solvent. GAE, gallic acid equivalents; QE, quercetin equivalents; DW, dry weight.

Table 1 shows numerical variation in the total phenolic, tannin, and flavonoid contents of the three *Cinnamomum verum* bark extracts. CVB 50 exhibited the highest measured total phenolic and tannin

contents, whereas CVB DW showed the highest measured total flavonoid content. Because one extract was prepared per solvent and each extract was measured in technical triplicate, these results are interpreted descriptively and do not establish statistically significant differences in extraction efficiency among the solvents.

The phytochemical profile confirmed the presence of measurable phenolic, tannin, and flavonoid contents in *C. verum* bark extracts. The observed numerical variations among the extracts may contribute to differences in enzyme-inhibitory activity; however, the phytochemical measurements are interpreted descriptively because one extract was prepared per solvent and analyzed in technical triplicate.

Table 2 summarizes the extracts that exhibited the highest measured values for enzyme inhibitory activity and phytochemical contents among the three extraction solvents. For phytochemical measurements, these comparisons are descriptive because one extract was prepared per solvent and analyzed in technical triplicate.

Table 2. Extracts exhibiting the highest measured values for enzyme inhibitory activity and phytochemical contents among the three extraction solvents.

Assay	Extract with the Highest Measured Value
α -amylase inhibition	100% methanol
α -glucosidase inhibition	50% methanol
Total phenolic content	50% methanol
Total tannin content	50% methanol
Total flavonoid content	Distilled water

For phytochemical measurements, the entries indicate the extracts with the highest measured values and should be interpreted descriptively, as one extract was prepared per solvent and analyzed in technical triplicate.

DPPH radical scavenging activity

Table 3 presents the DPPH radical scavenging activity of *C. verum* bark extracts (CVB 100, CVB 50, and CVB DW) over a concentration range of 6.25–200 $\mu\text{g/mL}$. DPPH radical scavenging activity increased with concentration for all three extracts up to 100 $\mu\text{g/mL}$. At the lowest concentration (6.25 $\mu\text{g/mL}$), CVB 100 exhibited the highest measured inhibition ($39.94 \pm 0.09\%$), followed by CVB 50

(33.75 ± 1.23%) and CVB DW (19.54 ± 0.05%). A similar numerical pattern was observed at 12.5 and 25 µg/mL.

At 50 µg/mL, the measured inhibition values were 92.08 ± 0.13%, 90.75 ± 0.09%, and 74.26 ± 0.12% for CVB 100, CVB 50, and CVB DW, respectively. At 100–200 µg/mL, all extracts showed high radical scavenging activity, with values approaching or exceeding 90%. The IC₅₀ values were 10.33 ± 0.41 µg/mL for CVB 100, 13.47 ± 0.59 µg/mL for CVB 50, and 27.58 ± 0.33 µg/mL for CVB DW. Thus, CVB 100 showed the lowest measured IC₅₀ value, followed by CVB 50 and CVB DW. Because one extract was prepared per solvent and mea

surements were performed in technical triplicate, these results are interpreted descriptively.

Table 3. DPPH radical scavenging activity and IC₅₀ values of *Cinnamomum verum* bark extracts.

Concentration (µg/mL)	Inhibition (Mean ± SD) %		
	CVB 100	CVB 50	CVB DW
6.25	39.94±0.09	33.75±1.23	19.54±0.05
12.5	69.39±0.24	55.96±0.27	31.17±0.26
25	88.37±0.44	80.46±0.05	50.91±0.17
50	92.08±0.13	90.75±0.09	74.26±0.12
100	93.99±0.26	93.33±0.11	89.89±0.16
200	91.42±0.19	90.18±0.15	90.94±0.46
IC ₅₀ (µg/mL)	10.33±0.41	13.47±0.59	27.58±0.33

Values are presented as mean ± SD of three technical measurements performed on one extract per solvent. IC₅₀ represents the concentration required to achieve 50% DPPH radical scavenging activity.

ABTS radical scavenging activity

Table 4 presents the ABTS radical scavenging activity of *C. verum* bark extracts (CVB 100, CVB 50, and CVB DW) over a concentration range of 3.125–100 µg/mL. ABTS radical scavenging activity increased with concentration in all three extracts. At 3.125 µg/mL, the measured inhibition values were 36.21 ± 1.28%, 39.19 ± 0.95%, and 29.26 ± 1.00% for CVB 100, CVB 50, and CVB DW, respectively. At 25 µg/mL, the corresponding values were 88.69 ± 0.86%, 88.29 ± 0.80%, and 87.50 ± 0.71%, respectively. At 50–100 µg/mL, all extracts exhibited high ABTS radical scavenging activity (>94%). The calculated IC₅₀ values were 5.44 ± 0.04 µg/mL for CVB 100, 6.11 ± 0.34 µg/mL for CVB 50, and

8.58 ± 0.10 µg/mL for CVB DW. Thus, CVB 100 exhibited the lowest measured IC₅₀ value, followed by CVB 50 and CVB DW. Because one extract was prepared per solvent and measurements were performed in technical triplicate, these results are interpreted descriptively.

Table 4. ABTS radical scavenging activity and IC₅₀ values of *Cinnamomum verum* bark extracts.

Concentration (µg/mL)	Inhibition (Mean ± SD) %		
	CVB 100	CVB 50	CVB DW
3.125	36.21 ± 1.28	39.19 ± 0.95	29.26 ± 1.00
6.25	54.86 ± 0.78	50.59 ± 1.37	40.97±0.84
12.5	71.93 ± 1.12	73.31 ± 0.74	65.18±0.59
25	88.69 ± 0.86	88.29 ± 0.80	87.50±0.71
50	94.74 ± 0.72	95.43 ± 0.48	94.64±0.51
100	97.02 ± 0.29	98.11 ± 0.63	97.42±0.38
IC ₅₀ (µg/mL)	5.44 ± 0.04	6.11 ± 0.34	8.58 ± 0.10

Values are presented as mean ± SD of three technical measurements performed on one extract per solvent. IC₅₀ values were calculated separately for each technical replicate by linear interpolation between the two concentrations surrounding 50% inhibition and are presented as mean ± SD.

Figure 6 illustrates the concentration-dependent DPPH and ABTS radical scavenging activities of the three *C. verum* bark extracts. In both assays, radical scavenging activity generally increased with increasing extract concentration. Numerical differences were observed among CVB 100, CVB 50, and CVB DW, particularly at lower concentrations, whereas the measured activities became more comparable at higher concentrations. Because one extract was prepared per solvent and measurements were performed in technical triplicate, these differences are interpreted descriptively and do not establish statistically significant differences in antioxidant activity among the extraction solvents (Figure 6a, b).

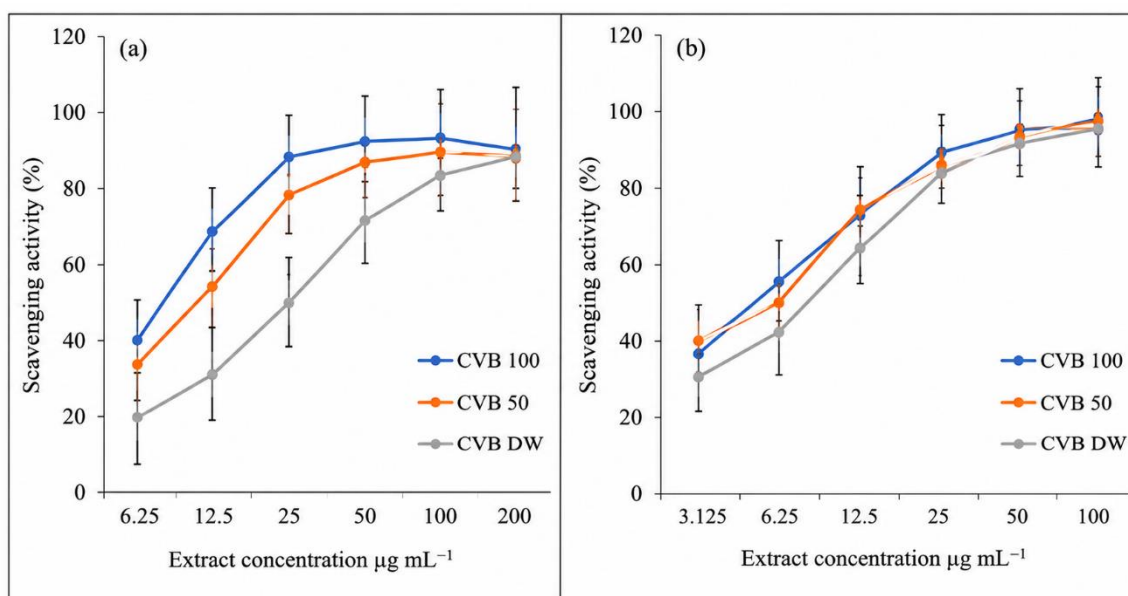


Figure 6. DPPH (a) and ABTS (b) radical scavenging activities of *Cinnamomum verum* bark extracts prepared using 100% methanol (CVB 100), 50% methanol (CVB 50), and distilled water (CVB DW).

Ferric reducing antioxidant power (FRAP)

Figure 7 presents the ferric reducing antioxidant power (FRAP) of *C. verum* bark extracts prepared using 100% methanol (CVB 100), 50% methanol (CVB 50), and distilled water (CVB DW), expressed as mg ascorbic acid equivalents per gram of dry weight (mg AAE/g DW). The three extracts exhibited relatively similar measured FRAP values, with CVB 100 showing the highest numerical value, followed by CVB DW and CVB 50. Because one extract was prepared per solvent and each extract was analyzed in technical triplicate, these results are interpreted descriptively and do not establish statistically significant differences in reducing capacity among the extraction solvents (Figure 7).

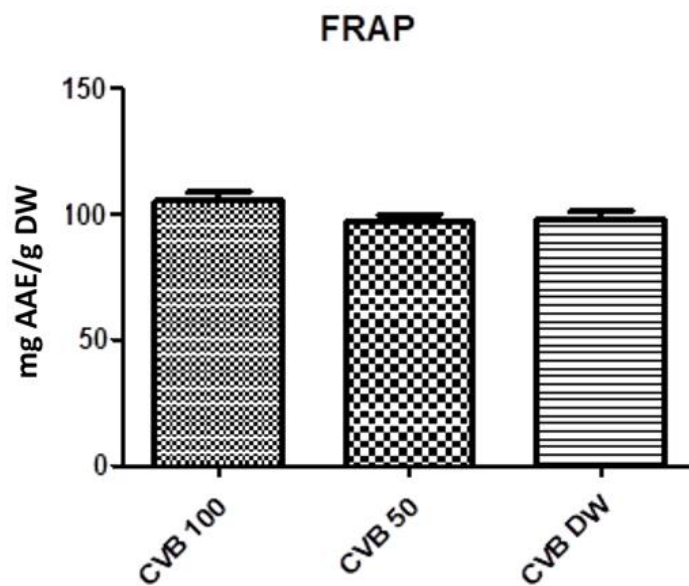


Figure 7. Ferric reducing antioxidant power (FRAP) of *Cinnamomum verum* bark extracts prepared using 100% methanol (CVB 100), 50% methanol (CVB 50), and distilled water (CVB DW), expressed as mg ascorbic acid equivalents per gram of dry weight (mg AAE/g DW). Values are presented as mean $\pm$ SD of three technical measurements performed on one extract per solvent.

The effects of *C. verum* bark extracts on HepG2 cell viability after 48 h of exposure are presented in Table 5. Cell viability generally decreased with increasing extract concentration over the tested range (3.125–100 μ g/mL). At 100 μ g/mL, the recorded cell viability values were 48.98%, 24.77%, and 23.78% for CVB 100, CVB 50, and CVB DW, respectively. Overall, the data show a concentration-dependent reduction in HepG2 cell viability across the tested extracts.

Table 5. Effects of *Cinnamomum verum* bark extracts on HepG2 cell viability after 48 h of exposure, as determined by the MTT assay.

Concentration μ g/mL	3.125	6.25	12.5	25	50	100
CVB 100	88.625	81.542	73.534	67.833	56.434	48.983
CVB 50	76.560	79.601	57.129	48.711	32.559	24.774
CVB DW	79.743	72.826	63.231	51.932	29.876	23.781

Values represent cell viability (%) relative to untreated control cells after 48 h of exposure.

Discussion

The present study demonstrates that *C. verum* bark extracts prepared using 100% methanol, 50% methanol, and distilled water exhibit measurable enzyme-inhibitory, phytochemical, and antioxidant activities. Numerical differences were observed among the three extracts, although these differences should be interpreted descriptively because one extract was prepared per solvent and the measurements were performed in technical triplicate. The methanolic extracts showed higher measured α -amylase and α -glucosidase inhibitory activities than the aqueous extract, while the measured phytochemical contents varied among the different compound classes. CVB 50 exhibited the highest measured total phenolic and tannin contents, whereas CVB DW showed the highest measured total flavonoid content. Previous studies have reported diverse biological properties of *C. verum*, including antioxidant and metabolic-related activities (Ranasinghe et al., 2013), while plant-derived phytochemicals have also been widely investigated for their α -glucosidase inhibitory potential (Khan et al., 2019).

The phytochemical composition recovered from plant materials can be influenced by several extraction parameters, including solvent type, extraction method, temperature, and extraction time (Kumar et al., 2023). Recent findings in another *Cinnamomum* species have similarly demonstrated that extraction method and solvent composition can influence polyphenol and flavonoid recovery as well as antioxidant and enzyme-inhibitory activities (Phyu et al., 2026). A systematic review of *C. burmannii* further highlighted the influence of extraction methods and solvent polarity on phytochemical profiles and the recovery of bioactive constituents (Rahwal et al., 2025). Accordingly, the numerical differences observed among CVB 100, CVB 50, and CVB DW may partly reflect differences in the solvent-dependent recovery of the measured phytochemical classes.

The DPPH and ABTS assays demonstrated concentration-dependent radical scavenging activity in all three extracts. In the DPPH assay, CVB 100 exhibited the lowest measured IC_{50} value, followed by CVB 50 and CVB DW. A similar numerical pattern was obtained in the ABTS assay, with IC_{50} values of 5.44 ± 0.04 , 6.11 ± 0.34 , and 8.58 ± 0.10 $\mu\text{g/mL}$ for CVB 100, CVB 50, and CVB DW, respectively. At higher concentrations, however, the scavenging activities of the three extracts became increasingly similar. FRAP measurements also showed relatively comparable reducing capacities among the extracts. These observations indicate that *C. verum* bark contains constituents capable of contributing to radical-scavenging and electron-donating reactions.

The antioxidant activity of cinnamon has been associated with multiple mechanisms involving radical scavenging and redox-related processes, with the magnitude and mechanism of activity depending on its bioactive constituents (Ju et al., 2023).

Previous studies have attributed the antioxidant properties of cinnamon to phenolic constituents such as procyanidins, catechins, cinnamic acid derivatives, and related compounds (Mathew and Abraham, 2006; Peng et al., 2008; Rao and Gan, 2014; Mazimba et al., 2015; Ashfaq et al., 2021; Madhushika et al., 2024). Recent phytochemical profiling of aqueous *C. verum* bark extract has also identified diverse polyphenols, flavonoids, terpenes, and related derivatives, together with measurable antioxidant and enzyme-inhibitory activities (Falade et al., 2025). However, the present study does not establish which individual compounds are directly responsible for the measured activities.

Phenolic compounds, tannins, and flavonoids have previously been associated with antioxidant activity and inhibition of carbohydrate-hydrolyzing enzymes (Sarian et al., 2017; Barrett et al., 2018; Shi et al., 2022; Sun and Shahrajabian, 2023).

The numerical variation in these phytochemical classes may therefore contribute to the differences observed in α -amylase and α -glucosidase inhibition and antioxidant activity. Nevertheless, the present data do not demonstrate a direct causal relationship between the concentration of any individual phytochemical class and a specific biological activity. In particular, the higher measured flavonoid content of CVB DW did not correspond to the highest measured enzyme-inhibitory or radical-scavenging activity, emphasizing that the biological responses are likely influenced by the combined composition of each extract rather than by a single phytochemical class.

The MTT assay further showed a concentration-dependent reduction in HepG2 cell viability following 48 h of exposure to the three *C. verum* bark extracts. At the highest tested concentration (100 μ g/mL), the recorded cell viability values were 48.98%, 24.77%, and 23.78% for CVB 100, CVB 50, and CVB DW, respectively. These findings indicate that the extracts were associated with reduced MTT-based metabolic viability of HepG2 cells at higher concentrations.

Previous in vitro studies have also evaluated cinnamon extracts in HepG2 cells using MTT-based assays. Kadan et al. (2013) reported cytotoxic effects of *C. verum* extract at concentrations above 100 μ g/mL, while Azimian et al. (2023) reported concentration-dependent effects of Ceylon cinnamon water extract on HepG2 cell viability together with changes in glucose uptake and metabolism-related gene expression.

Previous studies have also demonstrated antiproliferative effects of cinnamon extracts in cancer-cell models. For example, a water-soluble *C. cassia* extract was reported to inhibit tumor-cell proliferation

and promote apoptotic responses associated with suppression of NF κ B and AP1 signaling (Kwon et al., 2010). Cinnamaldehyde, a major bioactive constituent of cinnamon, has also been reported to exert anticancer effects in experimental models through mechanisms involving inhibition of cell proliferation, cell-cycle arrest, and induction of apoptosis (Han et al., 2024). Polyphenols have also been reported to influence cancer-cell bioactivity through mitochondria-associated mechanisms, including modulation of cellular redox status, mitochondrial function, and pathways involved in cell survival and death (Stevens et al., 2018).

However, the MTT assay alone does not establish the mechanism responsible for this reduction and therefore cannot distinguish among growth inhibition, metabolic suppression, or specific modes of cell death. Further studies incorporating appropriate mechanistic assays and normal-cell controls would be required to determine the biological basis and selectivity of this response.

Overall, the findings indicate multiple measurable in vitro bioactivities in *C. verum* bark extracts, including inhibition of carbohydrate-hydrolyzing enzymes, radical scavenging, reducing activity, and concentration-dependent effects on HepG2 cell viability.

The results also suggest that extraction solvent may influence the measured chemical and biological profiles; however, because only one extract was prepared per solvent, the observed differences should be regarded as descriptive trends rather than statistically established differences in extraction efficiency. Independent extraction replicates, detailed phytochemical characterization, and additional biological validation will be necessary to determine the reproducibility, active constituents, mechanisms of action, and potential biological relevance of these observations.

Conclusions

The present study demonstrates that *Cinnamomum verum* bark extracts prepared using 100% methanol, 50% methanol, and distilled water exhibit multiple in vitro biological activities, including α -amylase and α -glucosidase inhibition, antioxidant activity, and concentration-dependent effects on HepG2 cell viability. The extracts also contained measurable amounts of phenolics, tannins, and flavonoids, with numerical variations observed among the different extraction solvents.

Methanolic extracts generally showed higher measured enzyme-inhibitory activities and lower measured DPPH and ABTS IC₅₀ values, whereas CVB 50 exhibited the highest measured total phenolic and tannin contents and CVB DW showed the highest measured total flavonoid content.

Because one extract was prepared per solvent and measurements were performed in technical replicates, these differences should be interpreted descriptively and do not establish statistical

superiority of one extraction solvent over another. Overall, the findings support further investigation of *C. verum* bark as a source of bioactive phytochemicals with potential biological relevance. Further studies using independent extraction replicates, detailed phytochemical characterization, mechanistic investigations, in vivo validation, and comprehensive safety assessment are required to confirm these findings and evaluate their potential applications.

Consent for publication: Not applicable.

Conflict of interest: The authors declare that they have no conflicts of interest.

Availability of data and material: The data supporting the findings of this study are included within the article. Further inquiries can be directed to the corresponding author.

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