In vitro comparative study of enzyme inhibitory properties of herbal plants and their phenolic, tannin, and flavonoid content as natural inhibitors in public health

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Abstract

Medicinal herbs are used to treat numerous diseases all over the world. This study investigates the enzyme inhibitory potential, antioxidant properties, and phytochemical composition of methanolic extracts from *Cinnamomum verum*, *Nerium oleander*, *Laurus nobilis*, and *Eucalyptus camaldulensis* leaves. These plants, known for their healing properties, were tested to determine how effectively they inhibit α-amylase and α-glucosidase. These essential enzymes help break down carbohydrates, and their antioxidant capacities were evaluated using DPPH and ABTS assays. Phytochemical profiling revealed significant levels of phenolics (26.44–63.56 mg GAE/g), flavonoids (6.09–37.56 mg QE/g), and tannins (12.98–34.80 mg TAE/g), with *E. camaldulensis* and *L. nobilis* exhibiting the highest phenolic content. Methanolic extracts of *L. nobilis* and *N. oleander* demonstrated the strongest inhibition of α-amylase (0.586 ± 0.015 and 0.564 ± 0.0001 mmol acarbose/g) and α-glucosidase (7.570 ± 0.107 and 8.242 ± 0.113 mmol acarbose/g), outperforming synthetic controls. Antioxidant activity correlated with phenolic content, with *E. camaldulensis* showing the lowest IC_{50} in DPPH (8.83 µg/mL) and *L. nobilis* in ABTS (30.32 µg/mL). These findings highlight the therapeutic promise of these plants as natural sources of enzyme inhibitors and antioxidants, supporting their use in oxidative stress-related disorders. The study underscores the significance of plant-derived bioactive compounds in developing safer, cost-effective pharmaceuticals and functional foods.

Keywords: amylase, *Cinnamomum verum*, *Eucalyptus camaldulensis*, glucosidase, *Laurus nobilis, Nerium oleander*

Introduction

The pharmaceutical and drug industries have relied heavily on plant products for many decades. Approximately 20% of medications used worldwide are derived from plants, as they are more widely available, cost-effective, and often exhibit more potent and safer effects than synthetic pharmaceuticals (Chaachouay & Zidane, 2024). According to Rahman *et al*. (2021), numerous natural substances are being utilized as essential therapeutics for various chronic and degenerative health conditions that are prevalent globally. These include cancer, Alzheimer's disease, and diabetes mellitus. Context: There is a growing interest in the exploration of new natural compounds derived from plants (Akter *et al*., 2021).

Phenolic, tannin, and flavonoid compounds were among the many pharmacologically active substances extracted from the medicinal plants examined in this

study (Tungmunnithum *et al.*, 2021). These compounds are recognized for their pharmacological effects, including hypoglycemic, anti-inflammatory, hepatoprotective, antifungal, antibacterial, and hypolipidemic activities (Bourais *et al.*, 2023). Research conducted on four different plant species has demonstrated that various constituents of these plants exhibit anticancer properties against a wide range of tumors.

The pharmaceutical and drug industries have relied heavily on plant products for decades. Approximately 20% of medications used worldwide are derived from plants, as they are more widely accessible, cost-effective, and often exhibit more potent and safer effects than synthetic pharmaceuticals (Chaachouay & Zidane, 2024). The cinnamon plant, known as *Cinnamomum verum*, is particularly well-known for its medicinal and pharmacological properties (Singh *et al.*, 2020). The *Cinnamomum zeylanicum* plant has been used as a medicinal condiment since ancient times. Both Sri Lanka and the southern states of India are its native habitats. It belongs to the Lauraceae family (Gulcin *et al.*, 2019). It is a dry bark that has been stripped of its outer cork and the underlying parenchyma.

One of the most widely used medicinal herbs, *C. verum* can be utilized in various contexts (Chebabe *et al.*, 2025). It is frequently used in the commercial sector as a component of candies, chewing gum, mouthwash, and toothpaste (Kumar *et al.*, 2022). The plant contains many volatile oils, the most prominent of which are cinnamaldehyde, cinnamic acid, and cinnamate (Huang *et al.*, 2021). Eugenol, the primary active component, is associated with several biological functions. This herb is found in nearly every pharmaceutical system worldwide, and each of these attributes plays an essential role in advancing human health (Nisar *et al.*, 2021). The plant possesses several critical medical properties, including antimicrobial, wound healing, anti-inflammatory, anti-HIV, anti-anxiety, and anti-Parkinson's disease activities (Singh & Yadav, 2024). Eugenol, cinnamaldehyde, cinnamyl acetate, copane, and camphor are the principal constituents of the *C. verum* plant (Pathak and Sharma, 2021).

Nerium oleander (NO), often called oleander, is a member of the Apocynaceae family and is native to the Mediterranean regions of several continents, including Africa and Europe (Sanna *et al.*, 2019). It may grow as a small tree or an evergreen shrub with upright, coarse, dark green leaves. Despite its toxicity, it has been used as a medicinal agent for various conditions, including heart failure, malaria, dyspepsia, leprosy, and ringworm (Mouhcine *et al.*, 2019). Both hot and cold extracts of *N. oleander* have been evaluated for their potential to inhibit viral growth. Similarly, *N. oleander* has been reported to exhibit hypolipidemic activity and has been used in folk medicine as a cardiotonic (Rauf *et al.*, 2021).

Laurus nobilis (LN), which belongs to the family Lauraceae, is an evergreen tree cultivated in many warm regions worldwide, particularly in Mediterranean countries such as Morocco, Algeria, Spain, Portugal, Turkey, and Greece (Khodja *et al.*, 2023). Historically, it has been used to treat ailments including diabetes, rheumatism, dermatitis, gastrointestinal disorders, snakebites, and migraines (Ansari *et al.,* 2025).

Eucalyptus camaldulensis (EC) belongs to the Myrtaceae family and is commonly known as the river red gum. It is endemic to the continents of Africa and Australia. This tree has smooth, cream-colored or white bark; mature leaves that are lance-shaped or curved; flower buds arranged in clusters of seven or nine; white flowers; and hemispherical fruit with valves that extend above the rim (Kurek *et al.*, 2020). It is considered a source of compounds with physiologically active qualities (Elshafie, 2023). The leaf extract of *E. camaldulensis* has demonstrated several beneficial properties, including antioxidant and antimicrobial effects (Mahmoud Dogara et al., 2024).

Previous studies have demonstrated that *C. verum*, *N. oleander*, *L. nobilis*, and *E. camaldulensis* possess high nutritional value and considerable biological benefits. These effects include antioxidant, antiproliferative, and enzyme-inhibitory activities. However, to the best of our knowledge, no reports exist regarding the enzyme-inhibiting impact of the studied herbaceous plant species. Consequently, this study aimed to evaluate the enzyme inhibitory effects (anti-amylase and anti-glucosidase) of various extracts derived from different biological sources. Based on the results obtained, there may be potential interest in developing novel natural products for the pharmaceutical and food industries.

Materials and Methods

Collection of plant materials and preparation of extracts

The leaves of the herbaceous plants *C. verum* (*CV*) and *L. nobilis* (*LN*) were collected from spice markets in Riyadh; *N. oleander* (*NO*) was obtained from Riyadh Gardens, and *E. camaldulensis* (*EC*) from Al-Qassim. A taxonomist from the University of King Saud's Department of Botany verified the botanical identity of the plants after placing the voucher specimens in the herbarium. The leaves of the plant materials (*CV*, *NO*, *LN*, and *EC*) were dried at room temperature. A laboratory mill was used to grind the dried samples into a fine powder. Fifteen grams of each powdered component were extracted separately using methanol.

Preparation of the extract

Forty grams of the powdered components were extracted separately using 70% methanol, with a solid-to-liquid ratio of 1:5. The mixtures were then kept on a shaker for 3 days at +4 °C. The resulting mixtures were filtered through Whatman filter paper. The filtrates were concentrated and dried using a rotary evaporator at 50 °C (Yamato RE300, Japan) until a thick, dry substance was formed, following Yang's method (Yang *et al*., 2014). Distilled water was used to dissolve the powder for laboratory experiments.

α-Glycosidase inhibition studies

The leaf extract of *E. camaldulensis* has been shown to contain several advantageous properties, including antioxidant and antimicrobial effects (Mahmoud Dogara *et al*., 2024). To achieve this objective, a range of concentrations of WEC and EEC were introduced into phosphate buffer, which had a volume of 75 microliters and a pH of 7.4. Next, twenty microliters of α-glucosidase solution were added to the specified buffer, and the mixture was incubated for ten minutes. A 50 µL aliquot of p-nitrophenyl-D-glycopyranoside (p-NPG) was then added to the final mixture. The mixture was re-incubated at the physiological temperature of 37 °C, and absorbance was determined by spectrophotometric analysis at a wavelength of 405 nm.

α-Amylase activity

According to Visvanathan *et al.* (2020), the inhibitory effects of *E. camaldulensis* extract (EEC) on the α-amylase enzyme were tested at a specific concentration. Briefly, 1 g of starch was dissolved in 40 mL of a 0.4 M alkaline solution, and the mixture was heated at 80 °C for 30 minutes. After the solution was completely cooled and the pH adjusted to 6.9, deionized water was added to bring the total volume to 100 milliliters. Then, mixtures containing varying concentrations of EEC and an equal volume (35 µL) of starch and phosphate buffer (pH 6.9) were combined. Next, 20 µL of α-amylase solution was added to the final mixture, which was incubated at 30 °C for one hour. Finally, the reaction was quenched by adding 50 µL of hydrochloric acid (0.1 M), and the absorbance was measured at 580 nm.

Calculating the phenolic contents

The Folin–Ciocalteu technique (Nikolaeva *et al*., 2022) was used to determine the total phenolic content of each extract. Briefly, 2.5 mL of Folin–Ciocalteu reagent (10% w/v) was mixed with 1 mL of extract solution at concentrations ranging from 100 to 500 µg/mL. After 5 minutes, 2.0 mL of 75% Na_2CO_3 was added, and the mixture was incubated for 10 minutes at 50 °C with intermittent stirring. Subsequently, the sample was cooled before measuring its absorbance at 765 nm using a UV spectrophotometer (Shimadzu, UV-1800), compared to a blank sample without any extract. The results were expressed as milligrams of gallic acid equivalents per gram (mg GAE/g) of dry extract.

Calculating the tannin contents

The method described by Sharma *et al.* (2021) was used to determine the total tannin content (TTC). The procedure combined 1.5 mL of purified Milli-Q water, 0.1 mL of Folin–Ciocalteu phenol reagent, and 0.1 mL of the extracted samples. The mixture was allowed to stand for 8 minutes. Afterwards, the solution was neutralized by adding 0.3 mL of 30% Na_2CO_3. Subsequently, the components were mixed thoroughly and incubated at ambient temperature for 20 minutes in the dark. Absorbance was measured at 700 nm. The tannin content was calculated and expressed as milligrams per gram of dry weight (DW).

Calculating the flavonoid contents

The Dowd technique was used to measure the flavonoid concentration in each extract (Nikolaeva *et al*., 2022). A solution containing 0.2 mL of $AlCl_3$ in methanol (10% w/v), 0.2 mL of 1 M potassium acetate, and 5.6 mL of distilled water was mixed with either 1 mL of extract solution or 1 mL of quercetin, both with concentrations ranging from 25 to 200 µg/mL. After allowing the mixture to stand at room temperature for 30 minutes, absorbance was measured at 415 nm relative to the blank. The results were expressed as milligrams of quercetin equivalents per gram (mg QE/g) of dry extract.

DPPH radical scavenging assay

The free radical scavenging activity of the extracts was evaluated using the DPPH radical scavenging assay, following methodologies established by Murshed *et al.* (2024). This assay measures the ability of plant extracts to donate hydrogen atoms by decolorizing a methanol solution of DPPH (2,2-diphenyl-1-picrylhydrazyl). The violet/purple color of DPPH fades to yellow upon interaction with antioxidants. A 0.1 mM solution of DPPH in methanol was prepared and mixed with varying concentrations (12.5–150 µg/mL) of extract in methanol (2.4 mL DPPH solution combined with 1.6 mL extract).

The reaction mixture was vortexed thoroughly and incubated in darkness at room temperature for 30 minutes before measuring absorbance at 517 nm using a spectrophotometer. Butylated hydroxytoluene (BHT) served as the reference compound.

The percentage DPPH radical scavenging activity was calculated using:

$$\%\ \text{DPPH Radical scavenging activity} = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 represents the absorbance of the control, and A_1 is that of the extract or standard. The percentage inhibition versus concentration graph was used to calculate the IC_{50} value; experiments were performed in triplicate at each concentration.

ABTS radical cation decolorization assay

In this assay, color loss is measured when an antioxidant interacts with $ABTS^+$ radical cations, converting them into ABTS and causing decolorization. The method described by Kut *et al.* (Hirsch *et al*., 2022) outlines the assessment of antioxidant activity through this process. ABTS radical cations are generated by combining potassium persulfate (2.45 mM) with an aqueous stock solution of ABTS (7 mM). The working solution is prepared by mixing equal volumes of both stock solutions and incubating them for 16 hours at 25 °C in darkness before dilution with methanol to achieve an absorbance of 0.70 ± 0.2 at 734 nm, measured by spectrophotometry. Fresh solvent was used for each experiment, with Trolox as the antioxidant standard. The calibration curve included concentrations ranging from 0 to 500 µM. In test tubes, diluted samples (1 mL) were mixed with an equal volume of $ABTS^+$ radical cation solution, and absorbance was measured after seven minutes at 734 nm to calculate Trolox equivalent antioxidant capacity (TEAC), expressed as Trolox equivalents (µM).

Evaluation of cytotoxicity (MTT Assay)

Cell culture

The Hep-G2/2.2.15 human hepatoblastoma cell line was obtained from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany). The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) in a humidified atmosphere containing 5% CO_2 at 37 °C.

An MTT assay (3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide, cat#475989-1GM, Sigma-Aldrich, Germany) was used to assess the cytotoxicity of the plant extract *V. vinifera*. Briefly, cells were plated at a density of 5×10^4 cells/mL in a 96-well culture plate and incubated for 24 h. The cells were then treated with extract concentrations of 0, 2.5, 5, 10, 25, 50, and 100 µg/mL. Doxorubicin was used as a positive control. After 48 h of incubation, 10 µL of MTT solution (5 mg/mL in phosphate-buffered saline, PBS) was added to each well. Subsequently, 100 µL of acidified isopropanol was added to dissolve the formazan crystals. The plate was shaken for 10 minutes, and absorbance was measured at 570 nm using a microplate reader (BioTek, USA). Cell viability (%) was calculated as follows:

$$\text{Cell Viability (\%)} = \text{Mean absorbance}\frac{\text{treated cells}}{\text{untreated cells}}$$

The IC_{50} values (concentration of extract causing 50% inhibition) were determined from the dose-response curve of cell viability percentage using OriginPro software.

Statistical analysis

The experiment outcomes represent the average of three duplicate analyses. The mean ± standard deviation of the experimental data was calculated and analyzed. One-way analysis of variance (ANOVA) was performed. Duncan's multiple-range test was used to identify significant differences among means. Significance was defined as $P < 0.05$, and highly significant as $P < 0.01$.

Results and Discussion

Regulating blood glucose levels is a considerable challenge for individuals with diabetes. Inhibition of carbohydrate-digesting enzymes, such as α-amylase and α-glucosidase, is an effective strategy. α-Amylase catalyzes the hydrolysis of (1,4) glycosidic linkages in complex carbohydrates, such as starch or glycogen (Kaur *et al*., 2025), whereas α-glucosidase catalyzes the final step in carbohydrate hydrolysis (Lu *et al*., 2023). Consequently, the inhibitor acarbose was developed to reduce blood glucose levels in patients with diabetes mellitus. Novel and safe inhibitors derived from natural sources could serve as effective therapies for Alzheimer's disease (AD), skin disorders (SD), and diabetes mellitus (DM) (Mohd Zaid, 2023).

The inhibitory activities of *C. verum*, *N. oleander*, *L. nobilis*, and *E. camaldulensis* extracts against α-amylase and α-glucosidase were tested using a microplate reader (Table 1). The extracts exhibited inhibitory effects on the enzymes tested. Generally, methanolic extracts showed vigorous activity. Batiha *et al*., (2020) and

Chandorkar *et al*., (2021) reported similar results for *C. verum*, *N. oleander*, *L. nobilis*, and *E. camaldulensis* extracts and other plant extracts. These findings suggest that organic solvents may be suitable for enzyme inhibitory assays. Additionally, the enzyme-inhibitory activities of the extracts varied significantly according to the plant species used.

Table 2 presents the findings. All extracts showed inhibitory potential against the tested enzymes. The methanolic extracts of *L. nobilis* and *N. oleander* exhibited stronger inhibition of α-amylase activity than the other extracts, with values of 0.586 ± 0.015 and 0.564 ± 0.0001 mmol acarbose/g extract, respectively. *C. verum* and *E. camaldulensis* showed lower inhibition values of 0.230707 ± 0.017 and 0.369 ± 0.006 mmol acarbose/g extract, respectively (Figure 1).

Additionally, methanolic extracts of *N. oleander* and *L. nobilis* demonstrated stronger inhibition of α-glucosidase activity than the other extracts, with values of 8.242 ± 0.113 and 7.570 ± 0.107 mmol acarbose/g extract, respectively. In comparison, *C. verum* and *E. camaldulensis* showed lower inhibition values of 2.0476 ± 0.0468 and 1.4668 ± 0.08 mmol acarbose/g extract, respectively (Figure 2). Christoforidi *et al*., (2022) and Mutlu-Ingok *et al*., (2022) reported similar findings for some of these and other plant extracts. These results suggest that organic solvents may be appropriate for enzyme inhibitory assays.

The lowest α-amylase and α-glucosidase inhibitory potentials were observed in *C. verum* and *E. camaldulensis*, with values of 0.231 ± 0.02 and 1.466 ± 0.08, and 0.369_4 ± 0.006 and 2.048 ± 0.05 mmol acarbose/g extract, respectively.

The levels of total phenolics varied among the extracts. The highest phenolic content was observed in the methanolic extract of *E. camaldulensis* at 63.56 mg GAE/g of

Table 1. Antioxidant Activity of Concentrations with of line DPPH IC_{50} assay of extracts.

Concentration	DPPH Inhibition%			
	C. verum	*N. oleander*	*L. nobilis*	*E. camaldulensis*
3.90625	11.5195±1.264	26.8488±1.271	19.9637±1.98	43.2092±0.35
7.8125	24.5896±0.915	28.7020±0.684	28.0847±0.89	58.5385±0.24
15.625	56.0706±0.575	34.0528±0.467	63.0643±0.62	81.7900±0.046
31.25	87.6555±0.473	47.3232±0.766	76.3367±0.34	88.6049±0.47
62.5	89.7128±0.359	76.6450±0.443	83.9492±0.54	90.7654±0.49
125	90.6381±0.125	90.7401±0.275	86.4187±0.77	87.0123±0.44
250	90.1237±0.241	91.56246±0.545	84.7739±0.52	84.0806±0.66
500	89.0955±0.358	90.6394±0.611	82.0980±0.53	80.3888±0.24
IC_{50} (µg/mL)	13.9511±0.35	41.3920±0.29	12.5985±0.51	8.8355±0.037

CV: *Cinnamomum verum,* NO: *Nerium oleander,* LN: *Laurus nobilis,* EC: *Eucalyptus camaldulensis*.

Table 2. Antioxidant Activity of Concentrations with of line ABTS IC_{50} assay of extracts

Concentrations	ABTS Inhibition%			
	C. verum	*N. oleander*	*L. nobilis*	*E. camaldulensis*
3.90625	8.4059±1.129	4.5216±0.184	13.3306±1.424	13.099
7.8125	13.9820±0.205	12.4646±0.907	28.0875±1.063	17.40264
15.625	48.3392±0.882	18.8579±0.667	47.2369±1.848	43.50587
31.25	68.7187±0.228	36.8302±0.808	67.2966±1.351	63.3263
62.5	86.0181±0.205	57.3372±0.793	79.2940±0.706	83.36601
125	88.3877±0.345	85.4499±0.922	91.0747±0.857	86.12582
250	90.8760±0.349	94.9200±1.252	93.6141±0.381	88.87544
IC_{50} (µg/mL)	16.247±0.248	111.535±6.019	30.3200±3.218	38.6529±1.570

CV: *Cinnamomum verum,* NO: *Nerium oleander,*LN: *Laurus nobilis,* EC: *Eucalyptus camaldulensis*.

extract, followed by *L. nobilis* and *C. verum* with 57.397 and 54.324 mg GAE/g of extract, respectively. Conversely, the lowest phenolic content was recorded for *N. oleander* at 26.44 mg GAE/g of extract (Figure 3).

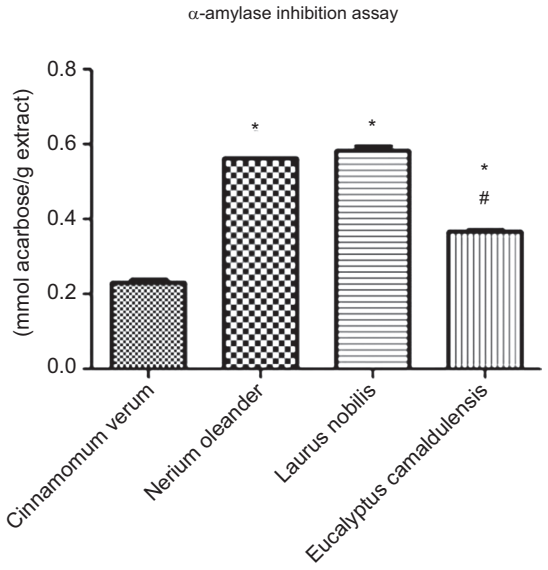

Figure 1. Enzyme inhibition α-amylase of *C. verum*, *N. oleander*, *L. nobilis*, and *E. camaldulensis* leaf extracts. *(*p*-value < 0.05) indicates significant differences in other plants compared to *Cinnamomum verum* plant. # (*p*-value < 0.05) indicates significant differences in *Eucalyptus camaldulensis* plant compared to other plants.

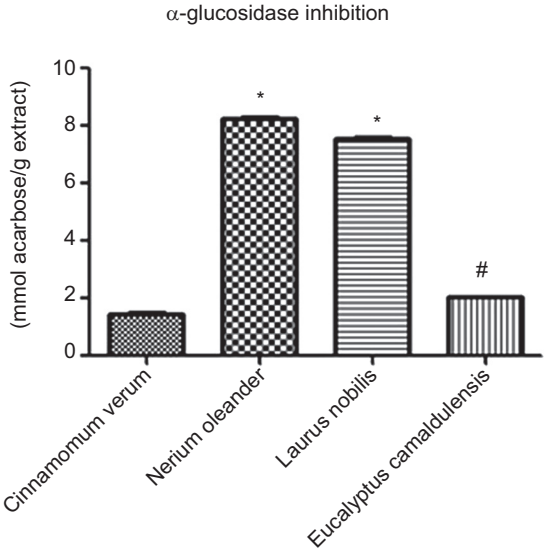

Figure 2. Enzyme inhibition α-glucosidase of *C. verum*, *N. oleander, L. nobilis,* and *E. camaldulensis* leaf extracts. *(*p*-value < 0.05) indicates significant differences in other plants in comparison to *Cinnamomum verum* plant. # (*p*-value < 0.05) indicates significant differences in *Eucalyptus camaldulensis* plant compared to other plants.

The differences found in the phenolic composition align with those reported in other comparable investigations (Limam *et al*., 2020). This outcome is likely due to the higher polarity of methanol, which enables a more efficient extraction of the highly polar antioxidant and phenolic compounds present in eucalyptus leaves (Johnson *et al*., 2020).

The phytochemical screening results in Figure 4 illustrate the presence of various components in the methanolic extracts. *C. verum*, *N. oleander*, *L. nobilis*, and *E. camaldulensis* extracts exhibited a weak to strong presence of essential phytochemicals. The levels of total tannins varied among the extracts. The highest tannin content was recorded at 34.80 mg GAE/g of extract for methanolic *E. camaldulensis*, followed by *L. nobilis* and *C. verum* with 30.84 and 29.37 mg GAE/g of extract, respectively. In contrast, the lowest tannin content was observed in *N. oleander* at 12.98 mg GAE/g of extract (Figure 4).

Tannins are associated with medicinal properties, including anti-inflammatory, antidiabetic, analgesic, and central nervous system effects (Omar *et al*., 2022). The identification of proteins, fatty acids, and carbohydrates highlights the nutritional value of cinnamon spice (*Cinnamomum verum*) (Al Dhaheri *et al*., 2023). Phytochemical screening of oleander (*Nerium oleander*) leaves confirms the presence of tannins. The phenolic and flavonoid content of oleander leaf extracts varied based on the polarity of the solvent (Redha, 2020). The tannin content recorded—12.98 mg GAE/g—is higher than the 10.89 mg/g DW found in the ethanolic extract of *Laurus*

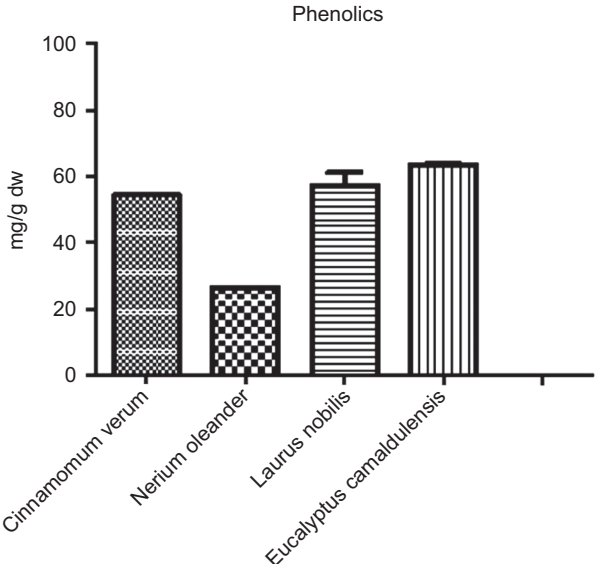

Figure 3. Total phenolics obtained, expressed as mg gallic acid/g of *C. verum, N. oleander, L. nobilis*, and *E. camaldulensis* leaf extracts.

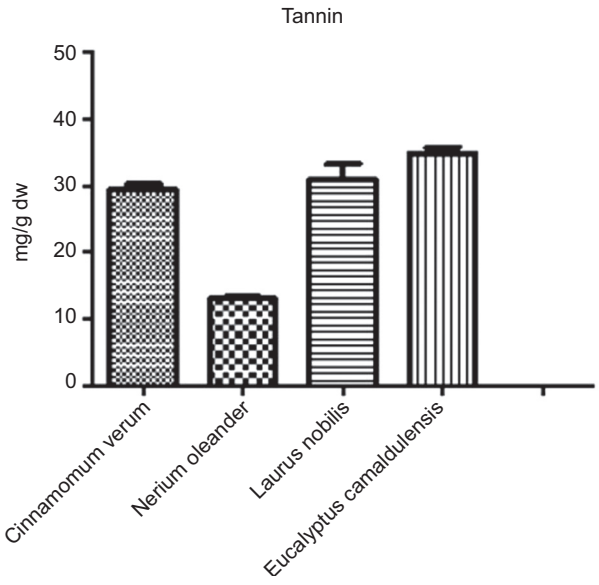

Figure 4. Total tannin obtained, expressed as mg gallic acid/g of *C. verum, N. oleander, L. nobilis,* and *E. camaldulensis* leaf extracts.

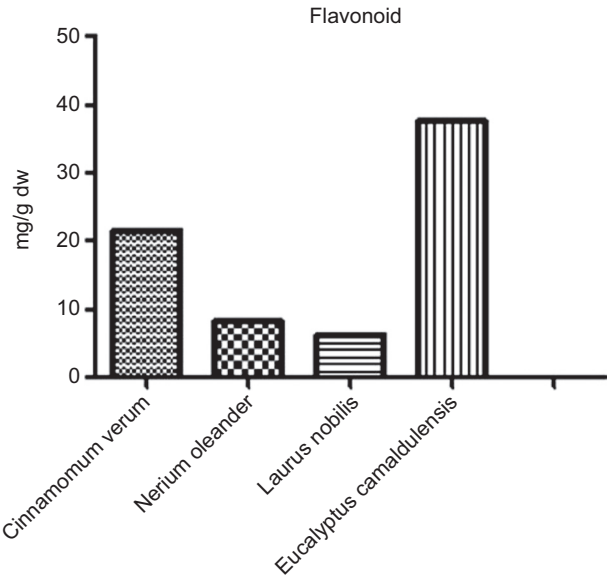

Figure 5. Total flavonoid was obtained and expressed as mg gallic acid/g of *C. verum, N. oleander, L. nobilis,* and *E. camaldulensis* leaf extracts.

nobilis leaves. Phytochemical screening of *Eucalyptus camaldulensis* also reveals a high tannin concentration in its leaves (Hussain *et al*, 2025).

The total flavonoid content varied across the extracts, as shown in Table 3. The results indicated that the methanolic extract of *Eucalyptus camaldulensis* had the highest flavonoid concentration, measuring 37.56 mg QE/g of extract. This was followed by *Cinnamomum verum*, with a flavonoid content of 21.51 mg QE/g of extract. Conversely, *Nerium oleander* and *Laurus nobilis* extracts exhibited the lowest flavonoid concentrations, measuring 8.13 and 6.09 mg QE/g, respectively (Figure 5). These findings are supported by several other studies (Ahmed *et al.*, 2020; Ayouaz *et al*., 2020).

C. verum, N. oleander, L. nobilis, and *E. camaldulensis* extracts exhibit antioxidant activity that can help protect against various diseases. In the current study, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was employed to evaluate the antioxidant potential of the extracts. Results showed that increasing the concentration of extracts enhanced their DPPH radical scavenging ability. At 500 µg/mL, the DPPH scavenging percentages were 89.10 ± 0.36, 90.64 ± 0.61, 82.10 ± 0.53, and 80.39 ± 0.24, respectively, with corresponding IC_{50} values of 13.95 ± 0.35, 41.39 ± 0.29, 12.60 ± 0.51, and 8.84 ± 0.04 µg/mL. For the ABTS assay at 250 µg/mL, scavenging percentages were 90.88 ± 0.35, 94.92 ± 1.25, 93.61 ± 0.38, and 88.88 ± 0.44, with IC_{50} values of 16.25 ± 0.25, 111.54 ± 6.02, 30.32 ± 3.22, and 38.65 ± 1.57 µg/mL, respectively (Tables 1 and 2). The radical-scavenging activity of the phenolic compounds is primarily responsible for the antioxidant effects observed in these plant extracts.

Laurus nobilis extracts have been reported to be rich in polyphenols. Our findings are consistent with those of Dobroslavic *et al*. (2022), who observed that L. nobilis extract exhibited significant antioxidant capacity, attributed to the high degree of hydroxylation in its phenolic compounds. This structural feature enhances the extract's ability to donate protons and stabilize free radicals such as DPPH. Notably, L. nobilis demonstrated strong antioxidant activity against DPPH radicals, as also reported by El Faqer *et al*. (2024). According to Stagos (2019), the antioxidant activity of these extracts is primarily driven by polyphenols acting as effective reducing agents.

The distinct chemical compositions—particularly in phenolics, tannins, and flavonoids—among *C. verum*, *N. oleander*, *L. nobilis*, and *E. camaldulensis* explain the observed variations in their enzyme inhibitory and antioxidant activities. While *L. nobilis* and *N. oleander* exhibited superior enzyme inhibition, *E. camaldulensis* consistently demonstrated higher total phenolic, tannin, and flavonoid contents, which contributed to its strong antioxidant capacity. These findings highlight the critical role of plant species in determining the therapeutic potential of natural extracts.

The superior antioxidant activity of *E. camaldulensis* and *L. nobilis*, demonstrated by their lower DPPH IC_{50} values

Table 3. Cytotoxicity (MTT) assay for testing of *C. verum, N. oleander, L. nobilis,* and *E. camaldulensis* extracts at different concentrations (µg/mL) against Hepatoblastoma (Hep-G2) after 48 h of incubation.

Concentration (µl/mL)	0	2.5	5	10	25	50	100	IC_{50} ± SD (µl/mL)
LNE	92.47028	91.65724	90.5328	84.72039	81.99031	61.55878	53.65728	137.48±12.30
CVE	83.4214	74.57067	75.38529	47.29194	38.71158	30.55923	26.7433	54.475±21.61
NOE	98.56787	91.82428	75.38556	64.95356	44.93201	36.31353	33.22215	44.20±0.0075
ECE	83.4214	74.57067	75.38529	47.29194	104.7116	30.55923	27.52976	54.475± 21.61

LNE: *Laurus nobilis,* CVE: *Cinnamomum verum,* NOE: *Nerium oleander,* ECE: *Eucalyptus camaldulensis extract*.

(8.83 and 12.50 µg/mL, respectively) and ABTS IC_{50} values (38.65 and 30.32 µg/mL), compared to *N. oleander* and *C. verum* (DPPH IC_{50}: 32.10 and 13.95 µg/mL; ABTS IC_{50}: 111.53 and 16.25 µg/mL), strongly correlates with their higher phenolic content. This finding aligns with established literature that links phenolic-rich extracts to enhanced radical scavenging capacity (Mohammed *et al.*, 2021; Ouattara *et al.*, 2024).

The Hep-G2/2.2.15 human hepatoblastoma cell line was treated with serial concentrations (0, 2.5, 5, 10, 25, 50, and 100 µg/mL) of methanolic extracts from *C. verum*, *N. oleander*, *L. nobilis*, and *E. camaldulensis* for 48 hours. The IC_{50} values for the extracts on the Hep-G2/2.2.15 cells were determined as 54.48 ± 21.61, 44.20 ± 0.0075, 137.48 ± 12.30, and 54.48 ± 21.61 µg/mL, respectively.

The superior α-amylase and α-glucosidase inhibitory activities observed in the methanolic extracts of *L. nobilis* and *N. oleander*—and to a lesser extent, *C. verum* and *E. camaldulensis*—are likely due to a complex interplay of their unique phytochemical compositions. While polyphenols such as flavonoids, tannins, and phenolic acids are consistently implicated in enzyme inhibition across many plants, other specialized metabolites also play significant roles. For instance, essential oil components like terpenoids and specific compounds such as cardiac glycosides in *N. oleander* contribute to the distinct inhibitory profiles and potency variations among these species. Further research focusing on the isolation and characterization of individual bioactive compounds from these potent extracts is essential to elucidate the precise mechanisms underlying their enzyme inhibitory effects.

Conclusions

This study underscores the significant therapeutic potential of *Cinnamomum verum*, *Nerium oleander*, *Laurus nobilis*, and *Eucalyptus camaldulensis* as rich sources of bioactive compounds. Methanolic extracts from these plants effectively inhibit α-amylase and α-glucosidase enzymes, with *L. nobilis* and *N. oleander* exhibiting particularly strong inhibitory activity, suggesting their promise as natural alternatives to synthetic inhibitors such as acarbose. Additionally, their potent antioxidant capacities, closely correlated with their phenolic content—especially notable in *E. camaldulensis* and *L. nobilis*—highlight their potential in mitigating oxidative stress-linked diseases. These findings open promising avenues for both industrial and clinical applications, positioning these plants as valuable natural antidiabetic agents that provide safer and more affordable options, particularly in developing regions. Furthermore, their antioxidant properties support the development of functional foods and dietary supplements aimed at preventing or alleviating chronic conditions such as cardiovascular diseases. The sustainability and widespread availability of these plant resources further enhance their appeal for pharmaceutical and food industry use, contributing to the advancement of a bioeconomy focused on natural product innovation.

Ethical Statement

The research was conducted in accordance with the "Guide for the Care and Use of Laboratory Animals." The study complied with the institutional guidelines for the use of animals or humans at King Saud University and met the standards set by the National Committee of Bio-Ethics (NCBE) in Saudi Arabia. The Royal Decree numbered M59 was issued on 14/9/1431H. The Research Ethics Committee of King Saud University (Approval No. KSU-Se-21-78) sanctioned all experimental methods.

Data Availability

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

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Author Contributions

Conceptualization, M.M. and J.T.; methodology, S.Q.; software, M.M.; validation, S.Q., J.T.; formal analysis, J.T.; investigation, M.M.; resources, M.M.; data curation, J.T.; writing—original draft preparation, S.Q.; writing—review and editing, M.M.; visualization, J.T.; supervision, S.Q.; project administration, M.M.; funding acquisition, M.M. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

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