BOLETIN LATINOAMERICANO Y DEL CARIBE DE PLANTAS MEDICINALES Y AROMÁTICAS
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Articulo Original / Original Article

In vitro evaluation of α-amylase and α-glucosidase enzyme, anti-coccidial and antioxidant activities of *Laurus nobilis* leaf extract

[Evaluación in vitro de la enzima α-amilasa y α-glucosidasa, actividades anti-coccidiales y antioxidantes del extracto de hoja de *Laurus nobilis*]

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Section Biological activity

Received: 9 August 2024
Accepted: 7 December 2024
Accepted corrected: 12 April 2025
Published: 30 July 2025

Citation:
Murshed M, Al-Tamimi J, Aljawdah HMA, Al-Quraishy S. *In vitro* evaluation of α-amylase and α-glucosidase enzyme, anti-coccidial and antioxidant activities of *Laurus nobilis* leaf extract
Bol Latinoam Caribe Plant Med Aromat
24 (4): 644 - 657 (2025)
https://doi.org/10.37360/blacpma.25.24.4.45

Abstract: Traditional medicine has long utilized medicinal plants to treat various diseases, leveraging their antioxidant and anticoccidian properties. This study investigates the potential effects of *Laurus nobilis* extract in inhibiting *Eimeria magna* oocysts, alongside its inhibitory capabilities on α-amylase and α-glucosidase enzymes. The research employed Fourier Transform Infrared Spectroscopy (FTIR) to identify functional groups within the extract, alongside assays for α-amylase and α-glucosidase inhibition, total phenolics, tannins, total flavonoids, and antioxidant activity using DPPH and ABTS tests. Results revealed the presence of 14 biologically active compounds. The extract exhibited significant inhibitory activity on α-amylase and α-glucosidase enzymes, with inhibition values of 2.047586 ± 0.05 and 0.369396 ± 0.00637, respectively. Additionally, the extract demonstrated high levels of phenolics, flavonoids, and tannins, correlating with a notable antioxidant capacity as assessed by DPPH and ABTS assays. Importantly, the *Laurus nobilis* extract proved effective against E. magna oocysts, highlighting its potential as an antioxidant and anticoccidial.

Keywords: *Laurus nobilis*; α-glucosidase; Anti-inflammatory effect; α-amylase enzyme; Traditional medicine.

Resumen: La medicina tradicional ha utilizado durante mucho tiempo plantas medicinales para tratar diversas enfermedades, aprovechando sus propiedades antioxidantes y anticoccidianas. Este estudio investiga los efectos potenciales del extracto de *Laurus nobilis* en la inhibición de ooquistes de *Eimeria magna*, junto con sus capacidades inhibitorias sobre las enzimas α-amilasa y α-glucosidasa. La investigación empleó espectroscopia infrarroja por transformada de Fourier (FTIR) para identificar grupos funcionales dentro del extracto, acompañada de ensayos para la inhibición de α-amilasa y α-glucosidasa, cuantificación de fenoles totales, taninos, flavonoides totales y actividad antioxidante mediante pruebas DPPH y ABTS. Los resultados revelaron la presencia de 14 compuestos biológicamente activos. El extracto mostró una actividad inhibitoria significativa sobre las enzimas α-amilasa y α-glucosidasa, con valores de inhibición de 2.047586 ± 0.05 y 0.369396 ± 0.00637, respectivamente. Además, el extracto demostró altos niveles de fenoles, flavonoides y taninos, correlacionándose con una notable capacidad antioxidante evaluada mediante ensayos DPPH y ABTS. Es importante destacar que el extracto de *Laurus nobilis* resultó efectivo contra los ooquistes de *E. magna*, resaltando su potencial como antioxidante y anticoccidial.

Palabras clave: *Laurus nobilis*; α-glucosidasa; Efecto antiinflamatorio; Enzima α-amilasa; Medicina tradicional.

INTRODUCTION

Traditional medicine remains a cornerstone of healthcare in many developing countries, where medicinal plants play a vital role in treating various ailments. Among these, *Laurus nobilis* (LN), commonly known as "sweet bay" or "bay laurel," is widely recognized for its therapeutic applications, including the treatment of liver disorders, diabetes, rheumatism, inflammation, indigestion, and the common cold (Sharififar *et al*., 2009; Shah & Shah, 2015; Zabihi *et al*., 2018). Additionally, LN has been used historically to alleviate pain associated with coughing, miscarriage, and pregnancy. Medicinal plants, including L. nobilis, are rich sources of bioactive compounds such as flavonoids, phenols, tannins, carotenoids, thiols, and anthocyanins. These compounds exhibit diverse pharmacological properties, including hypoglycemic, anti-inflammatory, hepatoprotective, antifungal, antibacterial, and hypolipidemic effects (Bahramikia & Yazdanparast, 2012; Qabaha, 2013; Gbenou *et al*., 2013). Despite the widespread use of plant-based remedies, only a fraction of medicinal plants has undergone comprehensive phytochemical and pharmaceutical analysis. Laurus nobilis belongs to the Lauraceae family, a group of aromatic angiosperms native to the Mediterranean region but now cultivated worldwide for culinary, medicinal, and ornamental purposes (Oliviera *et al*., 2009; Paparella *et al*., 2022). It is an evergreen shrub or tree with smooth, aromatic green leaves commonly used in cooking and traditional medicine. The plant is distinguished by its biological functionality, largely attributed to its extracts and essential oils. These have demonstrated antifungal (Caputo *et al*., 2017), antiviral (Simić *et al*., 2004), antibacterial (Derwich *et al*., 2009), acaricidal (Sırıken *et al*., 2018), and insecticidal (Fernandez *et al*., 2020) properties, among others. Extracts have also been used to treat helminths and other parasitic infections. In traditional medicine, *L. nobilis* leaves have been employed to treat neurological conditions such as epilepsy, neuralgia, and Parkinson's disease (Alizadeh *et al*., 2010; Caputo *et al*., 2017). The leaves and fruits are known for their aromatic, stimulating, and narcotic qualities (Abu-Dahab *et al*., 2014). Laurel extracts and essential oils have shown significant antibacterial and antioxidant activities (Santoyo *et al*., 2006; Derwich *et al*., 2009; Ozcan *et al*., 2010), and the leaves are traditionally used to alleviate gastrointestinal discomfort, including bloating and flatulence (Qnais *et al*., 2012). Additionally, the essential oil is widely used in the perfume and soap industries (Kosar *et al*., 2005) and has been utilized to treat hemorrhoids, rheumatic pain, and fungal infections (Alizadeh *et al*., 2010; Patrakar *et al*., 2012; Caputo *et al*., 2017). The present study explores the chemical composition of *L. nobilis* leaves, focusing on their inhibitory effects on α-amylase and α-glucosidase enzyme activities, antioxidant properties, and the content of total phenolics, flavonoids, and tannins. Furthermore, the study investigates the plant's anticoccidial activity against *Eimeria* oocysts in rabbits, highlighting its multifaceted therapeutic potential.

MATERIAL AND METHODS

Plant material and extraction:

Laurus nobilis leaves were sourced from the spice markets of Riyadh, Saudi Arabia. The botanical identity of the plant was authenticated by a taxonomist from the Department of Botany at King Saud University, with identification number 24649. The leaves were dried and finely ground into a powder. A 50 g portion of the powdered leaves was mixed with 80% methanol and placed on a shaker for three days to facilitate extraction. The resulting mixture was filtered using Whatman filter paper, and the filtrate was concentrated and dried using a rotary evaporator at 50°C (Yamato RE300, Japan) until a thick, dry residue was obtained, following the method described by Yang *et al*. (2014). The dried extract was reconstituted in distilled water for use in laboratory experiments.

Infrared spectroscopy

The dried extract powder was mixed with potassium bromide (KBr) and analyzed using Fourier Transform Infrared (FTIR) spectroscopy (Thermo Fisher Scientific, Waltham, MA, USA) to identify the bioactive components of *Laurus nobilis* leaves. The analysis focused on detecting and evaluating classes of compounds based on the presence of characteristic IR spectral bands. Spectral data were recorded for functional group identification within the resolution range of 400 to 4000 cm^{-1}.

In vitro α-amylase inhibition assay

The α-amylase inhibition assay was performed following the method described by Ali *et al*. (2006). Pig pancreatic α-amylase was prepared at a concentration of 4 U/mL by dissolving it in a sodium phosphate buffer (100 mM, pH=6.9). Potato starch (0.5% w/v) in the same buffer was used as the substrate. To conduct the assay, 250 µL of the sample or control was mixed with 250 µL of the enzyme solution and incubated for 5 minutes. Next, 250 µL of the starch solution was added, and the reaction mixture was incubated at 25°C for 15 minutes. The reaction was terminated by adding 500 µL of a dinitrosalicylic acid (DNS) color reagent, which contained 96 mM 3,5-dinitrosalicylic acid, 5.31 M sodium potassium tartrate, and 2 M NaOH. The mixture was then heated in an 85°C water bath for 20 minutes. After cooling, the absorbance of the reaction mixture was measured at 540 nm. Results were expressed as millimoles of acarbose equivalents per gram of dry extract (ACAEs/g extract).

In vitro α-glucosidase inhibition assay

The α-glucosidase inhibition assay was conducted following the method of Liu *et al*. (2013), with slight modifications. In this essay, 50 µL of extract solution at varying concentrations was mixed with 67 mM sodium phosphate buffer. Subsequently, 5 µL of α-glucosidase enzyme (2 U/mL) was added to the mixture. The reaction mixture was pre-incubated for 10 minutes at 37°C. The enzymatic reaction was initiated by adding 50 µL of 5 mM p-nitrophenyl-α-D-glucopyranoside (PNPG) as the substrate. The mixture was incubated at 37°C for 20 minutes. The reaction was terminated by adding 1 mL of 0.2 mM Na_2CO_3 solution. The activity of α-glucosidase was assessed by measuring the absorbance of the produced p-nitrophenol at 405 nm. The results were expressed as millimoles of acarbose equivalents per gram of dry extract (ACAEs/g extract).

Radical scavenging of DPPH

The antioxidant activity of the leaf extract was tested by 10 µg/mL added to a solution of DPPH containing 0.004% methanol. Following a 30-minute incubation period at room temperature, the absorbance of the samples was measured at a wavelength of 521 nm. Triple checks were performed on every concentration. Based on the following formula, the inhibitory of free radical scavenging activity was determined:

$$\text{Inhibition} = A_{bs1} - A_{bs2}/A_{bs1} * 100$$

where A_{bs1} (the absorbance of the negative),
A_{bs2} (the absorbance of the positive)

A graph displaying percentage inhibition versus extract concentration was used to calculate the concentration of the extract that causes 50% inhibition (IC_{50}). To create a calibration curve, Trolox was employed as a reference at concentrations ranging from 0-100 µg. DPPH radical-scavenging activities were measured in µg Trolox equivalents per mL of plant extract.

Evaluation of the entire antioxidant capacity

The total antioxidant activity (TAC) was determined by 0.15 mL of leaf extract combined with 1.5 mL of reagent solution that had 4 mM of ammonium molybdate, 28 mM of sodium phosphate, and 0.6 M of sulfuric acid. After that, the reaction mixture was kept warm for 90 minutes at 95°C. 695 nm was found to be the optical density of the samples when they were measured. The total antioxidant capacity was given the unit of measurement of milligrams of ascorbic acid equivalence (AAE) per gram of dry weight (DW).

$$\%\text{Inhibition} = [(A \text{ control} - A \text{ sample})/A \text{ control}] \times 100$$

The EC_{50} values were derived from the graph, which depicts the sample concentration that was necessary to scavenge 50% of either the ABTS or DPPH free radicals. These values were

determined. The EC_{50} value is frequently used to represent the number of extracts required at a certain concentration to scavenge 50% of the free radicals. ABTS and DPPH were both expressed in mg of GAE/L.

Calculating the overall content of phenols

The total phenolic content of the infusion was determined following the method described by Li *et al*. (2007). In brief, 0.50 mL of the extract, diluted tenfold with water, was combined with 2.5 mL of Folin-Ciocalteu reagent diluted 1:10 with water. After allowing the mixture to react for 4 minutes, 2 mL of a saturated sodium carbonate solution (approximately 75 g/L) was added. The mixture was incubated at room temperature for 2 hours, and its absorbance was measured at 760 nm. Gallic acid served as the reference standard, and the results were expressed as grams of gallic acid equivalent (g GAE) per liter of extract.

Calculating the overall content of the Tannin

The total tannin content (TTC) of the plant material was determined as follows: 0.1 mL of the extracted sample was combined with 1.5 mL of Milli-Q water and 1 mL of diluted Folin-Ciocalteu reagent. Subsequently, 0.8 mL of 7.5% sodium bicarbonate ($NaHCO_3$) solution was added. The mixture was incubated at 45°C for 45 minutes. After thorough mixing, the solution was stored in a dark space at room temperature for 20 minutes. The absorbance was then measured at 700 nm. The total tannin content was calculated and expressed as milligrams of tannic acid equivalent per gram of dry weight (mg TAE/g DW).

Determining the total amount of flavonoids

The flavonoid content was determined using the following method: 1 mL of the extract was mixed with 4 mL of distilled water, followed by the addition of 0.3 mL of 10% $AlCl_3$ and 0.3 mL of 5% $NaNO_2$. After 6 minutes, 2 mL of 1 N NaOH and 2.5 mL of distilled water were added to the mixture. The absorbance was measured at 510 nm, and the flavonoid content was calculated in milligrams of catechin equivalent per gram of dry weight (mg catechin/g DW). A calibration curve using various concentrations of catechin was prepared to determine the results.

Anticoccidial activity

Test of Eimeria oocysts

The *Eimeria magna* parasite was isolated from naturally infected rabbits, with its vitality maintained through successive passages in rabbits in the parasitology laboratory. In the experiment, 1×10^4 *E. magna* oocysts were incubated in 2 mL of potassium dichromate solution containing LNLE at concentrations of 5, 10, 20, 40, 80, and 150 mg/mL. Potassium dichromate solution ($K_2Cr_2O_7$) without LNLE was used as the control. Each treatment was performed in triplicate. The oocysts were incubated in six-well plates at 28°C with 70% relative humidity. The plates were semi-covered and shaken at regular intervals following the method of Lopez-Osorio *et al*. (2022). Observations of sporulated and non-sporulated oocysts were recorded at 24, 48, 72, and 96 hours (Murshed *et al*., 2022). A 5 µL sample of the solution was examined under a light microscope (BX51TF, OLYMPUS, Tokyo, Japan) at 40x magnification. Sporulation and inhibition of oocysts were assessed using a McMaster chamber and light microscopy. The percentages of sporulated and non-sporulated oocysts were calculated using the following formulas:

Sporulation % = Sporulated oocysts / Total oocysts *100
(Elsayed *et al*., 2014)

Inhibition %=sporulation of control-sporulation of medicine/sporulation of control*100
(Elsayed *et al*., 2014)

Statistical Analysis

The standard deviations (SD) of the given data in the tables and figures are the average across three replicates. The means of the separation and significant level determination at (p<0.05) were determined using Duncan's test, a one-way analysis

of variance (ANOVA), and SPSS software.

RESULTS

The FT-IR analysis and phytochemical examination of alcoholic extracts from *L. nobilis* leaves revealed 14 active chemical constituents, including phenols, flavonoids, and tannins, mostly responsible for anti-coccidia efficacy. Table No. 1 displays the absorption spectra of dried plant leaf extract obtained in the range of 4000–400 cm^{-1}. The spectra exhibit distinctive peaks (Figure No. 1).

Table No. 1
FT-IR spectra analysis of dried *L. nobilis* plant leaf extracts

Frequency (cm^{-1})	Peak Details	Transmittance (%)	Functional Group	Compound class
3409.71	medium, sharp	21	O-H stretching	alcohol
2927.1	weak, broad	29	O-H stretching	alcohol
1712.53	Strong	36	C=O stretching	aliphatic ketone
1606.44	medium	24	C=C stretching	aliphatic ketone
1515.18	strong	31	N-O stretching	nitro compound
1450.96	medium	28	C-H bending	Alkane
1368.61	strong	38	O-H bending	carboxylic acid
1270.36	strong	37	C-N stretching	aromatic amine
1152.72	strong	32	C-O stretching	aliphatic ether
1122.52	strong	26	C-O stretching	secondary alcohol
1066.69	strong	28	C-O stretching	primary alcohol
1033.40	strong	23	S=O stretching	Sulfoxide
818.35	medium	54	C=C bending	Alkene
795.06	medium	58	C-H bending	1,4-disubstituted
596.17	strong	63	C-Cl stretching	halo compound

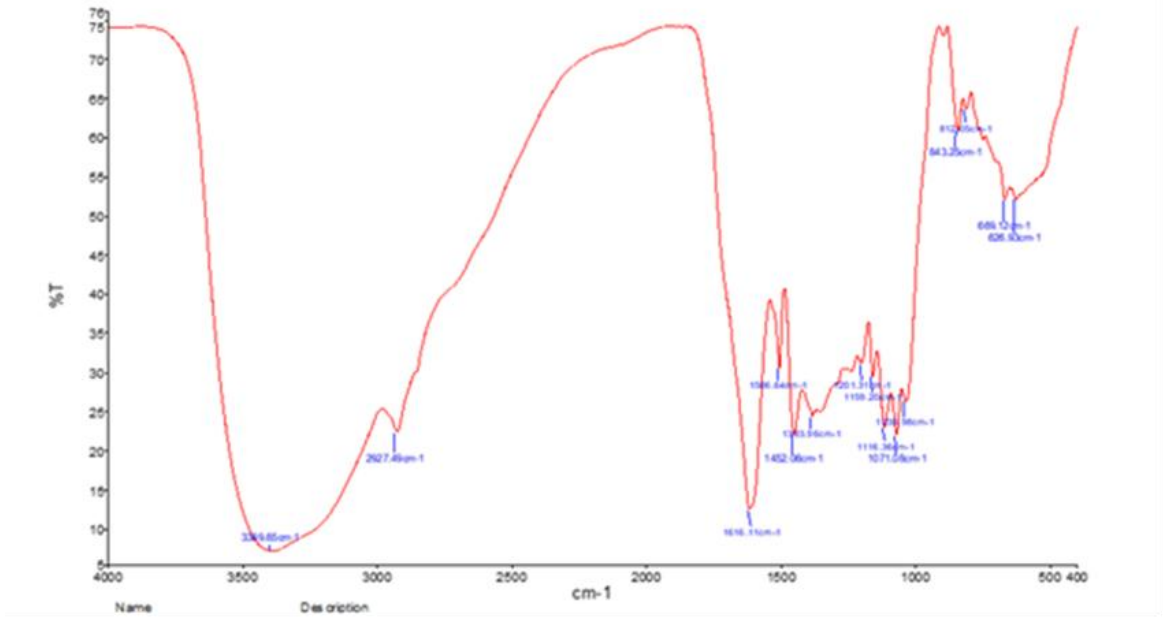

Figure No. 1
FT-IR spectrum of *L. nobilis* extracts based on the frequency range

α-amylase inhibition

α-amylase and α-glucosidase enzyme inhibitory activities of *L. nobilis* were tested in this study. The extract has remarkable enzyme inhibitory potentials. A significant difference in the potential inhibition against α-amylase and α-glucosidase (p<0.05) was observed (Table No. 2). For the herbal infusions studied, the inhibitory activities toward α-amylase were in a wide range of 7.57 ± 0.107.

α-glucosidase inhibition

Although the herbal plant appeared to have anti-α-glucosidase activities, our experiments showed that the studied herbal infusions are a weak inhibition against α-glucosidase. Figure No. 2 shows the α-glucosidase inhibition 0.59 ± 0.015.

Table No. 2
The enzyme inhibitory activity of a methanolic extract obtained from *Laurus nobilis* leaves

Extract	Solvent	Enzyme	(mmol acarbose/g extract)
Laurus nobilis	Methanol	α-amylase	7.57 ± 0.107
		α-Glucosidase	0.59 ± 0.015

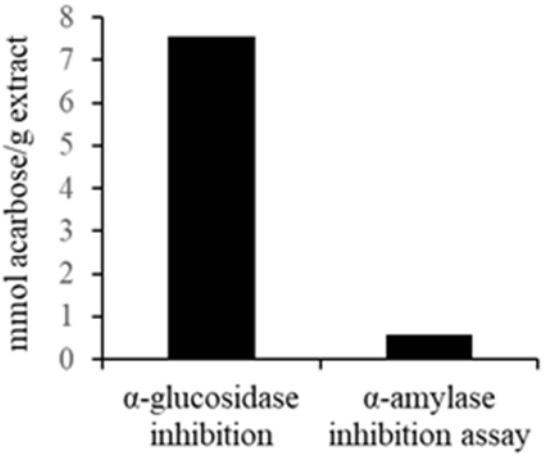

Figure No. 2
Potentials of *Laurus nobilis* leaf extract for α-amylase and α-Glucosidase enzyme inhibitory

Assessing the phenolic, tannin, and flavonoid content

In this study, the total phenolic content (TPC), total tannin content (TTC), and total flavonoid content (TFC), in the *L. nobilis* leaf extracts were estimated (Figure No. 3). The obtained results showed that the methanolic extract exhibited the highest amount of TPC (160.217 ± 0.652 mg/g DW), followed by TTC (65.872 ± 0.263 mg/g DW), while TFC (34.0463 ± 0.545 mg/g DW).

Laurus nobilis

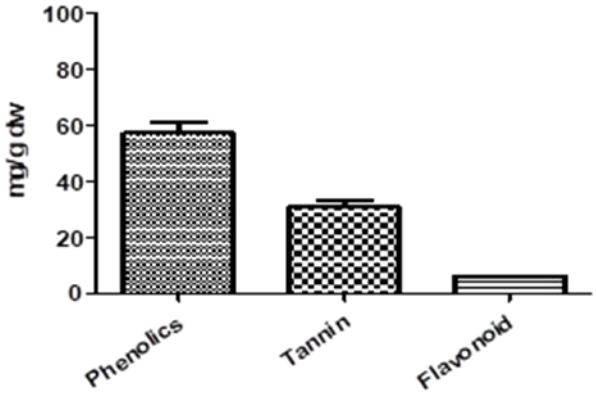

Figure No. 3
Flavonoids, tannin, and total polyphenols in the leaves methanolic extract of the *Laurus nobilis* leaf extracts plant

The results revealed significant differences (p<0.05) in the DPPH assay across various concentrations (Figure No. 4). The herbal *L. nobilis* extract exhibited a mean DPPH inhibition of 19.964 ± 2.42 at 3.91 mg/mL and 82.098 ± 0.65 at 500 mg/mL, demonstrating a 20-fold variation in its effectiveness. In the ABTS radical scavenging assay, a positive correlation was observed between the concentration of the methanolic extracts and their radical scavenging activity. The highest concentrations of *L. nobilis* samples demonstrated greater ABTS radical scavenging activity compared to the lower concentrations (Figure No. 5). The extract at 500 mg/mL showed the highest free radical scavenging capacity (93.614 ± 0.467 mg/mL), while the lowest concentration (7.81 mg/mL) exhibited the weakest scavenging capacity (13.33 ± 1.745 mg/mL).

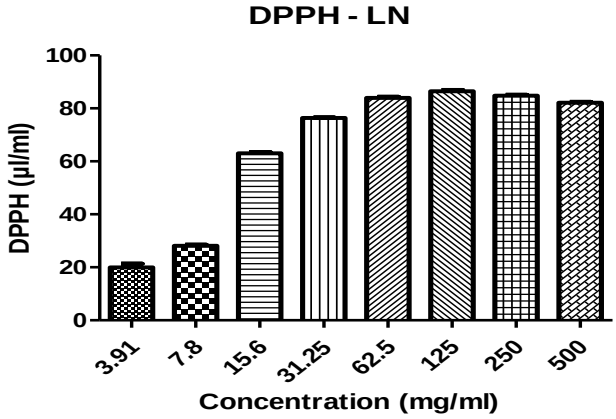

Figure No. 4
DPPH estimation: the data that were reported are the mean values based on three replicates ± SD

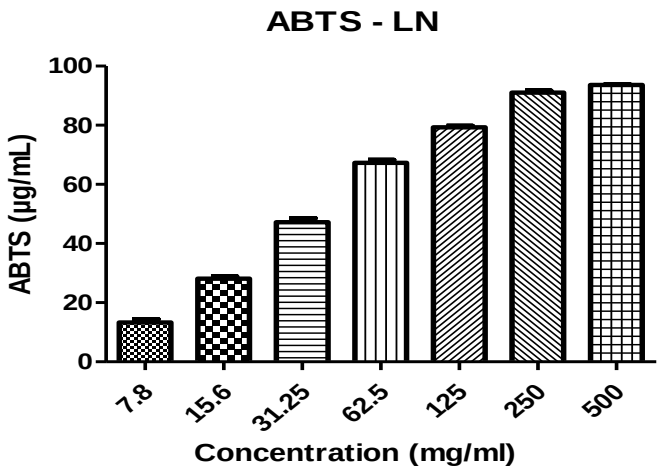

Figure No. 5
ABTS estimation: the data that were reported are the mean values based on three replicates ± SD

Figure No. 6 illustrates the relationship between the extract's total phenolic content and its antioxidant capabilities. The findings revealed a positive linear association (R_2 = 0.9663) between the extract's antioxidant capabilities and total phenolic content, suggesting that phenolic compounds may be a major factor in the extract's antioxidant properties (Figure No. 6).

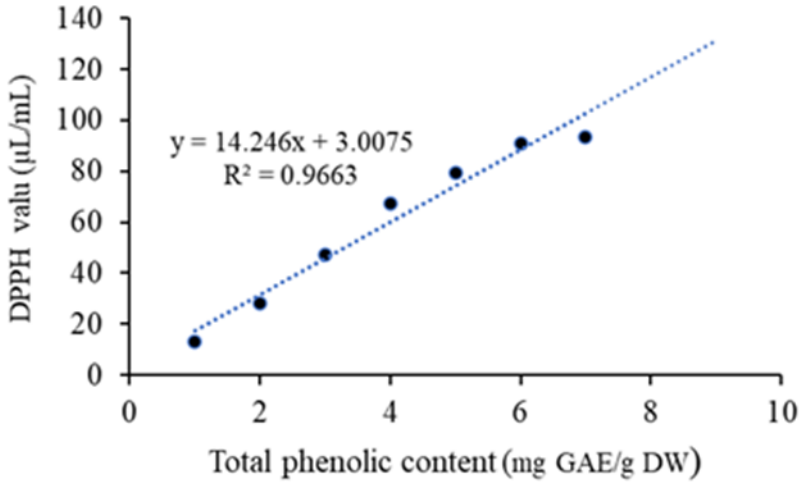

Figure No. 6
The correlation between total phenolic content and antioxidant capabilities was assessed by the DPPH experiment. GAE represents gallic acid equivalents

Figure No. 7 presents the effects of sporulation and treatment groups on the percentages of sporulation, non-sporulation, and inhibition in the in vitro studies of *Eimeria magna* oocysts. As the incubation time increased, the percentage of sporulation rose, while non-sporulation decreased (Figure No. 7). A significant difference was observed in the sporulation inhibition rate between the 24-hour and 96-hour exposures, with the inhibition rate increasing significantly over the incubation period, peaking at 96 hours ($p<0.05$). Figure No. 8 illustrates the significant impact of the experimental groups on the rates of sporulation (%), inhibition (%), and destructiveness (%). Oocysts exposed to 150 and 80 mg/mL concentrations of LNLE exhibited the highest inhibition rates and the lowest sporulation rates ($p<0.05$). Additionally, the inhibition of destructive sporulation was more pronounced in oocysts treated with higher concentrations of LNLE ($p<0.05$). Statistical analysis revealed significant differences in inhibition rates across experimental groups, particularly between the 150 mg/mL and 80 mg/mL concentrations compared to the control group ($p<0.01$). No other concentrations showed statistically significant differences (Figure No. 8).

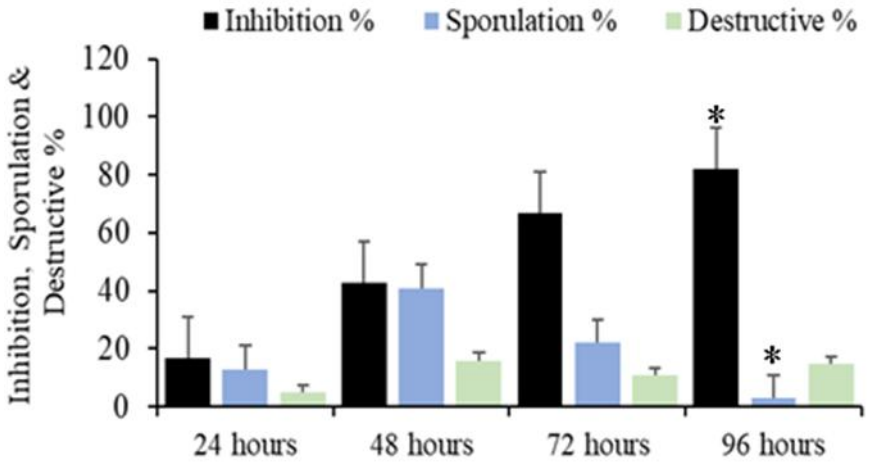

Figure No. 7
In-vitro effect of LNLE at different times on sporulation, inhibition, and destructed rates of *E. magna* oocysts

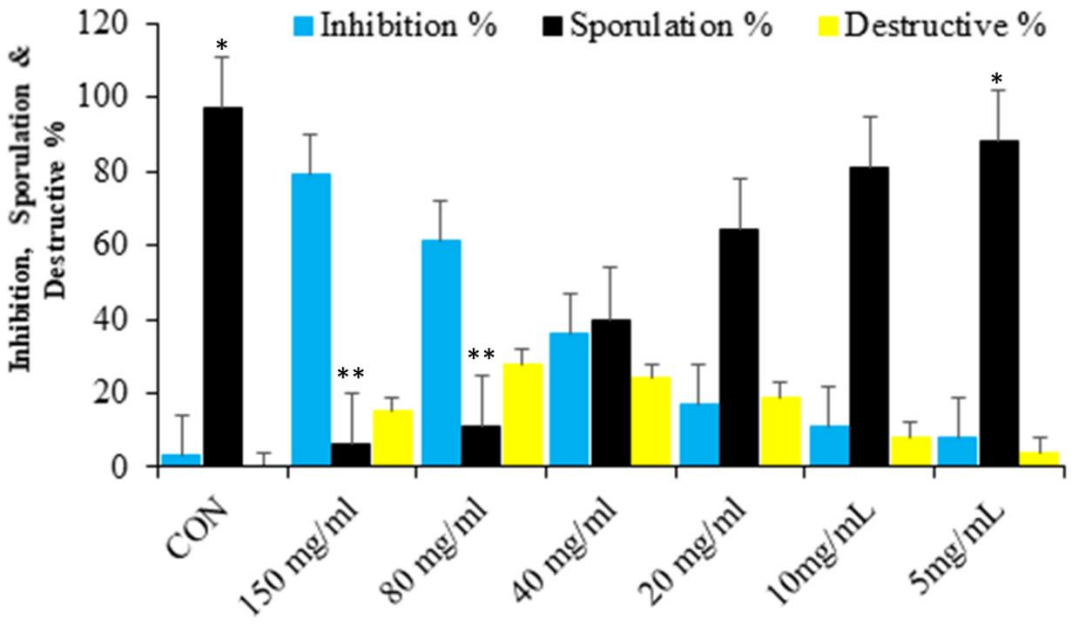

Figure No. 8
***In-vitro* effect of LNLE at different concentrations on sporulation, inhibition, and destructed rates of *E. magna* oocysts. **$p<0.001$ and *$p<0.05$**

DISCUSSION

The medicinal plant *Laurus nobilis* is known for its potent chemical components that can treat a variety of diseases (Taroq *et al*., 2021). Methanol was used to extract phytochemicals from the plant, as it serves as a solvent for both polar and medium-polar compounds. The volatiles from fresh *L. nobilis* leaves were isolated through methanolic solvent extraction and analyzed using FTIR. The analysis revealed the presence of halo compounds, 1,4-disubstituted alkenes, carboxylic acids, aromatic amines, and aliphatic ketones in the fresh bay leaves. This aligns with Mansour *et al*. (2018), who identified similar compounds, with variations attributed to differences in plant habitat, extraction methods, and solvent type and concentration. Enzyme inhibition has become a powerful strategy to address global health issues such as diabetes mellitus (DM) and Alzheimer's disease (AD) (Alzheimer's Association, 2012). Researchers have focused on developing advanced therapeutic strategies to manage these conditions. Controlling blood glucose levels is a key concern for individuals with diabetes, and inhibiting the digestive enzymes responsible for carbohydrate metabolism, such as glucosidase and amylase, is considered a promising approach. Alpha-amylase catalyzes the hydrolysis of glycosidic linkages (1,4) in complex carbohydrates (such as starch or glycogen), while alpha-glucosidase catalyzes the final stage of carbohydrate hydrolysis, which produces glucose (Tundis *et al*., 2010). Zengin (2016), suggested that *L. nobilis* extract might have potential applications in both food and medicine. In this study, the enzyme inhibitory activities of *L. nobilis* leaf extract were tested against alpha-amylase and alpha-glucosidase using a microplate reader. The methanolic extract demonstrated strong inhibitory activity, consistent with findings for other plant extracts, including Asphodeline (Custodio *et al*., 2013; Nouri *et al*., 2014; Sarikurkcu *et al*., 2015). These results indicate that methanol is a suitable solvent for enzyme inhibition assays. Furthermore, significant differences in enzyme inhibitory effects were observed at different concentrations of the *L. nobilis* leaf extract. The extract exhibited an inhibition of 7.57 ± 0.11 mmol acarbose/g for alpha-amylase and 0.59 ± 0.02 mmol acarbose/g for alpha-glucosidase. These results are comparable to the inhibitory activity reported in other studies (Lazarova

et al., 2015). The *L. nobilis* extract in this study showed a high concentration of phenolic compounds (57.397 mg GAE/g) and flavonoids (30.839 mg CA/g), as well as a significant number of tannins (6.0895 mg CA/g). These findings are consistent with previous research that identified alkaloids, flavonoids, terpenoids, and tannins in L. nobilis, which are crucial for treating various diseases (Al-Turfi *et al*., 2022). According to Tahiri *et al*. (2023), this study emphasizes the abundance of phenolic compounds in *L. nobilis*, which contribute to its resistance to inflammation, bacteria, cancer, and free radicals. Antioxidants play a key role in suppressing oxidation reactions and protecting cells from damage (Berendika *et al*., 2022). Medicinal plants produce numerous natural antioxidants, including ascorbic acid, carotenoids, and various phenolic compounds such as cinnamic acids, benzoic acids, flavonoids, coumarins, and lignans. The antioxidant activity of medicinal plant extracts is often assessed using various tests that measure their ability to scavenge free radicals, such as DPPH and ABTS. In this study, a dose-dependent relationship was observed between the concentration of the crude extract and the percentage of DPPH and ABTS inhibition. The DPPH assay showed that the ethanol extract of *L. nobilis* exhibited significant antioxidant activity, with a concentration of 125 µg/mL resulting in 86.41872 ± 0.95% inhibition. The high levels of phenols, tannins, and flavonoids in the extract support its strong antioxidant properties. Studies have shown that phenolic compounds, such as those found in *L. nobilis*, are powerful antioxidants that prevent the formation of radicals (Kallassy *et al*., 2017). Additionally, methanolic extracts of *L. nobilis* are known for their anti-inflammatory properties, which are supported by research from Rahmouni *et al*. (2017) and Amraei *et al*. (2018). The chemical composition of *L. nobilis* oils has been widely recognized for their antioxidant properties (Cherrat *et al*., 2014; Nieto, 2017). The study also assessed the *in vitro* antiparasitic activity of *L. nobilis* extracts (Tsantila *et al*., 2024), which effectively suppressed the sporulation of Eimeria magna oocysts infecting rabbits, especially at higher doses and longer incubation periods. The results indicated a significant reduction in oocyst sporulation, highlighting the potential of *L. nobilis* extracts in controlling parasitic diseases. These findings are consistent with previous studies on *E. magna* oocysts (Murshed *et al.*, 2024). The effectiveness of *L. nobilis* extract was also evaluated in mice naturally infected with *Aspiculuris tetraptera* (Mares *et al*., 2024).

CONCLUSION

Results suggest that *L. nobilis* could be considered a source of natural enzyme inhibitors in food and pharmaceutical areas. In addition, exhibited different bioactivities, which support their potential use as therapeutic medicinal plants having strong antioxidant and anti-inflammatory effects. However, further studies are needed for the characterization and isolation of phytochemicals in *L. nobilis* extracts.

ACKNOWLEDGMENT

The Researchers Supporting Project (RSP-2024R3), King Saud University, Riyadh, Saudi Arabia, supported the work financially.

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