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Effect of *Rumex nervosus* Leaf Extract on *Eimeria exigua*

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ABSTRACT

Background: Coccidiosis is a serious parasitic disease caused by protozoan parasites of the genus Eimeria, presents an important risk to rabbits' health and production.

Methods: Potential bioactive compounds in the extract were identified by FT-IR analysis and the total content of phenolics, tannins and flavonoids was calculated. In addition, enzyme inhibitory and antioxidant effects were investigated.

Result: The phytochemical results of methanolic extract of the leaves of *R. nervosus* showed the presence of 14 compounds of active chemical constituents. In addition, the results showed that the methanolic extract had the amount of TPC (54.33±0.198 mg/g DW), TFC (21.51±0.155 mg/g DW) and TTC (29.37±1.43 mg/g DW). The sporulation inhibition rate between 72- and 96-hour exposures, as the rate rose significantly with an increasing incubation period (p<0.05). The extract has inhibitory abilities on tested enzymes α-amylase and α-glucosidase.

Key Words: Antioxidants, *Eimeria exigua, Rumex nervous,* α- amylase, α- glucosidase.

INTRODUCTION

Coccidiosis is a significant parasitic disease caused by protozoan parasites of the genus *Eimeria* (Abd El-Ghany, 2020), poses a considerable threat to the health and productivity of rabbits (Bashar *et al*., 2024), leading to substantial economic losses in the livestock industry (Abebe and Gugsa, 2018). Among various species of *Eimeria exigua* is particularly relevant in the context of rabbit husbandry, as it can cause severe intestinal damage (Pilarczyk *et al*., 2020), resulting in clinical symptoms such as diarrhea, weight loss and, in severe cases, mortality (Bangoura and Daugschies, 2018). Traditional control measures primarily rely on synthetic anticoccidial drugs (Chapman, 2018). However, the increasing incidence of drug resistance and the growing consumer demand for organic and natural animal products necessitate the exploration of alternative control strategies.

Genus *Rumex* is found across the globe (Vasas *et al*., 2015). *Rumex nervosus* is a plant of the Polygonaceae family and contains more than 250 species, is often referred to as "Ithrib" on the Arabian Peninsula. The plants prevalent in Yemen, as well as in the Middle East and Africa and used as traditional herbal therapy. Historically, it has been used for treating many ailments and microbial infections (Al-Nowihi *et al*., 2020; Azzam *et al*., 2020; Hussein *et al*., 2008; Wachtel-Galor and Benzie, 2012). *R. nervosus* is widely distributed throughout several habitats, including mountains, overgrazed areas, roadsides, sandy areas, high-altitude regions, locations with reasonably high rainfall and rocky areas (Al-Aklabi *et al*., 2016; Al Yahya *et al*., 2018).

In Yemen, investigation of biological activities, antioxidant properties and enzyme inhibitory effects of *R. nervosus* leaves extract has been acquired a significant attention. Various studies have explored the potential health benefits of *R. nervosus* through detection the chemical composition and impact on health. Savran *et al*. (2016), highlighted the antioxidant, enzyme inhibitory and antimicrobial properties of edible *Rumex* species, shedding light on the potential health benefits of these plants. Similarly, (Khan *et al*., 2014) focused on the urease inhibitory potential of *R. nervosus*, emphasizing the importance of different plant parts in exhibiting bioactive properties. Furthermore, (Quradha *et al*., 2019) studied the antibacterial, antioxidant and anticancer properties of *R. nervosus* leaves, confirming that the plant multifaceted biological impacts. Furthermore, (Desta *et al*., 2016) investigated the antioxidant activity of *R. nervosus* leaves and stems, emphasizing the potential health benefits of plant. Furthermore, Tedila and Assefa (2019) explored the antimicrobial activity of *R. nervosus* against various pathogens, indicating its potential as a natural antimicrobial agent.

According to the World Health Organisation (WHO), herbal medicine is relied upon by 60% of the global population, with over 80% of people in poor nations depending heavily on it for their basic healthcare requirements (Khan and Ahmad, 2019). The preventive anti-inflammatory effects of *R. nervosus*, along with the effectiveness of high dosages of nanoparticles, provide a

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promising and cost-effective natural alternative for combating inflammation (Ibrahim *et al*., 2024). The plant *R. nervosus* has been extensively used in traditional medicine for the treatment of constipation, rheumatism, inflammation, scabies, gout, herpes and eczema (Kahraman and Yanardag, 2012; Tynybekov *et al*., 2013). The mentioned conditions include arthritis, diarrhea, jaundice, diuretic effects, laxative properties, wound healing, blisters and cancer (Bharti *et al*., 2010; Harshaw *et al*., 2010).

Recent studies have shown that extracts derived from several components of the *R. nervosus* plant, namely the roots, had strong antibacterial properties against various harmful bacteria and fungi (Al-Farhan *et al*., 2022; Al Yahya *et al*., 2018; Hussein *et al*., 2008). The antibacterial and fugal capabilities of *R. nervosus* are ascribed to the existence of several phytochemicals and bioactive substances inside the plant (Al-Garadi *et al*., 2022; Al Yahya *et al*., 2018). Furthermore, (Tawhid *et al*., 2023) provided evidence of the antidiarrheal effects of a methanolic extract derived from *R. nervosus*, suggesting its potential for therapeutic use.

Due to the rising occurrence of antibiotic-resistant bacteria, there is a growing inclination to investigate natural sources such as medicinal plants for new antimicrobial substances (Álvarez-Martínez *et al*., 2020; Schneider, 2021). In addition, Qaid *et al*. (2023) investigated the effects of supplementing broiler chickens with *R. nervosus* with offering valuable information on the possible physiological effects of the plant extract. These studies emphasize the need to assess the biological activity, antioxidant capacity and enzyme-inhibitory effects of *R. nervosus*. This contributes to expanding information on therapeutic qualities of this plant species.

This study aims to evaluate the biological properties, antioxidant potential and inhibitory effects on enzymes of root extracts derived from *R. nervosus* plants cultivated in Yemen. The results of this inquiry may aid in the advancement of novel herbal-derived antibacterial and therapeutic merchandise.

MATERIALS AND METHODS

Plant collection

Local farmers in the Ibb governorate (Republic of Yemen) were collected the leaves of *R. nervosus*. We then naturally dried the leaves at room temperature (25°C) for 15 days. We placed the voucher leaves in the Botany and Microbiology Department's herbarium at King Saud University to confirm their botanical compositions.

Preparation of extract

Rumex nervosus leaves drying by an oven (Binder, Bohemia, New York), the moisture content of dried and ground foliage of plant was determined using an electric blender (Manikandan *et al*., 2008). The ground material (50 g) was combined with 96% ethanol (500 mL) and agitated on a shaker for 4 days (Amer *et al*., 2015). The solution was filtered through a Whatman filter paper to separate the components.

Infrared spectroscopy

Infrared spectroscopy was utilized to determine the chemical composition of *R. nervosus* leaf extract. This approach reveals information on the functional groups and molecular structures found in plant extracts. The infrared spectrum of *R. nervosus* leaves has typical absorption bands for several phytochemicals such as flavonoids, phenolic acids and terpenoids (Al-Quraishy *et al*., 2020; Alshameri *et al*., 2022).

Evaluation of phenolic content

Total phenolic content (TPC) of *R. nervosus* leaf extract was determined according to (Mwamatope *et al*., 2020). A total of 300 µl sodium carbonate solution (20%) was added to 100 µl of the Folin-Ciocalteu reagent and 100 µl of the extracted leaf. The sample was stored and incubated in dark at an ambient temperature for 30 min and then measured using a UV-visible spectrophotometer (SHIMADZU, UV-1800) at a wavelength of 765 nm. The linear equation y = 0.0021x + 0.0021, which has an R_2 value of 0.9995, was used to determine the total phenol concentration in study samples. The equation was developed from a standard curve created using gallic acid with concentrations ranging from 25 to 400 g/mL. The total phenolic content was quantified in (mg RN/g) of dry weight.

Evaluation of flavonoid content

In this study, methodology of (Mazandarani and Ghafourian, 2017) was followed to determine the total flavonoid content (TFC) in *R. nervosus* leaf extract. Equal amount of an aqueous solution containing 2% AlCl3 with a 0.5-mL sample of methanol was measured at a wavelength of 420 nm after holding it at a temperature of 25°C for 2 hours. TFC was determined by creating a calibration curve using various concentrations (50-400 g/mL) of the quercetin standard. Then, computed TFC using the equation y = 0.0172x + 0.0507, which yielded an R_2 value of 0.995. The calculated TFC value is the amount of quercetin in (mg RN/g) of dry weight.

DPPH radical scavenging assay

The DPPH assay is based on the principle of measuring the ability of antioxidants to neutralize the free radicals, which is quantified spectrophotometrically. A volume of 1 mL of *R nervosus* leaf extract, with a concentration ranging from 31.25 to 1000 µg/mL, was combined with an equal amount of DPPH in methanol, which had a concentration of 0.135 mM. The optical density of both the extract and the control combination that consisting of 1 mL DPPH and 1 mL methanol, was quantified at a wavelength of 517 nm.

ABTS radical scavenging activity

ABTS free radical scavenging activity was evaluated in *R. nervosus* leavesusing the methodology described by (Re *et al*., 1999). We dissolved the compound 2,22 -casino-bis

(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) in distilled water at a concentration of 7 mM. We combined the ABTS stock solution with 2.45 mM potassium persulfate as the final concentration. We then maintained the combination in the dark at room temperature for 14 hours before using it. The ABTS+ solution was diluted with water until it reached an absorbance of 0.70 (±0.02) at a wavelength of 734 nm. The reaction involved using 0.07 mL of the extract and 3 mL of the ABTS radical. A spectrophotometer quantified the absorbance at 734 nm after an incubation period of 5-7 minutes.

Evaluation of tannin content

According to the method described by (Rodrigues *et al*., 2007), the total tannin content (TTC) was determined. with some slight modifications. This approach was used for the leaf extract. An amount of 0.1 mL of the extracted samples was added to a 2 mL Eppendorf tube already containing 1.5 ml of Milli-Q water and 0.1 ml of the Folin-Ciocalteu phenol reagent and allowed to stand for eight minutes. Then 0.3 ml of 30% of the solution of sodium carbonate were added to neutralize the solution. The ingredients were then mixed thoroughly and kept in a dark room at ambient temperature for twenty minutes. The measured value for the wavelength was 700 nm.

***In vitro* α-amylase inhibition assay**

The experiment was conducted according to the standard methodology with minor alterations (HANSAWASD, 2000). A suspension of 2 mg of starch azure was prepared in 0.2 mL of Tris-HCl buffer (0.5M, pH 6.9) containing 0.01 M $CaCl_2$ as the substrate solution. The ethanol extract of *R. nervosus* was solved. Next, a 0.2 mL aliquot of *R. nervosus* extract was introduced into the tube holding the substrate solution. The reaction was conducted at a temperature of 37°C for 10 minutes. The process was halted by introducing 0.5 mL of 50% acetic acid into each tube. The combination underwent centrifugation at a speed of 3000 rpm/ 5 minutes at a temperature of 4°C. The supernatant was analyzed for absorbance at a wavelength of 595 nm.

***In vitro* α-glucosidase inhibition assay**

The α-glucosidase inhibitory activity was assessed using the method published by (Ting *et al*., 2005), with minor modifications. Briefly, mixtures of 112 µl of PBS, pH 6.8, 20 µl of enzyme solution (1 unit/mL) and 8 µl of the sample dissolved in DMSO were put in a 96-well plate and incubated at 37°C for 15 mins. Afterward, 20 µl of pNPG (2.4 mmol/L) was added to each well and the plate was incubated again at 37°C for 15 mins. The reaction was stopped by adding 80 µl of a 0.2 mol/L solution of Na_2CO_3. The absorbance was measured at a wavelength of 405 nm. Instead of the sample solution, 8 µl of DMSO was added to the control and blank. Acarbose served as the positive control. The α-glucosidase inhibitory activity was quantified as the IC_{50} value, representing the concentration required to block 50% of the enzyme's activity.

Statistical analysis

Three rounds of analysis were performed on the *R. nervosus* samples. The data analysis was conducted using SPSS statistical software v23. The mean±SD was used to display the data that was collected. To determine the level of significance for the mean values at $p<0.05$, a One-Way ANOVA was conducted (Quirk and Quirk 2012).

Results and Discussion

Evaluation of bioactive phytochemical components

The study of phytochemicals has gained significant attention due to their potential health benefits and roles in various biological activities. Table 1 show bioactive phytochemical components of *R. nervosus* extracts (RNE), which contained 14 elements; the main components in the chemical group: were flavonoids, furanic aldehyde, pyranose, fatty acid, diterpene polyunsaturated fatty, acids (PUFA), monounsaturated fatty acids (MUFA), saturated

Table 1: Infrared spectroscopy (IR) spectrum of *R. nervosus* by frequency range.

Absorption (cm^{-1})	Peak details	Transmittance (%)	Functional group	Compound class
3425.60	Medium, sharp	27.5	O-H stretching	Alcohol
2925.24	Strong, broad	39	N-H stretching	Amine salt
2853.73	Medium	51.5	C-H stretching	Aldehyde
1613.73	Medium	38	C=C stretching	Cyclic alkene
1525.52	Strong	52	N-O stretching	Nitric compound
1445.71	Medium	47	C-H bending	Alkane
1367.22	Medium	8.4	O-H bending	Carboxylic acid
1284.79	Strong	45	C-N stretching	Aromatic amine
1223.51	Medium	50	C-N stretching	Amine
1106.65	Strong	49	C-O stretching	Aliphatic ether
1033.70	Strong	48	S=O stretching	Sulfoxide
870.22	Strong	75	C-H bending	1,2,4-trisubstituted
765.35	Strong	69	C-H bending	1,2,3-trisubstituted
591.37	Strong	74	C-H bending	Monosubstituted

fatty acids, adipic acid ester, glycidyl ester and fatty acyl chlorides. The infrared spectrum of *R nervosus* leaf extract using an FT-IR spectrometer revealed the presence of various functional groups (Table 1). The absorption bands at 3400 cm^{-1} and 1600 cm^{-1} indicated the presence of hydroxyl group (O-H), amino group (N-H), aldehydes group (C-H), cyclic alkenes group (C=C) groups and nitric compounds (N-O).

The obtained results showed that the methanolic extracts, which had the highest amounts of TPC, TFC and TTC. The methanolic extract had the greatest quantity of TPC at 54.33±0.198 mg/g DW (Table 2). The significant abundance of phenolic compounds is remarkable due to their well-known antioxidant qualities, which may aid in alleviating oxidative stress (Abidi *et al*., 2020). The methanolic extract's high TPC indicates that it might serve as a useful reservoir of antioxidants, perhaps leading to health advantages such as anti-inflammatory and anti-cancer properties (Pandey *et al*., 2020; Zheng *et al*., 2021).

The TFC was quantified as 21.51±0.155 mg/g DW after doing TFC analysis. Flavonoids, a kind of phenolic chemicals, are known for their ability to enhance cardiovascular health and have anti-allergic and anti-viral capabilities (Rakha *et al*., 2022). The considerably elevated TFC suggests that the methanolic extract may have potential benefits in improving general health (Kumar *et al*., 2023). The presence of flavonoids also implies that the extract might be potentially used in functional meals or dietary supplements, according with (Qaid *et al*., 2023).

The TTC was measured to be 29.37±1.43 mg/g DW. Tannins are polyphenolic chemicals recognized for their astringent qualities and possible health advantages, such as antibacterial activities and the capacity to bind and precipitate proteins (Crozier *et al*., 2006; Soares *et al*., 2020).

Enzyme inhibitory activities of *R. nervosus* extracts were tested against α-amylase and-glucosidase using a microplate reader (Table 2). The extract has inhibitory abilities on tested enzymes. Generally, the methanolic extract exhibited strong activity. It was observed that certain asphodeline and other plant extracts presented findings that were comparable (Custódio *et al*., 2013; Nouri *et al*., 2014; Sarikurkcu *et al*., 2015). The methanolic *R. nervosus* extract showed inhibitory activity α-amylase (0.230±0.017), less than α-glucosidase (1.467±0.076). These findings may suggest that organic solvent could be suitable for enzyme inhibitory assays. These results are consistent with the results of a studies conducted by (Ahmad *et al*. 2021; Collado-González *et al*., 2017; Mettupalayam and Packiam, 2020).

Evaluation of radical scavenging activities

The percentage of DPPH radical scavenging activity indicates the antioxidant capacity of the *R nervous* extracts under investigation. The samples are better antioxidants if they have higher percentages of DPPH radical scavenging activity (Fig 1). This means that they can neutralize free radicals and protect against oxidative stress. The data obtained from DPPH tests enables us to comprehend the *R. nervous* system's radical-scavenging capacities. The DPPH results provide a depiction of the antioxidant activity of the samples at varying concentrations. This information reveals their capacity to counteract oxidative stress and

Table 2: Phenolics, flavonoids, tannin and enzymes in *R. nervosus* leaves methanolic extract.

	Phenoles	Flavonoids	Tannin	α-amylase	α-glucosidase
Rumex nervous	54.33±0.198	21.51±0.155	29.37±1.43	0.230±0.017	1.467±0.076

Data is represented as Mean±SD.

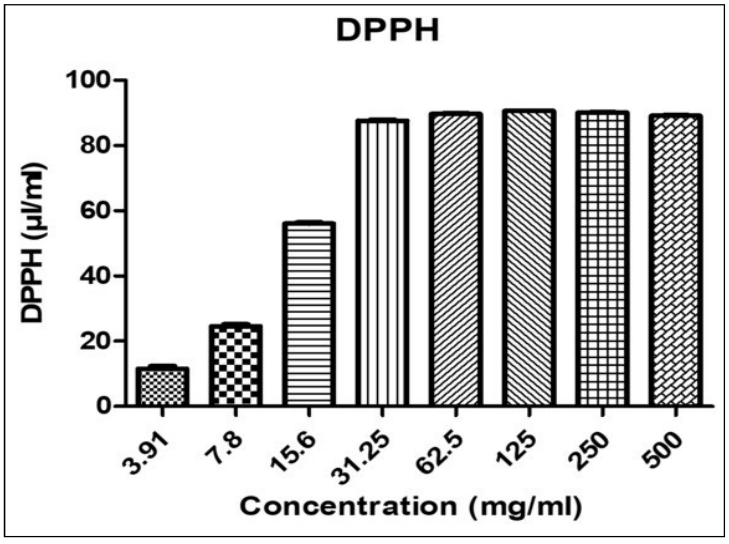


Fig 1: Concentration levels of DPPH in *R. nervosus* leaf methanolic extract.

safeguard cells from harm produced by free radicals. Our results are consistent with those of studies (Alasmari, 2020; Delgado *et al*., 2011; Sarikaya and Gulcin, 2013).

ABTS radical scavenging activity was positively correlated to the concentration of the methanolic extracts. In this analysis, the concentrations highest samples showed higher radical scavenging activity of ABTS than the lower concentrations (Fig 2). The results ABTS indicate a dose-dependent relationship between the concentration of *R. nervosus* extract and its antioxidant activity. As the concentration increased, the percentage inhibition of free radicals also increased, suggesting that the extract has significant antioxidant potential. These results are in line with what we already know about *R. nervosus's* antioxidant properties. Our results are consistent with the results of the following studies (Berillo *et al*., 2022; Chelly *et al*., 2020; Desta *et al*., 2016).

In vitro studies of *E.* exigua oocysts, Fig 3 and 4 showed the main effects of the sporulation and test groups on the percentages of sporulation and inhibition. As the incubation time increased, the sporulation percentage decreased, while the inhibition percentage increased. There was a significant difference in the sporulation inhibition rate between the 72- and 96-hour exposures, as the rate rose significantly with an increasing incubation period up to 96 hours (p<0.05).

Moreover, the sporulation of *E. exigua* oocysts was effectively reduced, especially at higher doses and longer

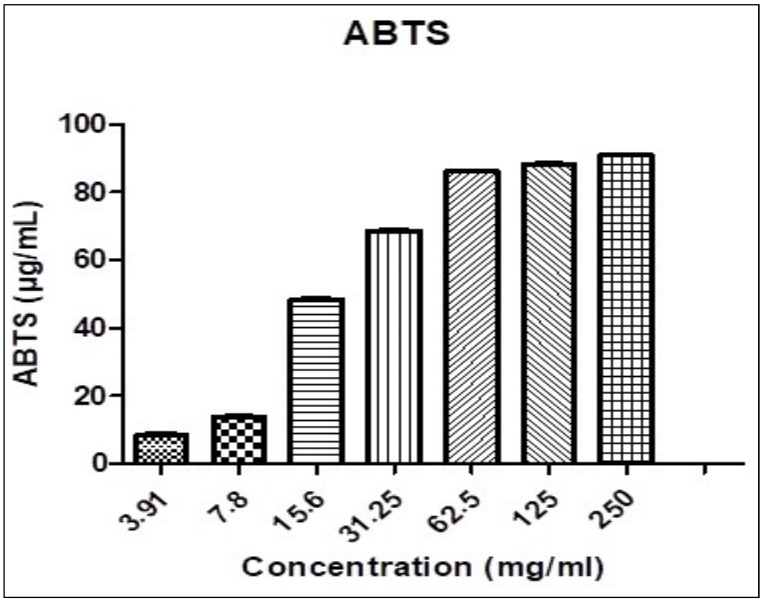


Fig 2: Concentration levels of ABTS in *R. nervosus* leaf extract.

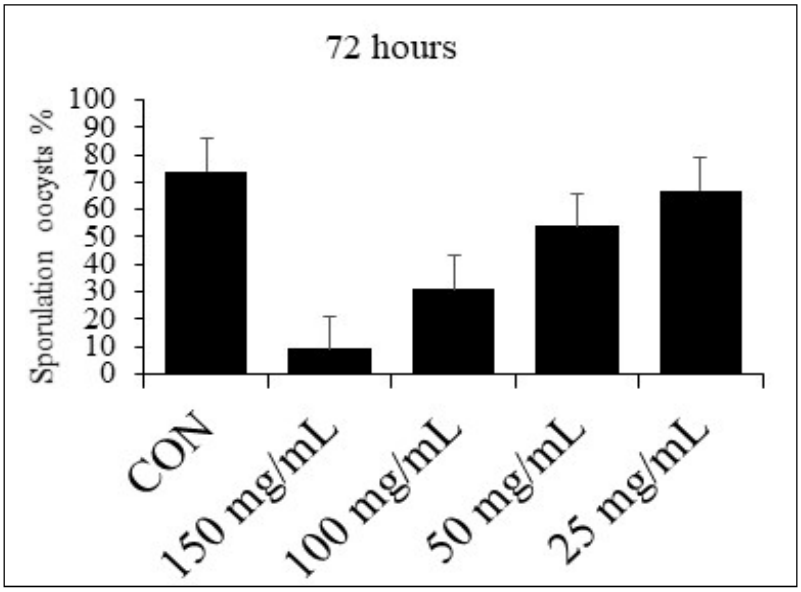


Fig 3: *In-vitro* effect of *R. nervosus* leaf extract at 72 h on sporulation and inhibition of *E. exigua* oocysts.

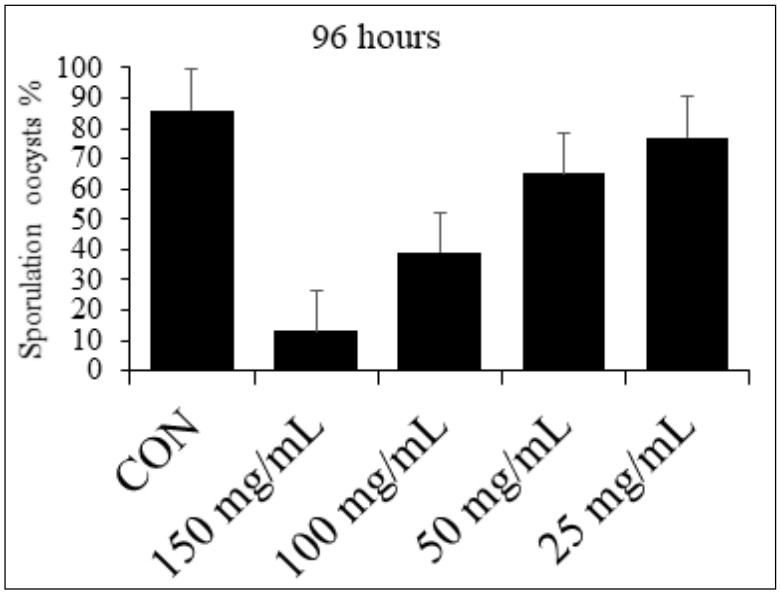


Fig 4: *In vitro* effect of *R. nervosus* leaf extract at 96 h on sporulation and inhibition of *E. exigua* oocysts.

incubation times. The results showed a noteworthy increase in oocyst inhibition, highlighting the possible application of *R. nervosus* leaf extracts in the management of parasite diseases. When evaluating *E. exigua* oocysts, this result is in line with what is shown in this extract (Ismail *et al*., 2020; Murshed *et al*., 2024).

CONCLUSION

Our findings revealed that *R. nervosus* extracts have potential health benefits. Antioxidant activity showed that the extract could remove free radicals, highlighting its role in protecting against oxidative stress. Overall, the results show that *R. nervosus* could be a source of bioactive compounds that can protect cells from damage and stop enzymes from working. Future research should focus on isolating specific phytochemicals responsible for these activities and exploring their mechanisms of action.

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Conflict of interest

The authors state that there are no conflicts of interest related to the publication of this work.

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