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Antioxidant and Anticoccidial Activity of Aqueous Methanol Extract of *Capparis spinosa* L. Plant Collected in Riyadh, Saudi Arabia

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ABSTRACT

Background: Traditional medicine has utilized plant bioactive compounds for the treatment of prevalent diseases. Antioxidants play a protective role against free radicals. The high concentration of phytochemicals in *C. spinosa* establishes it as a model species for the extraction of bioactive compounds. The genus Coccidia significantly contributes to intestinal issues in conventional rabbit husbandry.

Methods: The total of phenols, flavonoids, flavonols and tannins in the extract of *C. spinosa* was quantified. The antioxidant activity of CS extract was evaluated *in vitro* through DPPH assays and the IC_{50} values were established. *In vitro* anticoccidial activity was assessed using five concentrations of CS extract. The inhibition of sporulated oocysts of the extract was assessed at 96 hours.

Result: Chemical analysis of extract of *C. spinosa* showed that it contains a good percentage of the phenols, flavonoids, flavonols and tannins, the highest concentration of which was phenols. The extract had good antioxidant properties, with inhibition rates ranging from 79.142% to 7.952% at concentrations from 1000 to 31.25 µg/mL. The IC_{50} value was found to be 440.237 µg/mL. The anticoccidial effects were evaluated *in vitro*, revealing that oocysts exhibited the highest level of inhibition at concentrations of 100 mg/mL, 50 mg/mL and the reference drug.

Key words: Anticoccidial, Antioxidant, *Capparis spinosa*, Eimeria magna, Phytochemical.

INTRODUCTION

The abundance of bioactive phytochemicals in medicinal plants is a major contribution of nature (Kandpal *et al.,* 2023). Phytotherapeutic phytochemicals exhibit a wide range of structural variations. This variation is aimed at discovering new bioactive compounds and structures for the development of advanced pharmaceuticals. As other approaches have limitations, scientists are now turning to phytochemicals for drug discovery (Halder and Jha, 2023; Sharma *et al.,* 2021).

In the body, redox homeostasis is maintained by built-in antioxidant mechanisms based on enzymes (Alscher *et al.,* 2002; Alvarez and Radi, 2003) and non-enzymatic compounds (Alexandrova *et al.,* 2004; Ames *et al.,* 1993). Some antioxidants have been reported to have a protective role against free radicals, thereby slowing the progression of oxidative damage (Amorati *et al.,* 2003; Amorati *et al.,* 2006).

Rabbits are utilized as a source of protein, as well as for ecological balance. Coccidiosis is a common disease of rabbits (Lohkamp *et al.,* 2024; Murshed *et al.,* 2024b) and are infected once during their life. Coccidia of the genus *Eimeria* are common parasites in rabbits and one of the main causes of intestinal and hepatic disorders in conventional rabbit farms (Murshed *et al.,* 2024a).

Plants of the genus Capparis, family Capparidaceae, are drought-tolerant shrubs known since ancient times for their high nutritional value. Among the 250 species of Capparis, *Capparis spinosa* L. has long been the object of interest due to its antioxidant, cardiovascular, antibacterial, anti-inflammatory, hepatoprotective and hypoglycemic properties attributed to different parts of the plant (Boga *et al.,* 2011; Kazemian *et al.,* 2015). *Capparis spinosa* cultivated in tropical and subtropical regions of 60 countries worldwide, with the Mediterranean, Spain, Morocco, Turkey and Italy being the main producers (Tesoriere *et al.,* 2007). *Capparis spinosa* is known for its richness in phytochemicals, with particularly interesting nutritional and therapeutic potential, mainly polyphenolic compounds

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responsible for the antioxidant spectrum (Mollica *et al.,* 2019).

To the best of our knowledge, very little data has been published on *C. spinosa* L. conducted on the phytochemical analysis, antioxidant and antiparasitic properties of the aqueous methanol extract of the *C. spinosa* plant, which is utilized in traditional medicine and is collected in Riyadh, Saudi Arabia. The purpose of this study is to examine the differences between the extracts examined in other regions and the Riyadh region, Saudi Arabia. This, in turn, may reveal supplementary information about the character of pharmaceutical preparations and the extent to which active chemical compounds vary among plants and environments, even within the same species of plant. Plants of the same species have specific morphological and differences in chemical composition due to variation in geographical location, growing conditions, maturity, harvest time and genetic makeup (Hong *et al.,* 2023).

MATERIALS AND METHODS

Collection and extract of experimental plant material

The experimental plant *C. spinosa* (CS) was collected in June 2024 from the Riyadh region, Kingdom of Saudi Arabia, at latitude 24°42′50.9"N and longitude 46°31′35.2"E. The classification of the plant was conducted by a herbalist at King Saud University.

In the present study, only leaves of CS, were used. The plant samples were first cleaned with distilled water, air-dried in the shade for 15-25 days and then ground into a fine powder using a grinding machine. Then, 160 g of CS plant powder immersed in 70% methanol for 48 h at room temperature with shaken. The extract was filtered using Whatman No. 3 filter paper (Sigma, Germany) to remove plant debris. The extract was then dried and concentrated at 40°C under reduced pressure using a rotary evaporator (Yamato RE300, Tokyo, Japan) for further use.

Total phenolic content (TPC)

100 µl of sample extract was added to 2.5 ml of Na_2CO_3 (20%) and 500 µl of Folin-Ciocalteu reagent (1:1 with distilled water). The solution was mixed well and then left in the dark for 40 min to develop a specific color. The absorbance was then measured at 725 nm. A calibration curve of standard gallic acid (GA) was constructed and linearity was observed between 100 and 500 µg/mL. TPC was expressed as GA equivalents per 50 g of powdered plant material (µg GA/50 g of powdered plant material) (Çetin and Sağlam, 2022), using the following formula:

$$C = \frac{c \times V}{m}$$

Where,

C = Represents the total plant extract content (µg/g).

c = Denotes the concentration determined from the standard curve.

(y)= V indicates the extract volume (mL).

m = Signifies the weight of the pure sample extract (g).

Total flavonoid content (TFC)

Approximately 0.1 mL of the sample extract was dissolved in 200 mL of distilled water and subsequently mixed with 150 mL of a 5% w/v $NaNO_2$ solution. 150 µL of a 10% w/v AlCl3 solution was added and the mixture was subsequently incubated for an additional six minutes. Subsequently, 2 mL of NaOH (4% w/v) solution was added and the total volume was adjusted to 5 mL with distilled water. The mixture was shaken thoroughly and then maintained at room temperature for 15 minutes, after which the absorbance was measured at 510 nm. A standard graph of rutin (RUT) was constructed, with (TFC) expressed as RUT equivalents per 50 g of powdered plant material (µg RUT/50 g powdered plant material) (Zhishen *et al.,* 1999).

Total flavonol content

The total flavonol content was assessed using a colorimetric method involving aluminum chloride (Almaraz-Abarca *et al.,* 2007). Aliquots were prepared by combining 0.75 mL of methanol extract solutions (0.1 mg/ ml) with 0.75 mL of aqueous AlCl3 (20% w/v). Absorbance was measured at 425 nm following a 10-minute incubation against a blank, consisting of 0.75 ml methanol extract solutions and 0.75 ml methanol, using a UV/visible light spectrophotometer. All tests were conducted in triplicate. A calibration curve was established for Quercetin, represented by the equation below with a R^2 value of 0.996. The results were presented as mg of Quercetin Equivalents (QE) per 100 mg of extract.

$$y = 0.211\times - 0.193$$

Total tannin content (TTC)

A 0.5 ml sample extract was mixed with 0.5 ml methanol, 0.5 ml Folin-Denis reagent and 1 ml 35% Na_2CO_3 solution. The reaction mixture was made up to a total volume of 10 ml with distilled water. The solution was mixed well and left for 30 min at room temperature, after which the absorbance of the resulting solution was measured at 700 nm. A tannic acid (TA) standard plot was created, with TTCs expressed as TA equivalent per 50 g of powdered plant material (µg TA/50 g of powdered plant material) (Haile and Kang, 2019).

In vitro antioxidant activity

The antioxidant activity of extracts from CS leaves was evaluated in vitro through DPPH radical scavenging assays. The DPPH free radical scavenging assay was conducted using the method outlined by (Sangh Partap *et al.,* 2014). DPPH exhibits absorption at 517 nm in its radical form; however, its absorbance decreases upon reduction by another radical species or an antioxidant. Three milliliters of sample solution, consisting of stock solutions of CS leaf extracts prepared in methanol, were added to one milliliter of 0.1 mM DPPH solution. The DPPH solution was prepared by weighing 0.399 mg of DPPH and dissolving it in 50 ml of methanol, with sample concentrations ranging

from 31.25 to 1000 µg/ml. Absorbance was measured at 517 nm following a 30-minute interval. Increased free radical scavenging activity was associated with reduced absorbance of the reaction solution (weighed at 0.399 mg of DPPH and dissolved in 50 ml of methanol) and the absorbance was recorded as a blank measurement. DPPH radical scavenging activity (%) is calculated using the formula:

$$\frac{(Ac - As)}{Ac} \times 100$$

Where,

Ac = Represents the absorbance of ascorbic acid (control).
As = Denotes the absorbance in the presence of the extract.

The IC_{50} values were determined from the graph representing the concentration of the sample needed to scavenge 50% of the DPPH. The IC_{50} is commonly utilized to indicate the concentration of extracts required to neutralize 50% of free radicals.

In vitro antiparasitic activity

The experiment was performed on the *Eimeria magna* parasite that infects rabbits. This parasite was acquired from the Parasitology Laboratory inside the Department of Zoology at King Saud University**.**

Eimeria magna (oocysts) samples kept in a potassium dichromate solution ($K_2Cr_2O_7$), were cleaned using phosphate-buffered saline (PBS, pH 7.5). The centrifuge used Falcon tubes containing 10 mL of liquid, which were subjected to a centrifugal force of 1008 × g for approximately 15 min. This process was repeated 3 - 6 times until the $K_2Cr_2O_7$ solution was eliminated. The parasite suspension, consisting of 1,000 oocysts, was divided into Seven parts. Each portion included extract of CS concentrations at 6.25, 12.5, 25, 50 and 100 mg/mL, A standard treatment of toltrazuril at 30 µg/mL was used to enable comparison and potassium dichromate solution was used as a negative control. These components were placed in a 3 mL petri plates containing 24 compartments. The exact conditions were maintained for each treatment, which was carried out in triplicates. Sporulated oocysts were determined at 24, 48, 72 and 96 h. For the purpose of counting, it was prepared Sample preparation with the McMaster Egg Counting Method (Long and Rowell, 1958). An aliquot of the generated material was transferred to a McMaster Egg Counting chamber (Chalex LLC, Park City, UT) and let to stand for 5 minutes before viewing under a light microscope. Slides were analyzed with a light microscope (PX51, Olympus Co., Tokyo, Japan) at 10X magnification. antiparasitic efficacy of each treatment was calculated using the following equation (Wang *et al.,* 2009).

$$\text{Antiparasitic efficacy (in \%)} = \frac{(B - T)}{B} \times 100$$

Where,

B = Mean Sporulated oocysts number of control.
T = Mean Sporulated oocysts number of treatment.

Statistical calculations

Data are presented as mean±SD derived from three independent observations. *In vitro* antioxidant assays employed one-way ANOVA, followed by Tukey's test ($P<0.05$), to analyze the differences. A probability of $P<0.05$ was deemed significant.

RESULTS AND DISCUSSION

Total phenolics, flavonoids, flavonols and tannins

The quantification of phenols, flavonoids, flavonols and tannins in the aqueous methanol extract of *C. spinosa* leaves collected in Riyadh, Saudi Arabia quantified (Table 1) indicate that the concentration of phenols (34.698±0.5554) was higher than that of flavonoids, flavonols and tannins (22.866±0.3271, 11.4114±0.5311 and 18.933±0.3591 respectively).

The results align with prior studies, though variations in the concentration of certain compounds may be attributed to environmental differences affecting plant growth. (Hoste *et al.,* 2005; Mollica *et al.,* 2019; Tesoriere *et al.,* 2007; Alkhaibari and Alanazi, 2022). The extract of *C. spinosa* was found to contain a high concentration of flavonoid compounds, specifically rutin and quercetin (Tlili *et al.,* 2010). Phytochemicals such as Cappariloside A, stachydrin, hypoxanthine,uracil 1H-indole-3-acetonitrile 4-O-β-(62 -O-β-glucopyranosyl)-glucopyranoside, 1H-indole-3-acetonitrile 4-O-β-glucopyranoside, capparine A, capparine B, flazin, guanosine, 1H-indole-3-carboxaldehyde, 4-hydroxy-1H-indole-3-carboxaldehyde apigenin, kaempferol thevetiaflavone and alkaloids including capparisine A, capparisine B, capparisine C were identified from mature fruits of *C. spinosa* (Çaliş *et al.,* 1999; Fu *et al.,* 2007; Zhou *et al.,* 2010; Yang *et al.,* 2010a). In addition, tetrahydro quinoline acid was isolated from the fruits and stems of *C. spinosa* using column chromatorgraphy (Zhang *et al.,* 2014).

DPPH radical scavenging assay

The extract demonstrated radical scavenging activity, exhibiting inhibition rates of 79.142% to 7.952% at concentrations ranging from 1000 to 31.25 ìg/mL, with an IC50 value of 440.237 µg/mL. Statistically significant differences were observed in the DPPH scavenging assay among the various concentrations of the *C. spinosa* extract utilized (Table 2). The suppression of free radicals by the extract increased at the maximum tested levels (79.142%

Table 1: Total phenolics, flavonoids, flavonols and tannins of aqueous methanol extract of *C. spinosa* leaves.

Compounds	(mg/g DW)
Phenolic (mg GAE/g DW)	34.698±0.5555
Flavonoid (mg RUT /g DW)	22.867±0.3271
Flavonol (mg QE /g DW)	11.411±0.5311
Tannin (mg TAE/g DW)	18.934±0.3592

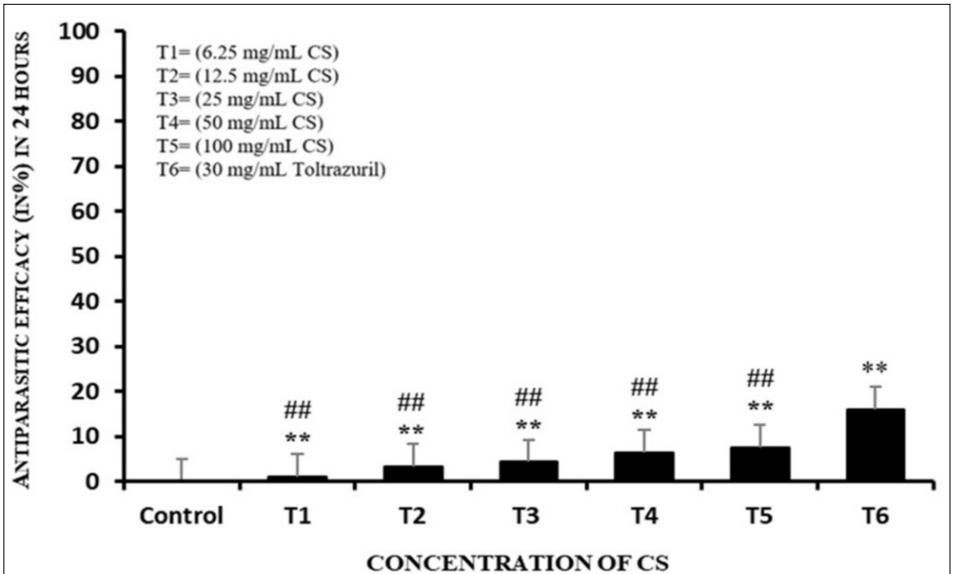
at 1000 µg/mL), with the extract exhibiting significant DPPH free radical scavenging activity and inhibition. These data indicate that *C. spinosa* functions as a natural antioxidant source.

The results align with prior studies, though variations in the inhibition rate may be attributed to differences in the concentration of specific active chemical compounds. Various studies have documented the DPPH scavenging activity of distinct parts of *C. spinosa*, (Germano *et al.,* 2002). The scavenging effect of lyophilized extract of buds of *C. spinosa* was concentration-dependent giving an EC_{50} value of 68.36 mg/ml (Bonina *et al.,* 2002). In contrast, lower concentration (0.32 mg/ml) was needed to give IC_{50} by methanolic extracts of fruits of *C. spinosa* from Turkey

Table 2: DPPH radical scavenging assay of different concentrations of phytochemicals isolated from the aqueous methanol extract of *C. spinosa* leaves.

Concentration (µg/ml)	DPPH radical Inhibition (%)	Concentration pair comparison	IC_{50} (µg/ml)
31.25 (T1)	7.952±0.7000	T1 vs T2**	
		T1 vs T3**	
		T1 vs T4**	
62.5 (T2)	13.328±1.0759	T1 vs T5**	
		T1 vs T5**	
		T2 vs T3**	
125 (T3)	19.268±1.1507	T2 vs T4**	440.237±11.0583
		T2 vs T5**	
		T2 vs T6**	
250 (T4)	31.370±1.9107	T3 vs T4**	
		T3 vs T5**	
		T3 vs T6**	
500 (T5)	55.371±1.0287	T4 vs T4**	
		T4 vs T6**	
		T5 vs T6**	
1000 (T6)	79.143±0.8243		

(*p≤0.05; **p≤0.01), shows significant differences compared between concentrations. Data are presented as mean±SD, (n=3).



Against *E. magna,* one day after the experiment. (*) Significant differences Concentration of (CS) and reference treatment vs. control p<0.05, (**) Significant differences were minor Concentration of (CS) and reference treatment vs. control p<0.01, (#) Significant differences Concentration of (CS) vs. reference treatment p<0.05, (##) Significant differences were minor Concentration of (CS) vs. reference treatment p<0.01.

Fig 1: *In vitro* study estimation of antiparasitic efficacy (in per cent) of aqueous methanol extract of *C. spinosa* leaves.

(Aliyazicioglu *et al.,* 2013). isolated several compounds from caper fruits with a strong DPPH scavenging activity (Yang *et al.,* 2010b). Direct comparison of data from the current study with those reported in the literature is difficult since different parts of plant were used.

In vitro antiparasitic activity of *C. spinosa* against *Eimeria magna*

The *in vitro* assessment of the antiparasitic efficacy of aqueous methanol extracts from the leaves of *C. spinosa* showed that concentrations of 6.25, 12.5, 25, 50 and 100 mg/mL resulted in inhibition rates of sporulated oocysts of 24.731%, 34.409%, 52.688%, 77.419% and 87.097%, respectively, during a 96-hour period. The results Antiparasitic efficacy demonstrated variability in sporulation and inhibition at various doses during an incubation time of up to 96 hours, with oocyst test results recorded every 24 hours. The results indicated the greatest inhibition of oocysts at concentrations of 100 mg/mL, 50 mg/mL and the reference drug, in contrast to lower concentrations where the inhibition rate diminished. Additionally, the inhibition increased with prolonged exposure time (Fig 1 to 4).

To our knowledge, no prior studies have elucidated the potential role of aqueous methanol extracts from the leaves of *C. spinosa* on Coccidia. *C. spinose* contains many active chemical ingredients such as glycosides, myrosinase,

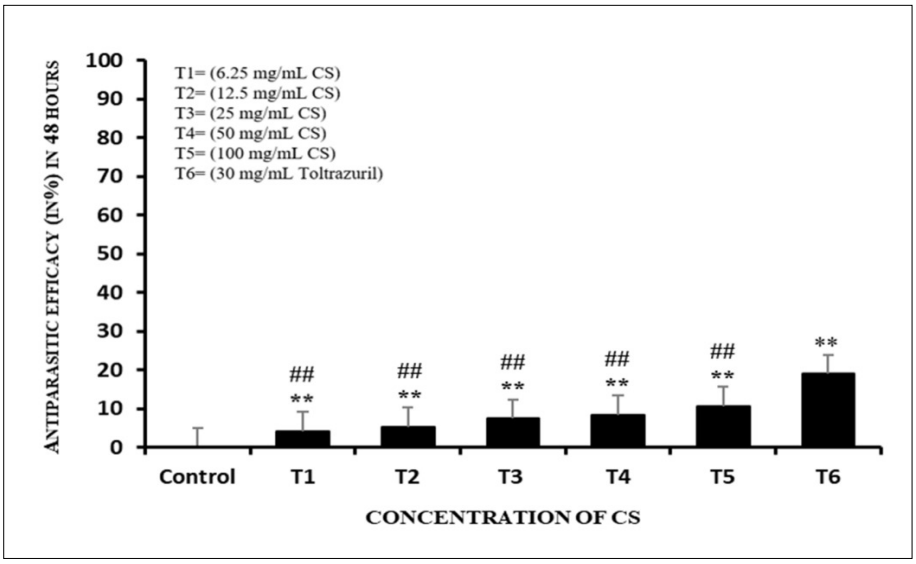


Fig 2: *In vitro* study estimation of antiparasitic efficacy (in per cent) of aqueous methanol extract of *C. spinosa* leaves against *E. magna*, two days after the experiment.

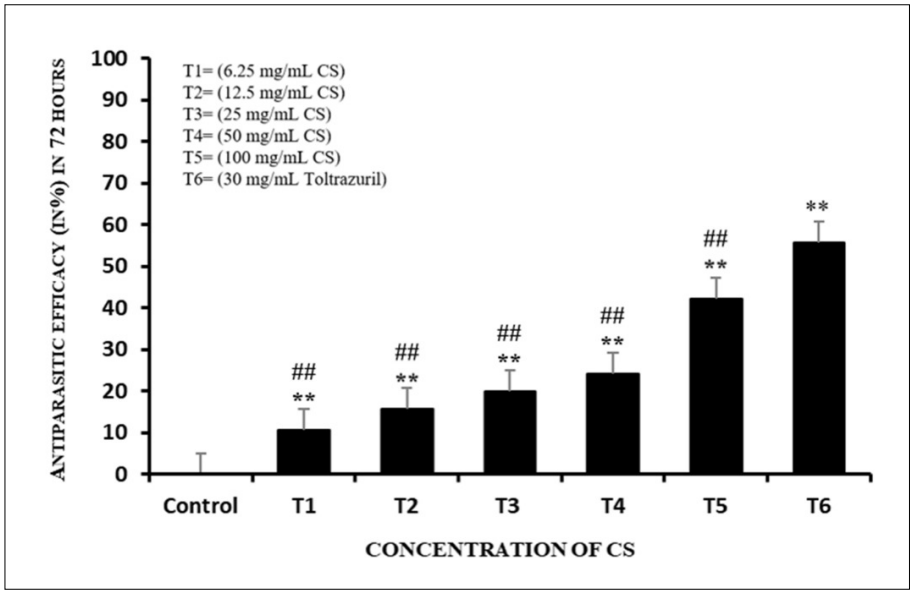


Fig 3: *In vitro* study estimation of antiparasitic efficacy (in per cent) of aqueous methanol extract of *C. spinosa* leaves against *E. magna*, three days after the experiment.

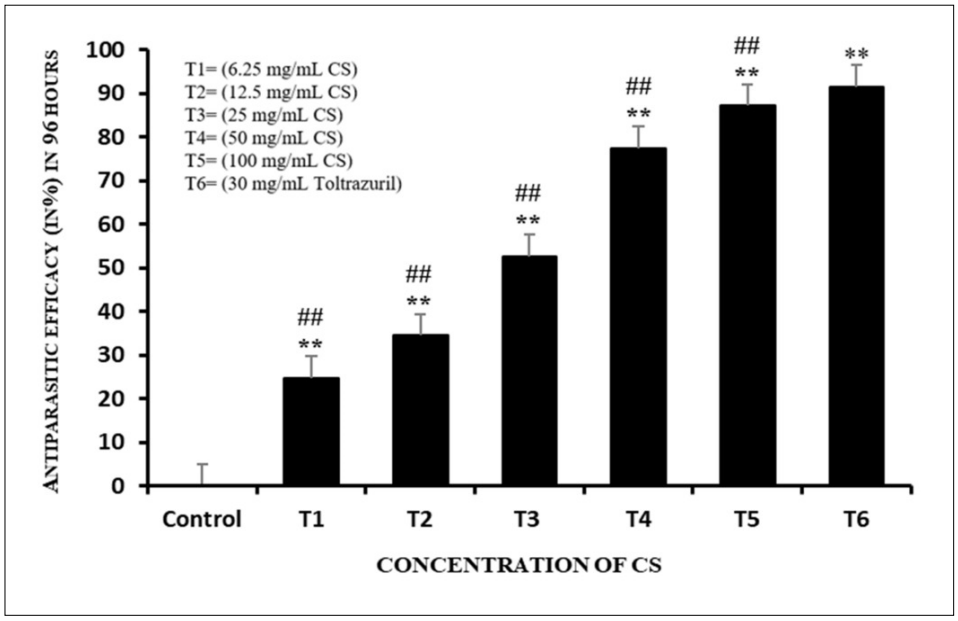


Fig 4: *In vitro* study estimation of antiparasitic efficacy (in per cent) of aqueous methanol extract of *C. spinosa* leaves against *E. magna*, four days after the experiment.

rustic acid, caproic acid, pectic acid, saponin, alkaloid substances such as stachydrine and flavonoids such as lerpenes and flavonoids, secondary metabolites that lead to pharmacological or toxic effects (Harsha *et al.,* 2013). These compounds act as analgesics and anti-inflammatories, as well as inhibit the growth and reproduction of microorganisms (Tlili *et al.,* 2017). Also, the pulp contains more flavonoids and phenolic than the roots, which affect energy metabolism and plasma membrane permeability (Hendra *et al.,* 2011) and alkaloids that can do the same action (Moghaddasian *et al.,* 2012).

CONCLUSION

Habitat has a significant effect on the biosynthesis and variability of plant secondary metabolites, different geographical and ecological harvesting areas result in different chemical compositions even in the same plant species.

Extracts from the leaves of *C. spinose* contains many active chemical and antioxidant compounds that have antiparasitic activity including phenols, flavonoids, flavonols and tannins.

It is generally agreed that *C. spinose* has a great impact antiparasitic the findings of the present study indicate that the leaf extract of *C. spinose* has potential oocysticidal characteristics, which may be utilized in coccidial treatment, the present study provides a significant basis for use of *C. spinose* solution for treatment of *Eimeria* spp. on Rabbits. Further studies are needed to evaluate the efficacy of the extract in living organs and to elucidate the molecular mechanisms underlying its activity.

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Disclaimers

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Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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