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Phytochemical Analysis, Antioxidant and Antiparasitic Activity of Extract of *Alhagi graecorum* Plant Used in Traditional Medicine: Collected in Saudi Arabia

Hossam M. Aljawdah[1], Mutee Murshed[1], Aiman A. Ammari[1], Saleh N Maodaa[1], Mohammed Mares[1], Saleh Al-Quraishy[1]

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ABSTRACT

Background: Plants within the same species vary in chemical composition. Phytochemicals serve as a source of novel therapeutic compounds owing to their structural variety and abundant availability. *A. graecorum* serves as an herb, fodder and alternative medicine source. The genus Coccidia, which is prevalent in rabbits, is a primary contributor to intestinal problems in traditional rabbit rearing.

Methods: An analysis was conducted on phytochemical composition in the extract of *A. graecorum*. The antioxidant activity of AG extract was evaluated *in vitro* through ABTS assays and the IC_{50} values were established. *In vitro* antiparasitic activity was assessed using five different concentrations of AG extract. The inhibition of sporulated oocysts of the extract was assessed at 72 hours.

Result: The FT-IR test of the *A. graecorum* extract showed that it contained 13 different compounds. Also, the GC-MS analysis revealed approximately 9 principal biologically active compounds. The extract had strong antioxidant properties, with inhibition rates ranging from 88% to 10% at concentrations from 500 to 15.625 µg/mL. The IC50 value was found to be 167.52 µg/mL. The antiparasitic effects were tested in vitro and showed that oocysts were most of inhibition at concentrations of 50 mg/mL, 25 mg/mL and the reference drug.

Key words: *Alhagi graecorum*, Antioxidant, Antiparasitic, Coccidia, Phytochemical.

INTRODUCTION

In recent years, herbal medicines and their pure components have emerged as a valuable source for the development of new antimicrobial agents. The high safety profile and minimal side effects associated with herbal medicines contribute to this outcome. The structural diversity and the vast resources of plant compounds made them valuable source for new therapeutic compounds (Mares *et al.,* 2024; Vaou *et al.,* 2021).

Plants of the same species exhibit certain morphological variations. Yet, differences in chemical composition have been reported due to various factors like geographical origin, growing conditions, maturity, harvest time and genotype (Al-Snafi, 2024).

Rabbits are distributed globally and have a significant relationship with human existence. Humans utilize them as a source of protein, as well as for ecological balance (Chen *et al.,* 2024). Humans gain immensely from this diversified group, but there are also a number of concerns involved. In contrast to other animals that offer comparable advantages, as such pigs, horses, cattle, or sheep, rabbits are more likely to spread illnesses to people while providing a same quantity of protein (Chen *et al.,* 2024). These organisms act as reservoirs for numerous pathogens, with coccidiosis ranking among the most common diseases affecting rabbits (Lohkamp *et al.,* 2024; Murshed *et al.,* 2024b).

[1]Department of Zoology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia.

Corresponding Author: Hossam M. Aljawdah, Department of Zoology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia. Email: haljawdah@ksu.edu.sa
ORCIDs: 0009-0002-7484-0398, 0000-0003-3717-6424, 0000-0002-1900-8446, 0000-0002-0662-2113, 0000-0002-1160-3072, 0000-0003-4204-3124

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Coccidia of the genus *Eimeria* are common parasites in rabbits and one of the main causes of intestinal disorders on conventional rabbit farms (Murshed *et al.,* 2024a).

Alhagi plant belongs to the family Fabaceae, has a natural distribution across the globe. Members of this genus inhabit desert and semi-desert environments. *Alhagi graecorum* Boiss is occurs naturally across Saudi Arabia (Beitsayahi *et al.,* 2024; Boulos, 2009). The phytochemical analysis of *Alhagi* species has identified the presence of various bioactive compounds, including sterols, glycosides

and fatty acids (Hamed *et al.,* 2012; Moustafa *et al.,* 2023), phenols (El-Saayed *et al.,* 1993; Singh *et al.,* 1999), alkaloids and flavonoids (Atta and El-Sooud, 2004; Saleh and Madany, 2013) (Akram and Mahmood, 2024) and other chemical components (Marashdah and Farraj, 2010). Biological studies of *Alhagi* species included anti-diarrheal (Rajeev Kumar *et al.,* 2010), gastroprotective, anti-inflammatory, antiulcer, antipyretic (Neamah, 2012), antimicrobial (Zain *et al.,* 2012), antioxidant (Al-Saleem *et al.,* 2019).

Environmental conditions have a significant impact on the biosynthesis and variability of secondary metabolites in plants (Verma and Shukla, 2015). However, to the best of our knowledge, no study has been conducted on the phytochemical analysis, antioxidant and antiparasitic properties of the aqueous methanol extract of the *A. graecorum* plant, which is utilized in traditional medicine and is collected in Riyadh, Saudi Arabia. As a result, the purpose of this study is to examine the differences between the extracts examined in other regions and the aforementioned. 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.

MATERIALS AND METHODS

Experimental plant material collection and extract

In August 2024 (summer), the plant *A. graecorum* (AG) was collected from the Riyadh region, Kingdom of Saudi Arabia, at latitude 24°86′33.8"N and longitude 46°46′33.8"E. a botanist at King Saud Uni. identified the plant species.

This study utilized only the stems and leaves of AG. The plant samples were initially washed with distilled water and subsequently air-dried in the shade for 20 to 25 days and then made into fine powder by grinding machine and stored for further use. 150 g of AG plant powder was individually dissolved in 70% methanol for 72 h at room temperature with continuous shaking. 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.

Infrared spectroscopy of extract from the stems and leaves of *A. graecorum* (AG)

The extract of AG stems and leaves was analyzed using Fourier-transform infrared spectroscopy (FT-IR) (Thermo Scientific, USA), following a defined procedure within the scanning wave number range of 4000 to 500 cm^{-1} at a resolution of 4 cm^{-1} to produce IR spectra. The spectra data were compared with references to identify the functional groups in the test samples, allowing for the interpretation of IR spectra obtained from the extract (Mabasa *et al.,* 2021).

Gas chromatography-mass spectrometry (GC-MS) of extract of *A. graecorum* (AG)

The extract of AG stems and leaves was analyzed using Gas chromatography-mass spectrometry (Thermo Scientific, TSQ 8000 Evo; Waltham, MA, USA). The analysis employed gas chromatography with Elite-5 Mass spectroscopy and a fused silica column. The carrier gas was 1 mL/min helium. The injector was calibrated at 260°C during chromatography. To analyze a 1 µL sample, the oven was heated to 60°C for 2 minutes, then ramped to 300°C/min for 6 minutes. The mass detector used electron impact at 70 eV for ionization, 0.2 seconds for scanning and 0.1 seconds for pieces 40 to 600 Dalton. 240°C was the transfer line and ion source temperature. Comparisons were made using the GCMS NIST (2008) library's database of known spectral components (Monika *et al.,* 2022).

Estimation of the *In vitro* antioxidant activity

ABTS assay

The ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) free radical scavenging assay was performed according to the method described in (Re *et al.,* 1999). Three milliliters of ABTS cation solution were combined with 30 microliters of methanol extract solution, with sample concentrations ranging from 15.625 to 500 µg/ml, in a 1 cm path length disposable microcuvette. The reduction in absorption was recorded over a duration of six minutes. Each assessment was carried out in duplicate. The calculation of antioxidant activity was performed using the following equation:

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

A control = Absorbance of negative control at the moment of solution preparation.

A sample = Absorbance of a sample after 5 min.

IC_{50} values were calculated from the graph illustrating the concentration of the sample required to scavenge 50% of the ABTS. The IC_{50} is frequently used to denote the concentration of extracts necessary to neutralize 50% of free radicals. ABTS was quantified as mg GAE/L.

Estimation of the *in vitro* antiparasitic activity

Parasite organisms

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.

Execution of the experiment on antiparasitic activity

Eimeria magna (oocyst) samples kept in a potassium dichromate solution ($K_2Cr_2O_7$) were cleaned using phosphate-buffered saline (PBS) with a pH of 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 AG concentrations at 3.125, 6.25, 12.5, 25, 50 mg/mL, A standard treatment of toltrazuril at 25 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 by recording the findings at 12, 24, 36, 48, 60 and 72 h. For the purpose of counting, it was prepared Sample preparation with the McMaster Egg Counting Method (Long and Rowell, 1958). An aliquot of 5 µL (parasite suspension and AG concentrations) 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 (P×51, Olympus Co., Tokyo, Japan) at 10X magnification.

Antiparasitic efficacy of each treatment was calculated using the following equation (Wang *et al.,* 2009). Antiparasitic efficacy.

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

Where,

B = Mean sporulated oocysts number of control.

T = Mean sporulated oocysts number of treatment.

Statistical calculations

Data were 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

FT-IR analysis of *A. graecorum* extract

The FT-IR analysis of the aqueous methanol extract from the stems and leaves of *A. graecorum* revealed the presence of 13 compounds. It exhibited many unique peaks that were explicitly attributed to the presence of particular functional groups or phytochemical substances. *i.e.*, a band which occurred at 3400.26 cm^{-1} might be attributed to the presence of primary amine (N-H); a band which occurred at 2950.66 cm^{-1} could be attributed to the presence of alkane (C-H); a band which occurred at 2842.24 cm^{-1} could be attributed to the presence of amine salt (N-H); a band which occurred at 2522.56 cm^{-1} could be attributed to the presence of carboxylic acid (O-H); *etc.* (Table 1) (Jacox, 2003; Powell *et al.,* 1966).

GC-MS Analysis of *A. graecorum* extract

The GC-MS analysis of the aqueous methanol extract from *A. graecorum* stems and leaves revealed approximately 9 principal biologically active compounds (Table 2). 3-Methyl butyl acetate (28.39%), d-glycero-d-ido-heptose (0.24%), Mome inositol (46.67%), Decanoic acid (0.66%), 11,14,17-Eicosatrienoic acid (0.78%), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (16.1%), (2R,3R)-2,3-Epoxyoctadec-4-yn-1ol (1.56%), Di(2-Ethylhexyl) adipate (2.88%) and 1,2-Benzenedicarboxylic acid (2.71 %) were identified as the phytocompounds present in significant quantities, while compounds were detected in lesser amounts (Table 2).

These results are similar to previous studies with differences in the concentration of some compounds, which may be due to differences in the environment in which plants grow. investigations concerning *A. maurorum* reveal the presence of sterols, fatty acids, alkaloids and flavonoids (Marashdah and Farraj, 2010; Samejo *et al.,* 2012). *A. graecorum* ethanolic extract was shown to contain sixteen phenolic compounds (Shaker *et al.,* 2022). It was observed that

Table 1: Analyze *A. graecorum* stems and leaves aqueous methanol extract to identify potential active chemical compounds using FT-IR.

Absorption (cm^{-1})	Transmittance (%)	Appearance	Group	Compound class
3400.26	1.14	medium	N-H stretching	primary amine
2950.66	14.82	medium	C-H stretching	alkane
2842.24	21.52	strong	N-H stretching	amine salt
2522.56	45.41	strong	O-H stretching	carboxylic acid
2123.37	41.67	strong	N=C=N stretching	carbodiimide
1651.01	14.62	medium	C=N bending	imine / oxime
1452.70	29.64	medium	C-H bending	Alkane/ methyl group
1411.82	29.43	strong	S=O stretching	sulfate
1113.47	34.74	strong	C-O stretching	secondary alcohol
1031.93	10.40	strong	S=O stretching	sulfoxide
1017.41	7.60	strong	C-F stretching	fluoro compound
749.41	22.65	strong	C-H bending	1,2-disubstituted
691.61	20.02	strong	C=C bending	alkene

Alhagi is a prolific source of physiologically active phytochemical substances, including phenolic compounds, flavonoids, polysaccharides and alkaloids (Gulzar Muhammad *et al.,* 2015). It was observed that the aqueous extract of *A. maurorum* had significant levels of caffeic acid, vanillic acid, ferulic acid, sinapic acid and rutin. *A. graecorum* serves as a substantial source of potent antioxidant flavonoid glycosides and several alkaloids (Ahmed, 2019). The aerial components of *A. graecorum* comprise tamarixetin 3-O-dirhamnoside and isorhamnetin 3-O-glucosyl neohesperidoside (El-Sayed *et al.,* 1993).

Antioxidant activity *in vitro*

The radical scavenging activity of the aqueous methanol extract from the stems and leaves of *A. graecorum* was assessed using the ABTS scavenging assay. The extract exhibited radical scavenging action, showing inhibition rates between 88% and 10% at concentrations from 500 to 15.625 µg/mL, with an IC_{50} value of 167.52 µg/mL. Statistically significant changes were noted in the ABTS scavenging assay across the different concentrations of the *A. graecorum* extract employed (Table 3). This investigation found that the suppression of free radicals by the extract increased at the maximum tested levels, with the extract exhibiting significant ABTS free radical scavenging activity and inhibition. These data indicate that *A. graecorum* functions as a natural antioxidant source.

These results are similar to previous studies with the difference in the inhibition rate, which may be due to the difference in the concentration of some active chemical compounds. A study evaluated the antioxidant activity of the aqueous extract of *A. maurorum*, demonstrating its antioxidant properties. Both doses (250 and 500 µg) demonstrated significant antioxidant activity, with inhibition rates of 82.88% and 86.70%, respectively (Buege and Aust, 1978). A separate study demonstrated that the extract (500 µg) significantly decreased malondialdehyde levels from 1.03±0.05 to 0.49±0.07, suggesting considerable

Table 2: Analyze *A. graecorum* stems and leaves aqueous methanol extract Identification of phytochemical compounds by GC-Mass.

Retention Time	Phytochemicals	Molecular formula	Molecular weight	Peak area %
7.81	3-METHYL BUTYL ACETATE	C7H14O2	130.18	28.39
14.68	D-GLYCERO-D-IDO-HEPTOSE	C7H14O7	210.18	0.24
16.48	MOME INOSITOL	C6H12O6	180.16	46.67
18.94	DECANOIC ACID	C10H20O2	172.26	0.66
20.26	11,14,17-EICOSATRIENOIC ACID	C20H34O2	306.5	0.78
20.37	3,7,11,15-TETRAMETHYL-2-HEXADECEN-1-OL	C20H40O	296.5	16.1
20.65	(2R,3R)-2,3-EPOXYOCTADEC-4-YN-1OL	C10H8O2	160.17	1.56
22.81	DI(2-ETHYLHEXYL) ADIPATE	C22H42O4	370.6	2.88
23.97	1,2-BENZENEDICARBOXYLIC ACID	C8H6O4	166.13	2.71

Table 3: ABTS radical scavenging assay of different concentrations of phytochemicals isolated from the leaves of *A. graecorum* stems and leaves aqueous methanol extract.

Concentration (µg/ml)	ABTS radical Inhibition (%)	Concentration pair comparison	IC_{50} (µg/ml)
15.625 (A)	9.980±0.3053	A vs B**	
		A vs C**	
		A vs D**	
31.25 (B)	23.389±0.3098	A vs E**	
		A vs F**	
		B vs C**	167.527±1.5650
62.5 (C)	38.046±0.5016	B vs D**	
		B vs E**	
		B vs F**	
125 (D)	57.905±0.9428	C vs D**	
		C vs E**	
		C vs F**	
250 (E)	79.516±1.2291	D vs E**	
		D vs F**	
		E vs F**	
500 (F)	88.050±1.0009		

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

antioxidant potential (Neamah, 2012). A study indicated that leaf extracts (20 µg/mL) exhibit superior free radical scavenging activity (83.5%) compared to flower extracts (72.3%), as assessed by ABTS free radical scavenging. In contrast, the antioxidant potential of quercetin standard was lower (63.41%) than that of both leaves and flowers (Laghari *et al.,* 2012).

In vitro antiparasitic activity of *A. graecorum* against *Eimeria magna*

The *in vitro* assessment of the antiparasitic efficacy of aqueous methanol extracts from the stems and leaves of *A. graecorum* showed that concentrations of 3.125, 6.25, 12.5, 25 and 50 mg/mL resulted in inhibition rates of 35%,

Table 4: *In vitro* study estimation of antiparasitic efficacy (in per cent) of *A. graecorum* aqueous methanol extract against *E. magna,* in 12 hours.

Treatments	Mean sporulated oocysts (B) in12 hours	Antiparasitic efficacy (in%) = (B - T) × 100/B in 12 hours
Control	47	0
T1 (3.125 mg/mL AG)	45	4.255[a-c]
T2 (6.25 mg/mL AG)	43	8.511[a-c]
T3 (12.5 mg/mL AG)	40	14.894[a-c]
T4 (25 mg/mL AG)	36	23.404[a-c]
T5 (50 mg/mL AG)	32	31.915[a-c]
T6 (25 mg/mL Toltrazuril)	28	40.426[a]

Table 5: *In vitro* study estimation of antiparasitic efficacy (in per cent) of *A. graecorum* aqueous methanol extract against *E. magna,* in 24 hours.

Treatments	Mean sporulated oocysts (B) in 24 hours	Antiparasitic efficacy (in%) = (B - T) × 100/Bin24 hours
Control	46	0
T1 (3.125 mg/mL AG)	42	8.696[a-c]
T2 (6.25 mg/mL AG)	38	17.391[a-c]
T3 (12.5 mg/mL AG)	35	23.913[a-c]
T4 (25 mg/mL AG)	30	34.783[a-c]
T5 (50 mg/mL AG)	25	45.652[a-c]
T6 (25 mg/mL Toltrazuril)	23	50.000[a]

Table 6: *In vitro* study estimation of antiparasitic efficacy (in per cent) of *A. graecorum* aqueous methanol extract against *E. magna,* in 36 hours.

Treatments	Mean sporulated oocysts (B) in 36 hours	Antiparasitic efficacy (in%)= (B - T) × 100/B in 36 hours
Control	46	0
T1 (3.125 mg/mL AG)	40	13.043[a-c]
T2 (6.25 mg/mL AG)	34	26.087[a-c]
T3 (12.5 mg/mL AG)	30	34.783[a-c]
T4 (25 mg/mL AG)	24	47.826[a-c]
T5 (50 mg/mL AG)	20	56.522[a-c]
T6 (25 mg/mL Toltrazuril)	19	58.696[a]

Table 7: *In vitro* study estimation of antiparasitic efficacy (in per cent) of *A. graecorum* aqueous methanol extract against *E. magna,* in 48 hours.

Treatments	Mean sporulated oocysts (B) in 48 hours	Antiparasitic efficacy (in%) = (B - T) × 100/B in 48 hours
Control	46	0
T1 (3.125 mg/mL AG)	37	19.565[a-c]
T2 (6.25 mg/mL AG)	32	30.435[a-c]
T3 (12.5 mg/mL AG)	27	41.304[a-c]
T4 (25 mg/mL AG)	22	52.174[a-c]
T5 (50 mg/mL AG)	17	63.043[a-c]
T6 (25 mg/mL Toltrazuril)	17	63.043[a]

Table 8: *In vitro* study estimation of antiparasitic efficacy (in per cent) of *A. graecorum* aqueous methanol extract against *E. magna,* In 60 hours.

Treatments	Mean sporulated oocysts (B) in 60 hours	Antiparasitic efficacy (in%)=(B - T) × 100/B in 60 hours
Control	45	0
T1 (3.125 mg/mL AG)	32	28.889^{a-c}
T2 (6.25 mg/mL AG)	28	37.778^{a-c}
T3 (12.5 mg/mL AG)	20	55.556^{a-c}
T4 (25 mg/mL AG)	15	66.667^{a-c}
T5 (50 mg/mL AG)	10	77.778^{a-c}
T6 (25 mg/mL Toltrazuril)	9	80.000^{a}

Table 9: *In vitro* study estimation of antiparasitic efficacy (in per cent) of *A. graecorum* aqueous methanol extract against *E. magna,* in 72 hours.

Treatments	Mean Sporulated oocysts (B) in 72 hours	Antiparasitic efficacy (in%) = (B - T) × 100/B in 72 hours
Control	44	0
T1 (3.125 mg/mL AG)	29	34.091^{a-c}
T2 (6.25 mg/mL AG)	24	45.455^{a-c}
T3 (12.5 mg/mL AG)	12	72.727^{a-c}
T4 (25 mg/mL AG)	7	84.091^{a-c}
T5 (50 mg/mL AG)	4	90.909^{a-c}
T6 (25 mg/mL Toltrazuril)	2	95.455^{a}

Notes: Means with distinct superscripts within a column are significantly different, (a) Significant differences Concentration of (AG) and reference treatment vs. control p<0.01, (ab) Significant differences were minor Concentration of (AG) and reference treatment vs. control p<0.05, (c) Significant differences Concentration of (AG) vs. reference treatment p<0.01, (cd) Significant differences were minor Concentration of (AG) vs. reference treatment p<0.05.

45%, 73%, 84% and 91%, respectively, during a 72-hour period. The results Antiparasitic efficacy demonstrated variability in sporulation and inhibition at various doses during an incubation time of up to 72 hours, with oocyst test results recorded every 12 hours. The results indicated the greatest inhibition of oocysts at concentrations of 50 mg/mL, 25 mg/mL and the reference drug, in contrast to lower concentrations where the inhibition rate diminished. Additionally, the inhibition increased with prolonged exposure time (Table 4 to 9).

To our knowledge, no prior studies have elucidated the potential role of aqueous methanol extracts from the stems and leaves of *A. graecorum* on parasites. Consequently, this study was essential in elucidating this role. Prior research has demonstrated the antifungal efficacy of *A. maurorum* extract against *Alternaria alternata*, Candida albicans and Cladosporium cladosporioides (Al-Snai *et al.,* 2019). The bioactive pharmaceuticals of *Alhagi* spp. have been extensively reviewed, A review has been conducted on various pharmacological and biological activities, including antimicrobial, antioxidant, anti-ulcer, cardiovascular, antipyretic, anti-inflammatory, antiurolithic, depigmenting, antidiarrheal and additional activities (Gulzar Muhammad *et al.,* 2015).

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 stems and leaves of *A. graecorum* contains many active chemical and Antioxidant compounds that have antiparasitic activity including phenols, flavonoids, flavonols and tannins.

It is generally agreed that *Alhagi* species have a great impact antiparasitic the findings of the present study indicate that the leaf extract of *A. graecorum* has potential oocysticidal characteristics, which may be utilized in coccidial treatment, the present study provides a significant basis for use of *A. graecorum* solution for treatment of *Eimeria sp.* 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

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

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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