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Phytochemical Analysis of *Rhazya stricta* Collected from Riyadh, Saudi Arabia as Antioxidant and against *Eimeria perforans* Activity

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ABSTRACT

Background: Saudi arabian plants have a rich biodiversity and are an important source of medicinal plants. The environmental conditions of Saudi Arabia have forced plants to develop adaptive mechanisms. This results in production of the numbers of secondary metabolites. *Rhazya stricta* contains glycosides, alkaloids, tannins and triterpenes, which have anti-cancer, anti-inflammatory, antioxidant and antibacterial properties.

Methods: GC-MS and FT-IR analyses were used in this study to detect the phytochemical composition of the extract of *R. stricta*. The antioxidant activity of *R. stricta* extract was assessed *in vitro* using ABTS assays and the IC_{50} values were determined. The *in-vitro* antiparasitic activity was evaluated using five distinct concentrations of *R. stricta* extract. The extract's inhibition of sporulated oocysts was evaluated after 72 hours.

Result: The GC-MS analysis identified approximately 20 primary biologically active compounds. The FT-IR analysis of the *R. stricta* extract revealed the presence of 10 distinct compounds. The extract exhibited significant antioxidant properties, with inhibition rates varying from 92.41% to 22.76% across concentrations of 500 to 15.625 µg/mL. The IC_{50} value was determined to be 45.3658 µg/mL. The antiparasitic effects were evaluated *in vitro*, revealing that oocysts exhibited the highest level of inhibition at concentrations of 50 mg/mL and 25 mg/mL.

Key words: Antioxidant, *Eimeria perforans*, Parasites, Phytochemical, *Rhazya stricta*.

INTRODUCTION

Natural medicinal plants have been used for decades as medication for treating diseases. However, researchers have debated the bioactive molecules, plant-derived molecules and mechanisms of action of natural medicines for many years and they have used these treatments, which show promising potential, to treat various diseases in humans and animals (Hassannia *et al*., 2020; Schweitzer, 2021). In the Arabian Peninsula, Saudi Arabian plants have a rich biodiversity and are an important source of genes for agriculture and medicinal plants. Due to its geographical location and dry climate (Choudhury *et al*., 2018; Ebrahim *et al*., 2020).

Primary metabolites are present in all plants, while secondary metabolites help a particular plant species interact with its environment (Budiastuti *et al*., 2022). The environmental conditions of Saudi Arabia strained plants to develop adaptive mechanisms. According to phytochemical analysis, such plants have a large number of secondary metabolites such as polyphenols, flavonoids, tannins, terpenes, alkaloids, saponins (El-Seedi *et al*., 2022). The potential antioxidant activity of plants is directly proportional to the amount of cell-enhancing chemicals present, such as phenolic compounds that are capable of catalyzing the fight against free radicals (Kumar *et al*., 2023).

The genus Coccidia, which is prevalent in rabbits, is parasite a primary contributor to intestinal problems in traditional rabbit rearing, especially *Eimeria perforans* (Murshed *et al*., 2023).

Rhazya stricta is one of the most popular medical shrubs on the deserts of the Arab peninsula, including Saudi Arabia and is used in plant -based drugs to treat various diseases (Redwan *et al*., 2016). Recently, scientists have used its extract in the production of silver nanoparticles, which plays a role in controlling mosquito vectors and many pathogens (Alshehri *et al*., 2020). *R. stricta* contains glycosides, alkaloids, tannins and triterpenes, considered a rich source of indole (Ahmed *et al*., 2018). Alkaloids often have anti-hypertensive, anti-cancer, anti-inflammatory,

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antioxidant and antibacterial properties (Rosales *et al*., 2020; Yaghmoor *et al*., 2015).

According to our knowledge, few data have been published on *R. stricta* conducted on chemical analysis, antioxidant properties and the principle of methanol extract of *R. stricta*, which is used in traditional medicine and collected in Riyadh, Saudi Arabia. Therefore, the purpose of this study is to check the difference between extracts tested in other areas and mentioned above. This, in turn, can reveal additional information regarding the characteristics of pharmaceutical preparations and to which extent the chemical compounds operate, which varies depending on plants and the environment, even in the same plant.

MATERIALS AND METHODS

Collection and preparation of *R. stricta* extract

In March 2024, the experimental plants *R. stricta* were collected from the Riyadh region, Kingdom of Saudi Arabia, at latitude 24°96′35.7″N and longitude 46°46′33.7″E. A herbalist at King Saud University conducted the classification of the plant.

This study utilized only the leaves of *R. stricta*. The plant samples were first rinsed with distilled water and then left to dry in the shade for 25 to 30 days before being pulverized into a fine powder with a grinding machine. Afterwards, 100 grams of *R. stricta* plant powder was soaked in 70% methanol for 48 hours at room temperature with agitation. The extract underwent filtration with Whatman No. 3 filter paper (Sigma, Germany). Afterwards, the extract was dried and concentrated at 40°C under reduced pressure utilizing a rotary evaporator (Yamato RE300, Tokyo, Japan) for future applications.

Gas chromatography-mass spectrometry (GC-MS) of *R. stricta* extract

The *R. stricta* extract was analyzed using Gas chromatography- mass spectrometry (Thermo Scientific, TSQ 8000 Evo; Waltham, MA, USA). Gas chromatography with an Elite-5 mass spectrometer and a fused silica column was used to separate the contents for the analysis. Helium (He) served as the carrier gas at a constant flow rate of 1 mL/min. Throughout the chromatography run, we calibrated the injector at a temperature of 260°C. A 1 µL sample was introduced into the instrument, with the oven temperature set to 60°C for 2 minutes, followed by a ramp rate of 300°C/min to reach 300°C, maintained for 6 minutes. The mass detector operated under the following conditions: It was ionized by electron impact at 70 eV and the scanning time was 0.2 seconds, with a 0.1-second break between scans for fragments 40 to 600 Daltons in size. The temperatures of the transfer line and ion source were both 240°C used the GCMS NIST (2008) library's database of known spectral components to compare them (Baeshen *et al*., 2023; Monika *et al*., 2022).

Fourier-transform infrared spectroscopy of *R. stricta* extract

The *R. stricta* extract was analyzed using FT-IR (Thermo Scientific, USA) and followed a set of steps to make IR spectra. The scanning wave number range was from 4000 to 500 cm^{-1} and the resolution was 4 cm^{-1}. The spectral data were compared with references to find the functional groups in the test samples. This made it possible to figure out what the IR spectra from the extract meant (Al-Otibi *et al*., 2024).

Estimation of the in-vitro antioxidant activity

The antioxidant activity of the extract from *R. stricta* leaves was evaluated in vitro using ABTS radical scavenging assays.

The extract from *R. stricta* leaves was evaluated for its antioxidant activity in vitro through ABTS radical scavenging assays. The ABTS free radical scavenging assay was performed following the method outlined by (Re *et al*., 1999). Three milliliters of ABTS cation solution (2,22 -azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) were mixed with thirty microliters of methanol extract solution. The sample concentrations ranged from 15.625 to 500 µg/ml in a disposable microcuvette with a path length of 1 cm. The absorption decrease was monitored for six minutes. All evaluations were performed in duplicate. The antioxidant activity was then calculated 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 determined based on the graph representing the concentration of the sample necessary to scavenge 50% of the ABTS. The IC_{50} is commonly utilized to indicate the concentration of extracts required to neutralize 50% of free radicals. ABTS was expressed as mg GAE/L.

Estimation of the *in vitro* antiparasitic activity

The experiment was performed on the *Eimeria perforans* parasite that infects rabbits. In the year 2024. This parasite was acquired from the Parasitology Laboratory inside the Department of Zoology at King Saud University.

Samples of *E. perforans* (Unsporulated oocyst) preserved in potassium dichromate solution were subjected to cleaning with phosphate-buffered saline (PBS) at a pH of 7.5. The centrifuge utilized Falcon tubes with 10 mL of liquid, subjected to a centrifugal force of 4000 × g for approximately 10 minutes. The procedure was conducted 3-6 times until the $K_2Cr_2O_7$ solution was fully removed. A suspension of the parasite was partitioned into seven segments. Each portion contained *R. stricta* extract concentrations at 3.125, 6.25, 12.5, 25 and 50 mg/mL.

A standard treatment of toltrazuril at a concentration of 30 µg/mL was employed for comparison, while potassium dichromate solution served as a negative control. Components were arranged in 3 mL petri plates with 24 compartments. Each treatment was conducted under precisely controlled conditions and performed in triplicate. Unsporulated oocysts were assessed by documenting observations at 12, 24, 36, 48, 60 and 72 hours. Sample preparation was conducted using the McMaster Egg Counting Method (Long and Rowell, 1958) for the purpose of counting. An aliquot of the generated material was transferred to a McMaster Egg Counting chamber (Chalex LLC, Park City, UT) and allowed to stand for 5 minutes prior to examination under a light microscope. Slides were examined using a light microscope (PX51, Olympus Co., Tokyo, Japan) at a magnification of 10X.

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 unsporulated oocyst number of control.

T = Mean unsporulated oocyst number of treatment.

Statistical calculations

Data are presented as the mean±SD derived from three independent observations. One-way ANOVA and Tukey's test ($p<0.05$ and $p<0.01$) were used to look for differences in the *in vitro* antioxidant and antiparasitic assays. A probability of $p<0.05$ was deemed significant and $p<0.01$ very significant.

RESULTS AND DISCUSSION

GC-MS analysis of *R. stricta* extract

The GC-MS analysis of the aqueous methanol extract from *R. stricta* leaves revealed approximately 20 principal biologically active compounds (Table 1). 2,2-dimethyl-1,3-dioxane-4,6-dione (0.54%), 3,4-benzisoxazol (1.67%), (e)-4-(5′-methyl-2′-furyl)-3-buten-2-one (0.17%), 3′,4′-dimethoxyacetophenone (0.62%), cytidine (6.57%), 4-methyl-4,3-borazaroisoquinoline (3.63%), mome inositol (63.41%), eburenine (2.13%), aspidospermidine (1.16%), guanethidine (3.08%), eutensol (0.5%), quebrachamine (2.84%), benzenemethanamine (0.31%), 4(z)-5-(formylmethylene)-4-methoxy-2(5h)-furanone (7.56%), 3-(.beta.,.beta.-dimethylpiperidyl)-1-propanol (2.22%), pleiocarpamine (0.64%), (s)-2- (z)-[2-(n-1-phenylethyl (0.11%), 3,3-dimethyl-2-exo-norbornyl-glyoxal (1.65%), sarpagan (1.19%) and 2,2-dimethyl-1,3-dioxane-4,6-dione (0.54%) were identified as the phytocompounds present in significant quantities, while other compounds were detected in lesser amounts.

FT-IR analysis of *R. stricta* extract

The FT-IR analysis of the water-methanol extract derived from *R. stricta* revealed the presence of 10 distinct compounds (Table 2). The analysis revealed various characteristic peaks, each uniquely attributed to the presence of specific functional groups or phytochemical compounds. An analysis using FTIR spectrometry revealed principal bands ranging from 691.32 to 3400.87 cm^{-1}. The stretching and bending vibrations of N-H, C-H, N=C=N, C=C, S=O, C-O and C-F were observed across various

Table 1: Analyze *R. stricta* leaves aqueous methanol extract Identification of phytochemical compounds by GC-Mass.

Retention time	Phytochemicals	Molecular formula	Molecular weight	Peak area%
8.22	2,2-dimethyl-1,3-dioxane-4,6-dione	$C_6H_8O_4$	144.12	0.54
9.3	3,4-benzisoxazol	C_7H_5NO	119.12	1.67
10.59	(e)-4-(5′-methyl-2′-furyl)-3-buten-2-one	$C_9H_{10}O_2$	150.17	0.17
14.12	3′,4′-dimethoxyacetophenone	$C_{10}H_{12}O_3$	180.20	0.62
16.14	cytidine	$C_9H_{13}N_3O_5$	243.22	6.57
17.53	4-methyl-4,3-borazaroisoquinoline	$C_{10}H_9N$	143.18	3.63
19.34	mome inositol	$C_6H_{12}O_6$	180.16	63.41
21.69	eburenine	$C_{19}H_{24}N_2$	280.4	2.13
22.05	aspidospermidine	$C_{22}H_{30}N_2O_2$	354.5	1.16
23.09	guanethidine	$C_{10}H_{22}N_4$	198.31	3.08
23.3	eutensol	$C_{10}H_{22}N_4$	198.31	0.5
24.01	quebrachamine	$C_{19}H_{26}N_2$	282.4	2.84
24.19	benzenemethanamine	$C_{15}H^{12}BrClN_4$	363.64	0.31
24.51	(z)-5-(formylmethylene)-4-methoxy-2(5h)-furanone	$C_7H_6O_4$	154.12	7.56
24.81	3-(.beta.,.beta.-dimethylpiperidyl)-1-propanol	C_7H1_7NO	131.22	2.22
25.35	pleiocarpamine	$C_{20}H_{22}N_2O_2$	322.4	0.64
25.98	(s)-2-{(z)-[2-(n-1-phenylethyl	$C_{10}H_{13}N_3O_2$	207.23	0.11
26.93	3,3-dimethyl-2-exo-norbornyl-glyoxal	$C_{11}H_{16}O_2$	180.24	1.65
28.01	sarpagan	$C_{19}H_{22}N_2O$	294.39	1.19
8.22	2,2-dimethyl-1,3-dioxane-4,6-dione	$C_6H_8O_4$	144.13	0.54

bands, indicative of a diverse array of compound functionalities. A number of these are primary amines, alkanes, carbodiimide, Alkene/ monosubstituted, Alkane/ methyl group, sulfate, secondary alcohol, fluoro compound and Alkene/ disubstituted (Jacox, 2003; Powell *et al*., 1966).

Although certain chemical amounts vary, maybe as a function of the plant environment, these results are comparable to those of earlier research. According to research on *R. stricta*, the primary chemicals found by relative concentrations were 3-ethylpiperidine (5.63%), quebrachamine (11.96%), methyl aspidospermidine-3-carboxylate (14.27%) and 1,2-didehydroaspidospermidine (28.37%). Alkaloids made up the majority of the substances that were extracted using methanol (Baeshen *et al*., 2023). Similarly, earlier research revealed that *R. stricta* contains alkaloids (Ahmed *et al*., 2018; Bukhari *et al*., 2017). Furthermore, the amount of alkaloids in plants can be influenced by genetic variation (Abd-Elgawad and Alotaibi, 2019). Scientists have investigated several alkaloids for their anticancer and antiproliferative properties since they are a rich supply of ingredients utilized in drug development and formulation (Mondal *et al*., 2019; Wada and Yamashita, 2019). The results of another study elucidated their role in providing protection to animals subjected to UV radiation (Takshak and Agrawal, 2019).

Antioxidant activity *in vitro*

The radical scavenging activity of the aqueous methanol extract from the leaves of *R. stricta* was assessed using the ABTS scavenging assay. The extract was able to get rid of radicals, with an IC_{50} value of 45.3658 µg/mL and inhibition rates ranging from 92.41% to 22.76% at 500 to 15.625 µg/mL. The ABTS scavenging assay showed statistically significant changes with the different concentrations of *R. stricta* extract used (Table 3). This study discovered that the extract was more effective at stopping free radicals at the highest levels tested. The extract showed strong ABTS free radical scavenging activity and inhibition. These data indicate that *R. stricta* functions as a natural antioxidant source.

Table 2: Analyze *R. stricta* leaves aqueous methanol extract to identify potential active chemical compounds using FT-IR.

Absorption (cm^{-1})	Transmittance (%)	Appearance	Group	Compound class
3400.87	1.57	Medium	N-H stretching	Primary amine
2950.59	14.57	Medium	C-H stretching	Alkane
2840.58	21.28	Medium	C-H stretching	Alkane
2122.92	45.47	Strong	N=C=N stretching	Carbodiimide
1644.08	13.02	Strong	C=C stretching	Alkene/monosubstituted
1450.67	30.23	Medium	C-H bending	Alkane/methyl group
1407.90	30.05	Strong	S=O stretching	Sulfate
1111.71	36.47	Strong	C-O stretching	Secondary alcohol
1017.55	11.25	Strong	C-F stretching	Fluoro compound
691.32	22.39	Strong	C=C bending	Alkene/disubstituted

Table 3: ABTS radical scavenging assay of different concentrations of phytochemicals isolated from the leaves of *R. stricta* aqueous methanol extract.

Concentration (µg/ml)	ABTS Radical Inhibition (%)	Concentration pair comparison	IC_{50} (µg/ml)
15.625 (A)	22.765±0.2545	A vs B**	
		A vs C**	
		A vs D**	
31.25 (B)	41.165±0.3738	A vs E**	
		A vs F**	
		B vs C**	45.365±1.2573
62.5 (C)	58.942±0.6055	B vs D**	
		B vs E**	
		B vs F**	
125 (D)	82.329±0.6165	C vs D**	
		C vs E**	
		C vs F**	
250 (E)	87.418±0.7024	D vs E**	
		D vs F**	
		E vs F**	
500 (F)	92.411±0.5316		

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

Table 4: (*In vitro* study) estimation of antiparasitic efficacy (in per cent) of *R. stricta* aqueous methanol extract against *E. perforans*.

Treatments	Mean sporulated oocysts (B)						Antiparasitic efficacy (in%) = (B - T) ×100/B					
Time	12 hour	24 hour	36 hour	48 hour	60 hour	72 hour	12 hour	24 hour	36 hour	48 hour	60 hour	72 hour
Control	194	190	188	188	188	188	0	0	0	0	0	0
3.125 mg/mL RS	174	160	148	140	130	122	10.3	16	21.3	25.5	30.9	35.1
6.25 mg/mL RS	156	148	130	116	82	74	19.6	22	30.9	38.3	56.4	60.6
12.5 mg/mL RS	130	110	56	44	40	35	33	42	70.2	76.6	78.7	81.4
25 mg/mL RS	90	70	40	24	16	0	53.6	63	78.7	87.2	91.5	100
50 mg/mL RS	76	50	32	3	2	0	60.8	74	83	98	98.9	100
30 mg/mL Toltrazuril	77	53	33	8	4	0	60.3	72	82.4	95.7	97.9	100

In this study, results clearly indicate that the methanolic extract from *R. stricta* leaves exhibited good antioxidant activities, reaching a 92.41% inhibition when tested at a 500 µg/mL concentration. 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. Several studies reported the ABTS scavenging activity of different parts of *R. stricta*. The assessment of antioxidant potential was done on the root fractions of *R. stricta* using different antioxidant assays. Fractions obtained through solvent-solvent extraction of root crude extract of *R. stricta* exhibited significant ($p<0.001$) free radical scavenging activity with IC_{50} ranging from 400 to 776 µg/ml (Mahmood *et al*., 2020).

The results of antioxidant assays are supported by previous reports in which *R. stricta* extract was examined for its antioxidant potential and found to have significant antioxidant activity (Al-Busafi *et al*., 2007; Ali *et al*., 2000; Iqbal *et al*., 2006). The fractions obtained by solvent-solvent extraction of *R. stricta* root raw extract exhibited remarkable free radical scavenging activity, with an IC_{50} of 400-776 g/mL (Mahmood *et al*., 2020). Direct comparison of data from the current study with those reported in the literature is difficult since different parts and expression units were used.

Antiparasitic activity of *R. stricta* against *E. perforans In vitro*

The *in vitro* assessment of the antiparasitic efficacy of extract from the leaves of *R. stricta* showed that concentrations of 3.125, 6.25, 12.5, 25 and 50 mg/mL. resulted in inhibition rates of 35.1%, 60.6%, 81.4%, 100% and 100%, respectively, during a 72-hours period. The results demonstrated variability in antiparasitic efficacy by sporulation and inhibition at various doses during an incubation time of up to 72 hours, where 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 our knowledge, no prior studies have elucidated the potential role of aqueous methanol extracts from the leaves of *R. stricta* in parasites. Consequently, this study was essential in elucidating this role. Studies have reported that *R. stricta* contains a wide range of active ingredients, as glycosides and alkaloids isolated from *R. stricta* possess anti-leishmanial activities (Tariq *et al*., 2016). *In vitro*, *R. stricta* leaf and fruit extract demonstrated antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Streptococcus pyogenes* and *Salmonella typhi* (Sultana and Khalid, 2010). *R. stricta* leaf extract demonstrated a control of bacterial growth on locally isolated meningococcal strains that increased with concentration and treatment time (Abadi *et al*., 2011). Chloroformic and methanolic extracts of *R. stricta* roots exhibited antimicrobial activity toward *B. subtilis*, *E. coli*, *S. aureus* and *P. aeruginosa* (Bashir *et al*., 1994). *R. stricta* chloroformic and methanolic root fractions demonstrated antifungal activities against *A. terreus*, *A. flavus* and *C. albicans* (Bashir *et al*., 1994). Another study revealed that fractionated *R. stricta* methanol and chloroform samples showed antifungal activity against *T. longifusis*, *C. albicans*, *A. flavus* and *Fusarium solani* (Khan and Khan, 2007).

CONCLUSION

The production and diversity of secondary metabolites in plants are greatly influenced by their habitat; even within the same plant species, different chemical compositions are produced by different ecological and geographic harvesting zones. *R. stricta* leaf extracts have a number of antioxidant and active chemical components that have antiparasitic qualities. It is well known that *R. stricta* has a major impact on antiparasitic properties. The investigation's findings show that *R. stricta* leaf extract has possible oocysticidal qualities that might be used to treat coccidiosis. A strong basis for using the *R. stricta* solution to treat *Eimeria sp*. in rabbits is established by this study. Additional research is required to assess the effectiveness of the extract in live tissues and to clarify the molecular pathways that govern its action.

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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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