© 2023 Journal of Pharmacy & Pharmacognosy Research, 11 (4), 635-650, 2023
ISSN 0719-4250
https://jppres.com

DOI: https://doi.org/10.56499/jppres23.1635_11.4.635

Original Article

Phytochemical profile and *in vitro* protoscolicidal effects of *Juniperus phoenicea* L., *Calotropis procera* (Aiton) Dryand, and *Artemisia judaica* L. against *Echinococcus granulosus* cysts

[Perfil fitoquímico y efectos protoscolicidas *in vitro* de *Juniperus phoenicea* L., *Calotropis procera* (Aiton) Dryand y *Artemisia judaica* L. contra quistes de *Echinococcus granulosus*]

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Abstract

Context: *Echinococcus granulosus* (*E. granulosus*) is a significant zoonotic agent in veterinary and medicinal fields. Surgery and benzimidazole drugs are used as treatments but have significant drawbacks. Therefore, there is growing interest in ethnomedical approaches to investigating the scolicidal properties of plants traditionally as anthelmintics. *Calotropis procera*, *Juniperus phoenicea*, and *Artemisia judaica* are three plants traditionally used as anthelmintics.

Aims: To examine the phytochemical composition and scolicidal capability against *E. granulosus* cysts of *C. procera*, *J. phoenicea*, and *A. judaica* methanolic extracts.

Methods: Fresh *E. granulosus* cysts were isolated from livestock in southern Jordan and tested to determine the scolicidal potential of *C. procera*, *J. phoenicea*, and *A. judaica* aerial parts methanolic extracts, and their chemical compositions were examined using LC-MS.

Results: Although all treatments were protoscolicidal, that of *J. phoenicea* exhibited higher protoscolices mortality rates at lower concentrations and treatment times than the other extracts. The LC-MS analysis revealed several components with biologically active properties were present in the plant extracts, including terpenes and polyphenolic compounds. Only *C. procera* contained the steroid uzarin and the flavonoid isoquercitrin.

Conclusions: This study demonstrated the potent effects of *A. judaica*, *J. phoenicea*, and *C. procera* methanolic extracts against *E. granulosus* protoscoleces *in vitro*, indicating that these plants and/or their phytochemical components may be attractive sources of novel scolicidal medications.

Keywords: *Artemisia judaica*; *Calotropis procera*; hydatid cyst; *Juniperus phoenicea*; scolicidal.

Resumen

Contexto: El *Echinococcus granulosus* (*E. granulosus*) es un agente zoonótico importante en los ámbitos veterinario y medicinal. La cirugía y los fármacos benzimidazólicos se utilizan como tratamientos, pero presentan importantes inconvenientes. Por ello, existe un creciente interés por los enfoques etnomédicos para investigar las propiedades escolicidas de plantas tradicionalmente utilizadas como antihelmínticos. *Calotropis procera*, *Juniperus phoenicea* y *Artemisia judaica* son tres plantas utilizadas tradicionalmente como antihelmínticos.

Objetivos: Examinar la composición fitoquímica y la capacidad escolicida frente a quistes de *E. granulosus* de los extractos metanólicos de *C. procera*, *J. phoenicea* y *A. judaica*.

Métodos: Se aislaron quistes frescos de *E. granulosus* de ganado del sur de Jordania y se analizaron para determinar el potencial escolicida de los extractos metanólicos de partes aéreas de *C. procera*, *J. phoenicea* y *A. judaica* y se examinaron las composiciones químicas mediante LC-MS.

Resultados: Aunque todos los tratamientos fueron protoscolicidas, el de *J. phoenicea* mostró mayores tasas de mortalidad de protoscolices a menores concentraciones y tiempos de tratamiento que los otros extractos. El análisis LC-MS reveló la presencia de varios componentes con propiedades biológicamente activas en los extractos vegetales, entre ellos terpenos y compuestos polifenólicos. Sólo *C. procera* contenía el esteroide uzarina y el flavonoide isoquercitrina.

Conclusiones: Este estudio demostró los potentes efectos de los extractos metanólicos de *A. judaica*, *J. phoenicea* y *C. procera* contra las protoscoleces de *E. granulosus in vitro*, lo que indica que estas plantas y/o sus componentes fitoquímicos pueden ser fuentes atractivas de nuevos medicamentos escolicidas.

Palabras Clave: *Artemisia judaica*; *Calotropis procera*; escolicida; *Juniperus phoenicea*; quiste hidatídico.

ARTICLE INFO
Received: March 11, 2023.
Accepted: July 1, 2023.
Available Online: July 23, 2023.

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INTRODUCTION

Infectious epidemics are significant health-related issues currently faced by human communities. Contact between humans and animals in a variety of scenarios may represent additional risk factors owing to their ability to spread zoonotic diseases. Tapeworms *Echinococcus granulosus* are zoonotic organisms that can infect humans with cystic echinococcosis (CE), also known as the hydatid disease. *Echinococcus granulosus* is one of the largest tapeworm outbreaks ever documented, with more than one million reported cases disseminated on all continents except Antarctica (Deplazes et al., 2017). Cystic echinococcosis is a chronic illness with a mortality rate of 1.29 per 100 patients (Hombo et al., 2020). However, the World Health Organization (WHO) now lists it as one of the world's 17 neglected tropical diseases because of insufficient funding for research, which poses a significant obstacle in the development of new treatments. Cystic echinococcosis is medically and economically relevant because it substantially negatively impacts livestock quality, resulting in huge annual economic losses (Najjari et al., 2020). Numerous disease subtypes caused by similar *Echinococcus* spp. have been reported; however, only CE has been acknowledged in Jordan as a disease (Al-Qaoud et al. 2003; Nasrieh and Abdel-Hafez, 2004). Cystic echinococcosis infection begins when *E. granulosus* eggs are unintentionally swallowed by humans. Six-hooked oncospheres are released during their passage through the intestinal wall into the bloodstream. Protoscolice fluid-containing hydatid cysts typically develop in the liver and lungs, although they occasionally appear unexpectedly (Almutairi and Al Rajhi, 2018; Shuaibi et al., 2021). Coughing, nausea, vomiting, and soreness or discomfort in the upper abdomen or chest are symptoms of this disease; however, CE may persist for years without exhibiting any symptoms, which increases its pathological complexity. Moreover, the release of cystic fluid may cause allergies or even death (Khachatryan, 2017). Currently, changes in infection prevalence and intensity are the main metrics used to track the spread of CE infections, with ultrasonographic imaging is frequently used to track patient treatment progress but is a challenging and not entirely accurate reflection of patient health (Haghighi et al. 2021).

Cystic echinococcosis therapy can be difficult because the characteristics of the cyst and the patient's medical conditions may influence their pathology. Cysts are more common in the liver and lungs of adult people than in the other organs. However, the appearance of the cyst in a peculiar place on the body has been reported (Shuaibi et al., 2021). Typically, synthetic drugs such as benzimidazole are used with two therapeutic modalities: surgery and puncture aspiration (Shams-Ul-Bari et al., 2011; Arif et al., 2008), which have significant downsides (Daimari et al., 2018). Therefore, there is growing interest in the ethnoveterinary field to examine the scolicidal properties of plants traditionally used by local farmers to treat CE. The WHO estimates that 80% of the population of developing countries relies on traditional medicines, mostly derived from plants, for their primary healthcare needs. Evaluation of the effects of natural scolicidal drugs on CE has recently been added to therapeutic pathways that extend beyond standard interventions. Both *in vitro* and *in vivo* testing of several substances derived from medicinal plants and herbs has been conducted (Alvi et al., 2022), aiding in the ability to treat and control disease pathogenesis and improve patient quality of life.

Despite being a small country, Jordan is well known for its vast array of medicinal plants. Several of these are frequently used as health aids in Jordan's traditional and rural settings (Abdel-Qader et al., 2020). Favored for their therapeutic qualities, *Artemisia judaica* L. (*Compositae*), *Juniperus phoenicea* L. (*Cupressaceae*), and *Calotropis procera* (Aiton) Dryland. (*Apocynaceae*), these plant species thrive naturally in Southern Jordan. Dry, warm locations in the Mediterranean bioclimatic zone are desirable for *J. phoenicea* and *A. judaica* to flourish, while in contrast, *C. procera* is found in Wadi Araba and other hot tropical regions (Palmer, 2014). These plants have long been recognized for their anthelmintic effects. The Egyptian Papyrus Ebers, which dates to around 1500 B.C., cites the curative powers of junipers and provides instructions on how to use them to treat roundworms and tapeworms (Metwaly et al., 2021). The anthelmintic properties of boiled preparations of *A. judaica* and *J. phoenicea* are widely known in Bedouin and farm medicine in the southern villages of Jordan, where it is used as a deworming treatment for intestinal worms in children and adults. Locals in the region where *C. procera* grows know of its prospects for treatment, with preparations made primarily from its flowers and roots blended with honey or as dried powder for use as anthelmintics. The limited accessibility and high cost of commercial drugs, as well as the increasing risk of helminth infection, are health risks in these rural communities, and there is a growing interest in ethnomedical approaches to investigate the anthelmintic properties of plants traditionally used by local farmers.

In response to the traditional medicinal uses and the limited scientific investigations addressing the cellular processes responsible for the anthelmintic

actions of *J. phoenicea*, *A. judaica*, and *C. procera*, we aimed to evaluate the phytochemical makeup of these plants and validate the traditional applications connected to these varied species, especially the scolicidal activity of methanolic extracts against *E. granulosus* cysts.

MATERIAL AND METHODS

Collection of *E. granulosus* cyst protoscolices

Hydatid cysts were obtained from the organs of naturally infected animals during slaughter at the Al-Karak Abattoir. The hydatid cyst contents were transferred into sterile test tubes and allowed to settle for 30 min to facilitate the precipitation of protoscoleces as previously described (Smyth and Barrett, 1980). After removing the supernatant, the protoscoleces were washed three times with sterile physiological phosphate-buffered saline (PBS) solution (pH 7.2). To assess the viability of the protoscolices, 100 µL pooled protoscolices were mixed with 100 µl 0.1% eosin on a slide and incubated for 15 min. Dead protoscoleces were stained red, whereas living protoscoleces remained colorless when observed under a compound microscope. The viability test was conducted on days 1 to 6, with the medium replaced every two days. Only samples with 100% viable protoscoleces were used for the *in vitro* and *in vivo* studies (Al Qaisi, et al., 2022).

Plant collection and extraction

Fresh plant material from *J. phoenicea*, *A. judaica,* and *C. procera* was collected in the spring of 2021 from their natural locations in Jordan (Fig. 1). The Department of Herbarium and the National Center for Agricultural Research in Al-Balqa, Jordan, cooperatively identified the plant specimens.

Various plant parts from each species were used to prepare methanolic extracts (Table 1). The protocol was performed at room temperature. Every pallet was cleaned and dried in a shaded place with sufficient ventilation. After being weighed, the materials were ground into a fine powder in a grinder. The obtained weights (g) are illustrated in Table 3. The dried plant samples were soaked in methanol at a ratio of 10:1 (v/w) for 72 h with continuous shaking at room temperature. Then, the suspension was filtered through Whatman paper No.1, and the crude methanol extract was dried using a rotary evaporator at 45°C under reduced pressure. The resulting extracts were stored in airtight containers at -20°C. To calculate the percentage yield, the weight of the dried extract was measured and compared with that of the dried plant samples before extraction using the following equation as previously described (Pandey and Tripathi, 2014): Yield% = (weight of dry extract/weight of dry parts before extraction) × 100%.

Viability test

The viability assessment of the protoscolices was performed according to the method described by Miman et al. (2010). 100 µL of pooled protoscolices were combined with 100 µL of 0.1% eosin in a test tube for 15 min. Under a compound microscope, the dead protoscoleces stained red, whereas the living protoscoleces remained colorless. From the first to the sixth day of the viability test, the RPMI medium was replaced every two days. For the *in vitro* experiments, only samples with 100% viable protoscoleces were used.

Figure 1. Medicinal plants used to treat *Echinococcus granulosus* protoscolices *in vitro*.
(A) *Artemisia judaica*; **(B)** *Juniperus phoenicea*; and **(C)** *Calotropis procera.*

Table 1. Medicinal plants tested for scolicidal activity against *E. granulosus* cyst, and their collection data.

Plant name	Organs used	Collection site	GPS coordinates (decimal degrees)		Elevation (m)	Registration number
			Latitude	Longitude		
Juniperus phoenicea subsp *phoenicea* L.	Leaves and fruit	Mediterranean forest, Dana natural reserve, Jordan	35.5003031	30.6615746	1241	JOH4932
Artemisia judaica L.	Leaves and stalks	Irano-Turanian, Petra, Jordan.	36.0749980	33.96375	787	JOH4931
Calotropis procera (Aiton) Dryan	Leaves, flowers, and fruit	Wadi Araba and Dead sea, Jordan	35.41725313	30.81037	343	JOH4933

In vitro scolicidal assessment

Stock solutions of the methanolic extracts were prepared in 30% dimethyl sulfoxide (DMSO) at a concentration of 0.5 g/mL as recommended by Mahmoudvand et al. (2014b). The extract stocks were added to RPMI medium (10, 20, and 40 µL) to produce final concentrations of 5, 10, and 20 mg/mL. Protoscolices cultured in a final volume 1 mL culture medium containing 40 µL 30% DMSO served as controls. The hydatid cyst protoscoleces were treated with extracts of *A. judaica*, *J. phoenicea*, and *C. procera* at different doses (5, 10, and 20 mg/mL). These mixtures were thoroughly mixed and incubated at 37°C for 1, 3, 6, 12, and 24 h. After adding 10 µL 0.1% eosin solution to 10 µL protoscoleces solution for 15 min, the viability of the protoscoleces was evaluated under a microscope to measure the scolicidal activity of the extracts. One hundred protoscoleces were counted for each evaluation, and their viability was determined by their body movement, flame cell activity, and eosin impermeability, as observed under a compound microscope. Protoscoleces were deemed viable when their frame cells were motile and did not stain with eosin (Walker et al., 2004). The effectiveness of albendazole (ABZ) and methanolic extracts of the tested plants on the viability of the protoscoleces were compared.

The stock ABZ solution was created by combining 0.5 g ABZ with 1 mL 30% DMSO. The drug was filtered through a 0.22 mm filter prior to starting the experiment. We used 20 mg/mL ABZ as a positive control to compare the effect of methanolic plant extracts on the viability of protoscoleces. The details of the structuring procedure are described elsewhere (Taghipour et al., 2021). Normal saline was used as the negative control. The mortality rate was estimated using the following formula as described previously (Chai et al., 2021): Mortality rate = Number dead/Number live (control) × 100. Triplicates of each experiment were carried out independently at each time point.

LC-MS analysis of methanolic extracts

A Shimadzu LC-MS 8030 System (Kyoto, Japan) was used for HPLC separation in accordance with the manufacturer's operational methodology (LCMS QuickGuide_Shimadzu, 2003) to identify the chemicals contained in the plant extracts and compare their components. In a nutshell, the mobile phase contained solvents A and B in a gradient, with A composed of 0.1% (v/v) formic acid in water and B composed of 0.1% (v/v) formic acid in acetonitrile for the gradients of 5% B for 5 min, 5-100% B for 15 min, and 100% B for 5 min, at a flow rate of 0.5 mL/min. An Agilent Zorbax Eclipse XDB-C18 column (2.1*150 mm × 3.5 m), 350°C oven, and 50 L injection volume were used. The eluent was monitored using a Shimadzu LC-MS 8030 with electrospray ion mass spectrometry (ESI-MS) in positive ion mode and scanned from 100 - 1000 m/z. The ESI-MS was performed at a voltage of 125 V and SKIMMER at 65 V. High purity nitrogen (99.999%) was used as a drying gas at a flow rate of 10 L/min, using a fragment nebulizer at 45 psi and capillary temperature of 3500°C. As a blank, we used 0.1% formic acid.

Statistical analysis

The Statistical Package for Social Sciences (IBM SPSS) for Windows, Version 21.0 was used to perform all statistical computations using one-way variance analysis (ANOVA). The standard deviation (S.D.) and number of observations (n) were used to describe how the data from the three trials varied in triplicate. P-values less than 0.05 were considered significant.

RESULTS

Viability of protoscolices

Wet-mount drop analysis was used to determine the fertility of the hydatid cysts based on the presence of free protoscoleces in the cystic fluid (Fig. 2). The living protoscoleces that remained colorless after eosin staining displayed distinctive muscle contractions and flame cell activity were used for the experiments

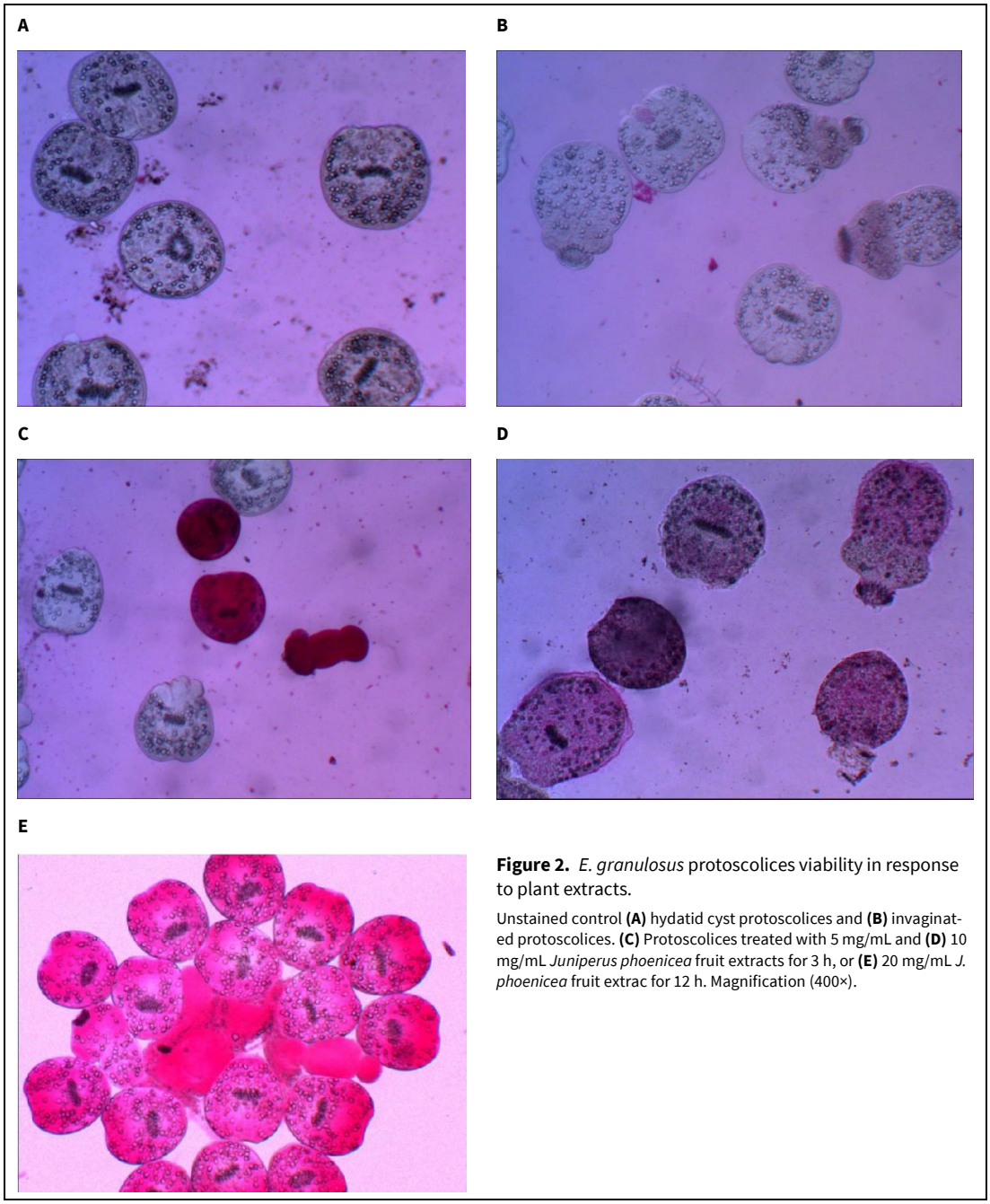

Figure 2. *E. granulosus* protoscolices viability in response to plant extracts.

Unstained control **(A)** hydatid cyst protoscolices and **(B)** invaginated protoscolices. **(C)** Protoscolices treated with 5 mg/mL and **(D)** 10 mg/mL *Juniperus phoenicea* fruit extracts for 3 h, or **(E)** 20 mg/mL *J. phoenicea* fruit extrac for 12 h. Magnification (400×).

(Fig. 2A). After 24 h of *in vitro* post-incubation, protoscoleces samples treated with normal saline contained at least 95% of their viable cells.

In vitro treatment of protoscolices

When compared with the ABZ and the negative control samples, those treated with the methanol extracts of *J. phoenicea*, *A. judaica*, and *C. procera* significantly ($p<0.05$) affected the mortality rate of the protoscoleces at the tested exposure times (Table 2). The *J. phoenicea* fruit and leaf extracts were used at doses of 5, 10, and 20 mg/mL, all of which showed strong toxicity against protoscoleces isolated from infected animal organs (Fig. 2C and D). When protoscoleces were administered a methanolic extract of the fruit and leaves of *J. phoenicea* at a concentration of 20 mg/mL, the mortality rate was dramatically reduced ($p<0.05$) (Fig. 3A and B). After 3 h, the *J. phoenicea* fruit extract increased the protoscolices mortality rate to 100%, whereas treatment with the *J. phoenicea* leaf extract increased this rate to 96%. When using 20 mg/mL ABZ as a positive control, a deadly effect of 10% was observed after 3 h of treatment, while 20 mg/mL *J. phoenicea* fruit and leaf methanolic extract achieved a substantially higher mortality rate in 3 h. The IC_{50} was 1.869 and 2.975 mg/mL for fruit and leaf methanolic extract, respectively. The scolicidal effect of 20 mg/mL ABZ enhanced the mortality rate of the

Table 2. Scolicidal effects of *Artemisia judaica*, *Juniperus phoenicea*, and *Calotropis procera* methanol extracts on protoscolices mortality compared with ABZ and negative controls.

Plant extract	Concentration (mg/mL)	Mortality rate (%)				
		1 h	3 h	6 h	12 h	24 h
Artemisia judaica	5	3.50 ± 0.50	5.86 ± 0.50	12.50 ± 1.30*	20.66 ± 2.08*	54.30 ± 2.51
	10	4.8 ± 0.40*	7.30 ± 0.50	57.00 ± 2.00*	90.00 ± 1.00*	100 ± 0.0
	20	7.16 ± 0.35*	63.16 ± 0.45*	87.00± 1.00*	100 ± 0.0*	100 ± 0.0
Juniperus phoenicea leaf extract	5	5.77 ± 0.47*	65.33 ± 0.25*	100 ± 0.0*	100 ± 0.0*	100 ± 0.0
	10	53.33 ± 4.16*	93.30 ± 0.21*	100 ± 0.0*	100 ± 0.0*	100 ± 0.0
	20	67.33 ± 6.80*	100 ± 0.0*	100 ± 0.0*	100 ± 0.0*	100 ± 0.0
Juniperus phoenicea fruit extract	5	7.00 ± 0.20*	85.00 ± 0.20*	100 ± 0.0*	100 ± 0.0*	100 ± 0.0
	10	70.50 ± 6.70*	100 ± 0.0*	100 ± 0.0*	100 ± 0.0*	100 ± 0.0
	20	85.13 ± 2.01*	100 ± 0.0*	100 ± 0.0*	100 ± 0.0*	100 ± 0.0
Calotropis procera leaf extract	5	4.26 ± 0.64*	24.33 ± 2.08*	33.33 ± 2.31*	51.33 ± 1.51	70.33 ± 0.57
	10	41.00 ± 1.52*	32.33 ± 1.15*	42.66 ± 3.60*	75.50 ± 7.27*	100 ± 0.0
	20	30.66 ± 1.52*	40.00 ± 0.50*	51.66 ± 3.05*	85.66 ± 0.65*	100 ± 0.0
Calotropis procera flowers and fruit mixed extract	5	3.30 ± 0.57	15.00 ± 1.00*	28.33 ± 1.52*	41.50 ± 2.29	61.00 ± 1.00
	10	7.00 ± 1.00*	20.00 ± 2.00*	30.66 ± 2.08*	42.83 ± 0.76	71.33 ± 1.65
	20	23.66 ± 4.16*	34.33 ± 2.08*	44.00 ± 2.64*	64.00 ± 4.52*	80.66 ± 1.15
Positive control (ABZ)	20	3.00 ± 0.20	9.70 ± 1.5	24.20 ± 3.68	51.00 ± 3.64	96.30 ± 2.49
Negative control		1.30 ± 0.60	1.30 ± 0.6	1.70 ± 0.6	2.30 ± 1.15	3.00 ± 0.0

Data represent the mean ± SD (n = 3). When compared to the positive control (ABZ) the (*) indicates a significant difference (p≤0.05).

protoscoleces up to 96.5% after 24 h exposure, demonstrating a potency of almost 79% of that attained by both methanolic extracts of the *J. phoenicea* fruit and leaf (Table 2). Compared to ABZ, the *J. phoenicea* extract had a very significant effect ($p<0.05$).

The mortality rate of *E. granulosus* protoscolices after exposure to *A. judaica* methanolic extracts was significantly different when compared to that of ABZ, which had a 50% reduction in protoscolices survival after 12 h exposure (Fig. 3C; Table 2). The greatest protoscolicidal effect, however, was observed at concentrations of 10 and 20 mg/mL *A. judaica* methanolic extract, which were 90 and 98%, respectively ($p<0.05$). These results showed that the effects of the *A. judaica* methanolic extract were dose-dependent, with a slight reduction in mortality rate (50%) at 5 mg/mL after 24 h of exposure to low doses. The IC_{50} was 17.696 mg/mL after 3 h of exposure to *A. judaica* methanolic extract.

The effects of different concentrations of the *C. procera* leaf and fruit extracts on protoscholeces mortality rates were next evaluated. The mortality rate was considerably higher when samples were treated with 20 mg/mL for 12 h when compared with ABZ treatment, with rates of 85% and 63%, respectively (Fig. 3D- E; Table 2). These results indicated that the *C. procera* methanolic extract effect was time-dependent ($p \leq 0.05$), with the maximum protoscolicidal effect observed at 10 and 20 mg/mL *C. procera* methanolic extract after 24 h of exposure ($p<0.05$) in contrast to ABZ, which demonstrated a mortality rate of up to 96.3% after 24 h of exposure. However, at low doses, the *C. procera* methanolic extract effects were not consistently significantly different from that of ABZ. The IC_{50} values were 27.2 and 32.215 mg/mL after 3 h exposure to *C. procera* leaf and fruit extracts, respectively. After 24 h of *in vitro* post-incubation, protoscoleces treated with normal saline still contained at least 95% viable cells.

The results of the mortality tests were consistent with the morphological and structural changes observed in the treated protoscoleces samples (Fig. 4A-B). However, after 24 h of exposure, protoscoleces incubated with the highest concentrations of *J. phoenicea*, *A. judaica*, and *C. procera* extracts, as well as ABZ, showed signs of rostellar disorganization, tegument bleb development, soma contraction, loss of microtrichomes, and hooks of the scolex. Throughout the course of the trial, the control protoscoleces main-

tained their viability, and there were no morphological or structural alterations to the tegument.

Phytochemical analysis of methanolic extracts

The total yields obtained from the methanolic extracts of *J. phoenicea*, *A. judaica*, and *C. procera* are shown in Table 3. The phytochemical compositions and chromatograms of these compounds are shown in Annex 1-6, which also include the component percentages. These extracts contained a variety of secondary metabolites, including terpenes, phenolic compounds, steroids, alkaloids, and glycosides. The main ingredients of the *J. phoenicea* extract were germacrene b (13.2%), tricyclene (12%), limonene (10%), myrtenol (8%), cadinene (8%) and cubebene (7%). The primary components of *A. judaica* were cinnamic acid esters (approximately 14%), isoferulic acid (10.3%), linalyl acetate (10%), citronellyl acetate (8.2%), and quercetin (7.5%). *Calotropis procera* contained uzarin (12%), isoquercitrin (10%), and lupeol (9.2%). The analysis revealed that the phytochemical categories retrieved from each plant vary. The terpene content of *J. phoenicea's* extract was considerably greater with monoterpenes (tricyclene 12%, α-phellandrene 6%, α-thujene 5%), sesquiterpenes (germacrenes 13%, cadinene 8%, cubebene 7%, α-campholenal 5.6 %,), and diterpenes (myrtenol 8%) being the most common. *Artemisia judaica* has a complex phytochemical composition that is characterized by polyphenolic components that contain both flavonoids and nonflavonoids. Whereas in *C. procera*, the cardenolide glycoside phytochemicals class—to which calotropin, calactin, and uscharin belong—are predominant.

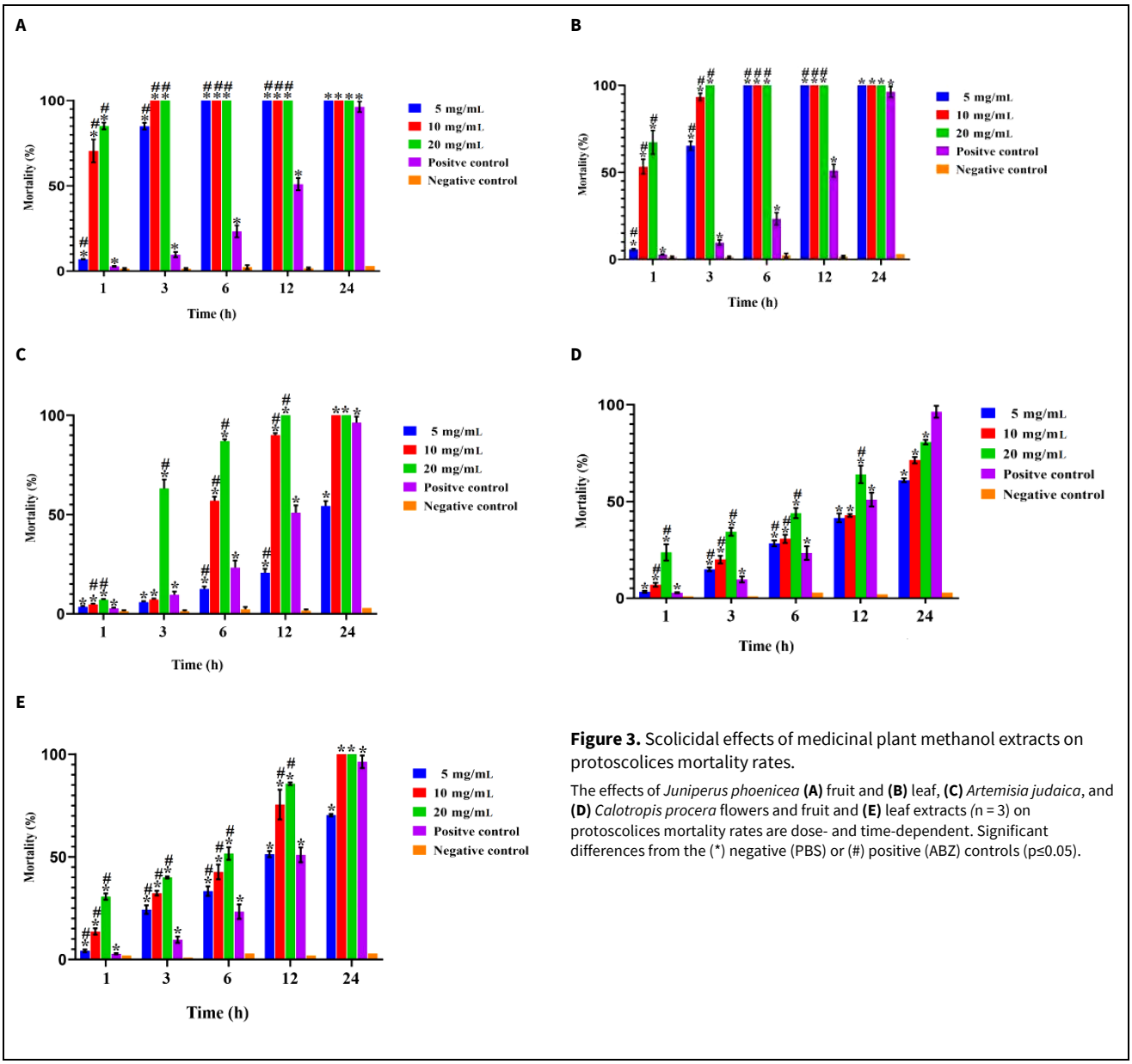

Figure 3. Scolicidal effects of medicinal plant methanol extracts on protoscolices mortality rates.

The effects of *Juniperus phoenicea* **(A)** fruit and **(B)** leaf, **(C)** *Artemisia judaica*, and **(D)** *Calotropis procera* flowers and fruit and **(E)** leaf extracts (n = 3) on protoscolices mortality rates are dose- and time-dependent. Significant differences from the (*) negative (PBS) or (#) positive (ABZ) controls (p≤0.05).

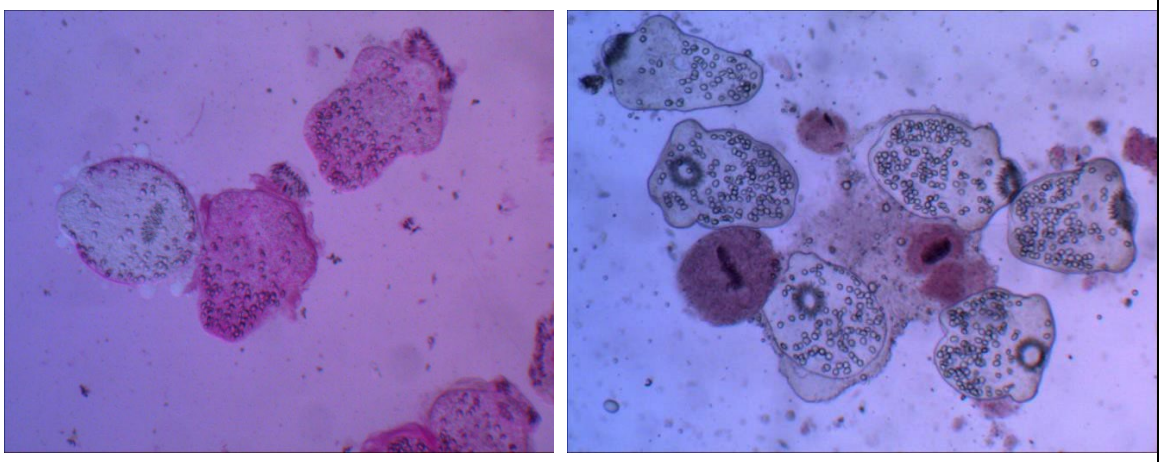

Figure 4. *Echinococcus granulosus* protoscolices morphologies after methanolic extract treatment.

(A) Exposure to 10 mg/mL *Juniperus phoenicea* leaf extract induced hooks separation and eosin staining, indicating nonviable cysts (6 h). **(B)** Partially dead protoscolices with contracted soma and tegument blebs after 20 mg/mL *Artemisia judaica* extract treatment (6 h). Magnification (400×).

Table 3. The total yields obtained from methanolic extracts of *Artemisia judaica*, *Juniperus phoenicea*, and *Calotropis procera.*

Plant	Dry weight of extract (g)	Dry weight of plant tissue before extraction (g)	Final yield (%)
J. phoenicea fruit extract	14.10	35	40.2
J. phoenicea leaf extract	17.64	35	50.4
A. judaica extract	16.00	35	45.7
C. procera fruit and flower extract	4.00	42	9.5
C. procera leaf extract	7.01	28	20.03

DISCUSSION

The only chemotherapies licensed for treating patients with CE are albendazole and mebendazole (Chai et al., 2021). Albendazole is the most frequently used drug because of its greater hydatid cyst penetration and stronger absorption qualities than mebendazole (Shams-Ul-Bari et al., 2011). However, their use can cause unfavorable side effects; several case reports have shown an increased risk of cyst rupture after albendazole therapy, which can lead to life-threatening complications (Daimari et al., 2018; Sheikhy et al., 2015). Thus, new treatment options are required to improve treatment safety and patient outcome. One such approach is to identify and assess novel chemical classes based on medicinal plants that can suppress protoscoleces, justifying the investment in *in vivo* and *in vitro* research in this area. The most frequently employed technique in such investigations is methanolic extraction (Bouaziz et al., 2021). Our literature review revealed that more than 65 plants have been studied, including *A. sativum* (Haji Mohammadi et al., 2018), *N. sativa* (Mahmoudvand et al., 2014a), *Z. officinale* (Houshmand et al., 2019), *R. graveolens*, and *P. harmala* (Al Qaisi et al., 2022) for their effect on deactivating hydatid cyst. We now provide an innovative and thorough examination of the *in vitro* scolicidal activity of methanolic extracts of *A. judaica*, *J. phoenicea*, and *C. procera* as a promising treatment of *E. granulosus* infection derived from naturally infected goats, as well as describe their chemical composition.

The effectiveness of several extracts varied significantly from the negative and positive control treatments of protoscoleces. A comparison of *J. phoenicea* extracts to those of *A. judaica* and *C. procera* showed that its effect against protoscoleces was substantially stronger. Specifically, 10 mg/mL ripe fruit extract had a protoscoleces mortality rate of 100% after 3 h. This is considered a modest dose in comparison to other treatments; for example, the maximum protoscolicidal activity of *R. graveolens* and *A. halimus* leaf extracts occurred after 3 h using a dose of 40 and 100 mg/mL, respectively (Al Qaisi et al., 2022; Bouaziz et al., 2021), while 100 mg/mL *Z. officinale* and 62.5 mg/mL *P.*

harmala extracts both demonstrated scolicidal efficacy after 24 and 48 h, respectively (Hammoshi et al., 2005; Houshmand et al., 2019). However, the mortality rate using shorter exposure times, such as those of less than 60 min, necessitated the use of relatively higher dosages of plant extracts, such as 100 mg/mL or more, in certain trials (Mahmoudvand et al., 2014b; Ranjbar et al., 2020). Therefore, higher doses are required to reduce exposure, which raises questions regarding the safety of these extracts under biological conditions.

There are no previous experiments on the scolicidal action of *J. phoenicia* on *E. granulosus*; however, there have been reports of its antimicrobial properties (Al khlifeh et al., 2021; Wu et al., 2022). It is locally known that Arar and constitutes a major portion of Jordan's southern vegetative cover that sheep and goats commonly graze upon. In addition to boosting blood circulation, these formulations have been used in conventional medicine as diuretics, abortives, and anti-diabetics (Afifi and Kasabri, 2013; Al Groshi et al., 2018). In this study, several types of terpenes were the only phytochemicals identified in the extracts of *J. phoenicea* fruit; this may explain why it was more effective than *A. judaica* and *C. procera* in killing the CE protoscoleces. Terpenes are naturally synthesized in a variety of plant species, including *Juniper* species, for their antibacterial defense (Falcão et al., 2018) and have been used in the investigation of CE treatments (Albani et al., 2014). The non-polarity of terpenes may disrupt membrane integrity as one of their scolicidal actions. This may be due to the presence of isoprene, which enhances the mechanism of scolicidal activity by modifying the membrane structure to make it more lipophilic, allowing for the delivery of additional phyto-constituents found in *J. phoenicea*. Previous studies have also demonstrated that terpenes from various plants can prevent DNA replication, at least in part, by inhibiting DNA topoisomerase (Baikar and Malpathak, 2010; Panter et al., 2018).

Artemisia judaica (family *Asteraceae* or *Compositae*), also called Baitharan in Jordan, is known as a highly effective treatment for helminthic infections in young infants (Lam et al., 2018). In southern Jordan, it is primarily planted as a perennial aromatic shrub widely available and reasonably priced in Jordanian homes. These herbs are the best anthelmintic options because of their accessibility and safety when used in humans. Additionally, because it grows abundantly on grazing lands, sheep and goats that eat it may have stronger resistance to nematode parasites. An extract of *A. judaica* has been shown to be non-toxic in mice, with less than 7.5 g/kg body weight of an ethanolic extract was orally administered (Soliman and Nofal, 2009). In our study, the scolicidal activity of *A. judaica* was investigated for the first time and attributed to its complex phytochemical makeup, the majority of which were polyphenolic compounds that included both flavonoids and nonflavonoids. Flavonoid molecules have been reported to impact CE and several other eukaryotic parasites (Idris et al., 2017; Salemi et al., 2021). We hypothesized that the scolicidal effects of phenolic compounds include antibacterial capabilities as their modes of action. For instance, it has been suggested that apigenin and quercetin, which make up about 13% of the phytochemicals in *A. judaica*, can interfere with plasma membrane function by forming complexes with extracellular soluble proteins or by causing membrane shrinkage (Lee et al., 2018). Additionally, the anti-parasitic mechanism of apigenin in *L. amazonesis* includes mitochondria dysfunctions (Fonseca-Silva et al., 2015). Microbial systems have demonstrated that nucleic acid-processing enzymes are severely impeded by phenolic compounds (Ohemeng et al., 1993), further showing the diverse effects of natural phenolic compounds. Therefore, the reactivity to these biological functional groups may result from the electron delocalization of the aromatic rings.

In this study, *C. procera* extracts showed time-dependent effects on the mortality rates of protoscoleces ($p<0.05$), which decreased more gradually with only the 20 mg/mL extract, reaching 100% mortality after 24 h. These findings are consistent with recent studies that showed how different plant extracts can have both time- and dose-dependent effects (Al Qaisi et al., 2022). Unlike *J. phoenicea* and *A. Judaica* extracts, *C. procera* is known for being toxic to livestock and man. It is a tropical tree and a member of the *Asclepiadaceous* subfamily of the larger *Apocynaceae* family (Endress et al., 2007). In Jordan, ashur is commonly used to describe *C. procera*, which is only in Jordan, where the Sudanese bioclimatic zone has migrated, particularly in the Wadi-Araba and El-Ghore regions. Aqueous leaf extracts can be applied topically to treat a variety of skin conditions and wounds, and latex extract-based remedies are particularly popular within local communities. Clinical studies have reported the toxic effects of *Calotropis* (Ghramh et al., 2021; Vahidi et al., 2021), including the induction of apoptosis associated with the production of ROS and ATP as part of this mechanism (Winitchaikul et al., 2021). A wide range of biological activities of *C. procera* has been demonstrated (Al-Dalahmeh et al., 2022), including antileishmanial and antimalarial characteristics (Ilaghi et al., 2021; Muthaura et al., 2015). It is also used to treat elephantiasis, a disorder caused by filarial worms (Kushwaha et al., 2018). Using GC-MS analysis, the active substances uzarin, quercitrin, uscharin, calactin, and calotropin were identified in *C. procera* extracts, consistent with previous findings (Al-Dalahmeh et al., 2022). It is well recognized that

calotropin, calactin, and uscharin are toxic substances (Iyadurai et al., 2020), and due to *C. procera*'s cytotoxicity in eukaryotic cells, it is an ideal anthelmintic substitute. However, this may have unfavorable effects, and to prevent toxicity, it is essential to develop mechanism-based (i.e., targeted) delivery methods for these types of plant extracts. In addition to their scolicidal action, the plant extracts tested in this study generated morphological alterations in protoscoleces at different phases of culture.

The efficiency of the plant extracts was determined by counting live protoscoleces using eosin staining. Furthermore, it was easy to distinguish between viable (unstained) and nonviable (stained) cells under low microscopic power fields (10×); however, higher power fields (40×) are necessary for analytical precision. Microscopic observation identified blebs that developed on the tegument, shrunken soma, and disorganized rostella. Morphological changes in the extract-treated protoscoleces also resulted in the loss of their microtrichomes and scolex hooks. These changes were clearly visible in the cultures after 24 h of extract treatment and indicated low viability of the parasite and potentially the start of a serial sequential dying process. Other plant extracts have been shown to elicit similar effects using scanning and transmission electron microscopy (Al Qaisi et al., 2022).

CONCLUSION

Artemisia judaica, *J. phoenicea*, and *C. procera* are sources of phytochemical components with scolicidal activity. However, additional research is required to improve our understanding of their purification, identification of bioactive components, and toxicology.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

The authors would like to acknowledge Al-Balqa Applied University- Deanship of Scientific Research (DSR) for funding this research (grant number DSR-2022#511). The authors would like to express their sincere appreciation to Dr. Khaled Abolaila (kabulaila@gmail.com), a senior botanist, conservation biologist, and the Director of Biodiversity at the National Agricultural Research Center (NARC), Jordan, for his assistance in the recognition and classification of the plant specimens.

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AUTHOR CONTRIBUTION:

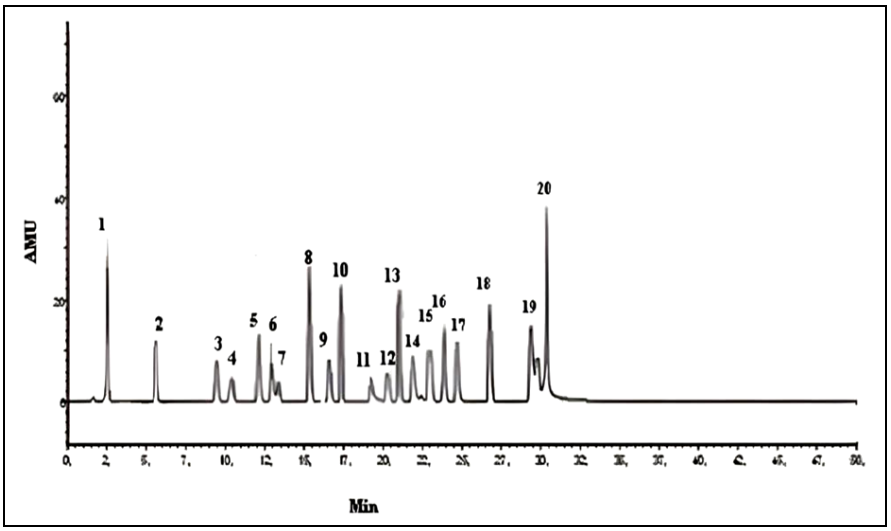
Contribution	Al khlifeh E	Saidat N	Al Qaisi Y	Khleifat K
Concepts or ideas	x			
Design	x		x	
Definition of intellectual content	x			
Literature search	x			
Experimental studies		x	x	
Data acquisition	x	x	x	
Data analysis	x			
Statistical analysis	x	x	x	
Manuscript preparation	x		x	
Manuscript editing	x			
Manuscript review	x	x	x	x

Citation Format: Al khlifeh E, Saidat N, Al Qaisi Y, Khleifat K (2023) Phytochemical profile and *in vitro* protoscolicidal effects of *Juniperus phoenicea* L., *Calotropis procera* (Aiton) Dryand, and *Artemisia judaica* L. against *Echinococcus granulosus* cysts. J Pharm Pharmacogn Res 11(4): 635–650. https://doi.org/10.56499/jppres23.1635_11.4.635

Annex 1. Phytochemical profile of *Juniperus phoenicea* L.

No.	Chemical compound	Molecular weight	Molecular formula	m/z	Percent	RT
1	Tricyclene	136.23	C10H16	93,91,92	12	2.0
2	α-Thujene	136.23	C10H16	93, 91, 77, 92, 98	5	5.2
3	α-Pinene	136.23	C10H16	93, 92, 77, 39, 79	2	9.8
4	α-Fenchene	136.23	C10H16	93, 79, 80	1	10.2
5	Verbenene	134.22	C10H14	91, 92, 93	5	11.3
6	Sabinene	136.23	C10H16	137, 122, 94, 66	3	12.2
7	Myrcene	136.23	C10H16	93, 69, 94, 79, 77	1	12.5
8	Limonene	136.23	C10H16	91, 55	10	15.2
9	Verbenone	150.22	C10H14O	109, 123, 133	2	16.6
10	Myrtenol	152.23	C10H16O	79, 91, 108, 41, 39	8	16.9
11	Humulene	204.35	C15H24	93, 80, 41	1	18.7
12	Cubenol	222.37	C15H26O	119, 41 ,43	1	20.0
13	δ-Cadinene	204.35	C15H24	161, 93 ,91, 41, 119	8	20.9
14	α-Terpineol	154.25	C10H18O	59, 93, 436, 121, 43	3	21.5
15	Terpinene	136.23	C10H16	93, 91, 136, 77, 121	2	22.0
16	α-Phellandrene	136.23	C10H16	93, 91, 77, 92, 136	6	23.2
17	α-Amorphene	204.35	C15H24	105, 106, 107	4	25.0
18	Cubebene	204.35	C15H24	105, 161, 81, 93, 120	7	26.8
19	α-Campholenal	152.23	C10H16O	108	5.6	28.0

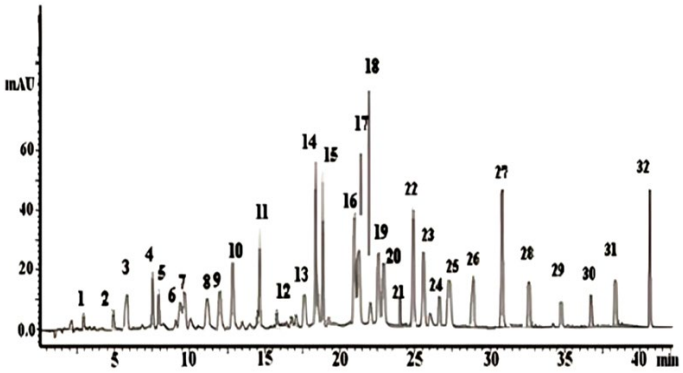
Annex 2. Chromatogram of *Juniperus phoenicea* L. methanolic extract.



Annex 3. Phytochemical profile of *Artemisia judaica* L.

No.	Chemical compound	Molecular formula	Molecular weight	%	RT
1	Furanone	$C_4H_4O_2$	84.07	0.4	2.9
2	3-Hexenol	$C_6H_{12}O$	100.16	0.5	5.0
3	Adamantane	$C_{10}H_{16}$	136.23	1.0	5.5
4	Cinnamic acid	$C_9H_8O_2$	148.16	3.2	7.5
5	Thymol	$C_{10}H_{14}O$	150.22	2.0	7.8
6	Camphor	$C_{10}H_{16}O$	152.23	0.1	9.0
7	Cyclodecan-1-one	$C_{10}H_{18}O$	154.25	1.2	9.5
8	Methyl cinnamate	$C_{10}H_{10}O_2$	162.18	0.6	11.0
9	Jasmone	$C_{11}H_{16}O$	164.24	2.2	11.6
10	Gallic acid	$C_7H_6O_5$	170.12	3.5	12.5
11	cis-Ethyl cinnamate	$C_{11}H_{12}O_2$	176.21	5.0	14.5
12	ethyl-3-Phenylpropionate	$C_{11}H_{14}O_2$	178.23	0.3	15.7
13	Caffeic acid	$C_9H_8O_4$	180.16	0.9	18.0
14	Isoferulic acid	$C_{10}H_{10}O_4$	194.18	10.3	19.0
15	Linalyl acetate	$C_{12}H_{20}O_2$	196.29	10	19.6
16	Citronellyl acetate	$C_{12}H_{22}O_2$	198.30	8.2	20.8
17	Isocaryophyllene	$C_{15}H_{24}$	204.35	4.0	21.0
18	Cadinene	$C_{15}H_{26}$	206.37	0.2	22.0
19	Spathulenol	$C_{15}H_{24}O$	220.35	3.0	22.5
20	beta-Eudesmol	$C_{15}H_{26}O$	222.37	2.5	23.0
21	Chrysin	$C_{15}H_{10}O_4$	254.24	1.5	24.0
22	Caryophyllene acetate	$C_{17}H_{28}O_2$	264.40	7.0	24.8
23	Apigenin	$C_{15}H_{10}O_5$	270.24	5.2	25.5
24	Luteolin	$C_{15}H_{10}O_6$	286.24	0.7	26.5
25	Kaempferol	$C_{15}H_{10}O_6$	286.24	1.2	27.0
26	Diosmetin	$C_{16}H_{12}O_6$	300.26	1.5	28.5
27	Quercetin	$C_{15}H_{10}O_7$	302.23	7.5	30.9
28	Cirsimaritin	$C_{17}H_{14}O_6$	314.29	3.3	33.5

Annex 4. Chromatogram of *Artemisia judaica* L. methanolic extract.



Annex 5. Phytochemical profile of *Calotropis procera* (Aiton) Dryand.

No.	Chemical compound	Molecular formula	Molecular weight	%	RT
1	Adamantane	$C_{10}H_{16}$	136.23	1.0	2.0
2	Camphor	$C_{10}H_{16}O$	152.23	0.5	3.1
3	Cyclodecan-1-one	$C_{10}H_{18}O$	154.25	4.2	4.6
4	Gallic acid	$C_7H_6O_5$	170.12	0.3	5.9
5	Caffeic acid	$C_9H_8O_4$	180.16	6.0	7.2
6	Giganticine	$C_{13}H_{19}NO_3$	237.29	3.3	8.3
7	Kaempferol	$C_{15}H_{10}O_6$	286.24	5.6	9.8
8	Quercetin	$C_{15}H_{10}O_7$	302.23	1.1	10.1
9	Isorhamnetin	$C_{16}H_{12}O_7$	316.26	3.1	12
10	Azaleatin	$C_{16}H_{12}O_7$	316.26	1.3	13.1
11	Rosmarinic acid	$C_{18}H_{16}O_8$	360.30	4.4	13.9
12	Lineolone	$C_{21}H_{32}O_5$	364.50	6.2	14.5
13	Coroglaucigenin	$C_{23}H_{34}O_5$	390.50	2.2	15.1
14	Stigmasterol	$C_{29}H_{48}O$	412.70	4.1	16.0
15	beta-Sitosterol	$C_{29}H_{50}O$	414.70	2.0	16.3
16	beta-Amyrin	$C_{30}H_{50}O$	426.70	3.2	17.1
17	Lupeol	$C_{30}H_{50}O$	426.70	9.2	17.9
18	Cyclosadol	$C_{31}H_{52}O$	440.70	1.9	18.5
19	Oleanolic acid	$C_{30}H_{48}O_3$	456.70	7.5	19.2
20	Isoquercitrin	$C_{21}H_{20}O_{12}$	464.40	10	20.1
21	Calotropeol	$C_{29}H_{40}O_9$	532.60	0.6	21.0
22	Calactin	$C_{29}H_{40}O_9$	532.60	0.8	23.0
23	Frugoside	$C_{29}H_{44}O_9$	536.70	0.4	23.4
24	Frugoside	$C_{29}H_{44}O_9$	536.70	1.1	23.6
25	Uscharin	$C_{31}H_{41}NO_8S$	587.70	8.0	26.0
26	Uzarin	$C_{35}H_{54}O_{14}$	698.80	12.0	27.0

Annex 6. Chromatogram of *Calotropis procera* (Aiton) Dryand.