

Molluscicidal and larvicidal activities of *Capparis spinosa* aerial parts against *Galba truncatula* intermediate host of *Fasciola hepatica*

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ABSTRACT

Objective: Fascioliasis caused by *Fasciola hepatica* L. (Fasciolidae), a digenetic trematode, is a parasitic disease infecting many people worldwide. The present study was carried out to evaluate the molluscicidal and larvicidal activities of *Capparis spinosa* L. (Capparaceae) aerial parts against *Galba truncatula* Müll. (Lymnaeidae) and *Fasciola hepatica* larval stages contaminating this snail in Tunisia. Accordingly, ethyl acetate, methanol and methanol-water were used as solvents of extraction. *n*-hexane, methylene chloride and methanol were used for the fractionation of the active extract. **Materials and Methods:** Phytochemical tests were conducted on extracts in order to establish a meaningful relationship the most active with molluscicidal and larvicidal activities. **Results:** ethyl acetate extracts showed potent activities, giving LC₅₀=8.03 mg/L for leaves and LC₅₀=8.79 mg/L for stems. All the fractions of leaf ethyl acetate extracts were active. The highest activity was detected in the methanolic fraction with LC₅₀=3.53 mg/L. Ethyl acetate extracts of leaves, stems and the methanolic fraction of leaf ethyl acetate extract an LC gave potent larvicidal activities with deterioration rates exceeding 30.39% (30.39; 91.52%). Phytochemical tests showed that these activities may be attributed to the presence of sterols/carotenoids/triterpenoids in ethyl acetate extracts and flavonoids/saponins in the methanolic fraction. **Conclusion:** The molluscicidal potential of *C. spinosa* has been proved in the present investigations and can be recommended for control of *G. truncatula* snails.

Key words: *Capparis spinosa*, *Fasciola hepatica*, *Galba truncatula*, Larvicidal activity, Molluscicidal activity.

INTRODUCTION

Fascioliasis is an important parasitosis of farm live-stock that results of a helminth species *Fasciola hepatica* L. (*F. hepatica*, Fasciolidae). It is spread worldwide and it causes serious economic losses in the industry of animal husbandry.¹ *Galba truncatula* Müll. (*G. truncatula*, Lymnaeidae) also called *Lymnaea truncatula* belongs to the *Lymnaeidae* family and was identified as the principal intermediate host of *F. hepatica*.²

In Tunisia, human fascioliasis is a rare disease; only 38 cases were reported between 1940 and 2007.³ The majority of patients come from the north and south west of Tunisia.

However, animal fluke is more common, it is 20% in Sejane⁴ and 44% in Tozeur.²

Treatment of fascioliasis requires high or multiple doses of drugs with numerous side effects. Therefore, snail control is essential; it is regarded as a rapid and efficient method for reducing or eliminating transmission and is among the methods to bring these diseases under an adequate control through the breakage of the parasite life cycle. The search for local molluscicidal plants was considered more sustainable than the use of synthetic ones.⁵

Capparis spinosa (*C. spinosa*) showed important biological activities against a large num-

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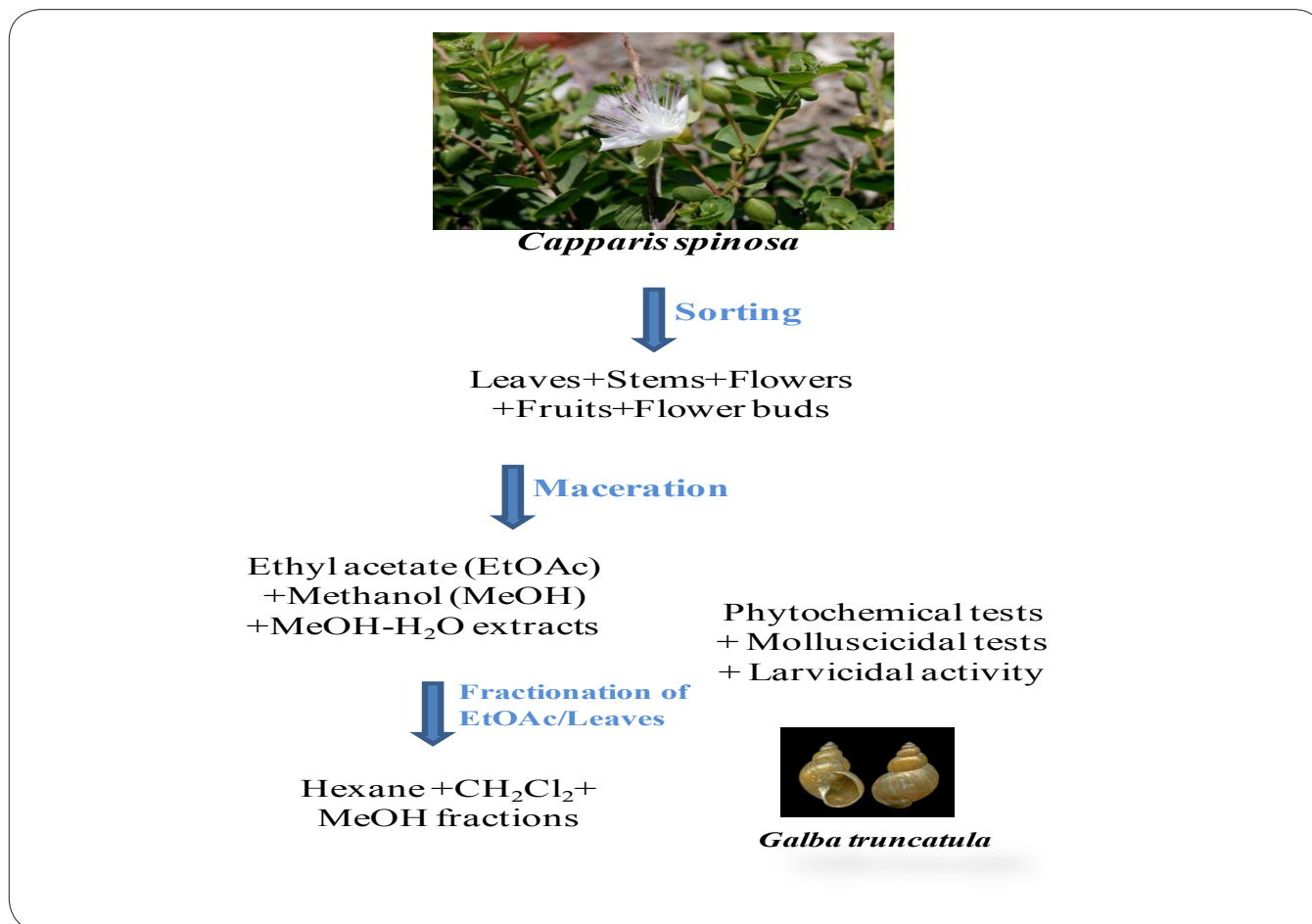
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Graphical Abstract

ber of pathogens. Aqueous extracts of its aerial parts had a significant antihepatotoxic activity against carbon tetrachloride- and paracetamol-induced hepatotoxicity *in vivo*. Antifungal, antibacterial, antihypertensive and diuretic activities were also demonstrated in *C. spinosa*.⁶ This plant also agglutinated *Leishmania* (parasite) and killed it in the vector *Phlebotomus papatasi*.⁷

Many compounds were identified in *C. spinosa*, including flavonoids,⁸ alkaloids,⁹ terpenoids,¹⁰ volatile oils and fatty acids.¹¹ Moreover, laboratory studies showed that this plant possesses pharmacological effects, such as anti-hyperglycemic,¹² hypolipidemic and antioxidant¹³ ones. Also, melon seeds of this plant has proved an antiproliferative effect and inhibited HIV-1 reverse transcriptase activities.¹⁴

Molluscicidal activity of *C. spinosa* against *Bulinus truncatus* and *Biomphalaria alexandrina* was investigated in literature. Aerial parts were extracted successively using petrol, *n*-hexane, methylene chloride, ethyl acetate, methanol and water then tested against the snail *Bulinus truncatus*; the highest lethal concentration that killed 50% of the snails was determined for the *n*-hexane extract (62.94 mg/L).¹⁵ Moreover, its dry powders were potent against *Biomphalaria alexandrina*.^{16,17} This study

aims to investigate the phytochemical composition of *C. spinosa* aerial parts and the molluscicidal and larvicidal potencies of samples from this plant against *Lymnaea truncatula*, the snail intermediate host of *F. hepatica* so as to open new areas of application of plants as eco-friendly molluscicides.

MATERIALS AND METHODS

Plant material

C. spinosa named “Kabbar” in Arabic belongs to the *Capparaceae* family. It was harvested in November 2011 in Sfax, Tunisia. The botanical identification was established by Pr. Mohamed Chaieb, Botanist at the Faculty of Sciences, Sfax, Tunisia. The voucher specimen's number LCSN114 was deposited at the Laboratory of Chemistry of Natural Substances of the Faculty of Sciences, Sfax.

Preparation of extracts

Shade-dried and powdered *C. spinosa* plants were sorted into leaves, stems, flowers, fruits and flower buds. Their powders were successively macerated using ethyl acetate, methanol and methanol-water (8-2: v-v), during 48 h each. Extracts were filtered, solvents were removed

using a rotary evaporator under reduced pressure, and the residues dried. These extracts were subjected to Thin Layer Chromatography (TLC) and chemical tests to elucidate their phytochemical composition.

Fractionation of the active extract

Leaf ethyl acetate the most active extract was successively fractionated using *n*-hexane, methylene chloride and methanol.

Phytochemical screening

The preliminary phytochemical analysis of the plant extracts and fractions was conducted in order to screen for bio-active components which exist in the aerial parts: sterols/triterpenoids, carotenoids/triterpenoids, alkaloids, quinones, coumarins, flavonoids, saponins, tannins, and tropolone nuclei. This was established either using color reactions or TLC plate revelation or both techniques.

Using color reactions: Plant extracts submitted chemical tests (T_1 to T_8) on the basis of their colors for the presence of some phytoconstituents. Consequently, 1 mg of each sample was dissolved in 1 mL of the requisite solvent to obtain solution E which was shaken to ensure a uniform mixture, then was added to various appropriate working standard solutions, as follows:

T_1 : Liebermann's reaction for sterols/triterpenoids.

T_2 : Carr and Price's reaction for carotenoids/triterpenoids.

T_3 : Wiustater's reaction for tropolone nuclei.

T_4 : Borntraeger's reaction for free quinones.

T_5 : test of flavonoids.

T_6 : Mayer's reaction for alkaloids.

Tests (T_1 to T_6) were performed as described by Harborne methods.^{18,19}

T_7 : Frothing test for saponins.²⁰

T_8 : test for coumarins.²¹

T_9 : test for tannins.²²

Using UV detection of TLC plates: TLC plates interpretation under UV/365 nm was established to demonstrate the previous tests, whether with or without spraying specific reagents.

***Detection of alkaloids:** Dragendorff's reagent (Munier and Macheboeuf's formula):

This reagent reveals alkaloids as red-orange spots in visible light.¹⁸

***Detection of saponins:** Total steroidal saponins appear red-purple after spraying plates with a chloroform saturated antimony trichloride solution, and a few minutes after heating the plate (100-110°). All steroidal nuclei are located without distinguishing saponins from glycoalkaloids (GA) which were already revealed as orange spots by Dragendorff's reagent.¹⁸

***Detection of sterols/triterpenoids:** A range of colors is produced, and is visible both in daylight and UV, on spraying heated plates for 10 m at 100° with Carr-Price reagent, i.e. 20% antimony chloride in chloroform.²³

***Detection of coumarins:** Natural coumarins exhibit fluorescence properties in UV/365 nm. Their spots can be easily detected on TLC plates, without using any chromogenic reagents. Purple and blue colors characterize them.²⁵

Concentration of the investigated phytochemicals was scored as follows:-(no reaction), + (weakly positive reaction), ++ (positive reaction), +++ (important positive reaction).

Snails

G. truncatula snails were used at a uniform size of 3-5 mm in length. They were collected locally from El

Table 1: Masses and yields of different extracts of *Capparis spinosa* after 48 h of extraction with increasing polarity solvents

		Ethyl Acetate extract (EtOAc)	Methanol extract (MeOH)	Methanol-Water extract (8:2, v-v)
Leaves (550 g)	Mass (g)	14.54	46.88	48.13
	Yield (%)	2.64	8.52	8.75
Stems (550 g)	Mass (g)	1.77	23.90	28.06
	Yield (%)	0.32	4.34	5.11
Flowers (550 g)	Mass (g)	13.76	115.2	42.78
	Yield (%)	2.50	20.94	7.78
Fruits (30 g)	Mass (g)	0.49	0.97	2.12
	Yield (%)	1.63	3.23	7.07
Flower buds (30 g)	Mass (g)	0.45	1.20	1.83
	Yield (%)	1.50	4.00	6.10

Table 2: Chemical compounds present in the extracts of the aerial parts from *Capparis spinosa*

Aerial part	Solvent of extraction	Fraction of EtOAc extract	Sterols/ triterpenoids		Flavonoids		Saponins		Alkaloids		Coumarins		Carotenoids/ triterpenoids	Tannins	Tropolone	Quinones		
			*	**	*	**	*	**	*	**	*	**						
Leaves	EtOAc	-	+	++	-	-	-	-	-	-	-	-	+	-	-	-		
		<i>n</i> -hexane	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	
		CH ₂ Cl ₂	+	++	-	-	-	-	-	-	-	-	-	++	-	-	-	
Leaves	MeOH	MeOH	-	-	+	++	+	+	-	-	-	-	-	-	-	-	+	
		MeOH-H ₂ O	-	-	+	+	+	-	-	+	+	+	-	-	-	-	-	+
		EtOAc	-	++	-	-	-	-	-	-	-	-	-	++	-	-	-	-
Stems	MeOH	MeOH	-	-	+	++	-	-	+	+	-	-	-	-	-	-	+	
		MeOH-H ₂ O	-	-	+	+	-	-	-	+	+	-	-	-	-	+	-	-
		EtOAc	-	++	-	-	-	-	-	-	-	-	-	+	-	-	-	+
Flowers	MeOH	MeOH	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	
		MeOH-H ₂ O	-	-	+	+	-	-	-	+	+	-	-	-	-	++	-	++
		EtOAc	-	++	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Fruits	MeOH	MeOH	-	-	++	+	-	-	-	+	+	++	-	-	-	-	+	
		MeOH-H ₂ O	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	++
		EtOAc	-	++	-	-	-	-	-	-	-	-	-	++	-	-	-	-
Flower buds	MeOH	MeOH	-	-	++	++	+	+	-	+	+	-	-	-	-	-	++	
		MeOH-H ₂ O	-	-	++	++	+	+	+	++	+	+	-	-	-	-	++	
		EtOAc	-	-	++	++	+	+	+	++	+	+	-	-	-	+	++	

-: No reaction; +: Weak presence; ++: Presence; +++: Richness. EtOAc: Ethyl acetate; MeOH: Methanol; MeOH-H₂O: Methanol-Water (8-2: v-v); CH₂Cl₂: Methylene Chloride; *: Color reaction results. **: TLC plate revelation results.

Table 3: Molluscicidal activities of *Capparis spinosa* extracts and fractions against *Galba truncatula*

Aerial part	Powder/ Solvent	Fraction of the leaf EtOAc extract After 48 h action	After 24 h action		After 48 h action	
			LC ₅₀ (mg/L) (95% CI)	LC ₉₀ (mg/L) (95% CI)	LC ₅₀ (mg/L) (95% CI)	LC ₉₀ (mg/L) (95% CI)
Leaves	Powder	-	20.64 (14.64; 26.11)	41.55 (31.97; 72.38)	16.83 (10.98; 21.79)	35.65 (27.03; 63.49)
	EtOAc	-	4.17 (3.19; 5.53)	8.28 (6.02; 29.67)	8.03 (0.070; 14.16)	7.51 (5.60; 19.81)
		n-hexane	26.38 (1.91; 363.33)	103.18 (28.88; 368.60)	24.96 (2.66; 233.85)	87.69 (32.55; 236.25)
		CH ₂ Cl ₂	21.55 (2.98; 155.63)	64.96 (34.05; 123.92)	20.87 (4.76; 91.40)	54.91 (32.72; 92.13)
		MeOH	15.62 (0.16; 1497.14)	55.15 (13.85; 219.52)	3.53 (1.65; 4.66)	29.93 (17.88; 483.22)
	MeOH	-	Inactive	Inactive	Inactive	Inactive
MeOH-H ₂ O	-	Inactive	Inactive	Inactive	Inactive	
Stems	Powder	-	23.94 (5.11; 111.99)	70.38 (34.66; 142.92)	19.00 (1.27; 284.23)	59.89 (28.99; 123.72)
	EtOAc	-	9.23 (7.83; 10.07)	11.80 (10.71; 15.68)	8.79 (6.65; 9.72)	11.71 (10.49; 17.40)
	MeOH	-	Inactive	Inactive	Inactive	Inactive
	MeOH-H ₂ O	-	55.72 (33.49; 92.71)	132.75 (31.81; 554.02)	44.14 (29.95; 65.03)	117.12 (30.00; 452.21)
Flower buds	Powder	-	20.89 (3.08; 141.39)	66.87 (36.51; 122.47)	16.70 (1.51; 184.34)	50.80 (20.52; 125.73)
	EtOAc	-	24.90 (1.70; 362.94)	94.38 (31.54; 282.37)	22.62 (3.20; 159.69)	70.15 (34.59; 142.29)
	MeOH	-	56.34 (30.68; 103.46)	176.76 (16.39; 1971.42)	53.46 (30.35; 94.15)	175.58 (16.06; 1919.01)
	MeOH-H ₂ O	-	Inactive	Inactive	Inactive	Inactive

CI: confidence interval; LC₅₀: 50% lethal concentration; LC₉₀: 90% lethal concentration; EtOAc: Ethyl Acetate; CH₂Cl₂: Methylene Chloride; MeOH: Methanol; MeOH- H₂O: Methanol-Water; CuCl₂ showed a molluscicidal activity against *G. truncatula* after 48 h with an LC₅₀ = 26.12 (19.35; 31.69) mg/L and an LC₉₀ = 62.71 mg/L (49.83; 96.50). Dechlorinated water (negative control) did not show any molluscicidal activity against *G. truncatula* snails.

Table 4: Deterioration rates (%) of cercariae, rediae and intraredial germinal masses in infected *Galba truncatula* after 48 h of exposure to molluscicidal products and in infected, untreated snails placed in dechlorinated water

	% deterioration				P value		
	MeOH Fraction of the leaf EtOAc extract (a)	Leaf EtOAc extract (b)	Stem EtOAc extract (c)	Dechlorinated water	a/b	b/c	a/c
Rediae	55.13 ± 13.51	43.59 ± 8.91	30.39 ± 11.98	0	0.089	0.041	0.010
Intraredial germinal masses	69.38 ± 14.52	52.88 ± 7.89	38.68 ± 11.79	0	0.028	0.027	0.003
Cercariae	91.52 ± 11.94	86.84 ± 19.54	58.07 ± 25.38	0	0.365	0.002	0.020

P: P value calculated comparing: a to b, b to c and a to c.

Melah river, Ain Soltan, Gafsa, southwest Tunisia, in April 2012, then transferred to the Fungal and Parasitic Molecular Biology Laboratory, Faculty of Medicine, Sfax, Tunisia. They were acclimatized for four days in aquaria containing aerated and dechlorinated tap water

and washed sand, before being exposed to the aqueous solutions of powders, extracts and fractions.

Molluscicidal tests

The evaluation of the molluscicidal activities of *C. spinosa* powders, extracts, fractions and CuCl₂ (used

as positive control) against snails was done as recommended by the World Health Organization WHO.²⁶ A series of aqueous solutions was prepared from each plant material for further use in the bioassays. Each series consists of 20 solutions containing amounts ranging from 5 to 100 mg of extract material in 5 mg increments. For CuCl_2 , weights ranged from 1 to 20 mg in 1 mg increments. We added a sufficient amount of dechlorinated water to each plant material to give a final volume of 1000 mL. Each solution was divided into equal volumes of 500 mL. For each test, a control of dechlorinated water without extract was used, having the same volume as the test solution. Snails were exposed in groups of ten (five replicates) for 48 h (exposure period) to 500 mL of each concentration of the material to be tested: powders, extracts or fractions as listed in Table 3. CuCl_2 was used as a positive control. After exposure, snails were rinsed thoroughly in dechlorinated water and left for 48 h (recovery period) inside before mortality was evaluated. Dead animals were removed immediately to avoid the contamination of the alive ones and snail mortality was established by the contraction of the body into the shell. No response to a needle probe is taken as an evidence of death.

Antiparasitic tests

Most potent molluscicidal extracts/fraction was then tested at LC_{90} on a group of contaminated snails (3.5-5 mm in length). The activity of each molluscicidal extract was interrupted after 48 h by placing snails in dechlorinated water. A group of 20 naturally infected snails not exposed to molluscicides was used as a control. After dissection, snail bodies were kept in 200 mL of dechlorinated water. The cercariae and rediae were assumed to be dead once they stop moving. Deteriorated larval stages were identified by their surface alteration and vesiculation.²⁷ The numbers of deteriorated and underdeteriorated larval stages (rediae, cercariae and intraradical germinal masses) were counted in each snail; then the deterioration rate was calculated.

Statistics

Concentrations that kill 50% (LC_{50}) and 90% (LC_{90}) of the mollusks were determined by WinDI software.²⁸ Student's t test was used to compare the alteration of different larval stages of the parasite after treatment with active samples.²⁹

RESULTS

Masses and yields of *C. spinosa* extracts after 48 h of extraction using increasing polarity solvents are given in Table 1. Yields vary between 0.32% and 2.64% for ethyl acetate extracts, 3.23% and 20.94% for metha-

nolic extracts and between 5.11% and 8.75% for hydromethanolic extracts. The most important yield was obtained for the methanolic extract of flowers (20.94%). Phytochemical tests performed on each extract/fraction are reported in Table 2. Molluscicidal activities of each tested plant material are shown in Table 3.

The methanolic fraction of leaf ethyl acetate extract revealed the strongest capacity to kill the mollusks with an LC_{50} =3.53 mg/L and was rich in flavonoids and/or saponins. Leaf and stem ethyl acetate extracts were also very potent on snails, with respective lethal concentrations of 8.03 mg/L and 8.79 mg/L. They were rich in sterols/triterpenoids/carotenoids.

Weaker molluscicidal activities were recorded in other samples, with lethal concentrations inferior to 40 mg/L, as recommended by WHO.³⁰ *n*-hexane and methylene chloride fractions of leaf ethyl acetate extract gave respective LC_{50} of 24.96 and 20.87 mg/L; they were rich in sterols/triterpenoids/carotenoids. Fruit and stem hydromethanolic extracts were active with respective LC_{50} of 12.86 and 32.26 mg/L. The first extract contained coumarins and quinones, while the second contained flavonoids, alkaloids and tropolone nuclei. Ethyl acetate extracts of fruits and flower buds gave respective LC_{50} of 27.91 and 22.62 mg/L, with the presence of sterols/ triterpenoids/ carotenoids inside. All the methanolic and hydromethanolic extracts were inactive, except for stem and fruit hydromethanolic ones.

All the powders were active but less potent than their corresponding extracts, except for flowers which didn't show any effect on snails. Copper chloride used as a positive control, showed molluscicidal activities against *G. truncatula* after 48 h with an LC_{50} of 26.12 mg/L. Snails were not affected by dechlorinated water (negative control) after 48 h of exposure. Haemolysis and hypersecretion of mucus were the common toxic responses of snails to the active tested materials.

Deterioration rates of larval stages in infected *G. truncatula* after exposure to molluscicidal samples are shown in Table 4. These three substances were toxic to the larval stages of *F. hepatica*. The methanolic fraction of the leaf ethyl acetate extract was significantly more toxic to cercariae than the others ($P<0.05$).

DISCUSSION

One of the major preventive steps against fascioliasis is the control of the vector snail populations. In recent years, several extracts of plant origin have been studied against snail transmitted parasitic diseases. The molluscicidal activity varies greatly from species to species and

even between different parts of the same plant.³¹ In parallel, scientists work on improving molluscicidal properties of known natural compounds, to make analogue new chemicals with better activities.³²

In the present study, some samples showed strong molluscicidal activities, namely the ethyl acetate extract of leaves and its methanolic fraction, the ethyl acetate extracts of stems, fruits and flower buds and the hydro-methanolic extracts of stems and fruits. Powders of all the organs were active, except for flowers. All these values fell well below the upper threshold of 40 mg/L set as a potential molluscicide by the WHO.³⁰ Consequently, *C. spinosa* leaves, stems, fruits and flower buds are considered noxious to *G. truncatula*, compared to the previous studies established on *C. spinosa*.¹⁵⁻¹⁷ As a result, working on specific organs helps better locate the lethal concentrations to kill the parasite, and that extracting the plant with as many as possible organic solvents helps estimate the nature of the products responsible for the molluscicidal activity. In previous studies, dry powders of the whole plant and its leaves were considered to be potent molluscicides against *Biomphalaria alexandrina*.^{16,17} Petrol, *n*-hexane, methylene chloride, ethyl acetate, methanol and water extracts of this plant aerial parts were tested against *Bulinus truncatus* and gave respective LC₅₀ of 70.58, 62.94, 106.70, 79.15, 170.58 and 2236.00 mg/L,¹⁵ besides, phytochemical tests established on these aerial parts showed the presence of coumarins, alkaloids, sterols and essential oils inside. In this study, the fractionation of leaf ethyl acetate extract (LC₅₀=8.03 mg/L) helped better locate the molluscicidal activity in the methanolic fraction (LC₅₀=3.53 mg/L). Stem extract was not fractionated (yield of 0.32%). Taking the study of the antiparasitic activity of *C. spinosa* into consideration, leaf and stem ethyl acetate extracts as well as the methanolic fraction of leaf ethyl acetate extract, used at lethal concentrations to the mollusk *G. truncatula* also gave potent larvicidal activities with deterioration rates exceeding 30.39%. Using this extracts/fraction would be very helpful in eliminating both the intermediate host and the larval stages of *F. hepatica*.

The methanolic fraction of the leaf ethyl acetate extract was rich in flavonoids/saponins. Previous studies on *C. spinosa* revealed the presence of flavonoids such as rutin, quercetin and its derivatives from *C. spinosa* aerial parts.^{8,33} Molluscicidal activities of rutin and quercetin were proved against *Lymnaea* snails.^{34,35} Nevertheless, quercetin derivatives weren't investigated yet, except quercetin 3-(2''-galloylglucoside). This compound was reported³⁶ as a molluscicidal agent against *Lymnaea natalensis*, but no literature data mentioned the presence of this flavonoid in *C. spinosa*.

Moreover, Mustafa³⁷ revealed through a preliminary screening on this plant the presence of saponins inside. These components are well known for their molluscicidal power against *Lymnaea* snails.^{35,38}

Hence, the molluscicidal potency found in the methanolic fraction of *C. spinosa* leaf ethyl acetate extract may be attributed to rutin, quercetin and saponins inside. Other leaf and stem ethyl acetate active extracts contained triterpenoids/sterols/carotenoids. The presence of carotenoids in leaves was justified.³⁹ however the presence of sterols and triterpenoids was not demonstrated yet in the aerial parts of *C. spinosa*. Besides, no data was provided about the molluscicidal activity of carotenoids and sterols, only a few triterpenoids were tested for their molluscicidal potency.⁴⁰

CONCLUSION

The molluscicidal potential of *C. spinosa* has been proved in the present investigation and it can be recommended for control of *G. truncatula* snails in Tunisia.

CONFLICT OF INTEREST

The authors report no declarations of interest.

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SUMMARY

- Fascioliasis infects many people world wide.
- Some extracts and fractions of *Capparis spinosa* possess phytochemical constituents known for their molluscicidal effects.
- Two ethyl acetate extracts and a methanolic fraction are the most potent against *Galba truncatula*.
- Active samples have considerable larvicidal activities.

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