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MOLLUSCICIDAL AND LARVICIDAL ACTIVITIES OF *Atriplex inflata* AERIAL PARTS AGAINST THE MOLLUSK *Galba truncatula,* INTERMEDIATE HOST OF *Fasciola hepatica*

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SUMMARY

Fasciolosis is a widespread parasitosis of farm live-stock in many developing countries. For this reason, it is necessary to search for new substances against parasitic diseases caused by flukes. Indeed, a wide variety of terrestrial plants have been subjected to chemical and pharmacological screening in order to discover their potential for human medicinal use. The molluscicidal and larvicidal activities of *Atriplex inflata* were tested on *Galba truncatula* and *Fasciola hepatica* larval stages infecting this snail in Tunisia. Phytochemical tests were conducted on extracts in order to establish a meaningful relationship with molluscicidal and larvicidal activities. The molluscicidal activity was evaluated by subjecting snails to sample aqueous solutions. Accordingly, hexane, ethyl acetate, methanol and methanol-water (8:2, v-v) were used as extraction solvents. As a result, hexane and ethyl acetate extracts showed potent activity, according to the World Health Organization, giving LC_{50} = 7.59 mg/L and 6.69 mg/L for hexane extracts of leaves and fruits, respectively. Ethyl acetate extracts gave LC_{50} = 5.90 mg/L and 7.32 mg/L for leaves and fruits, successively. Molluscicidal activities of powders were less potent on snails, but active according to the World Health Organization. Hexane and ethyl acetate extracts from leaves and fruits gave potent larvicidal activities with a delay rate exceeding 45.50% (45.50- 98.92%). Phytochemical tests showed that these activities may be attributed to the presence of triterpenoids and/or sterols.

KEYWORDS: Environmental Health; Molluscicidal activity; *Galba truncatula*; *Atriplex inflata*; Larvicidal activity; *Fasciola hepatica.*

INTRODUCTION

Fasciolosis is a serious parasitosis of farm live-stock that is caused by a helminth species *Fasciola hepatica* (*F. hepatica*). It is spread world-wide and causes serious economic losses in the industry of animal husbandry (MAGE *et al*. 2002). *Galba truncatula* (*G. truncatula*), also called *Lymnaea truncatula*, belongs to the *Lymnaeidae* family and has been identified as the major intermediate host of *F. hepatica* in southern Tunisia (AYADI *et al*. 1993, HAMMAMI & AYADI 1999).

In Tunisia, human fasciolosis is a rare disease; only 38 cases were reported between 1940 and 2007 (AYADI *et al*. 1991, HAMMAMI *et al*. 2007). The majority of patients come from the North and Southwest of Tunisia (AYADI *et al*. 1997). Animal fluke infection, however, is more common, being 20% in Sajnene (JEMLI *et al*. 1991) and 44% in Tozeur (HAMMAMI & AYADI 1999).

Treatment of fasciolosis requires high or multiple doses of drugs with numerous side effects (ABDUL-SAMIE *et al*. 2010). Therefore, snail control is considered not only complementary but also essential in fasciolosis control. It is regarded as a rapid and efficient method of reducing or eliminating transmission, as well as breaking up the parasite life cycle (MELLO-SILVA *et al*. 2006, JIGYASU & SING 2010). The search for local molluscicidal plants was considered more sustainable than the use of synthetic drugs (HAMMAMI *et al*. 2011).

Atriplex inflata (A. inflata) showed important biological activities against a large number of pathogens, but no previous investigations on the molluscicidal activity of this plant were established. However, other species of the genus *Atriplex* showed molluscicidal activities, such as *A. stylosa* and *A. hamilus* against *Biomphalaria alexandrina* (BAKRY 2009, TANTAWY 2002). Moreover, it was demonstrated that *A. leucoclada* and *A. nummularia lindleyi* had molluscicidal activities (SHOEB *et al*. 1987; CHRISTENSEN & OMAR 1985).

Based on these facts and since other species of the genus *Atriplex* have been described as potent molluscicide plants, rich in saponins, flavonoids and other components, the present study aims to investigate the phytochemical composition, and the molluscicidal and larvicidal activities of hexane, ethyl acetate, methanolic, as well as hydromethanolic extracts from this plant against *G. truncatula*, the intermediate host of *F. hepatica*, so as to contribute to the new area of application of these plant extracts as eco-friendly molluscicides (MELLO-SILVA *et al*. (2006)).

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MATERIALS AND METHODS

Chemicals: All solvents used for extraction and partitioning were fractionally distilled prior to use.

***Plant material*:** *A. inflata* F. Muell. = *A. lampifer* Buxb. = *A. lindleyi* Moq. = *Blackiella inflata* (F. Muell.) Aellen belongs to the *Chenopodiaceae* family. It is also called "Gtaf" in Arabic. This is an Australian plant introduced around 1895 in Tunisia as a forage species and, since then, it has been naturalized in southern Tunisia. It grows in saline soils with fine to medium texture (LE FLOC'H *et al.* 2010, CHAIEB & BOUKHRIS 1998).

The plant was harvested in November 2011, from Sfax, Tunisia. The botanical identification was established by Pr. Mohamed Chaieb, Botanist at the Faculty of Sciences of Sfax, Tunisia. The voucher specimen's number LCSN113 was deposited at the Laboratory of Chemistry of Natural Substances in the Faculty of Sciences, University of Sfax, Tunisia.

***Extractions*:** The aerial parts of *A. inflata* were collected from the coast of Sidi Mansour, Sfax (Latitude: 34.745; Longitude: 10.761) in November, 2011. Once sorted, leaves and fruits were dried in the shade and ground by a mechanical grinder; leaves (125 g) and fruits (270 g) were partitioned, each by sequential macerations with hexane, ethyl acetate, methanol and methanol-water (8-2: v-v), each for 48 h. A quantity of powder was kept aside for subsequent molluscicide tests. The extracts were filtered and concentrated under reduced pressure in a rotary evaporator, then dried. The dry extracts were subjected to various chemical tests and Thin Layer Chromatography (TLC) to detect the presence of different phytoconstituents.

Phytochemical tests: Plant extracts were tested by phytochemical qualitative reactions for usual plant secondary metabolites. The investigation included triterpenoids and/or sterols, flavonoids, saponins, alkaloids, coumarins, tannins, tropolone nuclei and quinones. Analytical responses were based on color reactions or TLC plate revelation.

Using color reactions: plant extracts were subjected to chemical tests (T_1 to T_8) based on their colors to check for the presence of some phyto constituents. Thus, 1 mg of each extract was dissolved in 1 mL of the suitable solvent to obtain solution E, which was shaken. Then, the appropriate standard solutions were added to test the presence of sterols and/or triterpenoids (T_1: Liebermann's reaction), tropolone nuclei (T_2: Wiustater's reaction), free quinones (T_3: Borntraeger's reaction), flavonoids (T_4: test of flavonoids) and alkaloids (T_5: Mayer's reaction).

These tests (T_1 to T_5) were performed according to HARBORNE (1964, 1973).

T_6: Frothing test for saponins according to ONWUKAEME *et al*. (2007).

T_7: test for coumarins by BÉKRO *et al*. (2007).

T_8: test for tannins by AKINJOGUNLA *et al*. (2010).

Using UV detection of TLC plates: additional TLC plate revelation which was done under UV light (365 nm), 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 (HARBORNE 1964).

* Detection of saponins: total steroidal saponins appear to be a reddish purple after spraying plates with a chloroform saturated antimony trichloride solution and, a few minutes after heating the plate (100-110 °C), all steroidal nuclei are located without distinguishing saponins from glycoalkaloids (GA) which were already revealed as orange spots by Dragendorff's reagent (HARBORNE 1964).

* Detection of sterols and/or triterpenoids: a range of colors is produced, visible in both daylight and UV, on spraying heated plates for 10 min at 100 °C with Carr-Price reagent, i.e. 20% antimony chloride in chloroform (HARBORNE 1998).

* Detection of flavonoids: 1% $AlCl_3$ in absolute ethanol reacts specifically with flavonoids yielding a fluorescing complex. It gives well colored yellow visible spots (MARKHAM 1982).

* 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 (GLOWNIAK 2009).

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

***Snails*:** Adult *G. truncatula* snails were used at a uniform size (3-5 mm in length). They were manually collected from El Melah river, in Ain Soltan oasis, Gafsa, a governorate in southwest Tunisia (34°27'13" N, 8°47' 44" E), in April 2012. About 4,800 uninfected snails used for molluscicidal tests were extracted from the upstream of El Melah river barrage, where snails are already known to be uninfected as it is a high river and hence out of livestock's reach (HAMMAMI & AYADI, 2008). However, about 210 naturally infected snails used for larvicidal activities were extracted downstream in the river. This downstream river was characterized by infested intermediate hosts, especially sheep and goats, and by snails showing two natural infection periods, in autumn and spring (HAMMAMI *et al*. 2007). All snails were transferred in plastic containers to the Fungal and Parasitic Molecular Biology Laboratory at the Faculty of Medicine in Sfax, Tunisia. They were cleaned and placed in holding tanks containing aerated, dechlorinated tap water and washed sand. They were kept alive under laboratory conditions: indoor temperature varying between 13 and 25 °C with attenuated natural light and were fed on green algae. The 210 naturally infected snails raised in Laboratory, in spring (April 2012) were examined for cercarial shedding that extends over a period of approximately seven to 30 days.

Molluscicidal tests: The evaluation of the molluscicidal activities of the extracts and powders of *A. inflata* and $CuCl_2$ (used as positive control) on snails was conducted as recommended by the World Health Organization (1965). Different concentrations of aqueous solutions were prepared for each extract, powder and $CuCl_2$. Disinfected snails were exposed, in groups of 10 (five replicates) for 48 h (exposure period), to 500 mL of each concentration of the material to be tested: powders and extracts as listed in Table 3. Similarly, five groups of ten snails immersed in dechlorinated water were used as negative controls. After exposure,

snails were rinsed thoroughly in dechlorinated water and left for 48 h (recovery period) inside. Mortality was recorded after 24 and 48 h. Dead animals were removed immediately to avoid the contamination of other animals. Snail mortality was established by the retraction of the body within the shell; no response to a needle probe was taken as evidence of death.

Larvicidal tests: Each snail that was assumed to be infested was placed individually in a Petri dish with algae and dechlorinated water. The positivity of each snail was confirmed by cercarial shedding observed under a stereomicroscope. Cercarial shedding was produced by thermal shock which is provoked according to RONDELAUD *et al*. 2013 and VIGNOLES *et al*. 2014.

The infected snails were exposed to the four potent LC_{90} molluscicidal extracts; corresponding to the leaves and fruits (ethyl acetate of leaves, hexane of leaves, ethyl acetate of fruits and hexane of fruits); in a group of 20 naturally infected 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 and placed only in dechlorinated water, was used as a control. After dissection, snail bodies were removed and placed in 200 mL of dechlorinated water. The cercariae, rediae, and intraredial germinal masses, which appear after dissection or leave the bodies of dissected molluscs, were observed under an optical microscope and then counted. Deteriorated larval stages were identified by their surface alteration and vesiculation (RUG & RUPPEL 2000). The number of the deteriorated and undeteriorated larval stages (rediae, cercariae and intraredial germinal masses) were counted in each snail; then the delay rate was calculated.

Statistics: Concentrations that would kill 50% (LC_{50}) or 90% (LC_{90}) of the exposed snails and the confidence intervals (95% CI) were determined by WinDL software (MARTIN *et al.* 2003). Student's t-test was used to compare the alteration of different larval stages of the parasite (cercariae, rediae and intraredial germinal masses) after treatment with active samples (ZIMMERMAN *et al*. 1993).

RESULTS

Masses and yields of different aerial part extracts of *A. inflata* after 48 h of extraction, using increasing polarity solvents, are given in Table 1. Yields vary between 0.05 and 1.17% for hexane extracts, between 1.20 and 3.73% for ethyl acetate extracts, between 1.89 and 26.51% for methanol extracts, and between 0.17 and 4.18% for hydromethanol extracts. The most important yield was obtained in the methanol extract from the leaves.

Phytochemical tests performed on each extract are reported in Table 2. Molluscicidal activities of each tested plant material are shown in Table 3.

Table 1
Masses and yields of different aerial parts extracts of *Atriplex inflata* after 48h of extraction with increasing polarity solvents

		Hexane extract	Ethyl acetate extract (EtOAc)	Methanol extract (MeOH)	Methanol- Water extract (8:2, v-v)
Leaves (125 g)	Mass (g)	1.46	1.50	33.14	5.22
	Yield (%)	1.17	1.20	26.51	4.18
Fruits (270 g)	Mass (g)	0.14	10.07	5.10	0.46
	Yield (%)	0.05	3.73	1.89	0.17

Table 2
Chemical compounds present in the extracts of aerial parts from *Atriplex inflata*

Aerial parts	Solvents of extraction	Sterols and/or triterpenoids		Flavonoids		Saponins		Alkaloids		Coumarins		Tannins	Tropolone	Quinones
		*	**	*	**	*	**	*	**	*	**	*	*	*
Leaves	Hexane	++	+	-	-	-	-	-	-	-	-	-	-	-
	EtOAc	+	+	-	-	+	-	-	-	-	-	-	-	-
	MeOH	-	-	+	+	-	-	-	-	+	+	-	-	-
	MeOH-H_2O	-	-	+	+	++	+	-	-	-	-	-	-	-
Fruits	Hexane	++	+	-	-	-	-	-	-	-	-	-	-	-
	EtOAc	+	++	-	-	-	-	-	-	-	-	-	-	-
	MeOH	-	-	+	+	+	+	-	-	-	-	-	-	-
	MeOH-H_2O	-	-	++	++	++	+	-	-	-	-	-	-	-

- : No reaction; +: Weak presence; ++: Presence; +++: Richness. EtOAc: Ethyl acetate; MeOH: Methanol; MeOH-H_2O: Methanol-Water (8-2: v-v). * : Colour reaction results. **: TLC plate revelation results.

Table 3
Molluscicidal activities of *Atriplex inflata* extracts against *Galba truncatula*.

Organs	Powder/Solvent	After 24 h action				After 48 h action			
		Equation of the line weighed regression	Slope	LC_{50} (mg/L) (95% CI)	LC_{90} (mg/L) (95% CI)	Regression	Slope	LC_{50} (mg/L) (95% CI)	LC_{90} (mg/L) (95% CI)
Leaves	Powder	Y= -5.22 + 3.65 X	1.87	26.79 (1.45; 37.48)	60.08 (44.36; 381.33)	Y= -4.45 + 3.35 X	1.98	21.39 (7.79; 58.73)	51.62 (34.61; 76.98)
	Hexane	Y= -2.75 + 2.61 X	2.41	11.31 (1.60; 79.85)	35.07 (17.29; 71.14)	Y= -3.42 + 3.88 X	1.80	**7.59 (4.70 ; 9.95)**	16.25 (12.26; 29.23)
	EtOAc	Y= -5.34 + 6.59 X	1.41	6.45 (4.46; 7.71)	10.08 (8.39; 15.69)	Y= -5.69 + 7.38 X	1.40	**5.90 (3.63; 7.13)**	8.80 (7.29; 13.51)
	MeOH	-	-	-	-	-	-	-	-
	MeOH-H_2O	Y= -5.74 + 3.22 X	2.04	61.64 (34.16; 111.22)	137.58 (34.07; 555.55)	Y= -5.50 + 3.17 X	2.06	54.12 (32.77; 89.38)	137.09 (29.17; 644.24)
Fruits	Powder	Y= -5.69 + 3.51 X	1.92	41.57 (25.95; 52.47)	96.27 (71.47; 248.18)	Y= -7.61 + 5.02 X	1.57	32.76 (24.30; 39.23)	58.95 (48.19; 91.08)
	Hexane	Y= -5.24 + 6.28 X	1.44	7.16 (4.51; 8.44)	10.28 (8.70; 17.16)	Y= -5.13 + 6.27 X	1.44	**6.69 (4.76; 7.68)**	8.58 (7.49; 13.00)
	EtOAc	Y= -5.91 + 6.32 X	1.44	8.61 (5.42; 13.69)	13.03 (9.88; 17.19)	Y= -5.63+ 6.36 X	1.43	**7.32 (3.11; 17.52)**	11.16 (8.06; 15.45)
	MeOH	-	-	-	-	-	-	-	-
	MeOH-H_2O	-	-	-	-	-	-	-	-
	Dechlorinated water	-	-	-	-	-	-	-	-

CI: confidence interval; LC_{50}: 50% lethal concentration; LC_{90}: 90% lethal concentration; -: no activity. $CuCl_2$ showed molluscicidal activity against *G. truncatula* after 48 h with: LC_{50} = 26.12 (19.35; 31.69) mg/L and LC_{90} = 62.71 mg/L (49.83; 96.50). EtOAc: Ethyl Acetate; CH_2Cl_2: Methylene Chloride; MeOH: Methanol; MeOH-H_2O: Methanol-Water.

Molluscicidal activity: The highest molluscicidal activities were detected for hexane and ethyl acetate extracts of *A. inflata* leaves and fruits giving closer respective LC_{50} of 7.59, 6.69, 5.90 and 7.32 mg/L after 48 h of treatment. Meanwhile, methanol and hydromethanol extracts of leaves and fruits were considered inactive because their LC_{50} exceeded 40 mg/L or was zero according to the WHO (1993). Weaker molluscicidal activities were recorded in powders with LC_{50} of 21.39 mg/L for leaves and 32.76 mg/L for fruits. Copper chloride used as positive control showed a molluscicidal activity with an LC_{50} of 26.12 mg/L against *G. truncatula* after 48 h of treatment (Table 3). *G. truncatula* negative control organisms were not affected by dechlorinated water after 48 h of exposure. Hemolysis and hypersecretion of mucus were the common toxic reactions of snails to the active tested materials.

Chemical tests performed on the extracts for sterols and/or triterpenoids were positive, particularly for ethyl acetate extract of leaves. Methanolic and hydromethanolic extracts were inactive against *G. truncatula* and did not contain any sterols and/or triterpenoids, but contained flavonoids instead.

Larvicidal activity: Delay rates (%) of larval stages: cercariae, rediae and intraredial germinal masses in infected *G. truncatula* after 48 h of exposure to molluscicidal extracts of *A. inflata* and in untreated infected snails placed in dechlorinated water are listed in Table 4. The four most potent *A. inflata* molluscicidal extracts were toxic to the larval stages of *F. hepatica*. The hexane extract from fruits was significantly more toxic to intraredial germinal masses than the others ($p < 0.05$).

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

	% deterioration					*p* value					
	EtOAc extracts		Hexane extracts		Dechlorinated water	a/b	a/c	a/d	b/c	b/d	c/d
	Leaves (a)	Fruits (b)	Fruits (c)	Leaves (d)							
Rediae	69.58±5.56	45.5±6.34	81.25±23.93	61.26±26.56	0	0.0039	0.2200	0.3100	0.0280	0.1850	0.1530
Intraredial germinal masses	78.47±7.33	59.38±15.86	98.92±1.86	92.73±8.94	0	0.0658	0.0047	0.0490	0.00639	0.0169	0.1520
Cercariae	89.32±22.57	65.09±27.97	59.92±26.89	54.48±22.10	0	0.0230	0.0081	0.0013	0.3390	0.1790	0.3130

p: *p* value calculated comparing: a to b; b to c and a to c.

DISCUSSION

The high cost of synthetic molluscicides, their toxicity to non-target organisms, and even human beings as well as the complex organization required in their application has hindered their continuous use in fasciolosis control programs. Many plants have been screened for their intrinsic molluscicidal properties in an attempt to find an alternative to synthetic ones (HASSAN *et al*. 2011). In previous studies, some species of *Atriplex* have been found to have biological activities such as anti-inflammatory potency (ATEYA *et al*. 2005), insecticidal activity against *Tribolium* spp. (TRABELSI 2004), and antiviral efficiency against *Herpes simplex* infection (BEN SASSI *et al*. 2008). Antifungal activity of the same plant against *Aspergillus fumigatus* and three *Fusarium* species was also proved. Besides, antibacterial activity (against *Bacillus subtilis* and *Staphylococcus aureus*), antidiabetic, and antioxidant activities was described (MOHAMMED *et al*. 2012). In addition, a hepato-renal protective effect of *A. lindleyi* Moq. aerial parts against bromobenzene (BB) intoxication in rats was described (MATLOUB *et al*. 2011). Other studies have investigated several *Atriplex* species for their cytotoxicity, such as *A. confertifolia* and *A. lindleyi* Moq (CAPUA *et al*. 2010; MATLOUB *et al*. 2014).

Some *Atriplex* species have been found to have a strong molluscicidal activity which varies greatly from one species to another and even among parts of the same plant (SILVA *et al*. 2005).

Taking these facts into consideration, together with the increase of morbidity, mortality and treatment cost, we decided in the present research to study *A. inflata* phytochemical composition, as well as its molluscicidal and larvicidal potencies.

Hexane and ethyl acetate extracts of *A. inflata* leaves and fruits showed remarkable molluscicidal activities which ranged between 5.90 and 7.59 mg/L. Ethyl acetate leaves extracts showed the highest molluscicidal activity with LC_{50} of 5.90 mg/L after 48 h of exposure. These values were significantly more important than the mortality caused by copper chloride. Powders were less active than extracts. These results belong to the reports of CHRISTENSEN & OMAR (1985), BAKRY (2009), TANTAWY (2002) SHOEB *et al.* (1987) who revealed that the molluscicidal activities of other *Atriplex* species vary from one species to another.

It was demonstrated that *A. stylosa* gave LC_{50} between 31 and 74 mg/L in various solvents against *B. alexandrina* (BAKRY 2009). This result is not that important compared to *A. inflata*, because lethal concentrations should fall well below the upper threshold of 40 mg/L according to the WHO guidelines (1993). Moreover, *A. ambriosides* was considered to be a potent molluscicide against *Bulinus truncatus* with LC_{50} varying between 1.41 mg/L and 14.12 mg/L after 24 h. These values are remarkable, compared to the molluscicidal potency of *A. inflata*. These differences in potency may be attributed to several factors including the location of the plant species, the collection time of the plant sample, the used part, the storage conditions, the method of extraction and the solvent type (BRACKENBURY & APPLETON 1997, HASSAN *et al*. 2010).

In addition to its remarkable molluscicidal potency, *A. inflata* reveals some interesting characteristics for an ideal molluscicide plant. It is a nontoxic plant that belongs to the genus *Atriplex*, which is widespread in different parts of the world. This genus is also regarded as a workable group on a generic level (MOHAMMED *et al*. 2012, OSMOND *et al*. 1980).

In this study, extracts with a notable molluscicidal activity contained sterols and/or triterpenoids. In a previous scientific research, triterpenoids and steroids were isolated from the petroleum ether extract of *A. inflata* (MATLOUB *et al*. 2011). We can deduce that the important molluscicidal activities observed in this study may be attributed to the presence of triterpenoids and/or steroids.

In our study, the hydroethanol extracts of the leaves and the fruits of *A. inflate*, rich in flavonoids, were not active. Likewise, ATEYA *et al*. (2005), EL SAKHAWY *et al*. (2013), and MOHAMMED *et al*. (2012) have isolated some flavonoids from the hydroethanol extract of the aerial parts of *A. inflata*. However, in other studies, it was reported that rutin and quercetin, isolated respectively from *Calendula officinalis* and *Bauhinia variegata* plants, were flavonoids having molluscicidal activities: rutin was found to be powerful against *Biomphalaria alexandrina*, *Bulinus truncatus* and *Lymnaea* snails (EL-SHEIKH *et al*. 2012); while, quercetin was active against *Lymnaea acuminata* with a LC_{50} of 9.86 mg/L (SINGH *et al*. 2012).

Taking the larvicidal activity of *A. inflata* into consideration, hexane and ethyl acetate extracts of both aerial organs, leaves and fruits, used at lethal concentrations to the mollusk *G. truncatula* also gave potent larvicidal activities with delay rates exceeding 45.5%. The use of these extracts seems to be very helpful in eliminating both the intermediate host and the larval stages of the parasite *F. hepatica*.

The high rate of toxicity of the fruits hexane extract to rediae, intraredial germinal masses and cercariae may be attributed to the high level of triterpenoids and/or steroids contained in these fruits.

CONCLUSION

In the present study, hexane and ethyl acetate extracts of *A. inflata* F. Muell. aerial parts showed potent molluscicidal and larvicidal activities against the snail gastropoda *G. truncatula* and larvicidal activity against *F. hepatica* larval stages, according to the World Health Organization guidelines for hexane extracts of leaves and fruits. Molluscicidal activities of powders were less potent on snails, but active according to the same guidelines. Phytochemical tests showed that these activities may be attributed to the presence of triterpenoids and/or sterols.

The use of these extracts may provide additional arsenal to the control methods of snail transmitting fasciolosis in both the tropical and the third world countries where fasciolosis is a common disease.

RESUMO

Atividade moluscicida e larvicida das partes aéreas de *Atriplex inflata* contra o molusco *Galba truncatula*, hospedeiro intermediário da *Fasciola hepatica*

Fasciolose é uma parasitose generalizada que ocorre em animais de fazendas em muitos países em desenvolvimento. Por esta razão, é necessária a busca de novas substâncias contra as doenças parasitárias

causadas por vermes. De fato, uma grande variedade de plantas terrestres foi objeto de testes farmacológicos e químicos a fim de descobrir o seu potencial para utilização em terapêutica humana. As atividades moluscicida e larvicida de *Atriplex inflata* foram testadas contra estágios larvários de *Galba truncatula* e *Fasciola hepatica* infectando este caracol na Tunísia. Testes fitoquímicos foram realizados com extratos a fim de estabelecer uma relação significativa com as atividades moluscicida e larvicida. A atividade moluscicida foi avaliada submetendo os caracóis a soluções aquosas. Conforme o caso, hexano, acetato de etilo, metanol e metanol-água (8:2, v-v) foram utilizados como solventes de extração. Como resultado, hexano e extratos de acetato apresentaram atividades potentes de acordo com a Organização Mundial de Saúde, resultando em LC50 = 7,59 mg/L e 6,69 mg/L para extratos de hexano de folhas e frutos, respectivamente. Extratos de acetato de etilo resultaram em LC50 = 5,90 mg/L e 7,32 mg/L para as folhas e frutos sucessivamente. Atividades moluscicidas das substâncias sob a forma de pó foram menos potentes em caracóis, mas ativas de acordo com a Organização Mundial de Saúde. Hexano e extratos de acetato de folhas e frutos apresentaram atividade larvicida potente, com uma taxa de atraso superior a 45,50% (45,50-98,92%). Testes fitoquímicos mostraram que estas atividades podem ser atribuídas à presença de triterpenóides e/ou esteróis.

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