Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 7, No. 6, p. 242-248, 2015
http://www.innspub.net

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RESEARCH PAPER
OPEN ACCESS

Molluscicidal activity of the Saharian medicinal plants *Limoniastrum feei* and *Launaea nudicaulis* against the fresh water snail *Lymnaea stagnalis*

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Article published on December 29, 2015

Key words: *Limoniastrum feei, Launaea nudicaulis,* Molluscicidal activity, *Lymnaea stagnalis,* Parasitic trematodes**.**

Abstract

In South Algeria, some of the plant species investigated in the present study is used in popular medicine to treat different diseases. In this paper, we present the screening of two species medicinal plants against *Lymnaea stagnalis* snails to finding molluscicidal plant extracts. A preliminary screen to detect extracts with molluscicidal activity was run using a simple bioassay according to WHO recommended the methodology. The molluscicidal activity of aqueous extract of *Launaea nudicaulis* and *Limoniastrum feei* against the snail *Lymnaea stagnalis* was studied. The molluscicidal activity of all the plant products was found to be both time and concentration dependent. The 72 h LC_{50} of *Limoniastrum feei* stem against *L. stagnalis* was 26,8 µg/ml, extraction by maceration were more toxic than *Launaea nudicaulis* by reflux extraction, that was 37,27 µg/ml. The 48 h LC_{50} of *Limoniastrum feei* stem and *Launaea nudicaulis* was 34,39 and 75,51 µg/ml, respectively. Thus, the highest molluscicidal activity, followed by *Limoniastrum feei* stem, whole *Launaea nudicaulis* (aerial part) and *Limoniastrum feei* leaves against *Lymnaea stagnalis*.

This preliminary study on the screening and biological evaluation of Saharan medicinal plants could offer possible alternative for sustainable and affordable use.

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Introduction

Many of freshwater snails are involved in parasitic trematodes cycle and parasitic diseases as intermediate host (Żbikowska, 2011) *Lymnaea stagnalis* is snail is an obligatory first intermediate (primary) host as well as the second intermediate host for a number of digenean trematode parasites (Esch *et al.,* 2002; 2001). Lymnaeidae family snails have place in order of Basomatomorpha and sub-class of Pulmonata from Gastropoda class (Dahane-Rouissat *et al.*,2015). In some cases, infection of some species of snails from Lymnaeidae family with miracidium of mammal's and bird's parasitic schistosoma leads to creation of cercaria which is creator of dermatitis in humans (Faltýnková *et al.,* 2008; Karruki *et al.,* 2004). The molluscicidal properties of numerous plant extracts have been studied with a view to developing an accessible and inexpensive technology for appropriate control of the snail vector by local communities (Mendes *et al.,* 1997) Risks and problems associated with the use of chemicals lead to increasingly stringent environmental regulation of pesticides (Pavela *et al.,* 2010); in this context, screening of natural products has received the attention of researchers around the world (Kebede *et al.,* 2010) .

The genus *Launaea* Cass. belongs to the tribe Lactuceae of the Asteraceae family, contains about 54 species, most of which are adapted to dry, saline and sandy habits. The genus *Launaea* is represented in the flora of Algeria by nine species including five endemics of North Africa (Ozenda, 2004; Cheriti *et al.,*2012). *Limoniastrum feei* is native to Southeast of Algeria, belongs to *Plumbagenaceae* family. It grows to a height of 10 –40 ft, it's possess long leaves and flowering palms, it's flower is entoured by a brickly bracts with a purplish red color (Ozenda ,2004 ; Maire, 1953). It is common plant known under the name vernacular "Melefet Khadem" and used as a common herbal drug in the south-western Algeria (Cheriti *et al.*,2004, Belboukhari and Cheriti, 2005). In the present study we evaluated the molluscicidal activity of *Launaea nudicaulis* (Asteraceae) and *Limoniastrum feei* (Plumbagenaceae) leaves, stem and twig against the snail *Lymnaea stagnalis*.

Materials and methods

Collection of snails

Adult *Lymnaea stagnalis* snails (2.50 ± 0.50 cm in length), collected locally from different irrigation canal in Kenadsa (Algeria), were used as the test animals. Snails were acclimatized for 72 h in distillated water or water source. Ten experimental animals were kept in Petri dishes containing 20ml distillated water at 24 ± 1 °C. Dead animals were removed at each observation to avoid any spoilage of the Petri dishes water.

The snails were identified by using the parasitological keys and for confirmation of identification, radula was studied. The soft parts of snail were placed in 10% KOH and after 4 h were investigated in a Petri dish under a loop. The radula was dissected from the other parts, and after staining with the radula, identifications were be dissected.

For the fixation, cercariae were heated in the 10% formalin, then (for stabilizing the external shape of the cerecariae) were mixed 1 cm^3 of warm formalin with 1 cm^3 of water contain cercariae, and then the cercariae were stained and identified by light microscopes (Rivaz *et al.,* 2014).

Plant materials and extraction

According to the results of ethno pharmacological study to several medical plants that are used in South west of Algeria in traditional medicine, it carries out by POSL *laboratory since 1998*. We are interested to deepen the investigation of the medicinal species: *Launaea nudicaulis* and *Limoniastrum feei*.

Launaea nudicaulis (vernacular name: Rghama) is an herbaceous plant belonging to the Asteraceae family, which is widely distributed in the Algerian Sahara. (Belboukhari & Cheriti, 2006; Cheriti *et al.*,2013). The whole plants were collected in March-April 2014 from Kenadsa (south Algeria. The botanical

identification and a voucher specimen are conserved at the Phytochemical Herbarium of the Phytochemical and Organic Synthesis Laboratory under accession number CA02/02.

The aerial parts were separated and dried and the plants were ground into powder using a grinder. The extraction of an aerial part of the plant by reflux for 2 h (Crude water extracts were prepared from these powders by mixing 20 g powder (aerial parts) with 200 ml of distilled water, boiling for 2 h and then filtering through a pre-weighed Whatman No. 1 filter paper. The residue was evaporated to done the phytochemical screening and to determines the molluscicidal activities of the obtained extracts.

Limoniastrum feei (vernacular name: Melefet l'khadem) was collected in March – April 2014 from Boukais-Lahmar (South-western Algeria). A voucher specimen (CA99/14) is deposited at the herbarium of Phytochemistry and Organic Synthesis Laboratory (Rahmani *et al.,* 2014; Boulenouar *et al.,* 2009).

All parts of *Limoniastrum feei* (leaves stem and twig) were individually ground to a fine powder .Extraction using reflux apparatus and extraction by maceration with water ; reflux for 2 hours was performed and maceration to ambient temperature for 24h. The residue was evaporated in rotavapory apparatus. The weighed extract was subject to phytochemical screening and to determines the molluscicidal activities (Boulenouar *et al.,* 2009; Rahmani *et al.,*2012).

Phytochemical screening

For the phytochemical screening, 20 grams of dried material was mixed with 200 mL of distilled water and it was macerated for 48 hours. The aqueous extracts were divided into fractions for subsequent determination of secondary metabolites which may be present in the aqueous and organics extracts (Boulenouar *et al.,* 2009 Miranda and Cuéllar,2002)

Molluscicidal assay

Tests were performed in duplicate using 10 snails for each test. Compounds were initially dissolved in a small amount of distillated water then the desired dilution was prepared with distillated water. The snails were exposed to different dilutions for 24 h followed by 24 h in distillated water as recovery period.

Aqueous extracts of *Launaea nudicaulis* (aerial part) and *Limoniastrum feei* (leaves stem and twig) were tested against adult *Lymnaea stagnalis* snails according to the method recommended by WHO (WHO,1983) Tests were carried out at incubator (with aeration and Temperature 24°C ± 2). After 24 h of exposure, the suspension was decanted, the snails were rinsed thrice with distilled water and transferred to the same extract and maintained there for another 24 h recovery period. Ten snails were immersed in separate concentration of aqueous extract with the same volumes that would serve as control. All groups were observed carefully after 24 h, the number and the percentages of death in each group were calculated. Snails were considered dead if they could not move or retracted well into or hanging out of the shell, with the body and shell discolored (Vijay,2010)
Eight groups of adult *Lymnaea stagnalis* (2.50 ± 0.50 cm in length), each of 10 snails, were exposed to the aqueous extract of each part of plant , at the previously determined LC50 and LC90, for 24 h and their mortalities recorded 48 h, 72h and 96h post-exposure.

Data analysis

Determination of LC50 and LC90

The effects of two molluscicides on *Lymnaea stagnalis* were expressed by LC50, LC90 (only after 96h), and their 95% confidence limit (95% CL). The regression coefficient between exposure time and different values of LC_{50} was determined by the method of Probit Analysis (Finney,1952; Sokal and Rohlf,1996).

Results and discussion

Table 1 shows the results obtained from the

phytochemical screening the aqueous extracts of *Launaea nudicaulis* and *Limoniastrum feei*. The test presence of alkaloids was negative for all plants. Presence of phenols and tannins were recorded in *Launaea nudicaulis*., and presence of flavonoids and reducing compounds in all the extracts. The presence of saponins was observed in extracts of *Launaea nudicaulis* and *Limoniastrum feei* (leaves).

Table 1. Phytochemical screening of the molluscicidal aqueous extracts.

	Launaea nudicaulis	*Limoniastrum feei (leaves)*	*Limoniastrum feei (Stems)*	*Limoniastrum feei (twig)*
Alkaloids	-	-	-	-
Phenols /Tannins	+++	+	+	+
Flavonoids	+++	+	+	+
Reducing compounds	++	++	-	-
Saponins	+++	+++	+	+

+++ Abundant; +Positive; - Negative.

Molluscicidal activities of *Launaea nudicaulis* and *Limoniastrum feii* (Leave, stem and twig) against *Lymnaea stagnalis* snails were determined by the bioassay. The LC50 and LC90 for *Launaea nudicaulis* after 24h, 48h, 72h and 96 h exposure are given in Table 2.

Table 2. Lethal Concentration (µg / ml) 50 % and 90 % of mortality accumulated by the aqueous extracts (maceration and reflux extract) on *Lymnaea stagnalis* snail.

Exposition time Hour	Plants	Extraction types	Equation	R^2	LC50 (µg/ml)	LC90 (µg/ml)
	Launaea nudicaulis (Aerial	Maceration	Y=9,646x -22,73	0,949	340,83	362,25
24	*parts)*	reflux	Y=8,003x -19,88	0,764	495,081	532,787
	Limoniastrum feei (Leaves)	Maceration	Y=8,017x -19,93	0,772	498,756	536,697
		reflux	Y=8,131x -20,22	0,774	496,282	533,486
	Limoniastrum feei (Stem)	Maceration	Y=8,017x -19,93	0,772	498,75	536,69
		reflux	Y=8,017x -19,93	0,772	498,75	536,69
	Launaea nudicaulis (AP)	Maceration	Y=5,051x -8,766	0,912	117,99	132,556
		A reflux	Y=2,542x -3.075	0,923	75,51	95,16
	Limoniastrum feei (Leaves)	Maceration	Y=8,017x -18,87	0,945	367,848	395,831
48		reflux	Y=7,494x -17,43	0,869	356,920	386,042
	Limoniastrum feei (Stem)	Maceration	Y=1,933x -1,271	0,849	34,39	46,61
		reflux	Y=8,057x -18,94	0,943	364,43	392,01
	Launaea nudicaulis (AP)	Maceration	Y=6,040x -11,03	0,869	128,069	141,159
		reflux	Y=2,284x -2,032	0,976	43,00	55,62
	Limoniastrum feei (Leaves)	Maceration	Y=3,091x -4,359	0,928	91,174	110,270
72		reflux	Y=2,743x -3,430	0,966	74,103	91,812
	Limoniastrum feei (Stem)	Maceration	Y=1,966x -1,109	0,878	26,80	36,15
		A reflux	Y=2,548x -2,690	0,963	52,78	66,48
	Launaea nudicaulis (AP)	Maceration	Y=5,538x -9,393	0,886	100,66	111,938
		A reflux	Y=2,506x -2,239	0,900	37,27	47,128
	Limoniastrum feei (Leaves)	Maceration	Y=2,839x -3,271	0,832	56,312	69,266
96		reflux	Y=3,027x -3,861	0,944	68,673	83,391
	Limoniastrum feei (Stem)	Maceration	Y=2,904x -3,572	0,896	65,32	79,97
		reflux	Y=2,931x -3,451	0,904	57,15	69,84
Control +	*Cu SO_4, 5H_2O*	Aq. solution	Y=3,526x+0,945	0.991	8.581	42.265
Control -	Source water	-	Y=7,636x-18,65	0.901	612.253	655.362

The activity of the reference molluscicide *Limoniastrum feei* (Leaves, stem and twig) against *Lymnaea stagnalis* snails was determined by using the same assay procedure and gave an LC50 of 26,80 µg/ml and an LC90 of 36,15 µg/ml. After 48 h exposure in *Launaea nudicaulis* and *Limoniastrum feei* showed that Molluscicidal activities of *Launaea nudicaulis* were different to those of *Limoniastrum feei* (Table 1), in other way, the molluscicidal potency of all tested parts of *Limoniastrum feei* against *Lymnaea stagnalis* were concentration dependent. Generally, mortality increased with the increase in concentration of the extracts.

The lethal concentrations for aqueous extracts of *Launaea nudicaulis*, *Limoniastrum feei* (Leaves stem and twig) that killed 50% (LC50) of adult *Lymnaea stagnalis* were 37.27, 74.10, 52.78 and 72.94 µg/ml, respectively, while the respective LC90 values were 47.12, 91.81, 66.48 and 90.06 µg/ml (Table 2).

The toxicity of different aqueous extract of *Launaea nudicaulis* (aerial part) and *Limoniastrum feei* (leaves stem and twig), against *L. acuminata* was time and concentration dependent. Treatment of 510 µg/ml of *Launaea nudicaulis* (aerial part) caused no mortality in treated snails. The LC_{50} of *Launaea nudicaulis* (aerial part) at 24 h was 340, 83 µg/ml (with maceration) and 495.08 µg/ml (with reflux extraction) (Table 2). There was a significant negative correlation between the LC50, LC90 and exposure time. Mortality was determined every 24 h. Each set of experiment was replicated six times.

Comparing the LC50 and LC90 values of the plant parts, showed the highest molluscicidal activity, followed by *Limoniastrum feei* stem, whole *Launaea nudicaulis* (aerial part) and then *Limoniastrum feei* leaves against *Lymnaea stagnalis*. (Table 2).

Lymnaea stagnalis snail is the biggest intermediate host *Lymnaea* in Iran whose shell has 45 mm length and 25 mm width, contains 7-8 spire and the throat of shell is alike to human's auricle (Hohagen and Jackson,2013) The mortality and reproductive ability of the nematodes, when incubated with each plant extract, were evaluated using the methods of (Rasoanaivo and Ratsimamanga-Urverg,1993 ; Bourmita,2013) respectively.

The data given above clearly demonstrate that *Launaea nudicaulis (Aerial parts)* and *Limoniastrum feei (leaves stem and twig) extracts* are potent molluscicides. It has been shown that toxicity of crude or purified fractions either plant or animal products are potent molluscicides, if the LC_{50} is less than 100 mg/l (Hostettmann and Lea, 1987 ; Singh *et al.,* 1996). In the present study, 96 h LC_{50} of all plants are less than 100 µg/ml.

The group of compounds tested has been identified that the saponins, tannins, and flavonoids have molluscicidal activity (Perry, 1980; McCullough, 1982).

The toxicity of different preparations of both plants is time as well as concentration dependent as evidenced by the negative correlation between exposure time and LC_{50} of different treatments. The time dependent effect of both plant products may be due to the uptake of the active moiety which progressively increases the amount of active component in snail body with increase in exposure period or it may be possible that the active component(s) could change into more toxic forms in the aquarium water or in the snail body by the action of different enzymes.

The results of the present study indicate that *Limoniastrum feei (Stem)* and *Launaea nudicaulis* (aerial part) are the potential molluscicides of plant origin. Their toxic effects are time and concentration dependent as evident from negative regression between exposure period and LC50 of different treatments.

Conclusion

This study conclusively, shows that *Limoniastrum feei (Stem)* and *Launaea nudicaulis* (aerial part)

may be used as potent molluscicides. Both plants have great potential as molluscicides. For proper utilization of these plant products as molluscicides, further investigations are required to elucidate the mechanism of action in snail body.

Acknowledgement

L. Dahane-Rouissat Thanks Pr Benzahra (INA, Algiers); Mme Sahnouni (Oran University), Dr. Bourmita Y. (Bechar University) for their help . Thanks are due to DGRSDT (Algeria) and NRF (South Africa) for financial support under contracts N° A/AS-2013-007.

References

Belboukhari N, Cheriti A. 2006. Phytochemical investigation of the bioactive extract from *Launaea arborescens*. Pakistan Journal of Biological Sciences **9,** 2930–2932.

Belboukhari N, Cheriti A. 2005. Antimicrobial activity of aerial part of limoniastrum feei. Asian Journal of plant sciences **4(5),** 496-498.

Boulenouar N, Marouf A, Cheriti A. 2009. Effect of Some Saharian Medicinal Plants Extracts on the Causal Agent of Bayoud. Asian Journal of Biology, **9(6),** 600.

Bourmita Y, Cheriti A, Ould El Hadj MD, Mahmoudi K, Belboukhari N. 2013. Anti-termitic Activity of Aqueous Extracts from Saharan Toxic Plants Against *Anacanthotermes ochraceus"* , Journal of Entomology **10,** 207-213.

Cheriti A, Belboukhari M, Belboukhari N, Djeradi H. 2012. Phytochemical and biological studies od Launaea Cass. Genus (Asteracea) from Algerian sahara, Current *Topics in* Phytochemistry*,* **11,** 67-80.

Cheriti A, Belboukhari N, Hacini S. 2004. Ethnopharmacological survey and phytochemical screening of some medicinal plants of Algerian Sahara. Ir.J.Pharm.Res. **3,** 51.

Cheriti A, Belboukhari N, Hacini S. 2013. Ethnopharmacological survey and phytochemical screening of some medicinal Asteraceae of Algerian Sahara. PhytoChem & BioSub Journal. **7(2),** 52-55.

Dahane-Rouissat L, Cheriti A, Marouf A, Reddy KH, Govender P. 2015. Bref aperçu sur quelques molluscicides naturels. Phytochem & Biosub. Journal **9(3),** 80-91.

Esch GW, Barger MA, Fellis KJ. 2002. The transmission of digenetic trematodes: style, elegance, complexity. Integrative and Comparative Biology **42,** 304–312.

Esch GW, Curtis LA, Barger MA. 2001. A perspective on the ecology of trematode communities in snails. Parasitology **123,** S57–S75.

Faltýnková A, Nasincová V, Kablá sková L. Larval. 2008. Trematodes (Digenea) of planorbid snails (Gastropoda: Pulmonata) in Central Europe: a survey of species and key to their identification. Syst Parasitol **69,** 155-178.

Finney IJ. 1952 Probit analysis: a statistical treatment ofthe sigmoid response carve.Cambridge: Cambridge University Press. 318 p.

Hohagen J, Jackson DJ. 2013. *An ancient process in a modern mollusc: Expert PDF Evaluationearly* development of the shell in L. *stagnalis*. BMC Dev Biol. **13,** 27.

Hostettmann K, Lea PJ. 1987. Biologically Active Natural Products. Oxford Science Publication, Oxford. 65–84 p.

Karruki HC, Clennon JA, Brady MS, Kitron U, Sturrock RF, Ouma JH. 2004. Distribution patterns and cercaria shedding of Bulinus nasutus and other snails in the Msambweni area, Coast

Province, Kenya. Am J Trop Med Hyg; **70(4),** 449-456.

Kebede Y, Gebre-Michael T, Balkew M. 2010. Laboratory and field evaluation of neem (Azadirachta indica A. Juss) and Chinaberry (Melia azedarach L.) oils as repellents against Phlebotomus orientalis and P. bergeroti (Diptera: Psychodidae) in Ethiopia. Acta Tropica **113,** 145–150.

Maire R. 1953. Flore de L'Afrique du Nord: (Maroc, Algerie, Tunisie, Tripolitaine, Cyrenaïque et Sahara) (5 v.), París: Paul Le chevaller.

Mc Cullough FS. 1982. Plant Molluscicides. J. Med. Plant Res. **46,** 195-209.

Mendes NM, Vasconcellos MC, Baptista D. 1997. Evaluation of the molluscicidal properties of *Euphorbia splendens* var. Hislopii (N. E. Br.) latex: experimental test in an endemic area in the state of *Minas Gerais*. Brazil Mem Inst Oswaldo Cruz, **92,** 719-24.

Miranda M, Cuéllar A. 2002. Farmacognosia y productos naturales. La Habana: Editorial Félix Varela.

Ozenda P. 2004. Flore et végétation du Sahara. CNRS, Paris, 662 p.

Pavela R, Sajfrtova M, Sovova H, Barnet M, Karban J. 2010. The insecticidal activity of Tanacetum parthenium (L.) Schultz Bip. extracts obtained by supercritical fluid extraction and hydrodistillation. Industrial Crops and Products **31,** 449–454.

Perry LM. 1980. Medicinal plants of East and Southeast Asia: Attributed properties and uses. London: MIT Press.

Rahmani S, Belboukhari N, Cheriti A. 2014. Phytochemical Investigation of Bioactive Extract from Endemic Medicinal Plant *Limoniastrum feei* (Girard) Batt (Plumbaginaceae)" Asian Journal of Chemistry. **26(2),** 365-368.

Rahmani S, Belboukhari N, Cheriti A. 2012. The Saharan medicinal *plant Limoniastrum feei*: Ethnomedical survey and preliminary phytochemical screening of antibacterial extracts. PhytoChem & BioSub Journal **6(2),** 83-87.

Rasoanaivo P, Ratsimamanga-Urverg S. 1993. Biological evaluation of plants with reference to Malagasy flora. Monograph for the IFS-NAPRECA workshop on bioassays. Antananarivo, Madagascar. Napreca, Madagascar.

Rivaz S, Nasiri V, Karimi G, Abdigoudarzi M, Paykari H, Motamedi G, Azizi H, Pirali K. 2014. Lymnaea stagnalis (Linnaeus, 1758) snails' infection to trematoda larval stages in Shahrekord city's springs. Asian Pacific Journal of Tropical Disease **4,** 246-249.

Singh A, Singh DK, Mishra TN, Agarwal RA. 1996. Molluscicide of plant origin. Biol. Agric. Hortic. **13,** 205–252.

Sokal RR, Rohlf FJ. 1996. Introduction to Biostatistics. W.H. freeman, San Francisco, 1–363 p.

Vijay P. 2010.Evaluation of molluscicidal activity of some Indian medicinal plants against Lymnaea acuminate. Int J Appl Biol Pharm Technol. **1,** 308-311.

WHO. 1983. Report of scientific working group on plant molluscicide and guidelines for evaluation of plant molluscicide, (TDR/SCH– SWE (4)/ 83.3). Geneva: WHO; **12,** 7136–7142.

Żbikowska E. 2011. One snail - three Digenea species, different strategies in host-parasite interaction. Ani Biolo. **61,** 1-19.