

The extracting of the toxin component in red squill plant (*Urgniae maritime*) of north Africa and comparing with some commercial poisonous baits for norwag rats and roof rats

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استخلاص المركب السام في نبات بصل العنصل الأحمر (*Urgniae maritime*) بشمال إفريقيا ومقارنته ببعض الطعوم السامة التجارية للجرذان النرويجية والجرذان المتسلقة

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الملخص:

تهدف هذه الدراسة إلى استخلاص المادة الفعالة من نبات بصل العنصل الأحمر (*Urgniae maritime*) التي تم جمعها من منطقة الجبل الأخضر - ليبيا، وتم إجراء اختبار السمية في برنامج علم السموم بجامعة عمر المختار. حددت الدراسة الحالية أيضاً التأثيرات السمية لنبات بصل العنصل الأحمر (*Urginea maritime*) بتركيزات مختلفة باستخدام طعم سام ضد الجرذان البيضاء التي ربيت في المختبر، أظهرت نتائج التحليل الكروماتوغرافي باستخدام اختبار TLC لمستخلص نبات البصل الأحمر مجموعة من الحزم الملونة التي تحتوي على حوالي 17 مركباً من جليكوسيدات السيلارينات. عند معاملة الجرذان البيضاء عن طريق الفم بالمستخلص المحتوي على سيلارينات بتركيزات مختلفة، وجد أن نصف الجرعة المميتة كانت (LD50 274.57 مجم/كجم) وتراوحت هذه الجرعة بين 250، 300 مجم/كجم. أظهرت نتائج تغذية الجرذان النرويجية والجرذان المتسلقة (*Muridae*) على الطعوم التي تحتوي على مستخلص سيلارينات بتركيزات مختلفة أن الطعم الذي يحتوي على أعلى تركيز بنسبة 0.05% انخفض من تغذية الجرذان، بينما حدثت الوفيات عند أدنى تراكيز خلال 24 ساعة للجرذان للنرويجية، ولم يكن لها أي تأثير مميت على الفئران المتسلقة، وعند مقارنة الطعم الذي يحتوي على مستخلص السلارينات مع الطعم الذي يحتوي على ديفيناكوم بتركيز (0.05%) على كل من الجرذان النرويجية والجرذان المتسلقة (*Muridae*)، حدثت الوفيات بعد 7-10 أيام في النوعين، بينما لوحظت وفيات في هذه الجرذان خلال يوم إلى يومين بعد إطعامهم طعم يحتوي على 5% فوسفيد الزنك.

الكلمات المفتاحية: *Urginea maritime*، كروماتوغرافي، سيلاريني، ديفيناكوم.

Abstract

This study aims to extract the active substance from red squill plant (*Urgniae maritime*) collected from the Al-Jabal Al-Akhdar region - Libya, toxicity testing was carried out at the toxicology program Omar Al-Mukhtar University. The present study

also determined the toxicological effects of red squill plant (*Urginea maritime*) at different concentrations, the use of a poisonous bait against white rats raised in the laboratory, the results of chromatographic analysis using TLC test of the extract of the red squill plant showed a group of colored bundles, which contained about 17 compounds of sellarinate glycosides. When albino rats were treated orally with the extract containing sellarinate at different concentrations, it was found that the half-lethal dose was LD₅₀ (274.57 mg/kg) and this dose ranged between concentration of 250, 300 mg / kg. The results of feeding norwag and roof rats (Muridae) on baits containing sellarinate extract at different concentrations showed that the baits that contained the highest concentration of 0.05% decreased from feeding the rats, while deaths occurred at the lowest concentrations within 24 hours for the Norwegian rats, and they had no lethal effect on the climbing rats, And when comparing the bait containing sellarinate extract with the bait containing difinacom at a concentration (0.05%) on both the norwag and roof rats (Muridae), deaths occurred after 7-10 days in the two species, while deaths were observed in these rats within one to two days. After feeding them baits containing 5% zinc Phosphide.

Keywords: *Urginea maritima*, chromatographic, sellarinate, difinacom.

Introduction:

Pest control is a major concern in agriculture and for underdeveloped agro-based countries (Ahmed *et al.*, 2009). Plants may provide potential alternatives to currently used control tools because they constitute a rich source of bioactive chemicals (Van, Beek and Breteler, 1993). Terrestrial plants produce natural substances that have demonstrated insecticidal bioactivity against several pests (Jilani and Su, 1983; Isman, 2000; Ben Hamouda *et al.*, 2015). The plant red squill plant products that have activity against a wide range of rodent control program in terms of toxicity due to the presence of compounds glycosides (Abdel-Hamid, 2002). Red squill (*Urginea maritima*) is a species of flowering plant in the family asparagaceae, subfamily Scilloideae (formerly the family Hyacinthaceae) (Chase *et al.*, 2009). This species is known by several common names, including squill, sea squill, and maritime squill. It may also be called red squill (USDA, ARS, GRIN, 2017). And well adapted to its type of climate. The plant grows through autumn to spring, and there after the leaves get dried up and the bulbs undergo through dormancy during the summer season. *Urginea maritima* it produces flowers after several years when the bulb attains a considerable size (Weinzierl, 1999; Regnault-Roger *et al.*, 2002; Aktar *et al.*, 2009). It has been studied that the bulbs of *U. maritima* has some potential antifungal (Khan and Abourashed, 2011), insecticidal (Hammiche *et al.*, 2013), rodenticide, The squill (*Urginea maritima*) It is native to southern Europe, western Asia, and northern Africa (USDA, ARS, GRIN, 2017). is a plant a perennial plant and is most prevalent in coastal areas of the Mediterranean basin, The red squill plants common naturally in the mountain area green and as there is in most parts of the Libyan coastline. The plant red squill rodenticides plant is used to poison rats and mice (Jilani and Su, 1983; Isman, 2000; Regnault-Roger *et al.*, 2002; Pascual-Villalobos *et al.*, 2002). The plant has also been used as a poison. It is very bitter, so most animals avoid it. Rats, however, eat it readily, and then succumb to the toxic sellarinate. This has made the plant a popular rodenticide for nearly as long as it

has been in use as a medicine (Isman,2000).

Materials and methods:

place of collection.

Red squill (*Urginae maritime*) plant were collected from the Al-Jabal Al-Akhdar region – north Libya and identify in the museum of faculty of sciences at Omar ALMukhtar University

Extraction red squill (*Urginae maritime*).

The red squill Collected, cleaned and peels. Red squill cut into small pieces then directly ammonium sulfate concentration (5%) added to stop the activity of enzymes, and left for 24 hours then drying by Placing them in the oven on the degree of 70 °C. after squill get dry blende in order to get the powder .

100 grams of powder were taken for extraction by adding ethanol (70%) and placed in a water bath at a temperature 60 °C under intense reflector and reiterated the reclamation process several times and collect the extract after filtration was then focused using a cylinder rotor (Rotatory evaporator). lead acetate 5% added to the filtrate to precipitate any other compounds in the solution. to get rid of the remaining lead acetate in the solution passed on hydrogen sulfide gas and concentrat the filtrate in a rotary cylinder. Ethyl acetate was added to the remaining class in order to get alsellarinat, then placed in a revolving cylinder (Balbaa *et al.*, 1981).

Chromatographic analysis of substances separated by TLC (Thin layer chromatography).

silica jell sheets were activated by placing them in the oven for one hour at 105°C, then 100 ml of the extract put in the base plate (repeated the process of developing the samples 3 times the interval of approximately 5 minutes) and then transferred plates containing samples to the pool containing (water, methanol chloroform) (3.5 ml 22 ml 70 ml). the sheet left for a period of time until it reached near the top edge, and then drove out and left at room temperature until dried, then plate sprayed by detector vanill / sulfuric acid and heating on the temperature of 100 ° C for 10 minutes to showing spots (Krenn *etal*,1994).

Determine the Median Lethal Dose (LD50).

rats laboratory experiments were used, divided to five group each group contain three rats and each group given doses 400, 300, 250, 200, 0.00 mg / kg, rats and left for 24 hours. Death was calculated then LD₅₀ was calculated using a probit analysis programme.

Effectiveness of the extract with attractive bait.

The norwag and roof rats used in this experiment was collected from Albaida city and left four week in the lab to acclimatized and divided to five group each group given several concentrations of 0.05%, 0.04%, 0.03%, 0.02% 0.000 mg / kg and mixed with the taste and calculate the number of deaths

Compared with the best concentration of extract squill with some trade bait.

Used in this experiment norwag and roof rats (3 rats for each type), and have been given two types of baits, the first difenacoum concentration 0.05 mg / kg, while the second bait use zinc phosphide powder concentration of 5%, were control rats, and calculate the number of deaths.

Results and discussion:

The results of chromatographic analysis of materials separated by using (TLC Thin layer chromatography).

The results show thin layer chromatography of the extract of the plant and after clarifying the bundles by spraying it with vanillin / sulfuric acid reagent and heating to 100% for 10 minutes , It was noted this purified extract contain a number of colored bundles, which about 17 compounds of at least alsellarinat glycosides. This result was in agreement with (Dias *et al.*, 2000) the bundles resulting from the TLC test were colored. Also, the results showed that the number of compounds produced from the extract of alsellarinat was about 17 compounds, and this result is close to the number of compounds that were obtained, It contains about 16 compounds of squill, found in Italy, and in Tunisia 12 compounds (fig1) (Kopp *et al.*, 1996).



figure (1): shows colored bundles than the extract of sellarinate

Median Lethal Dose (LD50).

After giving the extract content sellarinate of rats experiments bred laboratory doses 400, 300, 250, 200 mg sillarinat / kg of body weight by mouth, were the number of deaths 90%, 66%, 33%, 0%, respectively, and identified the dose of half lethal after 24 hours was equal to 274.75 mg / kg of body weight. were used analysis of variance ANOVA analysis (LD50). In this study, the results showed that the dose of half-lethal (LD50) to plant red squill in rats 274.75 mg sillarinat / kg of body weight and this dose ranged between concentration of 250, 300 mg / kg, and this result is close to what was stated by (Munch and Horn, 1929) that the dose of half lethal for oral 250, 500, Table(1).

Table(1): Results of the Median Lethal Dose (LD50).

Dose mg / kg	% Dead
0.000	0.00%
200	0%
250	33%
300	66%
400	90%

Mixing extracts sellarinate with attractive bait.

When norwag and roof rats fed to the rats norwag, and when you use the focus 0.03% led to death of rats by 30%, while the concentration 0.04% mg / kg causing the death of rats in a time of 24 hours, and the death rate is 66%, did not cause the concentration of 0.02% mg / kg in a toast mixed with extract alsellarinat concentrations (0.00, 0.05%, 0.04%, 0.03%, 0.02% mg / kg). Results showed that the grafts in which the concentration of 0.05% did not cause any deaths as shown in and this is bait shines (Table 2) while 0.03 and 0.04 cause a death 30% and 66% respectively . The results of this study showed that onion extract with all its concentrations had no effect on Roof rats. This result agree with (Berg *et al.*, 1984).

Table2: the toxicity study the effect of plant extract on rats the norwag and roof rats

Extracts red squill rats	%Dead 0.02%	%Dead 0.03%	%Dead 0.04%	%Dead 0.05%
norwag rats	0	30%	66%	0
roof rats	0	0	0	0

Ready-made commercial baits

The toast contains the active ingredient 0.05 mg / kg difnacoum, to fed the norwag and roof rats, results showed that it was toxic to the norwag and roof rats and deaths have occurred from 7 to 10 days. Agreed with the results (Gill and Redfern, 1979; Watt *et al.*, 2005) on the pesticide difnacoum has high toxicity on rats, and results showed that this pesticide is given the proportion of death from 8-9 days. When fed to the norwag and roof rats powder fed on toast containing commercial 5% concentration zinc phosphide, the results showed that this pesticide is highly toxic to rats and the norwag and Roof rats where deaths occurred in a period of 1, 2 days as mention by (Casteel and Bailey, 1986; Clarkson, 2001) that the pesticide zinc phosphide very toxic to a rodents. The most important differences between the best concentration of the red squill extract and the ready-made commercial baits and their effect on the norwag and Roof rats were clarified in Table (3).

Table (3): the comparison between the best concentration of Red squill extract and commercial baits

Pesticides	Rats	%Concentration	%Dead
Red squil	Norwegian rats	%0.04	1 day
	Roof rats	%0.04	No effects
Zinc phosphide	Norwegian rats	%5	1 day
	Roof rats	%5	1 day
Difinacom	Norwegian rats	% 0.05	7-10 day
	Roof rats	% 0.05	6-9 day

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