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INSECTICIDAL ACTIVITY OF THE PLANT *Calotropis procera* AGAINST THE INSECT PEST *Tribolium castaneum*

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ABSTRACT

The insecticidal activity of the plant *Calotropis procera* against the insect pest *Tribolium castaneum* was tested by using leave extract of the plant. Leaves were collected in the vegetative stage and were air dried, ground and stored until extraction. In the preliminary investigation, n-Hexane was found to be the most active with a mortality of 40% followed by methanol and acetone (30 %) each. However, the water extract was found to be the least active among all extracts. Fractionation with n-Hexane, Heptane, ethyl acetate, and chloroform was performed in order to gets the more purified fraction containing the active constituent. Heptane, fraction found to be the most active in terms of mortality percentage (70%) followed by n-Hexane, Chloroform and Ethyl acetate respectively. Adults of *Tribolium castaneum*. were used for bioassay and Soxlet method was used for extraction to get more extracts. Leaves only were used for extraction since they produced more extracts than flower and root.

INTRODUCTION

The use of chemicals as pesticides has become indispensable for crop protection, but they have become instrumental to many environmental hazards. For assured crop production as well as to protect the environment, search for alternative to these synthetic pesticides is continuing all over the world. Plants possessing pesticidal properties are considered to be one of the potential alternative sources for developing new pesticidal chemicals. Many sources of natural compounds have been suggested as alternatives for conventional chemical control Consoli and Oliveira (1994), Carvalho *et al*. (2003). Pesticidal activity of plant species have been reported by many authors, Maria *et al*. (1999) evaluated a group of 57 wild plant species from 21 different botanical families, harvested from southeastern Spain for anti-insect activity using the stored grain pest, Ali-Shtayeh *et al*. (1998) Investigated the ethanolic and aqueous extracts of 20 Palestinian plant species used in folk medicine for their antimicrobial activities against five bacterial species, Srinivasan *et al*. (2001) studied Antimicrobial activity of fifty medicinal plants belonging to 26 families for their antimicrobial activity. Scientific descriptions have mentioned relevant activities for the latex of *C. procera*, such as antibacterial, analgesic or possessing in vitro schizonticidal activity Jain *et al*. (1996), Dewan *et al*. (2000), Sharma and Sharma (2000), Alencar *et al*. (2004). The protection of agricultural products in storage against attach by pests is essential in many countries suffering from inadequate storage facilities and/or climatic conditions that favor deterioration of food commodities. *Calotropis procera*, have been observed to possess medicinal properties, it is a well-known medicinal plant with leaves, roots, and bark being exploited by popular medicine to fight many human and animal

diseases. But a little is known about its pesticidal properties. The present experiment was therefore conducted to investigate the insecticidal activity of this plant extracts against one important insect pests *Tribolium castaneum*. This work deals with the fractionation of the crude extract produced by the green parts of the plant and aims to evaluate its toxic effects.

MATERIALS AND METHODS

Collection and processing of plant

Fresh and healthy leaves of *C*. *procera* were collected from Sirwah at Marib County in the northern part of Yemen. The plant was in the vegetative stage during 2006, mainly when the plant was at the flowering stage. The plant was authenticated by the taxonomist at the department of plant protection, faculty of agriculture, Sana'a University. Plants (5 kg) were air dried under shaded condition for 3 days then followed by drying in oven at 40 ^{o}C for 48 hours yielding a bout (250 g). The dried material was ground to a fine powder and stored in airtight glass containers in the dark until extraction.

Preparation of crude extracts

For preliminary investigation of insecticidal activity, plant sample (30 g) was extracted by soaking in (300 ml each solvent) for one night and then by shaking with electric shaker at 135 r/m for 3 hours. Solvents used for extraction were of increasing polarity (n-Hexane, acetone, methanol and water) at room temperature (20^{o}C) and the extracts from each sample were stored at 5^{o}C. The extracts designated as E_1, E_2, E_3 and E_4 respectively and concentrated to dryness by rotary evaporator under vacuum at 40 ^{o}C, except for water which was concentrated by freeze drying. The residue of all solvents was weighed and re-dissolved in the same solvent, at a concentration of 2 mg of crude extract / ml of solvent. Based on the results of this bioassay, the rest of the plant material (130 g) was extracted with n-Hexane for 12 hours using Soxhlet apparatus and it was designated as E_5.

Fractionation of n-Hexane Extract (E_5)

In order to get the more purified fraction containing the active constituent, n-Hexane extract (E_5) was subjected to liquid-liquid partitioning. The extract was dissolved in acetone and transferred to a seperatory funnel. Water was added up to the maximum solubility limit and the mixture was partitioned successively with n-Hexane, Heptane, ethyl acetate, and chloroform. The different solvent fractions thus obtained, designated as F_1, F_2, F_3 and F_4 respectively. Fractions were concentrated to dryness using rotary evaporator under vacuum at 40 ^{o}C. The residue was weighed and re-dissolved, in the same solvent, at a concentration of 2 mg of crude extract / ml of solvent.

Bioassay for Pesticidal Activity

The insect pest *Tribolium castaneum.* were used for bioassay. The adults (same age) were placed in a glass petri dishes 9 cm in diameter and 2

cm in depth. Each container held 10 insects in triplicates. Flour (5g) was treated with 5 ml (0.2 %) final extract and left until the solvent fully evaporated (24 h). After drying, the flour was put in the dishes for feeding by the insects. The control group was fed with flour treated with the same solvent containing no extract. The dishes was covered with muslin and kept at 22^0C. Observations were made each 24 h for 72 hours and mortality were recorded and compared with the control.

Statistics

Scale depending on percentage of insects (0-100%) in which activity was detected was used. appropriate comparisons of treatment and control were done.

RESULTS AND DISCUSSION

Selection of Solvent for Extraction

The preliminary results on the pesticidal activity of *Calotropis procera*, extracts E_1, E_2, E_3 and E_4 are presented in Table 1. The n-Hexane extract (E_1) was found to be the most active with a significant mortality percentage (40%) followed by methanol (E_3) and acetone (30 %) each. However, the water extract (E_4) was found to be the least active among all extracts. Therefore, n-Hexane was selected as the best solvent for extraction of the pesticidal principle.

Table 1. Insecticidal activity of *Calotropis procera,* extracts against *Tribolium Castaneum* at a concentration of 0.2% for 72h

Extracting Solvent	% Mortality [a]
n-Hexane, (E_1)	40 ± 3.7
Acetone, (E_2)	30 ± 4.2
Methanol, (E_3)	30 ± 3.8
Water, (E_4)	20 ± 3.1

***a*: values are means of three replicates and standard deviation.**

Purification by Liquid-Liquid partitioning

The activity of the different fractions is presented in Table 2 which reveals Heptane, fraction (F_2) is significantly, the most active in terms of mortality percentage (70%) followed by n-Hexane (F_1), Chloroform (F_4) and Ethyl acetate (F_3) respectively. Therefore, n-Hexane extract of *Calotropis procera,* may be processed by solvent partitioning with Heptane to obtain a purified extract with considerable insecticidal activity.

Extraction method

Soxhlet was used for extraction since it yields more extracts than electric shaker, also leaves only were used for extraction since they produced more extracts and showed stronger bioactivity than flower and root compared to the control Umsalama *et al.* (2006)

Latex and dried extract activity comparison

By comparing the results in our experiment to those in the literature, latex found to be more toxic than dry extract. The abundance of latex in the green parts of the plant reinforces the idea that it is produced and accumulated as a defense strategy against organisms such as virus, fungi, and insects Larhsini *et al*. (1997), Haque *et al*. (2000). Most of the published studies dealt with the latex of *C. procera* , latex was found to be a suitable source of active compounds exhibiting larvicidal activity Girdhar *et al*. (1984), to have ovicidal activity Singhi *et al*. (2004), to display toxicity upon egg hatching and larvae Márcio *et al*. (2006).

Table 2: Insecticidal activity of the different fractions of n-Hexane extract of *Calotropis procera,* against *Tribolium Castaneum* at a concentration of 0.2%

Extracting and/or Fractionating Solvent	% Mortality [a]		
	24 h	48 h	72 h
n-Hexane, (F_1)	20 ± 6.2	31 ± 6.4	46 ± 6.8
Heptane, (F_2)	33 ± 3.6	51 ± 4.4	70 ± 7.3
Ethyl acetate, (F_3)	9 ± 8.3	11 ± 11.7	11 ± 9.4
Chloroform, (F_4)	11 ± 7.5	14 ± 9.7	17 ± 8.6

a: values are means of three replicates and standard deviation.

Insecticidal activity and Chemical composition of *Calotropis procera*

The preliminary study was conducted by investigating the insecticidal activity of the crude extract for several solvents of different polarity. The statistical results indicated that, n-Hexane; (E1) is the best choice, same time Heptane was the most effective fraction when purification by Liquid-Liquid partitioning was conducted. These results demonstrated that the chemical properties of the compound which responsible for the killing is non-polar active ingredient. This result doesn't agreed with Mueen *et al.*(2005), who found that the ethanol extract of *C. procera* was the most toxic, of all solvents used (acetone, ethanol, petroleum ether and water), Findings suggest that the ethanol extracts may produce larvicidal, pupicidal and adulticidal effects, (behaving like general toxicants) against the flesh fly. The difference between two results is justifiable due to the fact that, *C. procera* possessing so wide range of activities, suggesting that *C. procera* contains many different active ingredients with different physo-chemical properties. The latex in most plants is a complex mixture of biological compounds including free amino acids, secondary metabolites among others and free proteins Yeang *et al*. (2002). Various researchers have undertaken the chemical analysis of *C. procera* and several compounds have been identified such as, cardenolides, proteolytic enzymes, alkaloids and carbohydrates Dhar and Sungh (1973), Seiber *et al*. (1982), Morsy *et al*. (2001). A wide range of chemical compounds including cardiac glysocisdes, flavonoids, phenolic compounds, terpenoids, have been isolated from *C. procera* Mueen *et al*. (2005). *C. procera*, have been observed to have very wide effects against wide range of organisms. It is a well-known medicinal plant with leaves, roots, and bark to

fight many human and animal diseases, it also found to possess larvicidal, pupicidal, ovicidal and adulticidal effects. *C. procera* also found to have antibacterial, analgesic and to display toxicity upon egg hatching.

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دراسة فعالية الاباده الحشرية لنبات العشر (*Calotropis procera*) علي خنفساء الدقيق الصدائية (*Tribolium castaneum*)

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في هذا البحث تمت دراسة الفعالية الابادية لمستخلص اوراق نبات العشر (*Calotropis procera*) علي خنفساء الدقيق الصدائية (*Tribolium castaneum*). تمت عملية جمع اوراق نبات العشر في مرحلة النمو الخضري وتم تجفيفها علي درجة حرارة الغرفة في مكان مظلم ثم طحنها وتخزينها حتي وقت الاستخلاص. تمت عملية التقييم الاولي لاختيار اكثر المذيبات كفاءة, واستخلص من الدراسة ان مذيب الهكسان هو اكثر المذيبات كفاءة وفعالية وبنسبة قتل تصل الي ٤٠% متبوعا بالميثانول ثم الاسيتون وبنسبة قتل وصلت الي ٣٠% لكل منها. ومن ناحية اخري وجد ان مستخلص الماء هو اقل المستخلصات كفاءة بين كل المستخلصات في عملية التقييم الاولي. لغرض الحصول علي مكونات فعالة اكثر نقائا تمت عملية التجزئ باستخدام مذيبات مختلفة (الهكسان, الهبتان, خلات الايثيل و الكلوروفرم). اتضح من النتائج المتحصل عليها أن التجزئ بالهبتان هو اكثر الطرق كفائة من ناحية النسبة المئوية للموت والتي بلغت (٧٠%) متبوعة بالهكسان والكلوروفرم وخلات الاثيل علي التوالي. في هذة الدراسة, تم استخدام الطور الكامل للخنافس كما وتم استخدام طريقة سوكسلت في عملية الاستخلاص نظرا لكفائتها العاليه في الحصول علي كم اكبر من المستخلص. ايضا تمت عملية الاستخلاص من اوراق النبات فقط وذلك بسبب انتاجها لكم اكبر من المستخلص الفعال مقارنة بالأزهار والجذور.