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Characterization of cantharidin extracted from the Egyptian blister beetle *Meloe proscarabaeus* (Meloidae)

Sarah S. Hegazy[1], Manal Assem[2], Hanan Helmy[3] and Nadia M. Lotfy[1]
1- Entomology Department - Faculty of Science – Ain Shams University
2- Biochemistry Department - Faculty of Science – Ain Shams University
3- Research and Training Center for Vector Control – Ain Shams University

ABSTRACT

The aim of the current study was to characterize the cantharidin extracted from the Egyptian blister beetle (*Meloe proscarabaeus*). **C**antharidin was collected by electric shock method from adult blister beetles and it was characterized by FT-IR and TLC to determine its level of purity. The results showed an improved purity of the extracted cantharidin when compared to the crude blister beetle extract. These results indicated that the used purification resulted in an appropriate level of cantharidin purity.

Key words: Cantharidin, blister beetle, characterization, FT-IR, TLC.

INTRODUCTION:

The cantharidin (CA) is one of the oldest-known toxins from insects and has been known to humans for more than 2000 years due to its physiological activities such as blistering (Chen *et al*., 2012). CA is found in the hemolymph and all tissues of both CA-producing and cantharipilous taxa. Dang *et al*. (2012) indicated that CA (2,3-di-methyl-7-oxabicyclo [2.2.l] heptane-2.3- dicarboxylic acid anhydride) is the principal active ingredient amongst the various compounds present in blister beetle haemolymph. When attacked or disturbed, adults of blister beetles release haemolymph droplets in so called "reflex bleeding". The highly toxic CA in the haemolymph, is a well defensive reaction against the aggressive predators. According to Becerro *et al*. (2008), CA is considered responsible for the repellent properties of meloid haemolymph against a wide variety of predators. In nature, CA is offered during courtship as a pre-copulatory gift by male beetles and only those with high concentrations of CA are acceptable by the female for mating (Akdemir *et al.*, 2011). The female impregnates her eggs with CA to protect them from predators (Bei *et al*., 2012).

Chemically, CA is a bicyclic terpenoid or anhydride of cantharidic acid with chemical formula $C_{10}H_{12}O_4$. Although CA was purified and crystallized in 1810 by Robiquet from the Spanish fly *Lytta vesicatoria*. It took 150 years of research to be fully synthesized. The first attempt was carried out by the German chemist von Bruchhausen but the first effective synthesis of this compound was made in 1951 by the American chemist G. Stork who reported the first natural product synthesis featuring a [4+2] cycloaddition in the stereo-controlled synthesis of CA. Then, some other efforts had been done for the CA synthesis (Deng *et al*., 2013). Dettner *et al*. (2004) reported that the content of the total CA is higher than the content of free CA in the family Meloidae using gas chromatography, as well as bound CA may exist in the forms of magnesium cantharidate, potassium cantharidate and calcium cantharidate.

This study aimed to characterize the CA extracted from the Egyptian blister beetle *Meloe proscarabaeus*.

MATERIALS AND METHODS:

Collection and rearing of the black blister beetle:

The newly emerged adults (1-day old) of the black blister beetle, *Meloe proscarabaeus* were collected as described by Ghoneim *et al*. (2012) using pit-fall traps in the crops of the Egyptian faba beans (*Vicia faba*) and clover fields (*Trifolium alexandrinum*) in Al-Farafra oasis. The laboratory culture conditions were (23±2°C, 46±10% RH, 12L:12D photoperiod). The adults (50: 16 males and 34 females) were collected in mid-October. They were fed on the fresh leaves of clover, faba beans and lettuce. After 4 days of the collection of the adults, only 5 females produced eggs, while the others died without ovipositing. The eggs (90-153) were laid after 10-13 days under the laboratory conditions.

From the 6 larval instars, 5 of them were predatory on the eggs of *Schistocerca gregaria* (Orthoptera: Acrididae), puncturing the eggs and consuming the egg yolk by their mandibles, and developed rapidly and matured in a short time. The 6^{th} instar, also known as the coarctate larva, is unusual in that it does not feed and is immobile. It burrows upward of the surface of the soil and constructs the pupal chamber to transform into the pupal stage. The mean duration of larval development for the six instars was 4, 2, 2, 2, 12 and 48 days, respectively with a standard deviation of 2 days. The pupal stage (exarate type) required a mean of 18 ± 2 days to moult into the adult stage.

Collection of cantharidin (CA):

The CA was collected by the electro-shock method, commonly used to extract honey bee venom, according to Sanad and Mohanny (2013). The collector device depended on using electrical impulses to disturb and intimidate the blister beetles, so that they produce CA in an act of defense. A portion of CA was collected directly from the insect's body surface by a standard 0.3 ml syringe. The resulting CA was scrapped up for collection with a scraper, and it was stored at room temperature for later use.

Purification and characterization of extracted cantharidin:

The purification of CA was under the guidelines of Verma and Prasad (2012). The extracted CA was dissolved in absolute methanol. The accumulated extract was concentrated by evaporating the solvent using hot plate at 40^{o}C. The purity was confirmed by Fourier transform infrared (FT-IR). Also, thin-layer chromatography (TLC) was performed to both complete *M. proscarabaeus* extracts and purified CA. They were respectively loaded to a silica gel plate (10 cm × 20 cm) and developed with mobile phase of chloroform: acetone = 49:1*(v/v)*, 0.1 % bromocresol green alcohol solution was sprayed, heated, yellow spots appeared.

RESULTS AND DISCUSSION

Figure (1) showed the results obtained from the FT-IR test, which gave an indication and insight into CA's level of purity. The resultant peaks as a result of the aromatic characteristics of CA were in the 500, 700, 1000, 1400, 1600 and 1700 cm^{-1} regions. Figure (2) showed the TLC profiles of both the crude extract, and the purified CA. The purity of the CA was shown to be in an acceptable level. These results were in accordance with Ge *et al.* (2013); Verma and Prasad (2012); Liu *et al.* (2015).

In conclusion the present results indicated that the used purification resulted in an appropriate level of CA purity.

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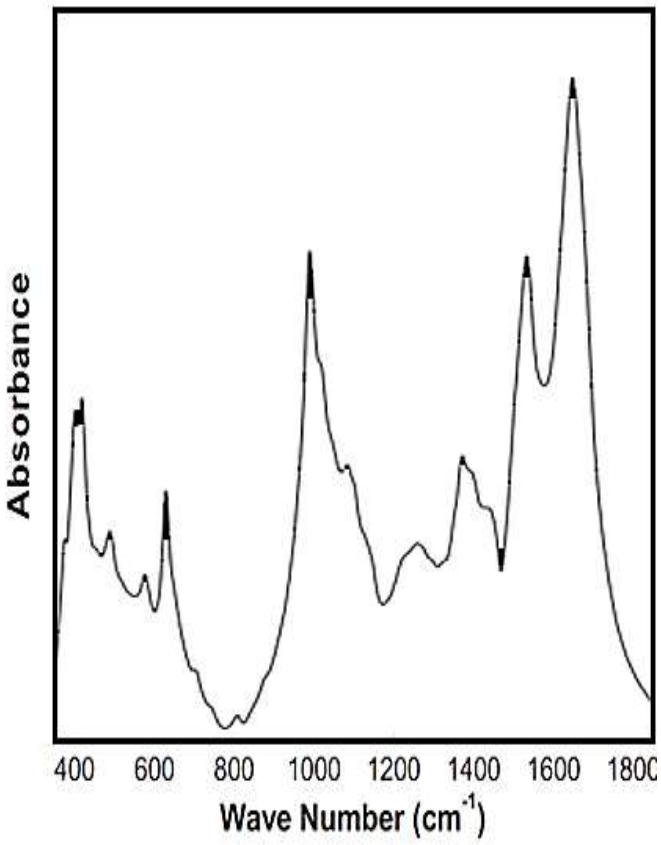

Fig. (1): FT-IR spectrograph of cantharidin.

Fig. (2): TLC profiles of: crude extract (left) and purified cantharidin (right).

REFERENCES

Akdemir, O.; Bilkay, U.; Tiftikcioglu, Y.O.; Ozek, C.; Yan, H.; Zhang, F. and Akin, Y. (2011). New alternative in treatment of callus. J. Dermatol., 38(2): 146-150.

Becerro de Bengoa Vallejo, R.; Losa Iglesias, M. E.; Gomez-Martin, B.; Gómez, R. S. and Crespo, A.S. (2008). Application of cantharidin and podophyllotoxin for the treatment of plantar warts. J. Am. Podiatric Medical Assoc., 98(6): 445-450.

Bei, Y.Y.; Chen, X.Y.; Liu, Y.; Xu, J.Y.; Wang, W.J.; Gu, Z.L. and Wang, Q. (2012). Novel norcantharidin-loaded liver targeting chitosan nanoparticles to enhance intestinal absorption. Int. J. Nanomed., 7: 1819-1827.

Dang, Y.J.; An, L.N.; Hu, C. and Zhu, C.Y. (2012). Inclusion complex of cantharidin with β cyclodextrin: Preparation, characterization, in vitro and in vivo evaluation. J. Appl. Polymer Sci., 123(3): 1557-1562.

Deng, L.P.; Dong, J.; Cai, H. and Wang, W. (2013). Cantharidin as an antitumor agent: A retrospective review. Current Medicinal Chem., 20(2): 159-166.

Dettner, K.; Schramm, S.; Seidl, V.; Klemm, K.; Gäde, G.; Fietz, O. and Boland, W. (2004). Occurrence of terpene anhydride palasonin and palasoninimide in blister beetle *Hycleus lunata* (Coleoptera: Meloidae). Biochemical Systematics and Ecology, 32(1): 115.

Ge, X.; Du, X.; Yu, Y.; Xin, X. and Tian, P. (2013). Extraction of cantharidin from *Mylabris phalerata* Pallas with compound enzyme and HPLC analysis. Asian J. Chem., 25(2): 749.

Ghoneim, K.K.; Abdel-Khaliq, A.A.; Bream, A. S. and Emam, D.M. (2012). Effect of food type on the adult performance of black blister beetle *Meloe proscarabaeus* (Coleoptera: Meloidae). Int. J. Biol. Sci., 1: 5-17.

Liu, R.; Cheng, Z.; Li, T. and Jiang, X. (2015). Investigation of two blood proteins binding to cantharidin and norcantharidin by multi-spectroscopic and chemometrics methods. J. Luminescence, 157: 398-410.

Sanad, R. and Mohanny, K. (2013). The efficacy of a new modified apparatus for collecting bee venom in relation to some biological aspects of honey bee colonies. J. Am. Sci., 9(10): 177-182.

Verma, A.K. and Prasad, S.B. (2012). Bioactive component, cantharidin from *Mylabris cichorii* and its antitumor activity against Ehrlich ascites carcinoma. Cell Biol. and Toxicol., 28(3): 133-147.

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توصيف مُركب الكانثرادين المستخلص من الخنفساء الحارقة المصرية ميلوبيروسكارابيوس

سارة سيد حجازي[1]، منال عاصم[2]، حنان حلمي[3]، نادية محمد لطفي[1]
1 - قسم علم الحشرات – كلية العلوم – جامعة عين شمس
2 - قسم الكيمياء الحيوية – كلية العلوم – جامعة عين شمس
3 - مركز التدريب والأبحاث لناقلات الامراض – جامعة عين شمس

المستخلص

هدفت الدراسة الحالية إلي توصيف مُركب الكانثرادين المستخلص من الخنفساء الحارقة المصرية (ميلوبيروسكارابيوس). تم فى هذه الدراسة جمع حشرة الخنفساء الحارقة من حقول الفول المصري والبرسيم من واحة الفرافرة بمصر، وتربيتها في ظروف معملية حتي اكتمال دورة حياتها وظهور الطور البالغ. تم استخلاص الكانثرادين من الخنفساء الحارقة باستخدام طريقة التحفيز الكهربي، حيث تم استثارة الحشرات بتوجيه شحنة كهربية ببسيطة أدت إلي افراز الحشرة لمادة الكانثرادين علي جسمها، والتي تم جمعها بمحقن قياسي. تم اذابة المادة في ميثانول مطلق ثم تبخيره حتي يتم تركيز الكانثرادين. تم فحص مدي نقاء الكانثرادين بتقنية الـ FT-IR والـTLC. أوضحت النتائج ان الكانثرادين المستخلص والمنقي قد اظهر تشابه شديد مع المنحنيات القياسية الخاصة بمادة الكانثرادين، وقد تم تأكيد هذه النتيجة بأختبار الـTLCالذي أوضح اختلاف كبير في مدي نقاء مادة الكانثرادين بعد تنقيتها مقارنة بالمسحوق الخام المأخوذ من جسم الحشرة.