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Full Length Research Article

IDENTIFICATION AND ISOLATION OF CANTHARIDIN AND OTHER IMPORTANT IN DEFENSIVE SECRETION OF BLISTER BEETLE *MYLABRIS PUSTULATA* (THUNBERG) (COLEOPTERA: MELOIDAE) FROM TAMIL NADU, INDIA

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Cantharidin ($C_{10}H_{12}O_4$) a potent vesicant and antifeedant agent, is produced by beetles belongs to families Meloidae and Oedemeridae (Coleoptera). Several studies to date have been focused on cantharidin secretions in blister beetles, and recently with a few reports on cantharidin-related compounds. The present investigation was carried out aiming to detect and isolate cantharidin and some other important compounds from *Mylabris pustulata* (Thunberg) from Tamil Nadu, India. Hit-spectrum of cantharidin had a peak in the chromatogram with the retention time (RT). RT-13.59 and ionization provided mass spectra with base peaks at m/z 96 and 128, methanone at m/z 81.0 and 99.1 (RT- 16.94), Alpha-terpinyl acetate at m/z 93.1, 121.1 and 136.1 (RT-9.96). As clearly indicated by the present results, the successful application of the proposed method is suitable for cantharidin analysis from the blister beetle *M.pustulata*.

Key words: Cantharidin; methanone; alpha-terpinyl acetate; meloidae; *Mylabris pustulata.*

INTRODUCTION

Secondary metabolites of plants and animals are the main constituents to study phytochemistry and organic chemistry as shown their centennial history that has successfully promoted the drug discovery and development. Nearly half of the drugs currently in clinical use belong to drugs of the natural origin. Blister beetles belongs to the family Meloidae and false blister beetles belongs to Oedemeridae in order Coleoptera. They secrete natural defensive secretion containing cantharidin, which has general importance in medical and pharmacological field. When these beetles were distributed by an external agent, they release yellow oily droplets of haemolymph from their leg joints or may be from antennal joints which causes blister on contact with human skin. Meloidae contains over 2500 species in approximately 125 genera of general biological significance because of their cantharidin and other relater secretions (for review Ghoneium, 2013a). This family is of general biological significance because of its hyper metamorphosis of larval development (for review Ghoneium, 2013b), the parasitoid biology of the larval phrases and the production of cantharidin. Males of these species can accumulate cantharidin in their reproductive glands, which is transferred to females during copulation and finally to the eggs to protect them against predators (Eisner *et al.,* 1996; Nikbakhtzadeh *et al.,* 2007; Ghoneium, 2013c). A few substances structurally similar to cantharidin known as Cantharidin-related Compounds in meloid beetle is not yet clear. Cantharidin has been shown to be toxic for mammals, birds and frogs (Schmidt, 2002; Ghoneium, 2013d).

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Cantharidin ($C_{10}H_{12}O_4$) a monoterpene anhydride molecule with molecular weight of 196.1 having the chemical name as [2-endo, 3-endo-dicarboxylic anhydride] is a vesicant compound secreted as a chemical defense by blister beetles (Meloidae) and false blister beetle (Oedemeridae). In china, dried bodies of some beetles *Mylabris* viz. *Mylabris phalerata*, *M.cichori* have been employed in Chinese traditional medicine for more than 2000 years and are still used as a folk medicine (Wang, 1989). Furthermore, cantharidin has important antitumor properties and has been used as an anticancer agent for the treatment of hepatoma and oesophageal carcinoma. Nowadays cantharidin has also used typically (0.7%) in the treatment of wartz (Moed *et al.,* 2001; Rauh *et al.,* 2007; Ghoneium, 2014). Cantharidin (CTD), nor cantharidin (NCTD) and cantharamide and its analogs represent the simplest natural models that could play important role in the search for new effective and selective anti cancer drugs (Garios *et al.,* 2013). Several studies to date have focused on cantharidin secretions of blister beetles worldwide however information on cantharidin reports from Indian meloid beetles is very scarce. Therefore the presence of cantharidin in the defensive secretion of Indian meloid *M.pustulata* was ascertained using the gas chromatography-mass spectrometry (GC-MS).

MATERIALS AND METHODS

Field collection of beetles: The blister beetle *M.pustulata* has black elytra with three orange or red band across, measuring about 1.00cm to 1.5cm in length. A sample of this species was collected from the host plant *Ipoma carnea* Jacq (Family-Convolvulaceae), in the vicinity of Annamalai university, Chidambaram, Tamil Nadu, during August to November. Collections was carried out by hand picking of the beetle from this host plant at early morning because of the very sluggish habit of this beetle in early morning with increasing activity as sunrises.

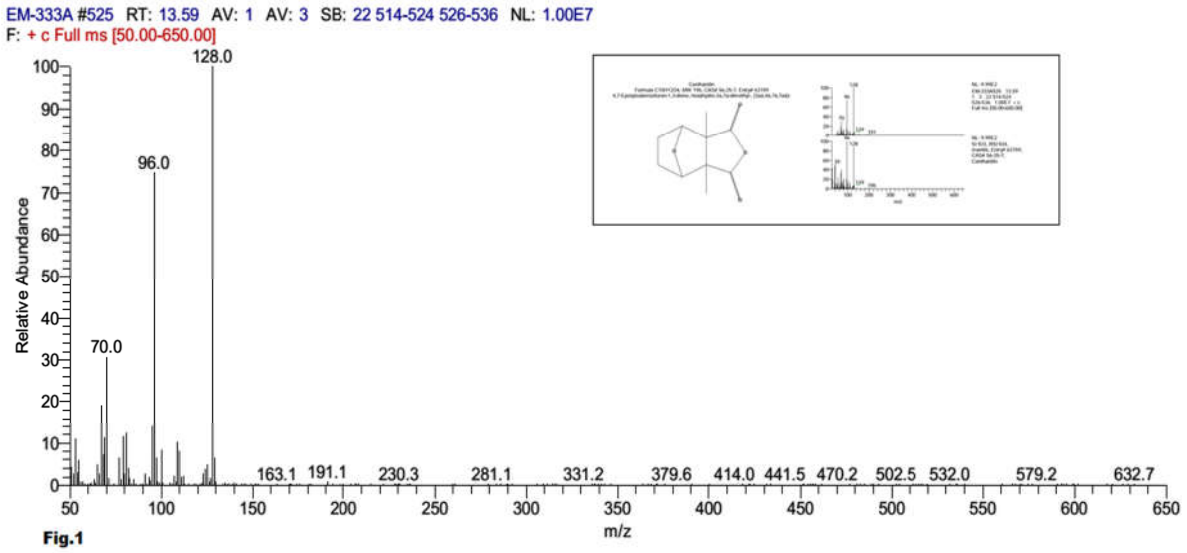

Fig.1. Mass spectra of cantharidin compound detected in *M.pustulata* (Meloidae), with base peak at m/z 96 and 128.

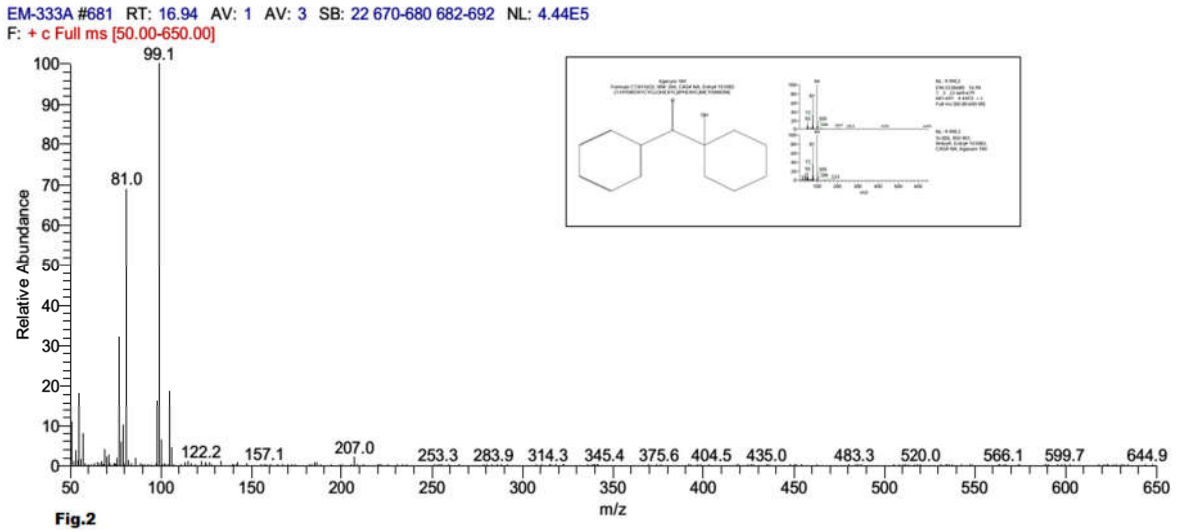

Fig. 2. Mass spectra of methanone compound, detected in *M.pustulata* (Meloidae), with base peak at m/z 81.0 and 99.1

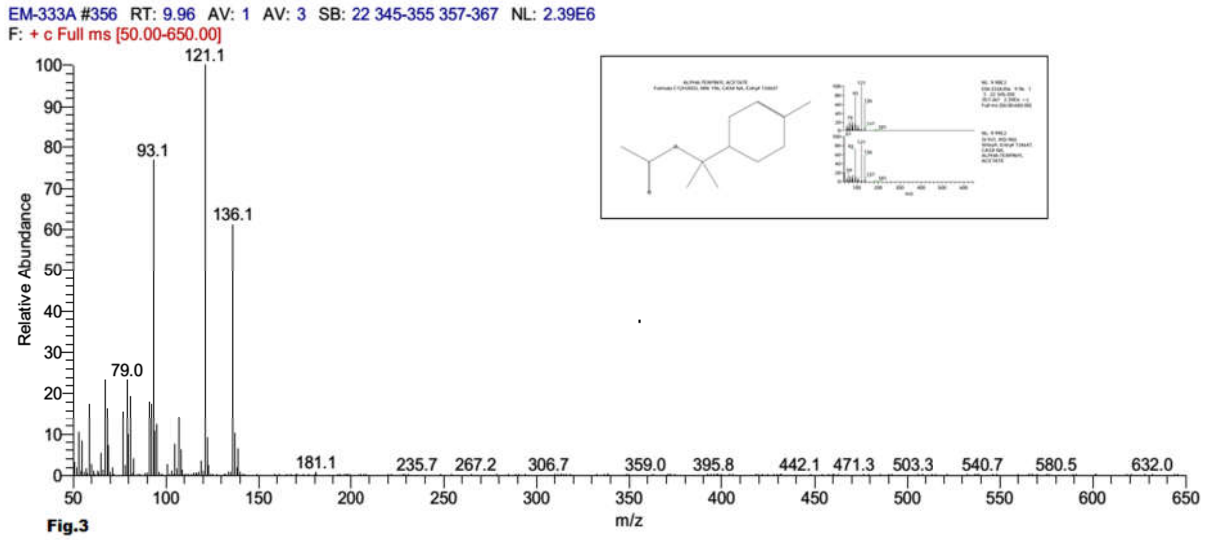

Fig. 3. Mass spectra of alpha-terpnyl acetate compounds, detected in *M.pustulata* (Meloidae), Alpha-terpinyl acetate with base peak at m/z 93.1, 121.1 and 136.1

Table 1. Physiochemical properties of cantharidin and other compounds detected in *M.pustulata* (Meloidae) based on qualitative gas chromatography-mass spectrometry

S.No	Species	Cantharidin Content		Reference
		Relative(%)	Total (µg)(mg)	
1	*Oxocopis thoracica*	0.34	35.2	Carrel et al.(1986)
2	*Heliocis repanda*	0.72	7.4	Carrel et al.(1986)
3	*Oedemera femorata*	No information avilable	8.4	Holz et al.(1994)
4	*Oedemera femorata*	0.15-0.21	21-31	Frenzel et al. (1994)
5	*Oedemera flavipes*	0.16	22	Frenzel et al.(1994)
6	*Oedemera lurida*	0.23	12.4	Frenzel et al. (1994)
7	*Oedemera podagrariae*	0.16	21.68	Abtahi et al. (2011)
8	*Mylabris quadripuctata*	-	1-9mg	Nikbakhtzadeh and Tirgari (2002)
9	*Hycleus polymorphos*	-	5-113mg	Nikbakhtzadeh and Tirgari (2002)
10	Several species	-	1-126mg	Nikbakhtzadeh and Tirgari (2002)
11	*Hycleus oculatus*	-	1mg	Mebs et al.(2009)
12	*Hycleus tinctus*	-	0.02mg	Mebs et al.(2009)
13	*Hycleus lunata*	-	0.01mg	Dettner et al.(2003)
14	*Pyrota insulate*	-	34 mg	Barr et al. (1998)
15	*Epicauta spp*	-	0.2 mg	Capinera et al.(1985)
16	*Epicauta funebris*	-	16.9mg	Carrel et al.(1993)
17	*Lytta polita*	-	0.62mg	Carrel et al.(1999)
18	*Berberomeloe majalis*	-	0.88mg	Sanchez-Barbudo et al.(2012)
19	*Hycleus scabiosae*	-	5.06mg	Nikbakhtzadeh et al.(2012)
20	*Mylabris pustulata*			Information are given in Table.2

Table 2. Cantharidin content in Meloidae or Oedemerid beetles in other authors study

S.No	Species	Cantharidin and other compounds					Reference
		RT	Molecular formula	Molecular weight	Diagnostic mass fragments	Relative (%)	
1	*Mylabris pustulata* (Cantharidin)	13.59	C10H1204	196	96,128	89.64	Present study
2	*Mylabris pustulata* (Methaone)	16.94	C13H1602	204	81.0,99.1	48.54	Present study
3	*Mylabris pustulata* (Alpha-terpinyl acetate)	9.96	C12H2002	196	93.1,121.1, 136.1	35.66	Present study

Extract Preparation: When the beetle is disturbed, an yellow colour, bad smelling defensive oily secretion exuded from the leg joints. This secretion was collected directly in a small vial and immediately an equal amount of chloroform was added. Then each sample was vigorously shaken on a Vortex mixer for 60s and centrifuged at 300rpm for 5min. The organic phase supernatant transferred into a conical 3-dram lip glass vial by Using Pasteur pipette for further analysis. All used glassware had been already silanized for 24h by dimethyldichlorosilane solution I in heptane 5% (C2H6Cl2Si, Fluka).

Qualitative Gas Chromatography-Mass Spectrometry: To detect cantharidin and other important compounds, Thermo GC-MS was used and 0.1µl of each sample splitlessly injected by a 1µl microsyringe into the injector. Relatively high volatility and good thermal stability are those characters of cantharidin which makes GC analysis the method of choice. Capillary GC sensitivities are very good and the typical high resolution achieved with capillary GC permits analyses of substances from biomatrices with minimal sample preparation. Instrumental analyses were performed using a gas chromatograph equipped with ZB 5-MS capillary standard non-polar column (film thickness: 0.25µm, inner diameter: 0.25mm, length: 30m) connected to a flame ionization detector. The temperature program used for analysis went from 70 to 260°C at a rate of 6°C/min, holding for 3min. Mass spectra were taken at 70eVwith scanning speed of 1 scan/s from m/z 50 to 650 while the detector delayed for 5min. Helium (carrier gas) flow was 1.0 ml/min and the injector and detector temperatures set at 70^0C and 260°C respectively. Integration of chromatographic peak areas were performed using Thermo GC - Trace Ultra Ver: 5.0.

Thermo Ms DSQ II. Cantharidin identification was performed by comparison with mass spectra available in 63159 main library entry search and conformation was achieved with the retention time and calibration curve from the injection of cantharidin standard analyzed by Sanchez-Barbudo *et al*., (2012).

RESULT AND DISCUSSION

Cantharidin was detected in *M.pustulata*. It had a peak in the chromatogram with the retention time RT-13.59 and mass spectra with base peaks at m/z 96 and 128 (Fig.1). Methanone and Alpha-terpinyl acetate compound had a peak in the chromatogram with the retention time 16.94 and 9.96. Mass spectra with base peaks methanone and Alpha-terpinyl acetate at m/z 81.0 and 99.1 and m/z 93.1, 121.1 and 136.1 respectively (Fig.2 and 3). The probability measurement (%), molecular weight and other information had been shown in Table.2. Cantharidin is an extremely powerful toxin produced by the members of meloidae and oedemeloidae families for their defence purpose. The present study revealed that cantharidin and other compounds were detected in the defence secretion of *M.pustulata* collected from Tamil Nadu, India. Many authors studied cantharidin content in meloidae or Oedemerid beetles as given in Table 1. These compounds are reported in this insect for the first time. However, the presence of cantharidin in the defence secretion had been reported earlier, such as of *Mylaris cichorii* from India (Verma and Prasad, 2012); *Hycleus* (*Mylabris*) *dicintus* from Zimbabwae (Tagwireyi *et al.,* 2000); *H. lunata* (Dettner *et al.,* 2003) and *M. phalerata* (Nakatani *et al.,* 2004; Huan *et al.,* 2006; Wang *et al.,* 2004) from France, Taiwan and China; *H. polymorphus* (Nikbakhtzadeh *et al.,* 2007) from Germany; *M.impressa stillata* and *H (M) Scabiosae* from Iran (Nikbakhizadeh *et al.,* 2007; 2012). *M. quadripunctata*, and *M. cichorii* (Wang *et al.,* 2008), as well as *H. oculatus* (Dietrich *et al.,* 2009) from South Africa, *H (M) maculiuentris* from Saudi Arabia (Al-Binali *et al.,* 2010) and *M. caregnea* from china (Galoris *et al.,* 2013). In the present investigation, apart from cantharidin some other compounds (*viz.,* methanone and alpha terpinyl acetate) (Fig 2 and 3) were also isolated and identified.

However, cantharidin-releated compounds (*viz.,* palasonin (dimethyl cantharidin and palasonomide) which were reported in the secretion of *H. lumatus, M. phalerata, H. oculatus* and *H. trinctus* were not detected in the defence secretion of *M. pustulata*. Cantharidin is also found in other coleopteran such as *Simus smyrnensis* and *O.melanopus* which co-occurs with the meloid (Nikbakhtzadeh and Tirgari, 2002). Cantharidin was previously known from a single species of cerambycid; *Phymatodes testaceus* of western Iran (Nikbakhtzadeh and Tirgari,2002). Cantharidin has important antitumor properties and has been used as an anticancer agent for treatment of hapetoma, oesophegeal carcinoma and leukemia. Cantharidin has been used for more than 2,000 years in both folk and traditional medicine. It was used in China for many medicinal purposes including turuncles, ulcers and tuberculosis, topically for abdominal masses, rabies and anticancer agent orally (Huan *et al.,* 2006; Ghoneium, 2014). On the other hand, 'Methanone' have been found in the stalk and leaves of *Achilea millefolium* (Asteraceae) (Ntalli, 2010). It is mainly used as an analgesic and anti-inflammatory drug in pharmacology. 'Alpha-terpinyl acetate' had been found in *Pycnanthemum albescens* commonly known as white-leaf mountain mint (Duke's, online), and also in *Citrus aurantium* commonly known as bitter orange. *C. aurantium* fruit peel, leaves, flowers and seeds are used in many folk traditions (Jyotsna, 2011).

Conclusion

In the Defensive secretion of *M.pustulata* (Coleoptera: Meloidae), GC-MS analysis revealed the presence of cantharidin as the major component, and methanone and alpha-terpinyl acetate as the minor components. The proposed method in the present study is a good alternative for classical solvent extraction method for Cantharidin in haemolymph to monitor the safe use and also provides the pharmacokinetic parameters for the coherent use of cantharidin in the future research.

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