FRACTION AND DETERMINATION OF CAPSAICINOIDS FROM RED PEPPER FRUITS WITH INSECTICIDAL ACTIVITY AGAINST MEALWORM, Tenebrio molitor, AND SUPERWORM, Zophobas morio

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ABSTRACT

Green chemistry has recently been an active area of research and it is expected to replace the chemical synthesis in agriculture. In this study, our attempt is to fractionate and determine the capsaicinoids from red pepper fruit to control mealworm Tenebrio molitor and superworm Zophobas morio. The methanol extraction of red pepper fruit (Capsicum annuum L.) effectively killed T. molitor and Z. morio at the concentration of 10000 ppm. The insecticidal activity of n-hexane, dichloromethane and aqueous layers derived from the extract by liquid-liquid partitioning were evaluated. Among them, only dichloromethane layer showed insecticidal activity; whereas n-hexane and aqueous layers were inactive against tested pests. The dichloromethane layer was successively fractionated by silica gel column chromatography to afford insecticidal compounds. The chemical structures of active compounds in dichloromethane layer were elucidated by the analysis of NMR and MS spectroscopic data. The active compounds were determined to be as capsaicinoids and their insecticidal activity was also evaluated using the in vitro residual contact bioassay method. The extracts containing capsaicinoids were formulated into a suspension concentrate (SC) biopesticide and evaluated for efficacy against T. molitor and Z. morio. The biopesticide caused mortalities by 95-100 % against tested pests at 1000 µg/mL. The study results suggested that extracts containing capsaicinoids could be used as active ingredients for the preparation of the biopesticide to control insect pests thereby preserving stored products in sustainable agriculture.

Keywords: capsaicinoids, insecticidal activity, Tenebrio molitor, Zophobas morio.

1. INTRODUCTION

Green chemistry technology is effective in reducing the impact of chemicals on human health and environment. There is a specific area within green chemistry that has direct
implications for sustainable agriculture: the field of biopesticides [1]. Biopesticides are the most likely important alternatives to some of the synthetic pesticides of the greatest concern with environmental problems. Biopesticides often are effective in very small quantities and often decompose quickly, thereby resulting in lower exposures and largely avoiding the pollution problems caused by conventional pesticides [1].

_T. molitor_ and _Z. morio_ have been used as a model for screening biopesticides [2]. _T. molitor_ are the larval forms of Darkling beetles from the order Coleoptera and belong to the phylum Arthropoda. Their larvae reach an average length of 1.8 - 2.5 cm, before moulting to obtain pupae form [2]. _Z. morio_ are also larvae of Darkling beetle. These insects are very similar to the _T. molitor_, but are larger about 5-6 cm long when full size. _T. molitor_ and _Z. morio_ are insect pests of stored products and used to feed for pet reptiles and fish in Vietnam. These insects can cause damage and contaminate stored products with their feces and cast skins. Severe infestations caused by _T. molitor_ and _Z. morio_ can destroy large quantities of stored products especially when unnoticed for a long period of time.

Capsaicin is the most common capsaicinoid (8-methyl-N-vanillyl-6-nonenamide) and generated through a condensation reaction between vainillilamina and 8-methyl-6-nonenoyl-CoA catalyzed by the coenzyme A-dependent acyltransferase [3]. Capsaicinoids are produced as secondary metabolites, probably as deterrents against certain mammals and fungi that infest and damage the host plants. Ethanol extract of _C. annuum_ and methanol (MeOH) extract of _C. frutescens_ containing capsaicinoids were previously reported to be toxic against IV instar larvae of _Anopheles stephensi_ and _Culexquinque fasciatus_ [4]. However, the insecticidal effect of _C. annuum_ against _T. molitor_ and _Z. morio_ has not yet reported.

In this study, we report the isolation of capsaicinoids from the fruits of _C. annuum_, structural determination of active components, and evaluation for _in vitro_ insecticidal activity of isolates and layers against _T. molitor_ and _Z. morio_.

2. MATERIALS AND RESEARCH METHODS

2.1. Materials

Fruits _C. annuum_ L. were collected in VinhPhuc province in November 2016 and were deposited in R&D Center of Bioactive Compounds – Vietnam Institute of Industrial Chemistry. Materials were dried at temperature 50 °C for 4 days to reach a moisture content of 8 %. The dried materials crushed to sizes ranging from 0.2 to 0.5 cm.

2.2. Extraction and isolation of constituents from the fruits of _Capsicum annuum_

The powdered dried fruits of _C. annuum_ (0.5 kg) were extracted intriplicates with 5 L of MeOH at ratio of 1:10 (kg/L), extraction time from 15 to 24 h at temperature room. The MeOH solution was filtered off and evaporated under reduced pressure using a rotary evaporator (BuchiRotavapor R-114) at 50 °C to yield MeOH extract. The MeOH extract (100 g) was suspended into 1 L of MeOH/ distillated water (8:2; v:v) and partitioned in triplicates with 1L of _n_-hexane (HEX). The aqueous layer was evaporated and re-suspended into 1L of distillated water and partitioned with 1L of dichloromethane (DCM). The resulting organic solutions were concentrated to yield HEX and DCM layers.

DCM layer (1.6 g) was applied on a silica gel column (20 g silica gel; 40-63 µm; 1.8 x 20 cm) and eluted with a gradient of HEX/ethyl acetate (9:1; 8:2; 7:3; 6:4; v:v). All fractions were

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monitored by using thin layer chromatography (TLC). Based on TLC profile, fractions with similar Rf values were pooled into 5 fractions F1-F5. Fraction F3 (0.4 g) was subjected on the top of a silica gel column (9 g silica gel; 40-63 µm; 1.5 × 10 cm). The elution was performed with mixtures of HEX / ethyl acetate (9:1; 8:2; 7:3; v/v; 30 ml for each) to give 3 fractions F3.1-F3.3. Fraction F3.2 (0.16 g) was subjected on top of a silica gel column (9 g silica gel; 40-63 µm; 0.8 × 10 cm) and eluted with mixtures of HEX/acetone (80:20; 79:21; 78:22; 76:24; v/v; 30 ml for each) to yield 3 fractions F3.2.1-F3.2.3. Fraction 3.2.3 is a mixture of capsaicinoids and named H943 (31 mg).

ESI-MS analysis was performed using a LTQ Orbitrap XL hybrid instrument (Thermo Fisher Scientific, San Jose, CA, USA). 1H- and 13C-NMR spectra were obtained on a Bruker AMX-500 (500 MHz) spectrometer (Bruker Analytische Messtechnik GmbH, Rheinstetten, Germany) operating at 500 MHz and 125 MHz, respectively. Acetone-d6 was used as the solvent and TMS as the internal standard.

2.3. Biopesticide formulation

The DCM layer (45 g) from the fruits of C.annuum was diluted with ethanol (14 mL), Propylene glycol (9 mL), and water (9 mL). Polypetyle glycol 4000 (PEG 4000; 23 g) was slowly added into the resulting suspenose and the mixture was stirred at 250 rpm for 4 hours to obtain a suspended concentration (SC) formula (MO3). The SC formula was tested for its insecticidal efficacy and solubility at a ratio of 1 ml/100ml and 2ml/100 ml of MO3 in water.

2.4. Bioassay and evaluation of insecticidal activity of extracts, isolates and SC formula

The insecticidal activity of HEX and DCM layers, and MeOH extract was tested against the larvae of T. molitor and Z. morio. Thirty insects were used per concentration and were placed individually in plastic dishes (Ø 60 mm × 15 mm) with a filter paper, fed with corn powder and maintained in the dark. After insects were transferred in to the test dishes and raised for 2 days, the test materials were sprayed on them at 10000 µg/mL. The insecticidal activity against T. molitor and Z. morio of fraction H943 and MO3 formula was evaluated at concentrations from 125-1000 µg/mL. Negative control was treated with water containing 2 % of MeOH. Positive control was treated with abamectin at 500 µg/mL. Mortality (M; %) was calculated according to the formula of ABBOTT [5] as below:

\[ M\% = \frac{C_a - T_a}{C_a} \times 100\% \]

where: \( C_a \) - the number of insects alive in the negative control after treatment; \( T_a \) - the number of insects alive in the experiment treated with test materials.

2.5. Statistics

Analysis of variance (ANOVA) was performed by one-way ANOVA following by Tukey’s post hoc test. Differences at the 5 % level of confidence were considered significant.

3. RESULTS AND DISCUSSION

3.1. Insecticidal activity of the extracts from the fruits of Capsicum annuum

The insecticidal activity of methanol extract and HEX and DCM layers against T. molitor
and Z. morio were evaluated at 10000 µg/mL and is presented in Table 2. MeOH extract caused mortalities ranging from 81 – 83 % for Z. morio 2 days after treatment, but it did mortalities ranging from 76 - 88 % for T. molitor.

HEX layer killed the larvae with mortalities less than 35% for both of T. molitor and Z. morio. While, DCM layer acted rapidly causing 100% mortality at 10000 µg/mL for T. molitor and Z. morio within 1 day after treatment.

Table 1. Insecticidal activities of MeOH extract and HEX and DCM layers against T. molitor and Z. morio

| The extract by | Concentration (µg/mL) | Mortality rate against T. molitor (%) | Mortality rate against Z. morio (%) |
|----------------|-----------------------|--------------------------------------|-----------------------------------|
| MeOH           | 10000                 | 76b                                  | 81b                               |
| HEX            | 10000                 | 25c                                  | 33c                               |
| DCM            | 10000                 | 100a                                 | 100a                              |

*Within each column, values followed by the same letter do not differ from each other according to Tukey test (P < 0.05); MeOH: methanol extract; HEX: n-hexane layer; DCM: dichloromethane layer; DAT: days after treatment.

3.2. Isolation and characterization of capsaicinoids from the fruits of Capsicum annum

Table 2. 1H-NMR and 13C-jmod- NMR data of fraction H943 a mixture of capsaicin and dihydrocapsaicin.

| Position | H943 | Capsaicin [7] | Dihydrocapsaicin [7] | Capsaicin [6] |
|----------|------|---------------|----------------------|---------------|
|          | 1H   | 13C          | 1H                  | 13C          |
| C-1      | –    | 129.51       | –                    | 131.42       |
| C-2      | 6.77 | 111.27       | 6.75                 | 111.65       |
| C-3      | –    | 146.21       | –                    | 148.35       |
| C-4      | –    | 144.84       | –                    | 147.05       |
| C-5      | 6.91 | 114.67       | 6.75                 | 113.71       |
| C-6      | 6.75 | 120.16       | 6.73                 | 120.05       |
| C-7      | 4.30 | 42.40        | 4.29                 | 42.87        |
| C-8      | –    | 172.56       | 168.51               | 170.88       |
| C-9      | 2.22 | 38.85        | 2.21                 | 36.70        |
| C-10     | 1.65 | 25.67        | 1.62                 | 25.87        |
| C-11     | 1.39 | 27.77        | 1.32                 | 29.32        |
| C-12     | 2.20 | 32.12        | 1.29                 | 32.89        |
| C-13     | 5.36 | 126.85       | 1.18                 | 129.71       |
| C-14     | 5.38 | 137.55       | 0.97                 | 140.56       |
| C-15     | 2.23 | 35.72        | 1.53                 | 32.12        |
| C-16     | 0.88 | 22.04        | 0.87                 | 22.12        |
| C-17     | 0.87 | 22.04        | 0.87                 | 22.12        |
| O-CH3    | 3.82 | 55.31        | 3.81                 | 55.93        |
| NH       | 5.73 | 5.61         | 5.84                 |
| OH       |      |              | 5.87                 |

* NMR data were recorded in acetone-d6 at 500 MHz for 1H-NMR and 125 MHz for 13C-NMR; 6NMR data were recorded in CDCl3.
Chemical structures of the capsaicinoids in **H943** was determined by ESI-MS, $^1$H-NMR and $^{13}$C-jmod-NMR. The NMR data were showed in Table 3, compared with the data given in references [6-8].

ESI-MS spectrum (Figure 1) of **H943** showed two major pseudo-molecular ion peaks at $m/z$ 306.22 and 308.23 [M+H], corresponding to molecular formulas C$_{18}$H$_{27}$NO$_3$ for capsaicin and C$_{18}$H$_{29}$NO$_3$ for dihydrocapsaicin, respectively. Another minor product ion detected at $m/z$ 137.16 indicated the presence of vanillyl ring fragment that was generated by the protonate vanilly head in the structure of both capsaicin and dihydrocapsaicin.

The $^{13}$C-jmod-NMR spectrum (Figure 2 and Table 2) displayed eighteen carbon signals. The signal at δ55.31 belongs to a methoxy group connecting to aromatic ring. Three signals at δ 127.51 (C-1), δ 146.21 (C-3), and δ 144.84 (C-4) are specific to three aromatic quaternary carbons. Two signals of trans-olefin (=CH) appear at δ126.85 and δ137.55. Three signals belong to carbons of an aromatic ring without substitution at δ 111.27 (C-2), δ 114.67 (C-5) and δ 120.16 (C-6). Two signals at δ 22.04 and δ 22.14 are showed to two carbons of methyl groups. Furthermore, there is a signal at δ 168.51 dedicating a carbon of carbonyl group. By comparing MS and NMR data with those previously reported [6-7], **H943** was determined to be a mixture of capsaicin and dihydrocapsaicin with a ratio of 0.9:1 [8] (Figure 1).

### 3.3. Insecticidal activity of isolates and MO3 formula

The solubility of MO3 formula was tested and the soluble possibility was observed phenomena delimitation, dispersion and changes the physical state of the mixture for 24 hours. The MO3 formula showed a good solubility and stability as well as dispersed well after 24 h. Therefore, MO3 was selected as a trial formula for the further study of insecticidal efficacy.

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**Figure 1.** ESI-MS spectrum of **H943** containing capsaicin and dihydrocapsaicin.

**Figure 2.** $^1$H-NMR (A) and $^{13}$C-jmod-NMR (B) spectra of **H943**.
The insecticidal activity against *T. molitor* and *Z. morio* of **H943** (capsaicin and dihydrocapsaicin with a ratio of 0.9:1) and MO3 formula with different concentrations was evaluated and presented in Figure 3. The insecticidal activity of **H943** containing capsaicin and dihydrocapsaicin with a ratio of 0.9:1 at concentrations ranging from 125-1000 µg/mL was tested against the larvae of *Z. morio* in *in vitro* condition (Figure 3 and 4). This mixture caused a mortality of 100 % at concentrations higher than 250 µg/mL; while it did mortalities by 89-92 % at 125 µg/mL 1 and 2 days after treatment. It seems that the capsaicinoids mixture at concentrations greater than 250 µg/mL displayed an efficacy equal to that of positive control with abamectin at 1000 µg/mL. For the tests against *T. molitor*, this capsaicinoid mixture also exhibited a good activity against *T. molitor* with mortalities ranging from 76 - 87 %; 91 - 95 %; 98 - 100 % and 100 %, respectively, at 1 day and 2 days after treatment (Figure 3). MO3 was effective against larvae of *Z. morio* with 96 % mortality at 500 µg/mL 1 day after treatment and gave 98 - 100 % mortality 2 days after treatment (Figure 3). The formulation also acted rapidly and caused mortality rate 100 % at 1000 µg/mL within 1 day after treatment (Figures 3 and 4).

![Figure 3](image3.png)

**Figure 3.** Mortality rates of MO3 and H943 against *T. molitor* and *Z. morio*. Means of three replicates were recorded at 1 and 2 days after treatment. Aba: abamectin at 1000 µg/mL. DAT: days after treatment.

![Figure 4](image4.png)

**Figure 4.** Insecticidal activity of H943 (capsaicin and dihydrocapsaicin with a ratio of 0.9:1) and MO3 against *Z. morio* *in vitro* in the laboratory. The mortalities were recorded 2 days after treatment.

Capsaicinoids are important in the food and pharmaceutical industries. Capsaicin is used in the development of new drugs because it has many beneficial properties, such as antioxidant, antimicrobial, anti-inflammatory and antitumor activities, and contributes to the control of diabetes and pain relief. Capsaicin and dihydrocapsaicin occur in varying ratios from plant to
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plant, from a 1:1 to 2:1 ratio. These compounds typically make up 80-90% of the total capsaicinoid content in the capiscium fruits [9]. Capsaicin was also reported to exhibits the effect of mammalian vanilloid receptor subtype 1 (TRPV1) on insect behavioral thermoregulation. At low concentrations (10⁻⁷ and 10⁻⁴ M), capsaicin induces seeking lower temperatures of T. molitor [10]. Our study demonstrated notable insecticidal property of capsaicin and dihydrocapsaicin against T. molitor and Z. morio. The result of our study suggested a use of capsaicinoids from C. annuum fruits to control the invasion of T. molitor and Z. morio with regard to store products.

4. CONCLUSION

MeOH extract, HEX and DCM layers of the fruits of C. annuum were evaluated for their insecticidal activity against T. molitor and Z. morio. A mixture of capsaicin and dihydrocapsaicin (1: 0.9) was isolated from the DCM layer of the fruits of C. annuum using column chromatography and chemical structures were identified using ESI-MS and NMR. The formula of DCM layer displayed a potent activity against T. molitor and Z. morio, implying its potential as an alternative to conventional pesticide and the further applications of the green technology. To apply this green technology in the future, insecticidal activity of individual components and their synergistic property will be evaluated and combined with other active products for the improvement of efficacy as biopesticides.

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REFERENCES

1. Isman M. B. - Plant essential oils as green pesticides for pest and disease management, Agricultural Applications in Green Chemistry, American Chemical Society (2004) 41-51.
2. Oppert B. - Rapid bioassay to screen potential biopesticides in Tenebrio molitor larvae, Biopesticides International 6 (2010) 67–73.
3. Ogawa K., Murota K., Shimura H., Furuya M., Togawa Y., Matsumura T., Masuta C. - Evidence of capsaicin synthase activity of the Pun1-encoded protein and its role as a determinant of capsaicinoid accumulation in pepper, BMC Plant Biology journal 15 (2015) 1–10.
4. Madhumathy A. P., Aivazi A., Vijayan V. A. – Larvicidal efficacy of Capsicum annuum against Anopheles stephensi and Culexquinquefasciatus, Journal of Vector Borne Diseases 44 (2007) 223-226.
5. Tran T.T., Nguyen T.D., Nguyen D.Q., Ngo T.N., Nguyen Q. T., Vu H.S., Vu D. H., Le Dang Q. - Extraction optimization of Annonaceae seeds for the preparation of a botanical pesticide against mealworm Tenebrionimolitor and super worm Zophobas morio, Viet Nam Journal of Chemistry 55 (3e) (2007) 52-58.
6. Lin L. Z., West D. P., Cordell G. A. - NMR assignments of cis- and trans- capsaicin, Natural Product Letters 3 (1) (1993) 5-8.
7. Aihua Peng, Haoyu Ye, Xia Li, Lijuan Chen - Preparative separation of capsaicin and dihydrocapsaicin from Capsicum frutescens by high-speed counter-current chromatography, Journal of Separation Science 32 (2009) 2967-2973.
8. Wagner C. E., Cahill T. M., Marshall P. A. - Extraction, purification, and spectroscopic characterization of a mixture of capsaicinoids, Journal of Chemical Education 88 (2011) 1574-1579.

9. Fattori V., Hohmann M. S., Rossaneis A. C., Pinho-Ribeiro F. A., Verri, W. A. - Capsaicin: Current understanding of its mechanisms and therapy of pain and other pre-clinical and clinical uses, Molecules 21 (7) (2016) 844.

10. Olszewska J., Tegowska E. - Opposite effect of capsaicin and capsazepine on behavioral thermoregulation in insects, Journal of Comparative Physiology A 197 (10) (2011) 1021-1026.