

## Supporting Information

*Rec. Nat. Prod. X:X (2018) XX-XX*

### **Insecticidal Activity of *Artemisia frigida* Willd. Essential Oil and Its Constituents against Three Stored Product Insects**

**Zhe Zhang<sup>1</sup>, Xue Pang<sup>1</sup>, Shanshan Guo<sup>1</sup>, Juqin Cao<sup>1,2</sup>, Yang Wang<sup>1</sup>,**

**Zhenyang Chen<sup>1</sup>, Yixi Feng<sup>1</sup>, Ning Lei<sup>3</sup> and Shushan Du<sup>1</sup>**

<sup>1</sup>*Beijing Key Laboratory of Traditional Chinese Medicine Protection and Utilization; Faculty of Geographical Science; Beijing Normal University, NO.19 Xijiekouwai Street, Beijing 100875, P.*

*R. China*

<sup>2</sup>*Medical Chemistry Department; School of Basic Medical Sciences; Ningxia Medical University, NO. 1160 Shengli Street, Xingqing District, Yinchuan 750004, P. R. China*

<sup>3</sup>*Department of Pharmacy; The General Hospital of the PLA Rocket Force, NO.16 Xijiekouwai Street, Beijing 100875, P. R. China*

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## S.1: Experimental Details

### S.1.1: Insects Culture

*L. serricorne* and *T. castaneum* adults were maintained on a 10:1 mixture of wheat flour and yeast and *L. bostrychophila* was reared on a 10:1:1 mixture of wheat flour, yeast and milk powder, in the dark incubators at 28-29 °C, 70-80% relative humidity for two preceding years before use. The 1-week-old insects were used for bioassay tests. All the containers housing *L. bostrychophila* and the Petri dishes used in experiments were coated with polyterafluoroethylene to prevent pests escape.

### S.1.2: Essential Oil Extraction

Fresh aerial parts of *A. frigida* were collected in August 2016 from Lanzhou City (36°01' N latitude and 103°45' E longitude), Gansu Province, China. The species was identified by Dr. Liu, Q.R. A voucher specimen (BNU-dushushan-20160808) was deposited at the Faculty of Geographical Science Beijing Normal University. *A. frigida* aerial parts were air-dried and then weighted. The sample was put into a modified Clevenger type apparatus, and hydrodistillation was carried out for 6 h to collect essential oil. The essential oil was dehydrated with anhydrous sodium sulphate and stored in a refrigerator at 4 °C

### S.1.3: Chemical Components Determination

GC-MS analysis of the *A. frigida* essential oil was performed on an Agilent 6890 gas chromatography, coupled to an Agilent 5973 N mass selective detector and equipped with a capillary HP-5MS (30 m × 0.25 mm × 0.25 µm) column and a gas chromatography-flame ionization detection (GC-FID). The oven temperature was programmed at 50 °C for 2 min, and then increased at a rate of 10 °C/min until the final temperature of 250 °C was reached. Helium was used as carrier gas at a flow rate of 1 mL/min. The injector temperature was maintained at 250 °C and the volume injected was 1 µL of 1% solution (diluted in n-hexane). The mass spectrum were scanned from 50 to 550 m/z. The retention indices were calculated for all volatile constituents using a series of n-alkanes C9-C17. Based on RI, the chemical constituents were identified by comparing with n-alkanes as reference. Further identification was made by comparison of their MS data with those stored in NIST 05 and Wiley 275 libraries or with mass spectra from literature [1]. Quantification of each compound was performed by averaging the GC-FID peak area% reports.

### S.1.4: Fumigant Toxicity

The main individual compounds were brought from Adamas Reagent Co., Ltd. ( $\alpha$ -terpinyl acetate), Tokyo Chemical Industry Co., Ltd. (verbenone), Sigma-aldrich Co., Ltd. (camphene) and Across organics Co., Ltd. (terpinen-4-ol). The method of fumigant toxicity of *A. frigida* essential oil and its compounds against *L. serricorne* and *T. castaneum* adults was conducted according to described by Liu and Ho [2] and that against *L. bostrychophila* adults was described by Zhou et al [3]. The appropriate testing concentrations were determined by running a lot of range-finding studies. Then dissolve the desired quantity of each material in n-hexane to obtain a graded series of five

concentrations respectively as testing solution. When tested on *L. serricornis* and *T. castaneum*, a Whatman filter paper (diameter 2.0 cm) was placed on the underside of the screw cap of a glass vial (diameter 2.5 cm, height 5.5 cm, volume 24 mL), each of which contained ten insects inside. A 10 µL sample solution treated with it. The cap was placed tightly on the glass vial right now to form a sealed chamber for *L. serricornis* and the solvent treated on *T. castaneum* was allowed to evaporate for 20 s before the cap was tightened. *n*-Hexane was used as a control. As for *L. bostrychophila*, a filter paper strip (3.5 cm × 1.5 cm) treated with 10 µL sample solution. The impregnated filter paper was then placed in the bottom cover of big glass bottle (250 mL). The small glass bottle (8 mL), containing 10 booklice, was put into the big glass bottle. *n*-Hexane was used as controls and dichlorvos was used as a positive control. Incubated under the same conditions as used for insect rearing. Mortality was determined for five replicates per treatment and control after 24 h and the LC<sub>50</sub> values were calculated using Probit analysis for all these three insects [4].

#### S.1.5: Contact Toxicity

The contact toxicity of the plant essential oils against *L. serricornis* and *T. castaneum* adults was determined according to the method of Liu and Ho [2] and against *L. bostrychophila* adults was measured as described by Zhou et al [3]. Range-finding studies were run to determine the appropriate testing concentrations. Then dissolve the desired quantity of each material in *n*-hexane to obtain a graded series of five concentrations as testing solution. Five replicates of each concentration were used. When it came to *L. serricornis* and *T. castaneum*, aliquots of 0.5 µL of the dilutions were applied topically to the dorsal thorax of the insects (10 insects per replicate, five replicates per dose). *n*-Hexane were used as negative controls and pyrethrins (pyrethrin I and II, 37%) were used as positive controls. The pretreated insects were transferred to glass vials and kept in incubators. The contact toxicity against *L. bostrychophila* was carried out with 5.5 cm diameter filter papers soaked with 300 mL of the testing solution. The filter paper after being treated with solid glue was placed in a 5.5 cm diameter Petri dish and 10 booklice were put on the filter paper. A cover was put and all the Petri dishes were kept in the incubator. *n*-Hexane was used as a negative control and pyrethrins were used as a positive control. Mortality of all these three insects was recorded after 24 h and the LD<sub>50</sub> values were calculated using Probit analysis (SPSS 19.0) [4].

## References

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