Chemical constituents from the aerial parts of Rhododendron thymifolium and its bioactivities against Tribolium castaneum, Lasioderma serricorne and Ditylenchus destructor

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Research Article

Keywords:

Posted Date: August 1st, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3193398/v1

Abstract

In this paper, we studied the isolation and identification of 13 compounds extracted from the aerial parts of R. thymifolium. These compounds include: one terpenoid (1), one alkane (2), one fatty acid ethyl ester (3), one thiol (4), two fatty alcohols (5 and 7), two coumarins (6 and 10), two flavonoids (11 and 12), three aromatics (8, 9 and 13). Compound (1) was isolated from R. thymifolium for the first time. The isolated compounds were tested for insecticidal activity against T. castaneum, L. serricorne and D. destructor. Apocynin (8) and Acetophenone (13) exhibited obvious contact activity against the storage pests with LD50 values of 6.46 and 8.72 µg/adult respectively. Acetophenone (13) showed excellent contact activity against D. destructor with LC50 values of 0.08 mg/mL. The study clearly showed the active fractions from R. thymifolium have might potential to be developed into potential compounds on storage insect prevention.

Introduction

Tribolium castaneum and *Lasioderma serricorne* are two important insect pest species commonly occurring in stored products.[1] Now evidence have shown that the annual loss of storage grain insects is about 1% and the insects also Caused serious economic loss.[2] *Ditylenchus destructor* is an important plant pathogenic nematode in agricultural production and is listed as a quarantine pest by many countries and regions.[3] Currently, plant-parasitic nematodes are major pests responsible for global agricultural losses amounting to an estimated $157 billion annually.[4] The widespread using of synthetic pesticides has resulted in the development of insecticide-resistant populations and harmful effects on human health and the environment.[5] Botanical insecticides have been used as alternatives to synthetic insecticides against crop pests because they are biodegradable and do not leave toxic residues or by-products to pollute the environment.[6]

Rhododendron thymifolium is widely distributed in China, mainly in Gansu, Qinghai, and Sichuan provinces. Most species of the *Rhododendron* genus have been reported to possess high medicinal value effective for the treatment of anti-tumor, anti-bacterial, anti-inflammatory, antivirus and analgesic.[7] The essential oil of *R. thymifolium* has been demonstrated to have insecticidal activity against *T. castaneum* and *L. serricorne*.[8] The present study was performed to isolate and identify compounds extracted from the aerial parts of *R. thymifolium*. Moreover, to investigate the biological activity of these compounds against *T. castaneum*, *L. serricorne* and *D. destructor*.

Materials and Methods

Plant material

The aerial parts of *R. thymifolium* were collected from Tianzhu city, Gansu Province (Longitude35°78'34" latitude, Latitude103°93'79", altitude 1950 m), China, in August 2021. The plant was identified by Dr. Liu, Q.R. (College of Life Sciences, Beijing Normal University, Beijing, China) and the voucher specimen

(voucher number: NWNU-20190901001) was deposited at the Herbarium of College of the Life Science, Northwest University.

Culture of *Tribolium castaneum* and *Lasioderma serricorne*

T. castaneum and *L. serricorne* were obtained from laboratory cultures in a permanent dark incubator at 29 ± 1℃ and 70% ± 10% relative humidity. These adults were reared in glass containers (0.5 L), containing wheat flour mixed with yeast (10:1, w/w) at 13% ± 1% moisture content. All adult beetles used in the experiment were considered as adults stage after an eclosion time of 1-2 weeks, regardless of gender.

Culture of *Ditylenchus destructor*

The nematodes were isolated from *A. sinensis* with Makou diseases by the Berman funnel method.[33] The isolated nematode suspension was disinfected and centrifuged with 3% (v/v) hydrogen peroxide, leaving about 2 mL of the liquid at the bottom to obtain a sterile nematode suspension. Botrytis cinerea was cultivated with potato dextrose agar (PDA). After 4 days, the sterile nematode suspension was vaccinated onto the PDA flat and placed in an incubator at 25°C for about 15 days with a relative humidity of 80%–90%. Then, 2 mL of sterile water was added to the bottom of the Petri dish, and the Petri dish was inverted. After 5 h, the sterile water containing nematodes was drawn. Then, a suspension with a large number of nematodes was obtained.

The isolation of thirteen compounds extracted from the aerial parts of *R. thymifolium*

The air-dried aerial parts of *R. thymifolium* (5 kg) were crushed into powder and extracted three times using 95% ethanol (boiling range: 60-90℃) (10 L) as the solvent by the heating reflux procedure at 78℃, where a slight boil state was maintained for 2 h. The supernatant extraction solution was separated and concentrated (under reduced pressure and temperature of 30℃) to give 656 g of the crude 95% ethanol extract. Then, the 95% ethanol extract was extracted with chloroform and ethyl acetate solvents to obtain chloroform extract (54.49 g) and ethyl acetate extract (150 g). The chloroform extract was chromatographed on a silica gel column (160-200 mesh) consisting of 54.49 g silica gel (160-200 mesh) and elution was carried out with a mixture of petroleum ether (PE) and ethyl acetate (EA) (100:0-0:100, v/v) to obtain 11 fractions. Through repeated silica gel column chromatography (CC), crystallization and recrystallization, eight compounds were isolated. The first fraction (4.60 g) was subjected to silica gel CC and eluted with a gradient of PE-EA (70:1-60:1, v/v) to obtain compounds **1** (108 mg) and **2** (92 mg). Compounds **3** (20 mg) was isolated from the second fraction (2.56 g) by the PE-EA (60:1-50:1, v/v) eluent. The third fraction (4.56 g) was subjected to silica gel CC and eluted with a gradient of PE-EA (50:1-40:1, v/v) to obtain compounds **4** (4.6 mg) and **5** (30 mg). Compounds **6** (20 mg), **7** (22.5 mg), **8** (137 mg), **9** (41.5 mg) were isolated from fifth fraction (10.23 g) by using PE-EA (30:1-10:1, v/v) as eluent. Compound **10** (27.1 mg) was separated from the sixth fraction (2.58 g) used to silica gel CC and eluted with a gradient of PE-EA (10:1-1:1, v/v). In addition, the ethyl acetate extract (150 g) mixed with 150 g silica gel of 160-200 mesh was chromatographed on a silica gel column (160-200 mesh) and eluted with

mixed solvents of dichloromethane-methanol (MeOH) (100:0-0:100, v/v) to obtain 11 fractions. The second (33.87 g) and third (11.32 g) fractions were further isolated with dichloromethane-MeOH (gradient, 30:1-10:1, v/v) and contained two impure compounds that were further separated on Sephadex LH-20 with an eluent of TCM-MeOH (1;1, v/v) to obtain compounds **11** (7.85 mg) and **12** (13.92 mg). The fifth fraction (23.21 g) was subjected to silica gel CC and eluted with a gradient of dichloromethane-EA (20:1-1:1, v/v) to obtain compounds **13** (28.65 mg).

Contact toxicity tests

Contact toxicity tests were performed on *T. castaneum* and *L. serricorne* according to the method described by Liu and Ho.[34] The isolated compounds were used as the testing samples. The compounds were dissolved in acetone to prepare a serial testing solution. Five replicates were implemented for all treatments, ten worms at a time. The dead insects were counted after 24 h. The data were corrected for control mortality using Abbott's formula. The LD_{50} values were calculated by using Probit analysis (IBM SPSS V22.0).

Determination of indoor toxicity of *Ditylenchus destructor*

The indoor toxicity tests were performed on *D. destructor* according to the method described by Barbosa.[35] The isolated compounds were prepared in a distilled water solution with 5% Tween-20 and 2% dimethyl sulfoxide (DMSO). Then, 90 μL of different concentrations of compound samples were injected into 96-well microplates. Further, a 10 μL suspension of *D. destructor* (containing 50–100 nematodes) was added to each well. The suspension was mixed well and placed in an incubator at 25°C, with a relative humidity of 80–90%. The mixture of 5% Tween-20 and 2% DMSO was used as a negative control, and carbofuran (purchased from Shanghai, China, purity = 98.4%) was used as a positive control. The same nematode experiment was replicated five times. All nematodes were evaluated under a light microscope, and the death/survival status of nematodes in each group was examined after 48 h (all nematodes were transferred to fresh distilled water, and nematodes that had not regained movement after being transferred to the water for 24 h were considered dead). The data were corrected for control mortality using Abbott's formula. The LC_{50} values were calculated by using Probit analysis (IBM SPSS V22.0).

Results and Discussion

Chemical compounds isolated from *R. thymifolium*

The isolation and identification of thirteen compounds extracted from the aerial parts of *R. thymifolium* have in the present study, including one terpenoid (1), one alkane (2), one fatty acid ethyl ester (3), one thiol (4), two fatty alcohols (5 and 7), two coumarins (6 and 10), two flavonoids (11 and 12), three aromatics (8, 9 and 13). The chemical structures of the obtained compounds were identified by mass spectrometry (MS), proton nuclear magnetic resonance (^{1}H-NMR), and Carbon-13 nuclear magnetic

resonance (^{13}C-NMR) spectra and were compared with the relevant results reported in the literature. These compounds were identified as ent-kaurane-16 *β*-ol (**1**),[9] pentacosane (**2**),[10] undecanoic acid, ethylester (**3**),[11] 1-Tridecanethiol (**4**),[12] 1-Dodecanol (**5**),[13] 4-Methyl-7-hydroxycoumarin (**6**),[14] tertradecan-1-ol (**7**),[15] apocynin (**8**),[16] 3-Methoxy-4-methylbenzaldehyde (**9**),[17] scopoletin (**10**),[18] hyperin (**11**),[19] quercetin (**12**),[20] acetophenone (**13**).[21] The structures of these compounds are shown in Fig.1.

Compound (1) has been isolated from R. thymifolium for the first time. Flavonoids have been reported to be presented in many genera of Rhododendron. Thirty flavonoids were reported in *Rhododendron genera*.[22] As is shown in these results, thrity flavonoid compounds were very rich in *Rhododendron genera*. Previously, compounds 11 and 12 have been reported in *R. mariesii* Hemsl,[23] *R. latoucheae* Franch,[25] *R. capitatum* Maxim,[26] *R. anthopogonoides* Maxim,[27] *R. concinnum* Hemsl,[28] *R. amesiae* Rehd,[29] *R. cerasinum* Tagg,[30] *R. anthopogon* D. Don,[31] respectively. Comparing this paper with previous studies on the chemical composition of flavonoids in Rhododendron spp. we conclude that flavonoids can be seated as marker compounds for the chemical classification of Rhododendron spp. and have a definite taxonomic value, but factors such as the geographical distribution of plants and convergent evolution can affect this chemical qualifications of species, which requires appropriate methods and interpret taxonomic conclusions more carefully when using flavonoids as a basis for chemical classification. In addition, terpenoids are also common chemical components in Rhododendron plants. To date, more than 130 diterpenes have been reported from Ericaceae family, the main types of which are derived from the enantiomeric shell finance diterpenes.[32]

Contact toxicity

The isolation and identification of thirteen compounds extracted from the aerial parts of *R. thymifolium* against two target insects were listed in Table S1 and S2. The isolated compound Apocynin (**8**) and 4-Methyl-7-hydroxycoumarin (**6**) showed strong contact toxicity against *T. castaneum* (LD_{50} = 6.46 and 6.78 μg/adult, respectively). Furthermore, the Acetophenone (**13**) showed significant contact toxicity against *L. serricorne* (LD_{50} = 8.72 μg/adult). Although there was no significant biological activity compared to the positive control, but compared with other isolated compounds of *R. thymifolium* tested using a similar bioassay in the literature, these compounds obtained in the present study exhibited significant contact toxicity against *T. castaneum* and *L. serricorne*, such as four compounds of *R. thymifolium* essential oil against *T. castaneum* adults were not observed, the contact activity (LD_{50} = 17.18 μg/adult) main composition germacrene of *R. thymifolium* against *L. serricorne*.

Indoor toxicity of *Ditylenchus destructor*

The isolation and identification of thirteen compounds extracted from the aerial parts of *R. thymifolium* against *D. destructor* were listed in Table S3. The isolated compounds Acetophenone (**13**) and Apocynin (**8**) showed strong contact toxicity against *D. destructor* (LC_{50} = 0.08 and 0.09 mg/mL, respectively),

which was approximate with the indoor toxicity of the positive control (Carbofuran). Compared with other compounds of tested using a similar bioassay in the literature, these compounds obtained in the present study exhibited significant contact toxicity against *D. destructor*, such as eucalyptus oil and verbenenol have certain insecticidal activity against *D. destructor*, with LC_{50} of 0.49 mg/mL and 1.09 mg/mL.[33]

Conclusion

In this study, the isolation and identification of thirteen compounds extracted from the aerial parts of *R. thymifolium* have in the present study, including one terpenoid (**1**), one alkane (**2**), one fatty acid ethyl ester (**3**), one thiol (**4**), two fatty alcohols (**5** and **7**), two coumarins (**6** and **10**), two flavonoids (**11** and **12**), three aromatics (**8**, **9** and **13**), While compound (**1**) has been isolated from *R. thymifolium* for the first time. The activity results showed that compounds exhibited strong contact activity against *T. castaneum* and *L. serricorne* and *D. destructor*, such as Apocynin (**8**) (LD_{50} = 6.46 μg/adult), Acetophenone (**13**) (LD_{50} = 8.72 μg/adult and LC_{50} = 0.08 mg/mL). In conclusion, the present study certainly enriches the chemical diversity of *R. thymifolium* and provides a reference for exploring in depth and subsequent development of *R. thymifolium*.

Declarations

Acknowledgments

This study was supported by Science & Technology Projects for People's Livelihood (No. 20CX9NA070) and Industry Pillar Program of Gansu Province (No. 2020C-21).

Data availability statement

Data available on request from the authors. The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Author Contribution Statement

J. Y. Liang, Y. Y. Yang and X. D. Wang conceptualized and designed the study, performed the experiment and statistical analysis, wrote the manuscript; H. S. Wu, J. L. Wang and W. B. Kong performed the experiment and statistical analysis; J. Zhang acquired the funds and revised the manuscript. All authors read and approved the final version of the manuscript.

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Figures

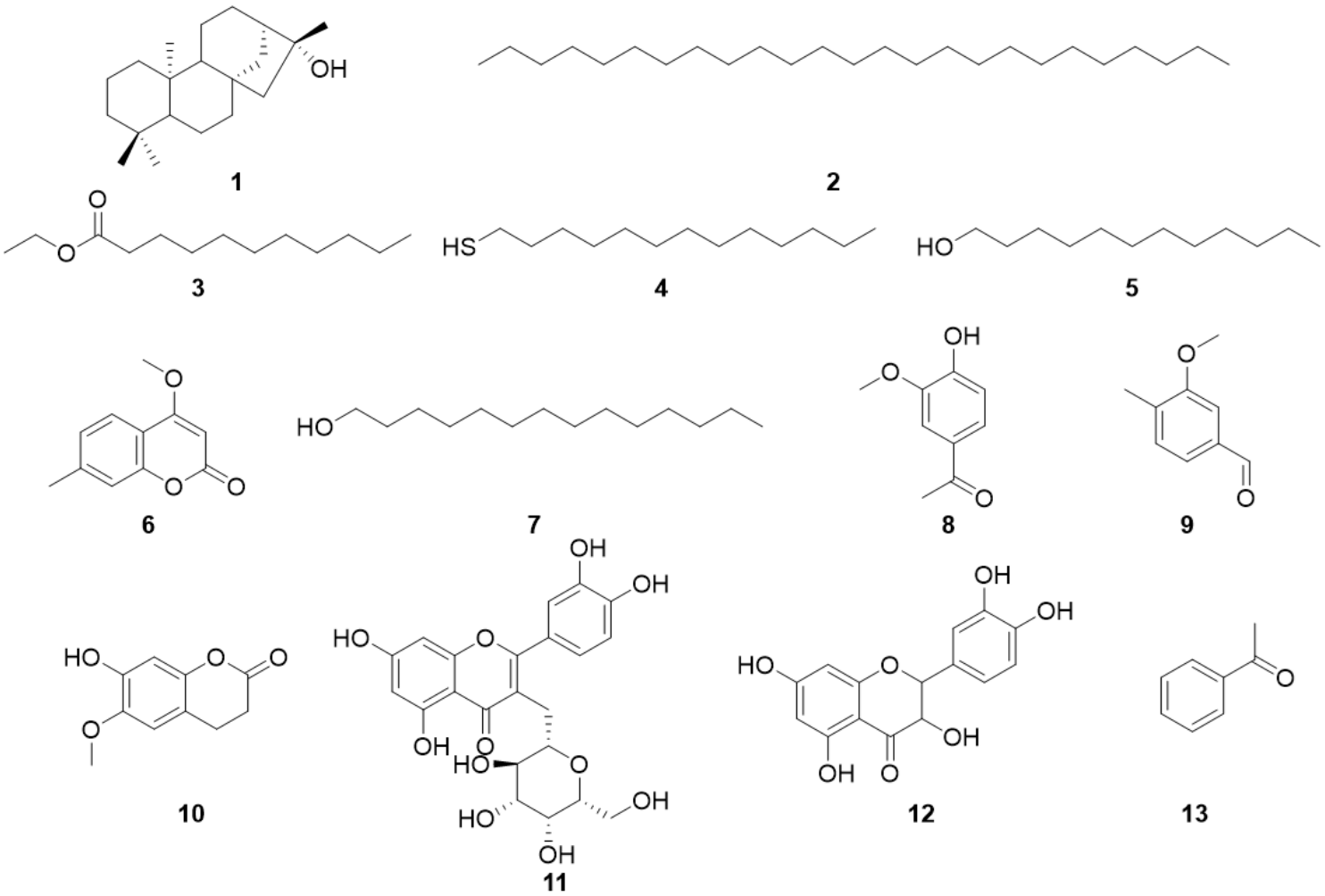

Figure 1

The structures of compounds 1-13.

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