Thymol Derivatives from *Eupatorium fortunei*

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(Received November 01, 2018; Revised February 01, 2019; Accepted February 10, 2019)

Abstract: The presence of thymol derivatives in the leaves and twigs of *E. fortunei* collected in Northern Vietnam was investigated. Five thymol derivatives, including new 9-O-angeloxy-10-hydroxy-8-methoxythymol, 10-acetoxy-9-chloro-8,9-dehydrothymol, 9-acetoxy-8,10-epoxythymol-3-O-isobutyrate, 8,10-epoxy-9-hydroxythymol-3-O-tiglate, and 9-acetoxy-8,10-epoxythymol-3-O-angelate together with six other compounds, including taraxasteryl acetate, *o*-coumaric acid, trans-melilotoside, (-)-loliolide, coumarin, and β-sitosterol were isolated. The structures of the isolated compounds were determined on the basis of MS and NMR spectroscopic data.

Keywords: *Eupatorium fortunei*; Asteraceae; thymol derivative. © 2019 ACG Publications. All rights reserved.

1. Plant Source

*Eupatorium fortunei* Turcz. (syn. *E. staechadosmum* Hance) of the family Asteraceae has been recorded as a medicinal plant under the name Man tuoi in Vietnam. The plant has been described in remedies for digestive benefits and for the treatment of fever [1].

The leaves and twigs of *E. fortunei* were collected in Gia Lam district, Hanoi, Vietnam, in December 2015. The plant material was identified by Dr. Nguyen Thi Kim Thanh, Faculty of Biology, VNU University of Science, Vietnam National University, Hanoi, Vietnam. A voucher sample (No. EF-12-15) has been deposited at the Laboratory of Chemistry of Natural Products, Faculty of Chemistry, VNU University of Science, Vietnam National University, Hanoi, Vietnam.

2. Previous Studies

Phytochemical reports showed the occurrence of monoterpenoids, sesquiterpenoids, triterpenoids, thymol derivatives, benzofurans derivatives, flavonoids, trihydroxypiperidines, pyrrolizidine alkaloids, and an acetonaphone from different plant collections in Vietnam [2, 3], China [4-8], and Japan [9-12]. Concerning pharmacological studies, antiviral activity against RNA viruses [13], reduced metastatic and angiogenic potency of malignant cancer via suppression of MMP-9 activity and VEGF production [14] of the aqueous extract of *E. fortunei* were reported. Thymol

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derivatives have been isolated from *Eupatorium* species, particularly *E. fortunei* [3, 15], *E. glechonophyllum* [15], *E. kiirunense* [15], and *E. cannabium* [16, 17]. They have been reported to exhibit cytotoxic activity against human cancer cell lines [5, 17], inhibitory activity on LPS-induced NO production [8], anti-inflammatory activity by suppressing fMLP/CB(formyl-L-methionine-L-leucyl-L-phenylalanine/cytochalasin B)-induced elastase release [16], and antibacterial activity [18].

3. Present Study

The dried powdered leaves (3.0 kg) or dried twigs (1.4 kg) of *E. fortunei* were extracted with MeOH at room temperature (three times, each time for 7 days). The combined MeOH extract from the leaves or the twigs was concentrated under reduced pressure. The residue was successively partitioned between water and organic solvents of increasing polarities to give n-hexane (120 g and 20.5 g, respectively), CH₂Cl₂ (12 g and 41.5 g), and EtOAc (12 g and 2.4 g) soluble fractions. *Separation of the leaf soluble fractions*: Part of the n-hexane-soluble fraction (55 g) was separated by CC on silica gel eluted with n-hexane-acetone 90:1, 29:1, 19:1, 6:1, 3:1, 1:1 to give twelve fractions. Fractions 2 (225.6 mg) and 3 (2.24 g) were washed with n-hexane to give 2 (586.8 mg). Fractions 7 (896 mg) and 8 (145.5 mg) were washed with acetone to give 3 (200.2 mg). Fraction 11 was separated by RP-18 CC with MeOH-H₂O 4:1, 9:1, and further purified by: 1) silica gel CC, n-hexane-EtOAc 29:1, 19:1, 6:1, 3:1, 1:1 and 2) preparative TLC, n-hexane-EtOAc 3:1 to give 1 (3 mg) and 4 (5 mg). The CH₂Cl₂-soluble fraction (12 g) was subjected to silica gel column chromatography eluted with n-hexane-acetone 70:1, 49:1, 29:1, 19:1, 15:1, 12:1 to give seven fractions. Fraction 2 (412 mg) was separated by silica gel CC, CH₂Cl₂-acetone 70:1, 49:1, 29:1, 19:1, 13:1, 12:1, and further purified by: 1) RP-18 CC with MeOH-H₂O 7:3, 9:1 and 2) silica gel CC with n-hexane-EtOAc 15:1, 12:1, 9:1, 6:1, 3:1 to give 5 (5 mg). Part of the EtOAc-soluble fraction (6 g) was separated by Sephadex LH-20 CC, MeOH, and further purified by: 1) silica gel CC, n-hexane-EtOAc-HCO₂H 20:5:1, 20:10:1, 20:19:1; 2) silica gel CC, 20:1:0.1, 20:5:0.1, 20:5:0.4, 20:5:1; and 3) silica gel CC, n-hexane-EtOAc-HCO₂H 20:5:0.1, 20:5:0.4, 20:5:0.8 to give 6 (12 mg). The water phase was concentrated under reduced pressure and the residue was subjected to RP-18 CC, MeOH-H₂O 4:1, 9:1, and further purified by: 1) silica gel CC, n-hexane-EtOAc-HCO₂H 10:10:1, 10:20:1, 10:40:1 and 2) silica gel CC, CH₂Cl₂-MeOH 29:1, 19:1, 15:1, 12:1, 9:1, 6:1, 3:1 to afford 7 (2.8 mg). *Separation of the twig soluble fractions*: The n-hexane-soluble fraction (20.5 g) was chromatographed on silica gel, n-hexane-acetone 90:1, 49:1, 29:1, 19:1, 9:1, 6:1, 3:1, 1:1 to give eleven fractions. Fraction 7 was purified by: 1) silica gel CC, n-hexane-EtOAc 99:1, 70:1, 50:1 and 2) silica gel CC, n-hexane-CH₂Cl₂ 99:1, 70:1 to give 8 (4.5 mg) and a mixture of 9 and 10 (4.5 mg). The EtOAc-soluble fraction (2.38 g) was subjected to Sephadex LH-20 CC, MeOH. Further purification by: 1) silica gel CC, n-hexane-EtOAc 49:1, 29:1, 19:1, 9:1, 6:1, 3:1, 1:1 to give 11 (6.5 mg).

**9-O-Angeloyx-10-hydroxy-8-methoxythymol (1):** White amorphous powder; [α]27
D 0.0 (c = 0.13, CHCl₃); IR νmax (film): 3396, 1703, 1697, 1649, 1629, 1462, 1231, 1159, 1045 cm⁻¹; HRESIMS (positive-ion mode): m/z 317.13605 [M + Na]⁺ (calcld. 317.13594 for C₁₆H₂₂O₃Na).

The structures of the known compounds, taraxasteryl acetate (2) [19], β-sitosterol (3) [20], 10-acetoxy-9-chloro-8,9-dehydrothymol (4) [21], (–)-loliolide (5) [22], o-coumaric acid (6) [23], trans-melililotoside (7) [24], 9-acetoxy-8,10-epoxythymol-3-O-isobutyrate (8) [25], 9-acetoxy-8,10-epoxythymol-3-O-angelate (9) [2], 8,10-epoxy-9-hydroxythymol-3-O-tiglate (10) [11], and coumarin (11) [26] (Fig. 1) were determined by comparing their spectroscopic data (MS, 1H, and 13C NMR) with reported literature values. The occurrence of 4, 5, and 7 in *E. fortunei* is reported for the first time in the present study.
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Figure 1. Chemical structures of compounds 1, 2, 4-11 and HMBC correlations of 1

Compound 1 was isolated as a white amorphous powder, \( [\alpha]_D^{27} 0.0 \) (c 0.13, CHCl₃). The molecular formula of 1 was determined to be C₁₆H₂₂O₅Na by positive-ion HRESIMS (m/z 317.1365 [M + Na]+). The IR spectrum showed absorption bands for a hydroxy group at \( \nu_{\text{max}} \) 3396 cm⁻¹, an ester carbonyl group at \( \nu_{\text{max}} \) 1703 and 1231 cm⁻¹, and an aromatic ring at \( \nu_{\text{max}} \) 1697, 1462, and 1159 cm⁻¹. The \(^1\)H and \(^{13}\)C NMR spectra of 1 showed signals for a ten-carbon thymol skeleton, a methoxy group \([\delta_H 3.37 (3\text{H, s}); \delta_C 51.4]\), and an angeloyloxy moiety \([\delta_H 1.88 (3\text{H, s}), 1.97 (3\text{H, d, } J = 7.0 \text{ Hz}), 6.12 (1\text{H, q, } J = 6.0 \text{ Hz}); \delta_C 15.8, 20.5, 127.1, 139.8, \text{ and } 168.0]\). Three oxygenated carbons were observed. The signal at \( \delta_C 82.7 \) was attributed to a tertiary oxygenated carbon, and the signals at \( \delta_C 63.3 \) \([\delta_H 3.92 (d, J = 12.0 \text{ Hz}), 3.97 (d, J = 12.0 \text{ Hz})] \) and 62.9 \([\delta_H 4.62 (d, J = 12.0 \text{ Hz}), 4.70 (d, J = 12.0 \text{ Hz})]\) were attributed to a hydroxymethyl group and an angeloyloxymethyl group, respectively. The presence of an aromatic methyl group at \( \delta_H 2.29 (3\text{H, s}); \delta_C 21.0 \) indicated the locations of the tertiary oxygenated carbon and two oxymethyl groups at C-8, and C-9 and C-10, respectively. Their locations were confirmed by \(^1\)H-\(^1\)H long-range correlations in the HMBC spectrum (Fig. 1) between H-5 \((\delta_H 6.90) \) and C-8 \((\delta_C 82.7) \), between H₂-9 \((\delta_H 4.62/4.70) \) and C-8, between H₂-9 and C-1′ \((\delta_C 168.0) \), and between 8-OC₃H₃ \((\delta_H 3.37) \) and C-8. Full assignment of the \(^1\)H and \(^{13}\)C NMR signals was established on the basis of \(^1\)H-\(^1\)H COSY, HSQC, and HMBC correlations. It has been reported that thymol derivatives possessing a chiral center at C-8 from *E. fortunei* may exist as racemic mixtures [11]. Having a chiral center at C-8, compound 1 showed no optical rotation and could be present in its racemic form.
Compounds 2, 5-7 were tested in an agar diffusion assay [27] for their antifungal and antibacterial properties toward *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 25923), *Bacillus subtilis* (ATCC 11774), *Staphylococcus aureus* subsp. *aureus* (ATCC 11632), *Aspergillus niger* (439), *Fusarium oxysporum* (M42), *Candida albicans* (ATCC 7754) and *Saccharomyces cerevisiae* (SH 20). No activity was observed at concentration of 50 µg/mL (5) or 100 µg/mL (2, 6, and 7).

More than 300 compounds were reported from the genus *Eupatorium* including flavonoids; monoterpenoids; guaiane, germacrane, and cadinane sesquiterpenoids; ent-labdane and ent-kaurane diterpenoids; taraxasterane, dammarane, epipodophyllane, ursane triterpenoids; pyrrolizidine alkaloids; phenylpropanoids; and quinonoids [15]. These diverse compounds form the chemical profile of *Eupatorium* species. Compounds isolated from the collection in Vietnam are in accordance with the *Eupatorium* chemical profile. The nor-terpene hydroxylactone (−)-loliolide (5) occurs in many plants and marine organism but has not been reported from any *Eupatorium* species. Chen et al. reported eudesmane sesquiterpenes and menthol monoterpenes as chemotaxonomic important compounds for *E. fortunei* [7]. However, eudesmanes were also isolated from some *Eupatorium* species such as *E. quadrangulare* [28] and *E. cannabinum* [29]. Thymol derivatives predominantly occur in *E. fortunei*, some of which may be considered as species-specific substances for the identification of *E. fortunei* among *Eupatorium* species judging from their plant-dependent characteristic oxygenated patterns. (−)-Loliolide (5) may also be considered as a useful marker to distinguish *E. fortunei* from the other *Eupatorium* species.

**Acknowledgments**

This research was funded by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 104.01-2017.41.

### Table 1. NMR spectroscopic data of 1 and reference compounds [11] (δ in ppm, J in Hz)

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Supporting Information


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**References**


