Labdane-type Diterpenes from Pinus eldarica Needles and Their Anti-Helicobacter pylori Activity

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ABSTRACT: Pinus eldarica is a medicinal tree used in traditional herbal medicine for the treatment of bronchial asthma and various skin diseases. As part of our ongoing search for bioactive phytochemicals with novel structures in natural products, we performed a phytochemical analysis of the methanol (MeOH) extract from P. eldarica needles collected in Iran. Phytochemical investigation of the MeOH extract, aided by liquid chromatography−mass spectrometry-based analysis, resulted in the isolation and identification of three labdane-type diterpenes (1−3), including a new and relatively unique norlabdane-type diterpene with a peroxide moiety, eldaricoxide A (1). The chemical structures of the isolated labdane-type diterpenes were elucidated by analyzing the spectroscopic data from 1D and 2D NMR and high-resolution electrospray ionization-mass spectrometry. The absolute configuration of eldaricoxide A (1) was established by employing a computational method, including electronic circular dichroism calculation and specific optical rotation. An anti-Helicobacter pylori test was conducted, where compound 3 exhibited the most potent antibacterial activity against H. pylori strain 51, inducing 72.7% inhibition (MIC$_{50}$ value of 92 μM), whereas eldaricoxide A (1) exhibited moderate antibacterial activity against H. pylori strain 51, inducing 54.5% inhibition (MIC$_{50}$ value of 95 μM). These findings demonstrated that the identified bioactive labdane-type diterpenes 1 and 3 can be applied in the development of novel antibiotics against H. pylori for the treatment of gastric and duodenal ulcers.

1. INTRODUCTION

Pinus eldarica is an evergreen tree, originally from the Transcaucasian region of Europe and Asia and also found in Iran, Afghanistan, and Pakistan. This species had been introduced to Iran several centuries ago, and it is particularly known for its ability to tolerate air contamination, dust, drought, and cold. P. eldarica belongs to the family "Pinaceae," characterized by a gray shell, needle-shaped leaves (pine needles), and single or paired cones. Needles of pines belonging to Pinaceae have been known to contain phenolic compounds, such as catechin, epicatechin, and taxifolin, and the extracts of pine needles have been shown to exhibit diverse physiological and pharmacological actions, such as anti-inflammatory, anti-oxidant, anti-neoplastic, and immune-modulatory properties. P. eldarica needles, buds, resins, and nuts have been widely used in traditional medicine for the treatment of bronchial asthma and various skin diseases, such as wounds, allergic rashes, and dermatitis.

Recent phytochemical analyses of P. eldarica showed that the essential oils extracted from its needles, fruit, bark, and pollen mainly consist of mono- and sesquiterpenoids, such as $\alpha$-pinene, carophyllene oxide, $\delta$-3-carene, (E)-$\beta$-caryophyllene, $\delta$-germacrene, longifolene, limonene, and myrtenal. Pharmacological studies on P. eldarica extracts showed the potentiality of diverse therapeutic efficacies that can be...
employed. For example, aqueous extracts from fruits exhibited anti-urolithiatic activity by inhibiting calcium oxalate deposition in rats with calcium oxalate nephrolithiasis;\(^2\) nut extracts lowered blood cholesterol levels and decreased aortic atherosclerosis in hypercholesterolemic rabbits;\(^3\) bark extracts exerted an anti-pseudomonas effect\(^4\) and cytoprotective and genoprotective effects on human umbilical vein endothelial cells (HUVECs) following exposure to cisplatin;\(^5\) and the needle extracts showed antidepressant activity in rats with reserpine-induced depression-like behavior,\(^6\) a neuroprotective effect in a mouse model with pentyleneetetrazole-induced seizure,\(^7\) and anti-inflammatory effects by decreasing acute and chronic pain and inflammation.\(^8\) Although there have been extensive pharmacological studies on P. eldarica extracts, the potential phytochemicals from diverse natural resources,\(^9\) we investigated a methanol (MeOH) extract of P. eldarica needles collected in Iran to explore antibacterial diterpenes using the liquid chromatography–mass spectrometry (LC–MS)-based analysis. The chemical structures of the isolated diterpenes were elucidated using the conventional spectroscopic data analysis, including 1D and 2D nuclear magnetic resonance (NMR) and high-resolution electrospray ionization-mass spectrometry (HR-ESIMS) and computational methods for electronic circular dichroism (ECD) calculations and specific optical rotation. Herein, we described the isolation and structural elucidation of the isolated diterpenes 1–3 and evaluation of their anti-Helicobacter pylori activity.

2. RESULTS AND DISCUSSION

2.1. Isolation of Diterpenes from P. eldarica Needles.

The MeOH extract of P. eldarica needles was fractionated by solvent partition, which afforded the hexane-, \(CH_2Cl_2\)-, EtOAc-, and BuOH-soluble fractions. Each fraction was further subjected to LC–MS analysis, referencing an in-house UV library database in our LC–MS system, which revealed that the hexane-soluble fraction mainly contains diterpenes, which was also confirmed by thin layer chromatography (TLC) analysis. In addition, recent studies have reported that labdane diterpenoids and clerodane diterpenes isolated from Andrographis paniculata and Polyalthia longifolia leaves, respectively, exhibited anti-\(H. pylori\) activities\(^9\)\(^{10\ a\ b}\) indicating the potential of diterpenes as antimicrobial agents against \(H. pylori\). Based on these results and preliminary data, the hexane-soluble fraction was subjected to phytochemical examination via repeated column chromatography and semipreparative high-performance liquid chromatography (HPLC) under the guidance of LC–MS analysis, resulting in the isolation of three labdane-type diterpenes (1–3), including a new norlabdane-type diterpene (1) (Figure 1).

2.2. Structural Elucidation of the Isolated Diterpenes 1–3. Compound 1 was isolated as an amorphous white powder, and its molecular formula was determined to be \(C_{29}H_{34}O_3\) by the positive-ion mode of HR-ESIMS, which revealed an \([M + Na]^+\) ion peak at \(m/z\ 331.2250\) (calcd for \(C_{29}H_{35}O_3Na\, 331.2249\)). The established molecular formula for compound 1 exhibits 4 degrees of unsaturation. The \(^1\)H NMR data (Table 1) of compound 1, assigned with the aid of a heteronuclear single quantum correlation (HSQC) experiment, revealed the presence of characteristic signals for one terminal vinyl group at \(\delta\_H\ 5.87\) (1H, dd, \(J = 17.5, 10.5\) Hz, H-14), 5.14 (1H, dd, \(J = 17.5, 1.5\) Hz, H-15a), 4.92 (1H, dd, \(J = 17.5, 1.5\) Hz, H-15b), and 4.22 (1H, dd, \(J = 10.5, 1.5\) Hz, H-16a) and another vinyl group at \(\delta\_H\ 6.87\) (1H, dd, \(J = 17.5, 10.5\) Hz, H-16b) and 5.14 (1H, dd, \(J = 10.5, 1.5\) Hz, H-16c). The remaining protons were assigned based on HSQC and HMBC experiments. The 

![Figure 1](https://doi.org/10.1021/acsomega.2c04147)

**Figure 1.** Chemical structures of compounds 1–3.

| position | \(\delta\_H\) (\(J\) in Hz) | \(\delta\_C\) (\(\delta\_H\) in ppm) |
|----------|------------------|------------------|
| 1\(\alpha\) | 1.65, m | 38.5 CH\(_2\) |
| 1\(\beta\) | 0.89, m | |
| 2\(\alpha\) | 1.43, m | 17.5 CH\(_2\) |
| 2\(\beta\) | 1.74, m | |
| 3\(\alpha\) | 2.13, m | 34.9 CH\(_2\) |
| 3\(\beta\) | 1.17, m | |
| 4 | | 84.0 C |
| 5 | 1.20, m | 55.9 CH |
| 6\(\alpha\) | 1.84, m | 19.7 CH\(_2\) |
| 6\(\beta\) | 1.47, m | |
| 7\(\alpha\) | 1.38, m | 43.1 CH\(_2\) |
| 7\(\beta\) | 1.84, m | |
| 8 | | 74.9 C |
| 9 | 1.32, m | 55 CH |
| 10 | | 37.1 C |
| 11\(\alpha\) | 1.58, m | 15.4 CH\(_2\) |
| 11\(\beta\) | 1.49, m | |
| 12\(\alpha\) | 1.77, m | 35.5 CH\(_2\) |
| 12\(\beta\) | 1.63, m | |
| 13 | | 73.5 C |
| 14 | 5.87, dd (17.5, 10.5) | 147.6 CH |
| 15\(a\) | 4.92, dd (10.5, 1.5) | 110.3 CH \(_2\) |
| 15\(b\) | 5.14, dd (17.5, 1.5) | |
| 16 | 1.27, s | 28.4 CH\(_3\) |
| 17 | 1.31, s | 25.1 CH\(_3\) |
| 18 | 1.28, s | 24.3 CH\(_3\) |
| 19 | 0.88, s | 14.9 CH\(_3\) |

\(^a\)Coupling constants (Hz) are given in parentheses. \(^b\)\(^1\)C NMR data are assigned based on HSQC and HMBC experiments.
The relative configuration of compound 1 was established via nuclear Overhauser effect spectroscopy (NOESY) analysis. The NOESY correlations from H-19 to H-16, H-17, and H-18 confirmed that the corresponding methyl groups have same orientation (Figure 3), and the peroxide group at C-4 has opposite orientation. The NOESY cross-peaks from H-9 to H-14 indicated that the corresponding protons have opposite configurations to the methyl groups at C-16, C-17, C-18, and C-19 (Figure 3).

Finally, the absolute configuration of compound 1 was confirmed by quantum chemical calculations for ECD simulations and comparison of optical rotation values. Two possible isomers, 1a (4R,5R,8R,9R,10R,13R) and 1b (4S,5S,8S,9S,10S,13S), were calculated for the ECD data, and the experimental ECD spectrum of compound 1 was compared with the obtained ECD data (Figure 4). The experimental ECD data highly correlated with the obtained ECD data of 1a, which confirmed the absolute configuration of compound 1 as 4R,5R,8R,9R,10R,13R. The stereochemistry of compound 1 was also confirmed by the optical rotation values obtained for 1 ([[\alpha]_D]^{25} + 8.9 (c 0.10, CHCl_3)) and 4R-hydroxy-18-normanoloy oxide ([[\alpha]_D]^{25} + 9.6 (c 0.08, CHCl_3)). Therefore, the chemical structure of 1, including its absolute configuration, was determined as shown in Figure 1, designated eldaricin A. Although the representative sesquiterpene with the peroxide group, artemisinin, has been noted, the norlabdane-type diterpene (1) with the peroxide group was one of the relatively unique natural products.

The other isolated compounds were identified as (+)-manool oxide (2)\(^\text{28}\) and manool oxide acid (3)\(^\text{29}\) (Figure 1) by comparing their NMR spectral and physical data with those reported earlier and the data from LC–MS analysis.

### 2.3. Evaluation of Antibacterial Activity of the Isolated Diterpenes against H. pylori

H. pylori infection is a major public health challenge, affecting approximately 50% of the global population.\(^\text{30}\) Eradication of H. pylori can help in the treatment of gastric and duodenal ulcers and gastric cancer since H. pylori is known to be the causative agent associated with several gastric and duodenal pathologies.\(^\text{31}\) As aforementioned, recent studies have reported that several labdane-type diterpenoids exhibit anti-H. pylori activity.\(^\text{25}\) Thus, the isolated labdane-type diterpenes 1–3 obtained in this study were evaluated for their antibacterial activity against H. pylori strain S1 (Table 2). Among the isolates, compound 3 exhibited the most potent antibacterial activity against H. pylori strain S1, inducing 72.7% inhibition at a final concentration of 100 \(\mu\)M, comparable to that of metronidazole (97.0% inhibition) as a positive control, and it showed an MIC\(_{50}\) value of 92 \(\mu\)M. The novel compound 1 exhibited moderate antibacterial activity against H. pylori strain S1, inducing 54.5% inhibition, which...
was higher than that of quercetin (34.4% inhibition) as a positive control, and it showed an MIC$_{50}$ value of 95 μM. Compound 2 exhibited weak activity against H. pylori strain S1, inducing 26.8% inhibition (Table 2). Based on these findings, the presence of carboxyl and peroxide groups at C-4 in the labdane-type diterpenes may be significant in the anti-H. pylori activity, with the carboxyl group appearing to have a more positive effect. Further studies are required to elucidate the mechanism by which compound 3 inhibits H. pylori growth.

3. CONCLUSIONS

In this study, phytochemical investigation of the MeOH extract of P. eldarica needles collected in Iran resulted in the isolation and identification of a novel norlabdane-type diterpene (1) with a peroxide moiety and two known labdane-type diterpenes inhibited the growth of H. pylori strain S1, inducing 72.7% inhibition (MIC$_{50}$). The most potent antibacterial activity against other pathogenic bacteria and toxicity mechanism of compounds 2 and 3 to inhibit the growth of H. pylori. In addition, specificity toward H. pylori including antibacterial activity against other pathogenic bacteria and toxicity of these compounds is also required in the following study. This study provides experimental evidence that bioactive labdane-type diterpenes can serve as natural antibacterial agents against H. pylori.

4. EXPERIMENTAL SECTION

4.1. General Experimental Procedures. Optical rotations were measured using a JASCO P-2000 polarimeter (JASCO, Easton, MD, USA). Ultraviolet (UV) spectra were obtained using an Agilent 8453 UV-visible spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). The ECD spectra were obtained using a JASCO J-1500 spectropolarimeter (JASCO). Infrared (IR) spectra were obtained using a Bruker IFS-66/S FT-IR spectrometer (Bruker, Karlsruhe, Germany). NMR spectra were obtained using a Bruker AVANCE III HD 800 NMR spectrometer with a 5 mm TCI CryoProbe operating at 850 MHz (1 H) and 212.5 MHz (13 C), with chemical shifts given in ppm (δ) for 1 H and 13 C NMR analyses. All HR-ESIMS data were obtained using an Agilent G6545B quadrupole time-of-flight mass spectrometer (Agilent Technologies) coupled to an Agilent 1290 Infinity II HPLC instrument using an Agilent Eclipse Plus C18 column (2.1 × 50 mm, 1.8 μm; flow rate: 0.3 mL/min). Preparative HPLC was performed using a Waters 1525 Binary HPLC pump with a Waters 996 photodiode array detector (Waters Corporation, Milford, MA, USA) and a Hector C18 column (250 × 21.2 mm, 5 μm; flow rate: 5 mL/min; Rstech Corporation, Korea). Semipreparative HPLC was performed using a Shimadzu Prominence HPLC System with SPD-20A/20AV Series Prominence HPLC UV-vis detectors (Shimadzu, Tokyo, Japan) and a Phenomenex Luna phenyl-hexyl column (250 × 10 mm, 5 μm; flow rate: 2 mL/min; Phenomenex, Torrance, CA, USA). The LC–MS analysis was performed using an Agilent 1200 Series HPLC system equipped with a diode array detector and 6130 Series ESI mass spectrometer using an analytical Kinetex C18 100 A column (100 × 2.1 mm, 5 μm; flow rate: 0.3 mL/min; Phenomenex). Silica gel 60 (230–400 mesh; Merck, Darmstadt, Germany) was used for column chromatography. TLC was performed using pre-coated silica gel F254 and RP-C18 F254s plates (Merck), and spots were detected under UV light or by heating following spraying with anisaldehyde-sulfuric acid.

4.2. Plant Material. P. eldarica needles were collected in May and June 2018 from the Tabriz district of Iran, which was identified by one of the authors (H. Hamishehkar). A voucher specimen (no.4036) was deposited in the Herbarium of the Pharmacy Faculty, Tabriz University of Medical Sciences.

4.3. Extraction and isolation. Finely ground P. eldarica needles were extracted in MeOH using a Soxhlet extractor for 6 h at 40 °C, and the extraction rate was 16%. The extracts were collected and filtered through Whatman filter paper no.1, and the filtrates were concentrated under vacuum at 35 °C using a rotary evaporator (Heidolph, Germany). The resulting crude MeOH extract (296.3 g) was suspended in distilled water (700 mL) and subjected to solvent partitioning with hexane, CH$_2$Cl$_2$, EtOAc, and n-BuOH (each 700 mL × 3), yielding its four corresponding solvent-partitioned fractions. Based on the LC–MS and TLC analyses of each fraction derived from solvent partitioning, we easily observed that the hexane-soluble fraction contains major non-polar compounds with characteristic colors indicating diterpenes following spraying with anisaldehyde-sulfuric acid via TLC analysis. The hexane-soluble fraction (19.9 g) was loaded onto a silica gel open column chromatography apparatus and fractionated using a gradient solvent system of hexane-EtOAc (30:1-1:1; v/v) to yield 13 subfractions (H1–H13). Subfraction H7 (260 mg) was further isolated by preparative HPLC (gradient solvent system from 80% MeOH/H$_2$O to 100% MeOH) to yield five subfractions (H71–H75). Subfraction H72 (55 mg) was purified by semipreparative HPLC using an isocratic system of 77% MeOH/H$_2$O, which further yielded four subfractions (H721–H724). Subfraction H721 (27.6 mg) was further purified by semipreparative HPLC with an isocratic system of 65% MeCN/H$_2$O to yield compounds 3 (t$_R$ 19.5 min, 5.8 mg) and 1 (t$_R$ 21.0 min, 2.1 mg). Subfraction H73 (46 mg) was purified by semipreparative HPLC with an isocratic system of 87% MeOH/H$_2$O to isolate compound 2 (t$_R$ 39.5 min, 1.0 mg).

4.4. ECD Calculation. Initial conformational searches were performed in the MMFF94 force field using the MacroModel (version 2021-4, Schrödinger LLC) program with a mixed torsional/low-mode sampling method, in which a gas phase
with a 50 kJ/mol energy window and 10,000 maximum iterations was employed. The Polak–Ribiere conjugate gradient algorithm was established with 10,000 maximum iterations and a 0.001 kJ (mol Å)^2 convergence threshold on the root-mean-square gradient to minimize conformers. The conformers proposed in this study (found within 5 kJ/mol in the MMFF force field) were selected for geometry optimization using TmoleX 4.3.2 with the density functional theory settings of B3-LYP/6-31+G (d,p). 32

ECD calculations for the 1a and 1b conformers (six conformers each) were performed at an identical theoretical level and basis sets. The calculated ECD spectra were simulated by superimposing each transition, where σ is the bandwidth at height 1/e and ΔE_i and R_i are the excitation energy and rotatory strength for transition i, respectively. 32 In this study, the value of σ was 0.2 eV. The excitation energies and rotatory strengths of the ECD spectra were calculated based on the Boltzmann populations of the conformers, and ECD visualization was performed using SigmaPlot 14.0.

\[ Δσ(ε) = \frac{1}{2.977 \times 10^{-35}} \frac{1}{\sqrt{2πσ}} \sum_α ΔE_i R_i e^{-(ε-ΔE_i)^2/(2σ)^2} \]

4.5. H. pylori Culture. The clinical strain of H. pylori 51 (HPKTCC B0006) isolated from a Korean patient with a duodenal ulcer was provided by the H. pylori Korean Type Culture Collection, School of Medicine, Gyeongsang National University, Korea. The strain was cultured and maintained on Brucella agar (BD Co., Sparks, MD, USA) supplemented with 10% horse serum (Gibco, New York, USA). The culture conditions were 37 °C, 100% humidity, and 10% CO_2 for 2–3 days.

4.6. Determination of Minimal Inhibitory Concentration (MIC) Values. Minimal inhibitory concentrations (MICs) were determined using the broth dilution method as previously reported. 33, 34 Twenty microliters of the bacterial colony suspension, equivalent to 2–3 × 10^8 CFU/mL and 20 μL of twofold diluted test samples and controls, respectively, was added to each well of a six-well plate containing Brucella broth medium supplemented with 10% horse serum. The final volume was made to 2 mL. After 24 h of incubation, the bacterial growth was evaluated by measuring the optical density at 600 nm using a spectrophotometer. The MIC_0 and MIC_90 values were defined as the lowest concentrations of samples at which bacterial growth was inhibited by 50 and 90%, respectively, and were computed using Microsoft Excel (Redmond, WA, USA). All values were obtained from two independent experiments.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c04147.

HR-ESIMS and 1D and 2D NMR spectra of compound 1 (PDF)

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**Notes**

The authors declare no competing financial interest.

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**REFERENCES**

1. Michelozzi, M.; Tognetti, R.; Maggino, F.; Radicati, M. Seasonal variations in monoterpene profiles and ecophysiological traits in Mediterranean pine species of group “halepensis”. iForest 2008, 1, 65–74.

2. Alizadeh, M.; Safae, N.; Shams-Bakhsh, M.; Mehrabadi, M. Neoscytalidium novaehollandiae causes dieback on Pinus eldarica and its potential for infection of urban forest trees. Sci. Rep. 2022, 12, 9337.

3. Ghaffari, T.; Kafil, H. S.; Asnaashari, S.; Farajnia, S.; Delazar, A.; Baek, S. C.; Hamishehkar, H.; Kim, K. H. Chemical composition and antimicrobial activity of essential oils from the aerial parts of Pinus eldarica grown in Northwestern Iran. Molecules 2019, 24, 3203.

4. Hames-Kocabas, E. E.; Yesil-Celiktas, O.; Isleten, M.; Vardar-Sukan, F. Antimicrobial activity of pine bark extract and assessment of potential application in cooked red meat. Gida 2008, 33, 123–127.

5. Rohdewald, P. A review of the French maritime pine bark extract (Pycnogenol), a herbal medication with a diverse clinical pharmacology. Int. J. Clin. Pharmacol. Ther. 2002, 40, 158–168.

6. Potta, S. P.; Doss, M. X.; Hescheler, J.; Sachinidis, A. Epigallocatechin-3-gallate (EGCG): A structural target for the development of potential therapeutic drugs against anti-proliferative diseases. Drug Des. Rev.-Online 2005, 2, 85–91.

7. Li, K.; Li, Q.; Li, J.; Gao, D.; Zhang, T.; Han, Z.; Zheng, F. Effect of procyanidins fromPinus koraiensis bark on growth inhibition and...
expression of PCNA and TNF-α in mice with U14 cervical cancer. *Therapy* 2007, 4, 685−690.

(8) Mamedov, N.; Craker, L. E. Medicinal plants used for the treatment of bronchial asthma in Russia and Central Asia. *J. Herbs, Spices Med. Plants* 2001, 8, 91−97.

(9) Mamedov, N.; Gardner, Z.; Craker, L. E. Medicinal plants used in Russia and Central Asia for the treatment of selected skin conditions. *J. Herbs, Spices Med. Plants* 2005, 11, 191−222.

(10) Irvani, S.; Zolfaghari, B. Phytochemical analysis of *Pinus eldarica* bark. *Res. Pharm. Sci.* 2014, 9, 243.

(11) Hosseinzadeh, H.; Khoei, A. R.; Khashayarmanesh, Z.; Motamed-Shariati, V. Anti-ulcerative activity of *Pinus eldarica* medw: fruits aqueous extract in rats. *Urol. J.* 2010, 7, 232.

(12) Huseini, H. F.; Anvari, M. S.; Rabban, S.; Sharifi, F.; Arzagh, S. M.; Fakhhradzeh, H. Anti-hyperlipidemic and anti-atherosclerotic effects of *Pinus eldarica* Medw. nut in hypercholesterolemic rabbits. *Daru* 2015, 23, 32.

(13) Sadeghi, M.; Zolfaghari, B.; Jahanian-Najafabadi, A.; Abtahi, S. R. Anti-pseudomonas activity of essential oil, total extract, and proanthocyanidins of *Pinus eldarica* Medw. bark. *Res. Pharm. Sci.* 2016, 11, 58.

(14) Sharifan, A.; Etebari, M.; Zolfaghari, B.; Alimomani, M. Investigating the effects of bark extract and volatile oil of *Pinus eldarica* against cisplatin-induced genotoxicity on HUVECs cell line. *Toxicol. Res.* 2021, 10, 223−233.

(15) Bolandghamat, S.; Moghimi, A.; Iranshahi, M. Effects of ethanolic extract of pine needles (*Pinus eldarica* Medw.) on reserpine-induced depression-like behavior in male Wistar rats. *Pharmacogn. Mag.* 2011, 7, 248.

(16) Mansouri, S.; Hosseini, M.; Behesthi, F.; Sobhanifar, M. A.; Rakhshandeh, H.; Anaiegoudari, A. Neuroprotective effects of *Pinus eldarica* in a mouse model of pentylenetetrazole-induced seizures. *Avicenna J. Phytomed.* 2021, 11, 610.

(17) Hajhashemi, V.; Zolfaghari, B.; Amin, P. Anti-nociceptive and anti-inflammatory effects of hydroalcoholic extract and essential oil of *Pinus eldarica* in animal models. *Avicenna J. Phytomed.* 2021, 11, 494.

(18) Lee, S.; Kang, H.; Yoo, M. J.; Yoon, U. J.; Ryoo, K. H.; Bae, H. Y.; Kim, K. H. Ergopyrone, a Styrlypyrone-Fused Steroid with a Hexacyclic 6/5/6/6/5 Skeleton from a Mushroom *Gymnopilus orientispectabilis*. *Org. Lett.* 2021, 23, 3315−3319.

(19) Khalil, A. A. K.; Park, W. S.; Lee, J.; Kim, H. J.; Akter, K. M.; Goo, Y. M.; Bae, J. Y.; Chun, M. S.; Kim, J. H.; Ahn, M. J. A new anti-Helicobacter pylori juglone from *Reynoutria japonica*. *Arch. Pharmacal Res.* 2019, 42, 505−511.

(20) Amin, M.; Anwar, F.; Naz, F.; Mehmoond, T.; Saari, N. Anti-Helicobacter pylori and urease inhibition activities of some traditional medicinal plants. *Molecules* 2013, 18, 2135−2149.

(21) Ybarra, M. I.; Popich, S.; Borkosky, S. A.; Asakawa, Y.; Bardón, A. Manoyl Oxide Diterpenoids from *Grindelia scorzonerifolia*. *J. Nat. Prod.* 2005, 68, 554−558.

(22) Decorzant, R.; Vial, C.; Nif, F.; Whitesides, G. M. A short synthesis of ambrox from sclareol. *Tetrahedron* 1987, 43, 1871−1879.

(23) Andersson, R.; Greff, R.; Lundgren, L. N. Manoyl oxide acid from resin of *Pinus sylvestris* needles. *Phytochemistry* 1990, 29, 1320−1322.

(24) Shaikh, R. U.; Dawane, A. A.; Pawar, R. P.; Gond, D. S.; Meshram, R. J.; Gacche, R. N. Inhibition of Helicobacter pylori and urease activities. *Comparative structural features, anti-histaminic and anti-Helicobacter pylori activities. Two clerodane diterpenes isolated from* *Polyalthia longifolia* leaves: Comparative structural features, anti-histaminic and anti-Helicobacter pylori activities. *Nat. Prod. Res.* 2021, 35, 5282−5286.

(25) Edmond, M. P.; Mostafa, N. M.; El-Shazly, M.; Singab, A. N. B. Comparison of the antibacterial effects of *Juniperus chinensis* Juniperus chinensis *Phytochemistry* 1995, 39, 391−394.