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HEMISYNTHESIS AND BACTERICIDAL ACTIVITY OF SEVERAL SUBSTITUTED BENZOIC ACID ESTERS OF 13(*S*)-LABDAN-8α,15-DIOL, A DITERPENE FROM *Oxylobus glanduliferus*

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*The diterpene 13(*S*)-labdan-8*α*,15-diol (**1**) was isolated in high yield from* Oxylobus glanduliferus*, a native species of Venezuelan Andean moorlands. Using this compound (**1**) as a starting material, it was possible to prepare 12 aromatic esters, which were structurally characterized by analysis of their spectroscopic data (IR, 1D and 2D NMR and MS). The bactericidal activity of these diterpene derivatives was evaluated against four bacterial strains [two Gram-positive and two Gram-negative]:* Staphylococcus aureus*,* Enterococcus faecalis*,* Escherichia coli*, and* Pseudomonas aeruginosa*.*

Keywords: labdanes, substituted benzoic acids, esterifications, NMR, bactericidal activity.

Labdanes are a group of bicyclic diterpenes, whose basic skeleton comprises a *trans*-decalin pentasubstituted system, in which the substituent at C-9 is a six-carbon isoprene chain [1]. The interest in studying labdanes is heightened due to the wide range of biological activities they exhibit, such as antibacterial, antifungal, anti-inflammatory, cardiotonic, cytotoxic, and various effects on the mammalian enzyme system, among others [2, 3].

Simple substituted benzoic acids (SBA) and their esters are widely distributed in nature. These compounds can occur in its free state or conjugated form in many fungi and plants [4, 5] and also in beverages and juices [6], fruits [7], berries [8] black and green tea [9], wines [10], and vegetable foods [11, 12]. It is likewise appropriate to emphasize that there are a diverse variety of natural bioactive terpenoids possessing a hydroxyl group esterified with SBA, such as *p*-hydroxybenzoic, anisic, gallic, genistic, vanillic, or protocatechuic acids [13–17]. QSAR studies have revealed the important role of the substituted benzoyl groups in the biological activity of these compounds [18, 19].

Literature on SBA and its derivatives is extensive; these compounds, well known as active substances, seem to exhibit a wide range of biological effects [4, 5, 20]. The antifungal activity of natural and semisynthetic gallates has been tested against *Aspergillus niger*, *Candida albicans*, *Saccharomyces cerevisiae*, and *Zygosaccharomyces bailii* [21].

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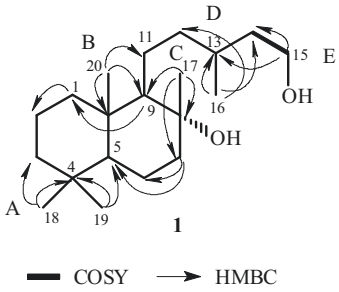


Fig. 1. Key ^{1}H–^{1}H COSY and HMBC correlations of **1.**

Significant antibacterial activity against Gram-positive and Gram-negative bacteria such as *Bacillus cereus*, *B. subtilis*, *Brevibacterium ammoniagenes*, *Escherichia coli*, *Micrococcus luteus*, *Propionibacterium acnes*, *Pseudomonas fluorescens*, *Salmonella* ssp., *Staphylococcus aureus*, and *Streptococcus mutans* has also been reported in several SBA esters [22–25]. In this sense, it is particularly noteworthy that esters of 4-hydroxybenzoic acid, also called parabens, are widely used as antimicrobial preservative agents in a large variety of food, beverages, and pharmaceutical and cosmetic products due to their excellent antimicrobial activities [26].

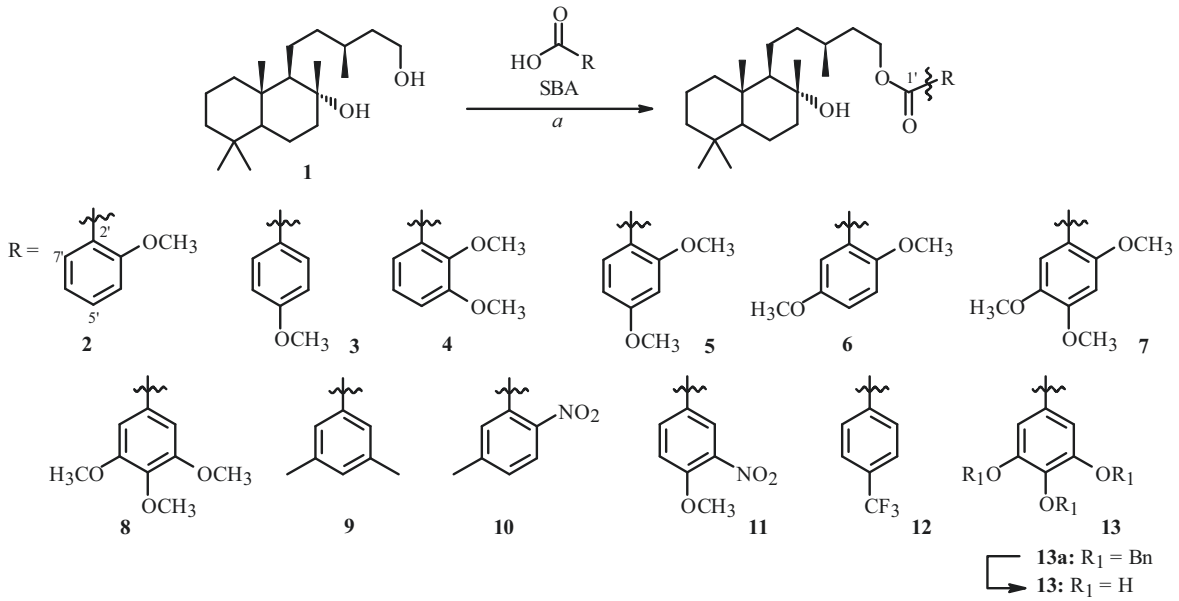
With the above, in this paper we approach the preparation of several aromatic esters from 13(*S*)-labdan-8α,15-diol (**1**), a diterpene isolated from *Oxylobus glanduliferus* [26], as well as the evaluation of their bactericidal activity against four bacterial strain.

Compound **1** was isolated as a white crystalline solid. Analysis of its ^{1}H and ^{13}C NMR spectral data allowed us to establish the molecular formula as $C_{20}H_{38}O_2$, which requires two degrees of unsaturation. Data of IR and ^{13}C NMR indicated that compound **1** has no double bonds or carbonyl groups, and therefore it must be bicyclic. The ^{1}H NMR spectrum shows a doublet and three singlets, assigned respectively to a secondary and four tertiary methyl groups. Expected correlations between ^{1}H NMR methyl group signals and DEPT-135/^{13}C-peaks observed in the HMQC spectrum allowed the assignment of ^{1}H and ^{13}C NMR signals of all methyl groups [δ_H 0.85 (d, J ≅ 6.3 Hz, H-16) ↔ δ_C 20.0 (C-16); δ_H 0.85 (s, H-18) ↔ δ_C 33.5 (C-18); δ_H 0.78 (s, H-19) ↔ δ_C 21.6 (C-19); δ_H 0.78 (s, H-20) ↔ δ_C 15.5 (C-20); δ_H 1.12 (s, HO-C-CH_3, H-17) ↔ δ_C 24.0 (HO-C-CH_3, C-17)]. In DEPT-135, three-methine peaks were detected [δ_C 56.2 (C-5), 62.5 (C-9), and 30.6 (C-13)]. In the negative phase of DEPT-135, nine peaks corresponding to methylenes were observed [δ_C 39.8 (C-1), 18.5 (C-2), 42.1 (C-3), 20.6 (C-6), 44.4 (C-7), 23.1 (C-11), 41.1 (C-12), 39.8 (C-14) and 60.8 (-CH_2-OH, C-15)] and three quaternary carbons, which are not detected in DEPT [δ_C 33.3 (C-4), 74.6 (>C-OH; C-8) and 39.2 (C-10)]. These data allowed us to establish that the compound under study is possibly a labdane or a halimane diterpene, because the number of methyl, methylene, methine, and quaternary carbons match only with skeletons of this type [27]. Correlations observed in the HMBC spectrum allowed us to establish the presence in the molecule of a *gem*-dimethyl group [H-18 ↔ C-4 ↔ H-19 (A)], an angular methyl [H-20 ↔ C-10 (B)], a methyl geminal to a hydroxyl group [H-17 ↔ C-8 (C)], a secondary methyl [H-16 ↔ C-13 (D)], and a terminal primary hydroxy group [H-13 ↔ C-15 ↔ H-14 (E)]. The position of the latter group was also supported by the ^{1}H–^{1}H COSY spectrum. The connectivity between fragments A, B, C, D, and E was established by their correlations in HMBC with methines and methylenes as indicated in Fig. 1.

Complete stereochemistry of the diol **1** was previously determined [26] by oxidation with Jones reagent to produce the corresponding diterpene acid, whose physical and spectral data were consistent with those reported in the literature for labdanolic acid [13(*S*)-labdan-8α,15-oic acid; (CAS No.10267-24-0)] [28]. Consequently, we reached the conclusion that compound **1** is the stereoisomer of labdan-8α,15-diol with configuration (*S*) at C-13.

Compound **1** was esterified using a variety of aromatic acids. The structure of each ester obtained was identified on the basis of spectroscopic analysis, including two-dimensional NMR. The signals of the ester derivative diterpene nucleus are practically unchanged (except for methylene C-15, whose signals are significantly deshielded); for this reason, spectral analysis is focused on the aromatic regions of the ^{1}H and ^{13}C NMR spectra. Also, they were particularly important in the HMBC spectra, i.e., the correlations between H-15 (oxymethylene protons of diol **1**) and the carbonyl carbon (C-1′) of each ester obtained; this fact is an important evidence to confirm the formation of the desired product.

Scheme 1 shows the overall esterification reaction and all derivatives obtained by the coupling between 13(*S*)-labdan-8α,15-diol (**1**) and aromatic acids. It should be emphasized that the hydroxyl group at C-8 (corresponding to tertiary alcohol) was never esterified.



a. CH_2Cl_2, DMAP, DIC, r.t.

Scheme 1.

The bactericidal activity was directly assessed using the method of minimum inhibitory concentrations (MIC) [29] and minimum bactericidal concentrations (MBC), which were determined by the microdilution method in liquid medium in 96-well plates (ELISA plates), where each compound was tested at a maximum concentration of 600 μg/mL. Plates were inoculated with 100 μL of a suspension of the microorganism to be tested, made from preinocula (prepared as described in the experimental section), so that it was at an initial cell density of $1–5 \times 10^5$ CFU/mL. Each test was performed in duplicate, and positive controls were inoculated under the same conditions, in DMSO, at a concentration equivalent to the maximum used in crop problems. A negative control (blank control) was prepared by adding 200 μL of medium to the wells in column 1. After 24 h incubation at 37°C in orbital shaking, the turbidity was observed. From those wells in which no visible growth was observed, aliquots were taken (100 μL) for a viable count on nutrient agar plates in order to establish the MIC (lowest concentration of the ester at which there is no visible growth of the microorganism) and MBC (minimal concentration of the ester required to kill 99.9% of the initial microbe population) using the microdilution method and based on a concentration of 600 μg/mL.

All compounds showed MIC higher than 600 μg/mL against each bacterial strain. Only gallate **13** was shown to have a moderate MIC between 600 and 300 μg/mL against *S. aureus*. These results are consistent with those reported in the literature, since all the derivatives of SBA that show bactericidal activity have hydroxy groups in the benzene ring [14, 15, 17].

The aerial parts of *Oxylobus glanduliferus* are an important source of 13(*S*)-labdan-8α,15-diol (**1**). The high yield of this diterpene in the plant ensures that the same is an appropriate source as starting material to continue performing chemical transformations for obtaining a wide group of derivatives in order to test other potential biological activities, such as lemanicide, fungicide, antialgal, cytotoxic, etc. Additionally, due to the high concentration of compound **1** in *O. glanduliferus*, it is recommended to carry out studies to establish the ecological role of this compound in the plant.

EXPERIMENTAL

General Procedures. All moisture-sensitive reactions were carried out in flame-dried glassware under argon atmosphere. The solvents used, tetrahydrofuran (THF) and dichloromethane (CH_2Cl_2), were treated immediately before use with the MBRAUN Solvent Purification System. Dimethylformamide (DMF) was distilled on CaH_2 under argon prior to use. Evaporations were conducted under reduced pressure at temperatures less than 40°C unless otherwise noted. Column chromatography was carried out under positive pressure using 40–63 μm silica gel (Merck) as adsorbent and the indicated solvents in each case. Melting points were determined with a Fisher–Johns instrument and have not been corrected. Optical activity was measured in $CHCl_3$ on a 60 Hz-Steeg & Reuter G.m.b.H. polarimeter. IR spectra were recorded on a PerkinElmer FT-1725X spectrophotometer as film or KBr pellets. ^1H, ^{13}C and two-dimensional NMR spectra, in the indicated solvents, were acquired with a Bruker-Avance DRX-300 instrument and calibrated using the residual solvent peak as internal standard.

Mass spectra were recorded on a Hewlett-Packard Spectrometer, model 5930A (70 eV). Analytical thin-layer chromatography (TLC) was developed on 0.25 mm layers of silica gel plates HF 254 (Merck), and spots were visualized by spraying with a mixture of water (235 mL), ammonium molybdate (12 g), ammonium cerium nitrate (0.5 g), and concentrated sulfuric acid (15 mL) and then heating with air flow at 100°C for a few seconds.

Plant Material and Extraction. Leaves and stems were collected at "Paramo El Aguila, Municipio Autonomo Rangel, Estado Merida, Venezuela." The plant was identified as *Oxylobus glanduliferus* (Sch.Bip. ex Benth. & Hook.f.) A. Gray by Eng. Juan Carmona Arzola, Department of Pharmacognosy and Organic Medicaments, Faculty of Pharmacy and Bioanalysis, University of Los Andes (ULA); a voucher specimen (J. M. Amaro & D. Villalobos, No. 1642) was deposited at the Herbario MERF of this faculty. The dried uncrushed leaves and stems (≅ 8.7 kg) were extracted with dichloromethane at room temperature for 5 min. Later, the material was dried under a hood and then extracted with methanol in a Soxhlet apparatus until exhaustion. Solutions from both extractions were filtered and then concentrated under vacuum in a rotary evaporator at temperatures no higher than 40°C. The extracts obtained were weighed (dichloromethane extract ≅ 665 g) and (methanol extract ≅ 2127 g) and stored in a refrigerator at temperatures below 4°C until use.

Isolation and Identification of 13(*S*)-Labdan-8α,15-diol (1). Dichloromethane extract was dissolved in hexane at 40°C to remove low-polarity products. Insoluble residue was dissolved in acetone at 40°C, preadsorbed on silica gel, and chromatographed (VLC, vacuum liquid chromatography) over silica gel 60, eluting with hexane and EtOAc in mixtures of increasing polarity. Fractions of 500 mL were collected and combined according to the TLC characteristics to afford 14 reunions (A–N). For reunions H, I, and J (hexane–EtOAc, 3:2 and 1:1), a white solid precipitated, which was recrystallized several times in ethanol; it was possible to recover about 18 g of pure compound **1**. White needles, mp 81–83°C; $[\alpha]_D$ –9.0° (*c* 0.13, $CHCl_3$). LR-EI-MS *m/z* 310 $[M]^+$ ($C_{20}H_{38}O_2$). IR (KBr, ν_{max}, cm^{-1}): 3360 (OH), 2926, 2869 (C–H), 1386, 1364 (C–H), 1062 (C–O). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 3.65 (2H, m, H-15), 1.84 (2H, dt, J = 12.0, 3.1, H-7), 1.12 (3H, s, H-17), 0.99 (1H, t, J = 3.1, H-9), 0.89 (3H, d, J = 6.3, H-16), 0.85 (3H, s, H-18), 0.77 (6H, s, H-19, 20). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 74.6 (C-8), 62.5 (C-9), 60.8 (C-15), 56.2 (C-5), 44.4 (C-7), 42.1 (C-3), 41.1 (C-12), 39.8 (C-1), 39.8 (C-14), 39.2 (C-10), 33.5 (C-18), 33.3 (C-4), 30.6 (C-13), 24.0 (C-17), 23.1 (C-11), 21.6 (C-19), 20.6 (C-6), 20.0 (C-16), 18.5 (C-2), 15.5 (C-20).

Preparation of Derivatives of 13(*S*)-Labdan-8α,15-diol (2–13).

General Procedure. A stirred mixture of SBA, 4-(dimethylamino)pyridine (DMAP), and labdane **1** dissolved in dry CH_2Cl_2 was put into an ice-water bath and *N*,*N*′-diisopropylcarbodiimide (DIC) was added. The stirred solution at room temperature was monitored by TLC until disappearance of the starting material. The reaction mixture was washed with a solution of 0.1 N HCl (the volume was calculated according to the number of equivalents of DMAP) and brine (100 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. Reaction products were purified by flash column chromatography.

13(*S*)-Labdan-8α-ol-15-yl 2-Methoxybenzoate (2). The general procedure was followed using 2-methoxybenzoic acid (100 mg, 0.658 mmol), DIC (0.1 mL, 0.644 mmol), DMAP (120 mg, 0.982 mmol), and 13(*S*)-labdan-8α,15-diol **1** (100 mg, 0.322 mmol) in dry-CH_2Cl_2 (7 mL). The crude was purified by flash column chromatography, eluting with cyclohexane–EtOAc (5:1), resulting in a colorless resin (yield 22%). MS *m/z* 444.4 $[M]^+$. IR (film, ν_{max}, cm^{-1}): 3516 (OH), 3076 (=C–H), 2934, 2870 (C–H), 1714 (C=O), 1602 (C=C), 1302, 1254, 1132, 1084 (C–O–C), 938, 756 (=C–H). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.77 (1H, dd, J = 7.9, 1.8, H-7′), 7.45 (1H, m, H-6′), 6.98 (2H, m, H-4′, 5′), 4.49–4.20 (2H, m, H-15), 3.89 (3H, s, 3′-OCH_3), 1.85 (2H, dt, J = 11.5, 2.8, H-7), 1.13 (3H, s, H-17), 1.00 (1H, t, J = 3.7, H-9), 0.96 (3H, d, J = 6.3, H-16), 0.85 (3H, s, H-18), 0.78 (6H, s, H-20, 19). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 166.4 (C-1′), 159.3 (C-3′), 133.4 (C-5′), 131.7 (C-7′), 120.7 (C-2′), 120.2 (C-5′), 112.2 (C-4′), 74.4 (C-8), 63.4 (C-15), 62.6 (C-9), 56.3 (C-5), 56.1 (OCH_3), 44.6 (C-7), 42.1 (C-3), 41.2 (C-12), 39.9 (C-1), 39.3 (C-10), 35.8 (C-14), 33.5 (C-18), 33.4 (C-4), 31.0 (C-13), 24.1 (C-17), 23.1 (C-11), 21.6 (C-19), 20.7 (C-6), 19.6 (C-16), 18.6 (C-2), 15.6 (C-20).

13(*S*)-Labdan-8α-ol-15-yl 4-Methoxybenzoate (3). The general procedure was followed using 4-methoxybenzoic acid (100 mg, 0.658 mmol), DIC (0.1 mL, 0.644 mmol), DMAP (120 mg, 0.982 mmol) and 13(*S*)-labdan-8α,15-diol (**1**) (100 mg, 0.322 mmol) in dry CH_2Cl_2 (7 mL). The crude was purified by flash column chromatography, eluting with cyclohexane–EtOAc (4:1), to provide in quantitative yield a colorless resin. MS *m/z* 444.4 $[M]^+$. IR (film, ν_{max}, cm^{-1}): 3510 (OH), 3078 (=C–H), 2934, 2870 (C–H), 1714 (C=O), 1608 (C=C), 1278, 1258, 1168, 1102 (C–O–C), 938, 848, 772, 698 (=C–H). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 7.97 (2H, d, J = 8.9, H-3′, 7′), 6.88 (2H, d, J = 8.9, H-4′, 6′), 4.42–4.18 (2H, m, H-15), 3.81 (3H, s, 5-OCH_3), 1.83 (2H, dt, J = 12.3, 3.1, H-7), 1.11 (3H, s, H-17), 0.99 (1H, t, J = 3.5, H-9), 0.95 (3H, d, J = 6.1, H-16), 0.83 (3H, s, H-18), 0.76 (6H, s, H-20, 19). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 166.4 (C-1′), 163.3 (C-5′), 131.6

(C-3′, 7′), 123.0 (C-2′), 113.6 (C-4′, 6′), 74.2 (C-8), 63.2 (C-15), 62.4 (C-9), 56.2 (C-5), 55.4 (OCH_3), 44.4 (C-7), 42.0 (C-3), 41.1 (C-12), 39.8 (C-1), 39.2 (C-10), 35.8 (C-14), 33.4 (C-18), 33.3 (C-4), 30.9 (C-13), 24.0 (C-17), 23.0 (C-11), 21.5 (C-19), 20.6 (C-6), 19.6 (C-16), 18.5 (C-2), 15.5 (C-20).

13(*S*)-Labdan-8α-ol-15-yl 2,3-Dimethoxybenzoate (4). The general procedure was followed using 2,3-dimethoxybenzoic acid (120 mg, 0.658 mmol), DIC (0.1 mL, 0.644 mmol), DMAP (120 mg, 0.982 mmol), and 13(*S*)-labdan-8α,15-diol **1** (100 mg, 0.322 mmol) in dry CH_2Cl_2 (7 mL). The crude obtained was purified by flash column chromatography, eluting with cyclohexane–EtOAc (4:1), generating a colorless resin (yield 42%). MS *m/z* 474.4 $[M]^+$. IR (film, ν_{max}, cm^{-1}): 3510 (OH), 3078 (=C–H), 2936, 2870 (C–H), 1708 (C=O), 1584 (C=C), 1266, 1236, 1154, 1062 (C–O–C), 938, 808, 754 (=C–H). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.29 (2H, dd, J = 7.2, 2.3, H-7′), 7.09–6.99 (2H, m, H-5′, 6′), 4.50–4.20 (2H, m, H-15), 3.87 (3H, s, 4′-OCH_3), 3.85 (3H, s, 3′-OCH_3), 1.83 (2H, dt, J = 12.1, 3.0, H-7), 1.11 (3H, s, H-17), 0.98 (1H, t, J = 3.7, H-9), 0.94 (3H, d, J = 6.2, H-16), 0.83 (3H, s, H-18), 0.76 (6H, s, H-20, 19). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 166.5 (C-1′), 153.6 (C-3′), 149.0 (C-4′), 126.6 (C-2′), 123.9 (C-7′), 122.3 (C-5′), 115.7 (C-6′), 74.2 (C-8), 63.6 (C-15), 62.4 (C-9), 61.5 (4′-OCH_3), 56.2 (C-5), 56.1 (3′-OCH_3), 44.4 (C-7), 42.1 (C-3), 41.0 (C-12), 39.8 (C-1), 39.2 (C-10), 35.7 (C-14), 33.4 (C-18), 33.3 (C-4), 30.8 (C-13), 24.0 (C-17), 22.9 (C-11), 21.6 (C-19), 20.6 (C-6), 19.6 (C-16), 18.5 (C-2), 15.6 (C-20).

13(*S*)-Labdan-8α-ol-15-yl 2,4-Dimethoxybenzoate (5). The general procedure was followed using *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDCI) instead of DIC and 2,4-dimethoxybenzoic acid (60 mg, 0.33 mmol), EDCI (60 mg, 0.339 mmol), DMAP (30 mg, 0.245 mmol), and 13(*S*)-labdan-8α,15-diol **1** (50 mg, 0.151 mmol) in dry CH_2Cl_2 (5 mL). The crude obtained was purified by flash column chromatography, eluting with cyclohexane–EtOAc (2:1), providing a colorless resin (yield 70%). MS *m/z* 474.4 $[M]^+$. IR (film, ν_{max}, cm^{-1}): 3508 (OH), 3086 (=C–H), 2936, 2854 (C–H), 1704 (C=O), 1610 (C=C), 1272, 1252, 1212, 1164 (C–O–C), 940, 836, 770 (=C–H). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.82 (1H, d, J = 9.0, H-7′), 6.50–6.43 (2H, m, H-4′, 6′), 4.49–4.12 (2H, m, H-15), 3.86 (3H, s, 5′-OCH_3), 3.82 (3H, s, 3′-OCH_3), 1.84 (2H, dt, J = 11.7, 2.9, H-7), 1.12 (3H, s, H-17), 0.99 (1H, t, J = 3.8, H-9), 0.94 (3H, d, J = 6.4, H-16), 0.84 (3H, s, H-18), 0.77 (6H, s, H-20, 19). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 165.7 (C-1′), 164.2 (C-3′), 161.4 (C-5′), 133.8 (C-7′), 112.8 (C-2′), 104.6 (C-6′), 99.1 (C-4′), 74.3 (C-8), 63.0 (C-15), 62.5 (C-9), 56.2 (C-5), 56.0 (5′-OCH_3), 55.5 (3′-OCH_3), 44.5 (C-7), 42.1 (C-3), 41.2 (C-12), 39.9 (C-1), 39.2 (C-10), 35.8 (C-14), 33.4 (C-18), 33.3 (C-4), 30.9 (C-13), 24.0 (C-17), 22.9 (C-11), 21.6 (C-19), 20.6 (C-6), 19.6 (C-16), 18.5 (C-2), 15.6 (C-20).

13(*S*)-Labdan-8α-ol-15-yl 2,5-Dimethoxybenzoate (6). The general procedure was followed using 2,3-dimethoxybenzoic acid (120 mg, 0.658 mmol), DIC (0.1 mL, 0.644 mmol), DMAP (120 mg, 0.982 mmol), and 13(*S*)-labdan-8α,15-diol **1** (100 mg, 0.322 mmol) in dry CH_2Cl_2 (7 mL). The crude obtained was purified by flash column chromatography, eluting with cyclohexane–EtOAc (4:1), affording a colorless resin (yield 41%). MS *m/z* 474.4 $[M]^+$. IR (film, ν_{max}, cm^{-1}): 3524 (OH), 3046 (=C–H), 2932, 2868 (C–H), 1716 (C=O), 1614 (C=C), 1286, 1246, 1218, 1182 (C–O–C), 938, 814, 736 (=C–H). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.31 (1H, d, J = 3.2, H-7′), 6.99 (1H, dd, J = 9.0, 3.2, H-5′), 6.89 (1H, d, J = 9.0, H-4′), 4.43–4.22 (2H, m, H-15), 3.83 (3H, s, 3′-OCH_3), 3.77 (3H, s, 6′-OCH_3), 1.84 (2H, dt, J = 12.1, 3.0, H-7), 1.12 (3H, s, H-17), 0.99 (1H, t, J = 3.7, H-9), 0.95 (3H, d, J = 6.3, H-16), 0.84 (3H, s, H-18), 0.77 (6H, s, H-19, 20). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 166.2 (C-1′), 153.6 (C-3′), 153.1 (C-6′), 121.2 (C-2′), 119.2 (C-7′), 116.3 (C-5′), 114.0 (C-4′), 74.3 (C-8), 63.6 (C-15), 62.5 (C-9), 56.9 (3′-OCH_3), 56.2 (C-5), 55.9 (6′-OCH_3), 44.6 (C-7), 42.1 (C-3), 41.2 (C-12), 39.9 (C-1), 39.3 (C-10), 35.7 (C-14), 33.4 (C-18), 33.3 (C-4), 31.0 (C-13), 24.0 (C-17), 23.0 (C-11), 21.6 (C-19), 20.7 (C-6), 19.6 (C-16), 18.5 (C-2), 15.6 (C-20).

13(*S*)-Labdan-8α-ol-15-yl 2,4,5-Trimethoxybenzoate (7). The general procedure was followed using 2,4,5-trimethoxybenzoic acid (140 mg, 0.659 mmol), DIC (0.1 mL, 0.644 mmol), DMAP (120 mg, 0.982 mmol), and 13(*S*)-labdan-8α,15-diol (**1**) (100 mg, 0.322 mmol) in dry CH_2Cl_2 (7 mL). The crude was purified by flash column chromatography, eluting with cyclohexane–EtOAc (4:1), providing a white solid (yield 22%), mp 98–100°C. MS *m/z* 504.4 $[M]^+$. IR (KBr, ν_{max}, cm^{-1}): 3394 (OH), 3046 (=C–H), 2924 (C–H), 1706 (C=O), 1612 (C=C), 1254, 1214 (C–O–C), 819, 793, 783 (=C–H). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.38 (1H, s, H-7′), 6.50 (1H, s, H-4′), 4.40–4.20 (2H, m, H-15), 3.91 (3H, s, 5′-OCH_3), 3.86 (3H, s, 3′-OCH_3), 3.85 (3H, s, 6′-OCH_3), 1.83 (2H, dt, J = 12.0, 2.9, H-7), 1.11 (3H, s, H-17), 0.98 (1H, t, J = 3.7, H-9), 0.95 (3H, d, J = 6.1, H-16), 0.83 (3H, s, H-18), 0.76 (6H, s, H-19, 20). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 165.8 (C-1′), 155.7 (C-3′), 153.5 (C-5′), 142.7 (C-6′), 114.6 (C-7′), 111.2 (C-2′), 98.1 (C-4′), 74.2 (C-8), 63.2 (C-15), 62.5 (C-9), 57.3 (5′-OCH_3), 56.5 (3′-OCH_3), 56.2 (C-5), 56.1 (6′-OCH_3), 44.5 (C-7), 42.1 (C-3), 41.2 (C-12), 39.9 (C-1), 39.2 (C-10), 35.8 (C-14), 33.4 (C-18), 33.3 (C-4), 31.0 (C-13), 24.0 (C-17), 23.0 (C-11), 21.6 (C-19), 20.6 (C-6), 19.7 (C-16), 18.5 (C-2), 15.6 (C-20).

13(*S*)-Labdan-8α-ol-15-yl 3,4,5-Trimethoxybenzoate (8). The general procedure was followed using 3,4,5-trimethoxybenzoic acid (140 mg, 0.659 mmol), DIC (0.1 mL, 0.644 mmol), DMAP (120 mg, 0.982 mmol), and 13(*S*)-labdan-8α,15-diol **1**

(100 mg, 0.322 mmol) in dry CH_2Cl_2 (7 mL). The crude was purified by flash column chromatography, eluting with cyclohexane–EtOAc (4:1), yielding quantitatively a colorless resin. MS *m/z* 504.4 $[M]^+$. IR (film, ν_{max}, cm^{-1}): 3530 (OH), 3052 (=C–H), 2954, 2870 (C–H), 1716 (C=O), 1590 (C=C), 1226, 1132 (C–O–C), 766, 736 (=C–H). ^1H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.26 (2H, s, H-2′, 7′), 4.41–4.22 (2H, m, H-15), 3.87 (6H, s, 4′, 6′-OCH_3), 3.86 (3H, s, 5′-OCH_3), 1.81 (2H, dt, J = 12.0, 3.1, H-7), 1.10 (3H, s, H-17), 0.97 (1H, t, J = 3.7, H-9), 0.94 (3H, d, J = 6.1, H-16), 0.81 (3H, s, H-18), 0.74 (6H, br.s, H-19, 20). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 166.3 (C-1′), 152.9 (C-4′, 6′), 142.1 (C-5′), 125.5 (C-2′), 106.9 (C-3′, 7′), 74.1 (C-8), 63.7 (C-15), 62.4 (C-9), 60.9 (5′-OCH_3), 56.2 (4′, 6′-OCH_3), 56.1 (C-5), 44.4 (C-7), 42.0 (C-3), 41.0 (C-12), 39.8 (C-1), 39.1 (C-10), 35.7 (C-14), 33.4 (C-18), 33.2 (C-4), 31.0 (C-13), 24.0 (C-17), 23.0 (C-11), 21.4 (C-19), 20.6 (C-6), 19.7 (C-16), 18.4 (C-2), 15.4 (C-20).

13(*S*)-Labdan-8α-ol-15-yl 3,5-Dimethylbenzoate (9). The general procedure was followed using 3,5-dimethylbenzoic acid (100 mg, 0.666 mmol), DIC (0.1 mL, 0.644 mmol), DMAP (120 mg, 0.982 mmol), and 13(*S*)-labdan-8α,15-diol **1** (100 mg, 0.322 mmol) in dry CH_2Cl_2 (7 mL). The crude was purified by flash column chromatography, eluting with cyclohexane–EtOAc (7:1), and a colorless resin was obtained (yield quantitative). MS *m/z* 442.4 $[M]^+$. IR (film, ν_{max}, cm^{-1}): 3524 (OH), 3052 (=C–H), 2928, 2868 (C–H), 1718 (C=O), 1608 (C=C), 1218, 1118 (C–O–C), 770, 738 (=C–H). ^1H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.63 (2H, s, H-3′, 7′), 7.14 (1H, s, H-5′), 4.44–4.22 (2H, m, H-15), 2.33 (6H, s, CH_3-C-4′, 6′), 1.85 (2H, dt, J = 12.0, 3.1, H-7), 1.13 (3H, s, H-17), 1.00 (1H, t, J = 3.7, H-9), 0.97 (3H, d, J = 6.1, H-16), 0.84 (3H, s, H-18), 0.77 (6H, br.s, H-19, 20). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 167.1 (C-1′), 137.9 (C-4′, 6′), 134.4 (C-5′), 130.4 (C-2′), 127.3 (C-3′, 7′), 74.2 (C-8), 63.4 (C-15), 62.4 (C-9), 56.2 (C-5), 44.4 (C-7), 42.0 (C-3), 41.1 (C-12), 39.8 (C-1), 39.2 (10), 35.7 (C-14), 33.4 (C-18), 33.3 (C-4), 30.9 (C-13), 24.0 (C-17), 23.0 (C-11), 21.6 (C-19), 21.2 (CH_3-4′, 5′), 20.6 (C-6), 19.7 (C-16), 18.5 (C-2), 15.5 (C-20).

13(*S*)-Labdan-8α-ol-15-yl 5-Methyl-2-nitrobenzoate (10). The general procedure was followed using 5-methyl-2-nitrobenzoic acid (120 mg, 0.662 mmol), DIC (0.1 mL, 0.644 mmol), DMAP (120 mg, 0.982 mmol), and 13(*S*)-labdan-8α,15-diol (**1**) (100 mg, 0.322 mmol) in dry CH_2Cl_2 (7 mL). The crude was purified by flash column chromatography, eluting with cyclohexane–EtOAc (4:1), affording a white solid (quantitative yield), mp 68–70°C. MS *m/z* 473.4 $[M]^+$. IR (KBr, ν_{max}, cm^{-1}): 3392 (OH), 3068 (=C–H), 2932, 2870 (C–H), 1732 (C=O), 1592 (C=C), 1530 (NO_2), 1348, 1292, 1206 (C–O–C), 938, 846, 750 (=C–H). ^1H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.80 (1H, d, J = 8.3, H-4′), 7.44 (1H, br.s, H-7′), 7.35 (1H, dd, J = 8.3, 1.1, H-5′), 4.43–4.22 (2H, m, H-15), 2.43 (3H, s, CH_3-6′), 1.82 (2H, dt, J = 12.0, 3.1, H-7), 1.11 (3H, s, H-17), 0.99 (1H, t, J = 3.7, H-9), 0.91 (3H, d, J = 5.9, H-16), 0.83 (3H, s, H-18), 0.76 and 0.75 (each 3H, s, H-20, 19). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 166.0 (C-1′), 145.7 (C-3′), 144.6 (C-6′), 131.8 (C-5′), 130.1(C-7′), 128.4 (C-2′), 124.1 (C-4′), 74.2 (C-8), 64.9 (C-15), 62.4 (C-9), 56.1 (C-5), 44.3 (C-7), 42.0 (C-3), 40.8 (C-12), 39.8 (C-1), 39.2 (C-10), 35.4 (C-14), 33.4 (C-18), 33.2 (C-4), 30.6 (C-13), 24.0 (C-17), 22.9 (C-11), 21.5 (C-19), 21.4 (CH_3-6′), 20.6 (C-6), 19.4 (C-16), 18.5 (C-2), 15.5 (C-20).

13(*S*)-Labdan-8α-ol-15-yl 4-Methoxy-3-nitrobenzoate (11). The general procedure was followed using 4-methoxy-3-nitrobenzoic acid (120 mg, 0.609 mmol), DIC (0.1 mL, 0.644 mmol), DMAP (120 mg, 0.982 mmol), and 13(*S*)-labdan-8α,15-diol **1** (100 mg, 0.322 mmol) in dry CH_2Cl_2 (7 mL). The crude was purified by flash column chromatography, eluting with cyclohexane–EtOAc (3:1), yielding quantitatively a white solid, mp 84–86°C. MS *m/z* 489.4 $[M]^+$. IR (KBr, ν_{max}, cm^{-1}): 3573 (OH), 3059 (=C–H), 2925, 2873 (C–H), 1714 (C=O), 1618 (C=C), 1535 (NO_2), 1350, 1277, 1239 (C–O–C), 957, 905, 760 (=C–H). ^1H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 8.43 (1H, d, J = 2.1, H-3′), 8.17 (1H, dd, J = 8.9, 2.1, H-7′), 7.1 (1H, d, J = 8.9, H-6′), 4.43–4.21 (2H, m, H-15), 3.97 (3H, s, 4′-OCH_3), 1.82 (2H, dt, J = 12.0, 3.1, H-7), 1.10 (3H, s, H-17), 0.98 (1H, t, J = 3.58, H-9), 0.94 (3H, d, J = 6.0, H-16), 0.82 (3H, s, H-18), 0.75 and 0.74 (each 3H, s, H-20, 19). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 164.5 (C-1′), 156.0 (C-5′), 139.3 (C-4′), 135.4 (C-7′), 127.1 (C-3′), 122.9 (C-2′), 113.2 (C-6′), 74.2 (C-8), 64.1 (C-15), 62.4 (C-9), 56.9 (5′-OCH_3), 56.1 (C-5), 44.5 (C-7), 42.0 (C-3), 41.0 (C-12), 39.8 (C-1), 39.2 (C-10), 35.6 (C-14), 33.4 (C-18), 33.2 (C-4), 30.9 (C-13), 24.0 (C-17), 23.0 (C-11), 21.5 (C-19), 20.6 (C-6), 19.6 (C-16), 18.4 (C-2), 15.5 (C-20).

13(*S*)-Labdan-8α-ol-15-yl 4-Trifluormethylbenzoate (12). The general procedure was followed using 4-trifluormethylbenzoic acid (120 mg, 0.631 mmol), DIC (0.1 mL, 0.644 mmol), DMAP (120 mg, 0.982 mmol), and 13(*S*)-labdan-8α,15-diol **1** (100 mg, 0.322 mmol) in dry CH_2Cl_2 (5 mL). The crude was purified by flash column chromatography, eluting with cyclohexane–EtOAc (2:1), providing a colorless resin (yield 82%). MS *m/z* 482.4 $[M]^+$. IR (KBr, ν_{max}, cm^{-1}): 3532, 3454 (OH), 3058 (=C–H), 2930, 2870 (C–H), 1726 (C=O), 1586 (C=C), 1326, 1278, 1134 (C–O–C), 938, 864, 776, 706 (=C–H). ^1H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 8.13 (2H, d, J = 8.1, H-3′, 7′), 7.68 (2H, d, J = 8.1, H-4′, 6′), 4.49–4.28 (2H, m, H-15), 3.97 (3H, s, 4′-OCH_3), 1.84 (2H, dt, J = 12.0, 3.1, H-7), 1.13 (3H, s, H-17), 1.00 (1H, t, J = 3.7, H-9), 0.97 (3H, d, J = 6.1, H-16), 0.84 (3H, s, H-18), 0.77 (6H, s, H-20, 19). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 165.5 (C-1′), 134.4 (q, J = 32.6, C-5′), 133.8 (C-2′), 130.1 (C-3′, 7′), 125.4 (q, J = 3.7, C-4′, 6′), 123.76 (q, J = 272.7, -CF_3),

74.3 (C-8), 64.2 (C-15), 62.5 (C-9), 56.3 (C-5), 44.6 (C-7), 42.1 (C-3), 41.2 (C-12), 39.9 (C-1), 39.3 (C-10), 35.7 (C-14), 33.4 (C-18), 33.3 (C-4), 31.0 (C-13), 24.1 (C-17), 23.0 (C-11), 21.6 (C-19), 20.7 (C-6), 19.6 (C-16), 18.6 (C-2), 15.6 (C-20).

13(*S*)-Labdan-8α-ol-15-yl-3,4,5-Trihydroxybenzoate (13). The general procedure was followed using 3,4,5-tris(benzyloxy)benzoic acid (211 mg, 0.479 mmol) (see supporting material), DIC (0.075 mL, 0.483 mmol), DMAP (59 mg, 0.483 mmol), and 13(*S*)-labdan-8α,15-diol **1** (100 mg, 0.322 mmol) in dry CH_2Cl_2 (20 mL). The crude was purified by flash column chromatography, eluting with cyclohexane–EtOAc (4:1) (yield 48%). The 13(*S*)-labdan-8α-ol-15-yl-3,4,5-tris(benzyloxy)benzoate (**13a**, 79.4 mg) dissolved in ethanol (10 mL) was introduced with a syringe into a reaction flask containing Pd/C (8 mg) under argon. Subsequently, argon was removed while bubbling hydrogen, and the reaction mixture was stirred. After 12 h of stirring under hydrogen atmosphere, the reaction mixture was filtered in a small column packed with cotton/Celite and concentrated under reduced pressure (yield quantitative): ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.22 (2H, s, H-3′, 7′), 4.44–4.06 (2H, m, H-15), 1.92 (2H, d, J = 12.2, H-7), 1.20 (3H, s, H-17), 1.09 (1H, br.s, H-9), 0.92 (3H, d, J = 6.3, H-16), 0.86 (3H, s, H-18), 0.79 and 0.78 (each 3H, s, H-20, 19). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 167.4 (C-1′), 144.2 (C-4′, 6′), 137.0 (C-5′), 121.5 (C-2′), 109.8 (C-3′, 7′), 76.4 (C-8), 63.9 (C-15), 62.6 (C-9), 56.1 (C-5), 44.0 (C-7), 42.1 (C-3), 41.2 (C-12), 39.8 (C-1), 39.4 (C-10), 35.9 (C-14), 33.5 (C-18), 33.4 (C-4), 32.0 (C-13), 24.0 (C-11), 23.7 (C-17), 21.6 (C-19), 20.6 (C-6), 20.4 (C-16), 18.6 (C-2), 15.7 (C-20).

Bacterial Strains. The bactericidal activity was determined for each compound against four selected bacterial strain, two Gram-positive (+) and two Gram-negative (–). Standard strain from the American Type Culture Collection (ATCC) of the following microorganisms were used: *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853).

Preparation of Bacterial Inoculants. Preinocula, consisting of 20 mL of nutritive broth taken from a solid culture medium, was prepared and then incubated at 37°C for 18 h under orbital stirring. These cultures were either diluted in physiological serum or in growth medium, to be used in each case as inoculum to perform cell density tests.

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REFERENCES

1. J. D. Connolly and R. A. Hill, *Dictionary of Terpenoids*, Vol. I, Chapman and Hall, London, 1991, 677 pp.
2. C. Demetzos and C. Dimas, *Labdane-type Diterpenes: Chemistry and Biological Activity*, in: Atta-Ur-Rahman (Ed.), Studies in Natural Product Chemistry, Vol. 25, Part F, Elsevier, Amsterdam, 2001, 235 pp.
3. M. Singh, M. Pal, and R. P. Sharma, *Planta Med*., **65**, 2 (1999).
4. S. A. Heleno, A. Martins, M. J. R. P. Queiroz, and I. C. F. R. Ferreira, *Food Chem*., **173**, 501 (2015).
5. S. Khadem and R. J. Marles, *Molecules*, **15**, 7985 (2010).
6. J. Gruz, O. Novak, and M. Strnad, *Food Chem*., **111**, 789 (2008).
7. P. Mattila, J. Hellstrom, and R. Torroten, *J. Agric. Food Chem*., **54**, 7193 (2006).
8. A. Szajdek and E. J. Borowska, *Plant Foods Hum. Nutr*., **63**, 147 (2008).
9. T. M. Rababah, N. S. Hettiarachchy, and R. Horax, *J. Agric. Food Chem*., **52**, 5183 (2004).
10. I. Mudnic, D. Modun, V. Rastija, J. Vukovic, I. Brizic, V, Katalinic, B. Kozina, M. Medic-Saric, and M. Boban, *Food Chem*., **119**, 1205 (2010).
11. A. Del Olmo, J. Calzada, and M. Nunez, *Crit. Rev. Food Sci. Nutr*., **57**, 3084 (2017).
12. F. A. Tomas-Barberan, and M. N. Clifford, *J. Sci. Food Agric*., **80**, 1024 (2000).
13. S. Awale, T. Kawakami, Y. Tezuka, J.-Y. Ueda, K. Tanaka, and S. Kadota, *Chem. Pharm. Bull*., **53**, 710 (2005).
14. J. Conrad, B. Vogler, I. Klaiber, G. Roos, V. Ulrich, and W. Kraus, *Phytochemistry*, **48**, 647 (1998).

15. A. M. Galal, E. A. Abourashed, S. A. Ross, M. A. ElSohly, M. S. Al-Said, and F. S. El-Feraly, *J. Nat. Prod.*, **64**, 399 (2001).
16. Y.-L. Lin, Y.-H. Kuo, M.-C. Cheng, and Y. Wang, *Chem. Pharm. Bull*., **36**, 2642 (1988).
17. B. Trusheva, I. Todorov, M. Ninova, H. Najdenski, A. Daneshmand, and V. Bankova, *Central Chem. J*., **4**, 8 (2010).
18. B. F. Rasulev, A. I. Saidkhodzhaev, S. S. Nazrullaev, K. S. Akhmedkhodzhaeva, Z. A. Khushbaktova, and J. Leszczynski, *SAR QSAR Environ. Res*., **18**, 663 (2007).
19. C. P. Reyes, F. Munoz-Martinez, I. R. Torrecillas, C. R. Mendoza, F. Gamarro, I. L. Bazzocchi, M. J. Nunez, L. Pardo, S. Castanys, M. Campillo, and I. A. Jimenez, *J. Med. Chem.*, **50**, 4808 (2007).
20. S. Kakkar and S. Bais, *ISRN Pharmacol.,* 952943 (2014).
21. K. Fujita and I. Kubo, *Int. J. Food Microb*., **79**, 193 (2002).
22. J.-G. Choi, S.-H. Mun, H. S. Chahar, P. Bharaj, O.-H. Kang, S.-G. Kim, D.-W. Shin, and D.-H. Kwon, *PloSOne*, **9**, e102697 (2014).
23. M. Gutierrez-Larrainzar, J. Rua, I. Caro, C. de Castro, D. de Arriaga, M. R. Garcia-Armesto, and P. Pilar del Valle, *Food Control*, **26**, 555 (2012).
24. I. Kubo, K. Fujita, K. Nihei, and A. Nihei, *J. Agric. Food Chem*., **52**, 1072 (2004).
25. B. Narasimhan, S. Ohlan, R. Ohlan, V. Judge, and R. Narang, *Eur. J. Med. Chem*., **44**, 689 (2009).
26. J. M. Amaro and M. Adrian, *Rev. Latinoam. Quim*, **13**, 110 (1982).
27. T. K. Devon and A. I. Scott, *Handbook of Naturally Occurring Compounds*, Vol. II. Terpenes, Academic Press, New York, 1972, 185 pp.
28. K. Bjamer, G. Ferguson, and R. D. Melville, *Acta Crystallogr*., **B24**, 855 (1968).
29. J. L. Rios, M. C. Recio, and A. Villar, *J. Ethnopharmacol*., **23**, 127 (1988).