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Synthesis and antimicrobial activity of new *ent*-kaurene-type diterpenoid derivatives

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ABSTRACT

This research consists in the synthesis of *ent*-kaurene-type diterpenoid derivatives from the new natural product *ent-kaur-3-acetoxy-15-ene,* to carry out structural modifications on the C_3 carbon of the *ent*-kaurene core by introducing different oxygenated groups, especially esters, in order to probe the structure-activity relationship (SAR) against microorganisms. The structure of the compounds was confirmed by FT-IR, ^{1}H NMR, ^{13}C NMR, and GC-MS. The antimicrobial activity of the synthesized derivatives was evaluated, e*nt*-kaur-3-*O*-(6',7'-bibenzyl-oxy-caffeoyl)-15-ene (4) exhibited activity against all tested microorganisms: *Staphylococcus aureus* (16 mm), *Enterococcus faecalis* (12 mm), *Escherichia coli* (13 mm), *Klebsiella pneumoniae* (10 mm), *Pseudomonas aeruginosa* (8 mm) and *Candida krusei* (10 mm). These results reveal a remarkable structure-activity relationship over the C_3 carbon of the *ent*-kaurene core, where the presence of oxygenated groups such as hydroxyl or alkyl esters enhances activity.

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1. Introduction

Natural products (NP) and their semi-synthetic analogues have played a vital role in the description and expansion of antimicrobial drugs, especially in the last 20 years [1]. Various terrestrial sources, such as plants, fungi, and lichens, present more than 80% of the described naturally occurring antibiotics. These natural products, together with new synthetic analogues, have confirmed their efficacy as alternatives to antimicrobial agents [2]. In addition, new natural and synthetic compounds have attracted considerable interest in replacing the potency of ineffective antibiotics. Diterpenes are one of the most important classes of NPs, due to their wide range of biological and ecological activities, such as antimicrobial [3], cytotoxicity with relative selectivity for cancer cells [4], antiparasitic [5], anti-HIV activity [6], anti-inflammatory [7], among others. In particular, there is a notable diterpene skeleton in this class: *ent*-kaurenes (Figure 1) [8,9].

Ent-kaurene diterpenoids represent an important group of tetracyclic diterpenes that have a long history of research and medical applications in traditional eastern remedies, and have attracted increasing interest since the last century due to their structural diversity and complexity, together with extensive bioactivity profiles such as antitumor, antibacterial, antiviral, and antiinflammatory effects [10]. *Ent*-kaurene was first isolated from the essential oil of the leaves of the New Zealand kauri (*Agathis australis* Salisb), a plant locally known as kauri pine [10]. However, plants of the genus *Espeletia* (subtribe Espeletiinae Cuatrec., Asteraceae), popularly known as "frailejón", which grow at an altitude of 2500 m in the northern Andes of South America and are used by high-moorland inhabitants with well-described medicinal attributes, provide an abundant source of natural products *ent*-kauranoid [11,12].

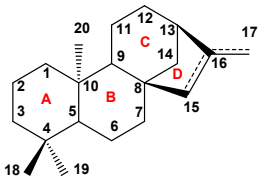

Figure 1. Carbon skeleton of an *ent*-kaurene-type diterpene.

Moreover, compounds with slight structural differences can be observed to show very different biological activities in

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several cases [13-16]. It should also be considered that these natural substances can be subjected to chemical modifications by chemical reactions or biotransformations, providing analogues that can be even more active [17,18]. Therefore, this is an interesting way to obtain new active compounds, which is important in the search for new antibiotics and treatments to defeat microbes. Natural products research in this field still represents a good prospect for finding new bioactive structures based on which new less expensive drugs can be developed; in recent decades, the use of additive and/or synergistic combinations of synthetic drugs and phytochemicals has been increasingly encouraged.

Part of the research focuses on the evaluation of biological activities of *ent*-kaurenes [19,20] considering the semi-synthesis approach [19-22], and some interesting previous results obtained in the research group [23], the decision was made to perform a search for active compounds against bacterial and fungal strains by obtaining semi-synthetic derivatives of the new natural product *ent*-kaurene: *ent*-kaur-3-acetoxy-15-ene (**1**), which was isolated from the extraction of neutral fractions from the leaves of *Espeletia semiglobulata* Cuatrec. (Asteraceae) [23].

Ent-kaur-3-acetoxy-15-ene (**1**) [23] was allowed to react with an excess of $LiAlH_4$, as a result of which the hydroxyl derivative was obtained (**2**), and from its different derivatives were synthesized (**3-8**). The results of the research on the synthesis, characterization, and antimicrobial activity against some microorganisms of novel *ent*-kaurane-type diterpenoid derivatives are presented here.

2. Experimental

2.1. Materials and instrumentation

All reagents and solvents were obtained from Sigma-Aldrich, Acros Organics, and Fisher Scientific and used as supplied unless otherwise stated. Glassware was oven-dried at 100 °C for 12 hours for moisture-sensitive reactions. Reactions were carried out under Argon using anhydrous solvents. Room temperature refers to ambient temperature. The reactions were monitored by thin layer chromatography (TLC) on aluminum-supported silica gel and visualized by exposure to 240-nm UV light and/or exposure to basic phosphomolybdic acid solution followed by heating. Flash column chromategraphy was performed with Merck PLC Silica Gel 60 using commercial solvents. All in vacuo evaporations were conducted at reduced pressure using a Büchi rotary evaporator.

Infrared spectra were recorded on a PerkinElmer Spectrum Two FTIR, 10.03.06 version, in KBr disks. Maximum absorption (ν) are reported in wavenumbers (cm^{-1}). GC-MS was performed on a Hewlett-Packard MSD 5973 instrument equipped with a fused silica column with 5% phenylmethyl polysiloxane (HP-5MS, 30 m, 0.25 mm, film thickness 0.25 µm). The initial analysis temperature was 250 °C, which was increased at 5 °C/min until reaching the final temperature of 300 °C and reported as *m/z*. NMR spectroscopy (^1H, ^{13}C, APT, COSY, HSQC, HMBC) was recorded at 25 °C with a Bruker-Advance Neo 400 (^1H at 400 MHz and ^{13}C at 100 MHz) in deuterated solvents, $CDCl_3$. NMR chemical shifts (δ) are quoted in parts per million (ppm) referencing the residual proton or the carbon peak of the solvent ($CDCl_3$: δ_H = 7.26 and δ_C = 77.1). The coupling constants are reported in hertz (Hz). Data for ^1H spectra are presented as chemical shift (δ, ppm), integration and multiplicity (*b*, broad; *s*, singlet; *d*, doublet; *t*, triplet; *q*, quartet; *quin*, quintet; *sept*, septet; *m*, multiplet; or as a combination (*e.g.*, *dd*, *dt*, *etc.*)). All carbon spectra were obtained by the attached proton test (APT).

2.2. Synthesis

Ent-kaur-3-acetoxy-15-ene (1) was allowed to react with an excess of $LiAlH_4$, as a result of which the hydroxyl derivative was obtained (**2**), and different derivatives were synthesized from it: one derivative by oxidation of this hydroxyl group (3) and the rest were acetoxy-alkylated derivatives (**4-8**) by esterification reactions with different organic acids. The synthetic pathway of these *ent*-kaurene derivatives is represented in Scheme 1.

Ent-kaur-3-acetoxy-15-ene (1) [23] was allowed to react with an excess of $LiAlH_4$, as a result of which the hydroxyl derivative was obtained (2) and its different derivatives were synthesized: one derivative by oxidation of this hydroxyl group (3), and the rest were acetoxy-alkylated derivatives (4-8) by esterification reactions with different organic acids. The results of the research on the synthesis, characterization, and antimicrobial activity against some microorganisms of novel *ent*-kaurane-type diterpenoid derivatives are presented here.

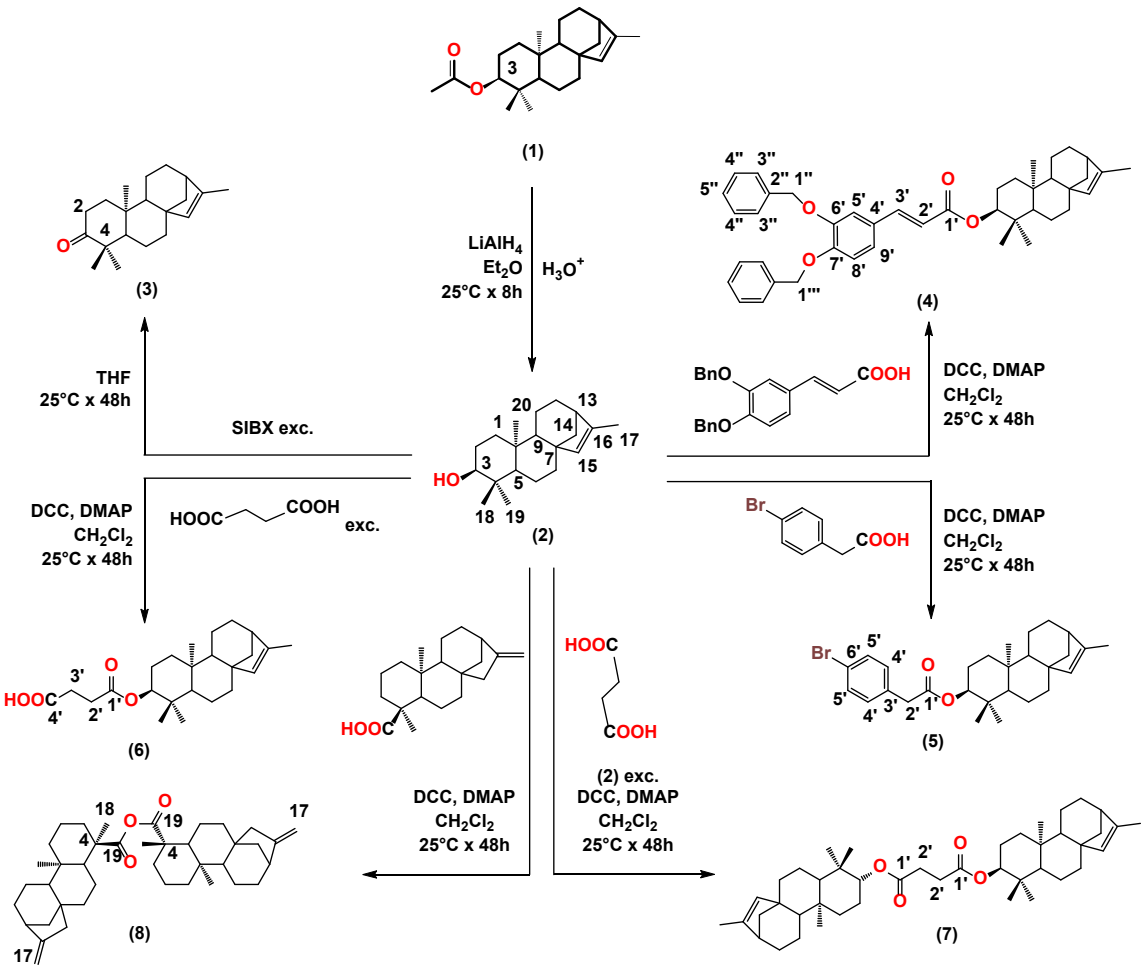
2.2.1. Synthesis of ent-kaur-3-hydroxy-15-ene (2) derivative

2.44 mmol of *ent*-kaur-3-acetoxy-15-ene (**1**) were introduced into a round bottom flask under an argon atmosphere and afterward dissolved in 160 mL of diethyl ether. Subsequently, an excess of $LiAlH_4$ (26.31 mmol), was added and it was left under continuous stirring in an argon atmosphere at room temperature for 8 h. Subsequently, the sample was carefully treated with a 1 M HCl solution until the appearance of a white precipitate. The mixture was filtered and the aqueous phase was extracted several times with diethyl ether (3 × 30 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated in vacuum. As a result, a white solid was obtained, which was purified by column chromatography using a mixture of hexane:AcOEt (4:1) as an eluent to obtain compound **2**.

Ent-kaur-3-hydroxy-15-ene (**2**): Color: White crystals. Yield: 792.00 mg, 99 %. M.p.: 127-129 °C. FT-IR (KBr, ν, cm^{-1}): 3392 (O-H, alcohol), 3040 (=C-H, alkene), 2925 (C-H), 1656 (C=C, alkene), 1045 (C-O, alcohol). ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 5.06 (*s*, 1H, CH, H_{15}), 3.57 (*s*, 1H, OH, H_{21}), 3.15 (*m*, 1H, CH-OH, H_3), 1.86 (*m*, 1H, CH, H_{13}), 1.80 (*s*, 3H, CH_3, H_{17}), 1.56 (*m*, 2H, CH_2, H_1), 1.54 (*t*, 2H, CH_2, H_2), 1.49 (*m*, 2H, CH_2, H_6), 1.48 (*m*, 2H, CH_2, H_{12}), 1.47 (*m*, 1H, CH, H_5), 1.43 (*m*, 2H, CH_2, H_{11}), 1.33 (*t*, 2H, CH_2, H_{14}), 1.29 (*m*, 2H, CH_2, H_7), 0.82 (*s*, 3H, CH_3, H_{18}), 0.80 (*s*, 3H, CH_3, H_{19}), 0.78 (*m*, 1H, CH, H_9), 0.73 (*s*, 3H, CH_3, H_{20}). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 139.78 (C, C_{16}), 124.47 (CH, C_{15}), 79.06 (CH-OH, C_3), 55.41 (CH, C_5), 52.40 (CH, C_9), 47.79 (CH, C_{13}), 41.68 (C, C_8), 41.49 (CH_2, C_{14}), 39.68 (C, C_{10}), 37.86 (C, C_4), 33.01 (CH_2, C_6), 32.69 (CH_2, C_7), 31.27 (CH_2, C_1), 28.22 (CH_3, C_{18}), 27.28 (CH_2, C_2), 26.75 (CH_2, C_{12}), 23.37 (CH_3, C_{17}), 22.70 (CH_3, C_{19}), 21.07 (CH_2, C_{11}), 17.66 (CH_3, C_{20}). MS (EI, *m/z* (%)): 288 (M^+, 15.0), 273.2 (M^+-CH_3, 40.2), 271.2 (M^+-OH, 38.1), 218.1 (M^+-C_5H_{11}, 80.7), 189.2 (M^+-$C_6H_{11}O$, 33.2), 161.2 (M^+-$C_8H_{15}O$, 29.4).

2.2.2. Synthesis of ent-kaur-3-oxo-15-ene (3) derivative

An equivalent of compound **2** (0.25 mmol) was dissolved in 10 mL of tetrahydrofuran (THF) and 4 equivalents of stabilized 2-iodoxybenzoic acid (SIBX) (1.01 mmol). The reaction mixture was continuously stirred in an argon atmosphere at room temperature for 48 hours. The mixture obtained was washed with water and extracted several times with diethyl ether (3 × 30 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated in vacuum. The crude product was purified through silica gel using hexane:AcOEt (99:1) as an eluent to give compound **3**.



Scheme 1. Synthetic pathway of *ent*-kaurene derivatives.

Ent-kaur-3-oxo-15-ene (**3**): Color: White crystalline solid. Yield: 72.00 mg, 98 %. M.p.: 131-132 °C. FT-IR (KBr, *ν*, cm^{-1}): 3041 (=C-H, alkene), 2927 (C-H), 1709 (C=O, ketone), 1639 (C=C, alkene). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 0.79 (*m*, 1H, CH, H_9), 0.81 (*s*, 3H, CH_3, H_{20}), 1.06 (*s*, 3H, CH_3, H_{19}), 1.08 (*s*, 3H, CH_3, H_{18}), 1.26 (*s*, 1H, CH, H_5), 1.28 (*m*, 2H, CH_2, H_7), 1.34 (*t*, 2H, CH_2, H_{14}), 1.43 (*m*, 2H, CH_2, H_{11}), 1.48 (*m*, 2H, CH_2, H_{12}), 1.49 (*m*, 2H, CH_2, H_6), 1.58 (*m*, 2H, CH_2, H_1), 1.90 (*s*, 3H, CH_3, H_{17}), 1.97 (*s*, 1H, CH, H_{13}), 2.71 (*t*, 2H, CH_2, H_2), 5.16 (*s*, 1H, CH, H_{15}). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 216.86 (C=O, C_3), 139.87 (C, C_{16}), 124.34 (CH, C_{15}), 55.40 (CH, C_9), 52.54 (CH, C_5), 48.05 (CH, C_{13}), 47.58 (C, C_4), 41.69 (C, C_8), 39.72 (CH_2, C_{14}), 37.68 (C, C_{10}), 35.72 (CH_2, C_7), 34.37 (CH_2, C_2), 33.58 (CH_2, C_1), 28.16 (CH_3, C_{18}), 26.89 (CH_2, C_{12}), 23.33 (CH_3, C_{17}), 21.67 (CH_2, C_6), 20.92 (CH_3, C_{19}), 19.79 (CH_2, C_{11}), 17.63 (CH_3, C_{20}). MS (EI, *m/z* (%)): 286 (M^+, 15.11), 257.2 (M^+-C_2H_6, 40.2), 247.2 (M^+-C_3H_4, 38.1), 218.2 (M^+-C_3H_7, 38.6), 189.2 (M^+-$C_6H_{10}O$, 69.4).

2.2.3. Synthesis of ent-kaur-3-acetoxyalkylated derivatives (4-8)

A compound **2** equivalent was placed in a round bottom flask and dissolved in 10 mL of CH_2Cl_2, with 1.5 equivalents of 3.4-bibenzyloxy-caffeic acid (0.52 mmol), 3 equivalents of dicyclohexylcarbodimide (DCC, 1.04 mmol) and 3 equivalents of *N*,*N*-dimethylaminopyridine (DMAP, 1.04 mmol). The reaction mixture was left stirring at room temperature for 48 hours under an atmosphere of argon, while being continuously monitored by TLC. The resulting mixture was washed with water and extracted with CH_2Cl_2 (3 x 20 mL). The organic phase was dried with $MgSO_4$, filtered, and concentrated. The crude was purified through silica gel using hexane:AcOEt (96:4) as eluent, yielding compound **4**: Color: solid white crystalline. Yield: 143.40 mg, 47.80 %. M.p.: 130-131 °C. A similar procedure was performed for the synthesis of compounds **5**-**8**, maintaining the same conditions and the purification procedure described above. For compound **5**: 1 equiv. compound **2** was dissolved in 5 mL of CH_2Cl_2, with 3 equiv. of *p*-bromophenylacetic acid (0.939 mmol), 3 equiv. DCC, and 3 equiv. DMAP. Color: white. Yield: 40.00 mg, 45 %. M.p.: 145-146 °C. Compound **6**: 1 equiv. compound **2** was dissolved in 10 mL of CH_2Cl_2, with 6 equiv. of succinic acid (2.08 mmol), 5 equiv. DCC, and 5 equiv. DMAP. Color: white. Yield: 57.00 mg, 57 %. M.p.: 185-186 °C. Compound **7**: 2 equiv. compound **2** (0.35 mmol) was dissolved in 10 mL of CH_2Cl_2, with 1 equiv. of succinic acid (0.17 mmol), 3 equiv. DCC, and 3 equiv. DMAP. Color: white. Yield: 49.00 mg, 50 %. M.p.: 190-191 °C. Compound **8**: 1 equiv. compound **2** was dissolved in 10 mL of CH_2Cl_2, with 4 equiv. of kaurenic acid (1.04 mmol), 6 equiv. DCC (1.04 mmol), and 6 equiv. DMAP (1.04 mmol). Color: white. Yield: 140.00 mg, 40 %. M.p.: 173 -174 °C. The synthetic pathway and structure of the compounds are given below (Scheme 1).

Ent-kaur-3-O-(6',7'-bibenzyl-oxy-caffeoyl)-15-ene (**4**): FT-IR (KBr, *ν*, cm^{-1}): 3090 (=C-H, alkene), 2946 (C-H), 1702 (C=O, ester carbonyl), 1633 (C=C, alkene), 1261 (C-O, ester), 843 (=C-H, alkene). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 0.73 (*s*, 3H, CH_3, H_{20}), 0.79 (*m*, 1H, CH, H_9), 0.80 (*s*, 3H, CH_3, H_{19}), 0.81 (*s*, 3H, CH_3, H_{18}), 1.30 (*m*, 2H, CH_2, H_7), 1.34 (*t*, 2H, CH_2, H_{14}), 1.43 (*m*, 2H,

CH_2, H_{11}), 1.47 (*m*, 2H, CH_2, H_{12}), 1.48 (*m*, 2H, CH_2, H_2), 1.50 (*m*, 2H, CH_2, H_6), 1.51 (*m*, 1H, CH, H_5), 1.55 (*m*, 2H, CH_2, H_1), 1.83 (*s*, 3H, CH_3, H_{17}), 1.87 (*s*, 1H, CH, H_{13}), 4.66 (*t*, 1H, CH-COO, H_3), 5.16 (*s*, 1H, CH, H_{15}), 5.21 (*s*, 2H, -O-CH_2-Ar, $H_{1'''}$), 5.22 (*s*, 2H, -O-CH_2-Ar, $H_{1''}$), 6.26 (*d*, 1H, -CH=CH-COO-, $H_{2'}$), 6.93 (*s*, 1H, -CH=CH-Ar, $H_{9'}$), 6.95 (*s*, 1H, -CH=CH-Ar, $H_{8'}$), 7.16 (*s*, 1H, -CH=CH-Ar, $H_{5'}$), 7.35 (*m*, 2H, 2CH-Ar, $H_{5''}$), 7.40 (*m*, 2H, 2CH-Ar, $H_{4''}$), 7.41 (*m*, 2H, 2CH-Ar, $H_{6''}$), 7.44 (*m*, 2H, 2CH-Ar, $H_{7''}$),7.45 (*m*, 2H, 2CH-Ar, $H_{3''}$), 7.56 (*d*, 1H, -CH=CH-COO-, $H_{3'}$). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 167.15 (-COO, $C_{1'}$), 151.03 (-C=, $C_{7'}$), 149.10 (-C=, $C_{6'}$), 144.12 (=CH, $C_{3'}$), 139.78 (=C-CH_3, C_{16}), 136.91 (2C, $C_{2''}$), 128.71 (2CH, $C_{3''}$), 128.70 (2C, $C_{7''}$), 128.19 (-C=, $C_{4'}$), 128.09 (2C, $C_{6''}$), 127.48 (2C, $C_{5''}$), 127.32 (2CH, $C_{4''}$), 124.35 (CH=CH, C_{15}), 122.98 (=CH, $C_{5'}$), 117.01 (=CH-, $C_{2'}$), 114.40 (=CH, $C_{9'}$), 113.81 (-C=, $C_{8'}$), 80.84 (CH-COO, C_3), 71.49 (CH_2-O, $C_{1''}$), 71,11 (CH_2-O, $C_{1'''}$).55.44 (CH, C_9), 52.50 (CH, C_5), 48.02 (CH, C_{13}), 40.18 (C, C_8), 39.85 (C, C_{10}), 39.67 (CH_2, C_{14}), 38.11 (C, C_4), 36.60 (CH_2, C_7), 33.00 (CH_2, C_1), 28.16 (CH_3, C_{18}), 26.66 (CH_2, C_{12}), 23.57 (CH_2, C_2), 23.41 (=C-CH_3, C_{17}), 22.70 (CH_3, C_{19}), 21.10 (CH_2, C_6), 18.47 (CH_2, C_{11}), 17.66 (CH_3, C_{20}). MS (EI, *m/z* (%)): 341.2 (M^+-$C_{20}H_{18}O_2$, 15.11), 289.1 (M^+-$C_{26}H_{41}O_2$, 10.21), 218.5 (M^+-$C_7H_{10}O_2$, 68.5), 189.2 (M^+-$C_9H_{14}O_2$, 38.6).

Ent-kaur-3-O-(carboxymethyl-2'-p-bromophenyl)-15-ene (**5**): FT-IR (KBr, *ν*, cm^{-1}): 3036 (=C-H, alkene), 2924 (C-H), 1733 (C=O, ester carbonyl), 1659 (C=C, alkene), 1255 (C-O, ester), 1136 (Ar-Br), 804 (*p*-Ar-H). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 0.76 (*s*, 3H, CH_3, H_{20}), 0.78 (*m*, 1H, CH, H_9), 0.80 (*s*, 3H, CH_3, H_{19}), 0.81 (*s*, 3H, CH_3, H_{18}), 1.32 (*m*, 2H, CH_2, H_7), 1.35 (*t*, 2H, CH_2, H_{14}), 1.44 (*m*, 2H, CH_2, H_{11}), 1.47 (*m*, 2H, CH_2, H_2), 1.48 (*m*, 2H, CH_2, H_{12}), 1.51 (*m*, 1H, CH, H_5), 1.52 (*m*, 2H, CH_2, H_6), 1.53 (*m*, 2H, CH_2, H_1), 1.91 (*s*, 3H, CH_3, H_{17}), 1.98 (*m*, 1H, CH, H_{13}), 3.59 (*s*, 2H, CH, $H_{5'}$).4.51 (*t*, 1H, CH-COO, H_3), 5.13 (*s*, 1H, CH, H_{15}), 7.20 (*d*, 2H, -CH_2-COO, $H_{2'}$), 7.45 (*d*, 2H, =CH-, $H_{4'}$). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 170.89 (COO, $C_{1'}$), 139.78 (=C-, C_{16}), 133.53 (C-CH_2-COO, $C_{3'}$), 131.73 (2CH-, $C_{5'}$), 131.17 (2CH-, $C_{4'}$), 124.42 (=CH-, C_{15}), 121.13 (Br-CH=, $C_{6'}$), 81.56 (CH-COO, C_3), 55.36 (CH, C_9), 52.51 (CH, C_5), 47.75 (CH, C_{13}), 41.52 (Ar-CH_2-COO, $C_{2'}$), 40.18 (C, C_8), 39.67 (CH_2, C_{14}), 38.52 (C, C_{10}), 37.94 (C, C_4), 35.65 (CH_2, C_7), 32.97 (CH_2, C_1), 28.16 (CH_3, C_{18}), 26.65 (CH_2, C_{12}), 23.67 (CH_2, C_2), 23.38 (CH_3-C=, C_{17}), 21.42 (CH_3, C_{19}), 21.01 (CH_2, C_6), 18.40 (CH_2, C_{11}), 17.65 (CH_3, C_{20}). MS (EI, *m/z* (%)): 405.2 (M^+-Br, 18.2), 367.2 (M^+-C_3H_4Br, 25.3), 297.2 (M^+-CH_5OBr, 47.2), 218.2 (M^+-$C_5H_{11}Br$, 80.5), 189.2 (M^+-$C_{14}H_{18}O_2Br$, 48.7).

Ent-kaur-3-O-succinyl-15-ene (**6**): FT-IR (KBr, *ν*, cm^{-1}): 3368 (-O-H, carboxylic acid), 3036 (=C-H, alkene), 2943 (C-H), 1710 (C=O, carboxylic acid carbonile), 1719 (-O-C=O, ester carbonyl), 1637 (C=C, alkene), 1378 (C-O), 1082 (C-O), 843 (=C-H). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 0.73 (*s*, 3H, CH_3, H_{20}), 0.79 (*m*, 1H, CH, H_9), 0.80 (*s*, 3H, CH_3, H_{19}), 0.81 (*s*, 3H, CH_3, H_{18}), 1.33 (*m*, 2H, CH_2, H_7), 1.34 (*t*, 2H, CH_2, H_{14}), 1.45 (*m*, 2H, CH_2, H_{11}), 1.46 (*m*, 2H, CH_2, H_2), 1.49 (*m*, 2H, CH_2, H_{12}), 1.51 (*m*, 2H, CH_2, H_6), 1.53 (*m*, 1H, CH, H_5), 1.54 (*m*, 2H, CH_2, H_1), 1.83 (*s*, 3H, CH_3-C=, H_{17}), 1.86 (*s*, 1H, CH, H_{13}), 2.57 (*t*, 2H, CH_2-COO, $H_{2'}$), 2.61 (*t*, 2H, CH_2-COOH, $H_{3'}$), 4.47 (*t*, 1H, CH-COO, H_3), 5.05 (*s*, 1H, -CH=, H_{15}), 11.4 (*s*, 1H, H-OOC, $H_{4'}$). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 177.94 (COOH, $C_{4'}$),171.96 (COO, $C_{1'}$), 139.77 (=C-, C_{16}), 124.42 (=CH-, C_{15}), 81.72 (CH-COO, C_3), 55.40 (CH, C_9), 52.50 (CH, C_5), 47.75 (CH, C_{13}), 40.15 (C, C_8), 9.67 (CH_2, C_{14}), 38.55 (C, C_{10}), 37.90 (C, C_4), 35.68 (CH_2, C_7), 32.97 (CH_2, C_1), 29.46 (CH_2-COO, $C_{2'}$), 29.15 (CH_2-COOH, $C_{3'}$), 28.15 (CH_3, C_{18}), 26.64 (CH_2, C_{12}), 23.65 (CH_2, C_2), 23.35 (CH_3-C=, C_{17}), 21.55 (CH_3, C_{19}), 21.06 (CH_2, C_6), 18.36 (CH_2, C_{11}), 17.66 (CH_3, C_{20}). MS (EI, *m/z* (%)): 388 (M^+, 17.7), 342.2 (M^+-HCOOH, 19.3), 218.2 (M^+-$C_8H_{13}O_3$, 89.6), 189.2 (M^+-$C_9H_{14}O_2$, 40.6), 161.0 (M^+-$C_8H_{16}O_2$, 18.4), 147.2 (M^+-$C_9H_{18}O_2$, 30.4).

Di-ent-kaur-3,3'-O-succinyl-15,15'-diene (**7**): FT-IR (KBr, *ν*, cm^{-1}): 2993 (=C-H, alkene), 2944 (C-H), 1731 (-O-C=O, éster carbonyl), 1631 (C=C, alkene), 1133 (C-O), 836 (=C-H). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 0.73 (*s*, 6H, $2CH_3$, H_{20}), 0.79 (*m*, 2H, 2CH, H_9), 0.80 (*s*, 6H, $2CH_3$, H_{19}), 0.81 (*s*, 6H, $2CH_3$, H_{18}),1.33 (*m*, 4H, $2CH_2$, H_7), 1.35 (*m*, 4H, $2CH_2$, H_{14}), 1.44 (*m*, 4H, $2CH_2$, H_{11}), 1.47 (*m*, 4H, $2CH_2$, H_{12}), 1.48 (*m*, 4H, $2CH_2$, H_2), 1.51 (*m*, 4H, $2CH_2$, H_6), 1.52 (*m*, 2H, 2CH, H_5), 1.54 (*m*, 4H, $2CH_2$, H_1), 1.82 (*s*, 6H, $2CH_3$, H_{17}), 1.88 (*s*, 2H, 2CH, H_{13}), 2.57 (*s*, 4H, $2CH_2$, $H_{2''}$), 4.47 (*t*, 2H, 2CH, H_3), 5.05 (*s*, 2H, 2CH=, H_{15}). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 172.16 (2COO, $C_{1'}$), 139.78 (2C=, C_{16}), 124.46 (2CH=, C_{15}), 81.37 (2CH, C_3), 55.40 (2CH, C_9), 52.51 (2CH, C_5), 47.99 (2CH, C_{13}), 40.17 (2C, C_8), 39.67 ($2CH_2$, C_{14}), 38.58 (2C, C_{10}), 37.94 (2C, C_4), 35.68 ($2CH_2$, C_7), 33.00 ($2CH_2$, C_1), 29.80 ($2CH_2$, $C_{2'}$),28.16 ($2CH_3$, C_{18}), 26.63 ($2CH_2$, C_{12}), 23.57 ($2CH_2$, C_2), 23.38 ($2CH_3$, C_{17}), 21.55 ($2CH_3$, C_{19}), 21.06 ($2CH_2$, C_6), 18.38 ($2CH_2$, C_{11}), 17.66 ($2CH_3$, C_{20}). MS (EI, *m/z* (%)): 468 (M^+-$C_{14}H_{21}$, 34.6), 426.2 (M^+-$C_{17}H_{27}$, 35.5), 342.2 (M^+-$C_{21}H_{30}O_2$, 40.7), 327.2 (M^+-$C_{22}H_{33}O_2$, 39.3).

Ent-kaurenic anhydride (**8**): FT-IR (KBr, *ν*, cm^{-1}): 3069 (=C-H, alkene), 2934 (C-H), 1789 (-O-C=O, asymmetric vibration of ester carbonyl), 1729 (-O-C=O, symmetric vibration of ester carbonyl), 1657 (C=C, alkene), 1378 (C-O, anhydride), 887 (=C-H). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 0.73 (*s*, 6H, $2CH_3$, H_{20}),0.78 (*m*, 2H, 2CH, H_9), 0.80 (*s*, 6H, $2CH_3$, H_{18}), 0.83 $a^{\beta}_{(axi)\ x\ 2}$ - 1.95 $b^{\alpha}_{(equ)\ x\ 2}$ (*m*, 4H, $2CH_2$, H_1), 1.11 (*d*, 2H, 2CH, H_5), 1.30 (*m*, 4H, $2CH_2$, H_7), 1.40 (*m*, 4H, $2CH_2$, H_{11}), 1.41 (m, 4H, $2CH_2$, H_2), 1.50 (*m*, 4H, $2CH_2$, H_6), 1.59 (*m*, 4H, $2CH_2$, H_{12}), 2.05 (*m*, 4H, $2CH_2$, H_{15}), 2.08 (*m*, 4H, $2CH_2$, H_{14}), 2.19 (*m*, 4H, $2CH_2$, H_3), 2.64 (*s*, 2H, 2CH, H_{13}), 4.74 $H_{a\ x2}$- 4.80 $H_{b\ x2}$ (*s*, 4H, 2=CH, H_{17}). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 173.55 (2COO-, C_{19}), 155.77 (2C=CH_2, C_{16}), 103.10 ($2CH_2$=C, C_{17}), 57.27 (2CH, C_5), 54.98 (2CH, C_9), 48.92 ($2CH_2$, C_{15}), 45.39 (2C-COO-, C_4), 43.96 (2CH, C_{13}), 43.78 (2C, C_8), 41.31 ($2CH_2$, C_1), 41.30 ($2CH_2$, C_{14}), 40.60 ($2CH_2$, C_7), 39.60 (2C, C_{10}), 33.10 ($2CH_2$, C_{12}), 33.00 ($2CH_2$, C_3), 28.06 ($2CH_3$, C_{18}), 21.92 ($2CH_2$, C_6), 18.97 ($2CH_2$, C_2), 18.44 (2CH, C_{11}), 16.40 ($2CH_3$, C_{20}). MS (EI, *m/z* (%)): 256.2 (M^+-$C_{21}H_{30}O_3$, 37.6), 241.2 (M^+- $C_{22}H_{33}O_3$, 39.9), 157.0 (M^+- $C_{28}H_{45}O_3$, 10.6).

2.3. Antibacterial activity

Antibacterial activity was determined using the paper-disk-diffusion agar method [24,25]. The following microorganisms were tested: *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Klebsiella pneumoniae* (ATCC 23357), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). An 18-hour culture of each microorganism in 2.5 mL of Müeller-Hinton (MH) broth at 37 °C was used. The bacterial inoculum was adjusted with physiological saline solution to the Mc Farland turbidity standard N ° 0.5 (1×10^8 CFU/mL). Each inoculum was spread with a swab on the surface of a plate containing Müeller-Hinton agar and then a filter paper disk (6 mm diameter) previously impregnated with 10 µL of the compound dissolved in DMSO at a concentration of 2 mg/mL for each (1, 2, 3, 4, 5, 6, 7, 8) was placed on the surface. A disc impregnated with DMSO was included as a negative control. Furthermore, the standard disc of the reference antibiotic was placed as a positive control for each microorganism (Oxacillin® 1 µg for *Staphylococcus aureus*; Vancomycin® 30 µg for *Enterococcus faecalis*; Tobramycin® 10 µg for *Escherichia coli*; Aztreonam® 30 µg for *Klebsiella pneumoniae*; Cefepime® 30 µg for *Pseudomonas aeruginosa*). After placing the discs on the Petri plates, they were refrigerated at 4 °C for 24 h [24]. Inhibition halos were read at 24 h and measured (mm) around the disc. All tests were performed in duplicate. The measurement of inhibition halos for antibacterial activity was done as follows: C = A - B (C = Size of the inhibition halo; A = Size of the halo plus the disk of filter paper; B = Size of the filter paper disk, 6 mm).

2.4. Antifungal activity

Antifungal activity was determined by the paper-disk-diffusion agar method [24]. The microorganism tested was *Candida krusei* (ATCC 6558), using Fluconazole 100 µg as a

positive control, and dimethylsulfoxide (DMSO) as a negative control. The strain had been repacked 12 hours before it came to a salted dextrose agar and it was used to prepare the inoculum which was compared to the Mc Farland Turbidity Standard N° 1.5 (10^8 CFU/mL). Each inoculum was spread with a swab on the surface of a plate containing Müeller-Hinton agar and then 6 mm diameter holes were opened in it, where 20 µL of the compounds dissolved in DMSO were placed at a 2 mg/mL concentration for each one (**1**, **2**, **3**, **4**, **5**, **6**, **7**, **8**). One well with DMSO was included as a negative control and one well with the reference antibiotic as a positive control. The Petri plates were then refrigerated at 4 °C for 24 h [26]. Inhibition halos were read at 24 h and measured (mm) around the well. All tests were performed in duplicate.

3. Results and discussion

3.1. Chemistry

In recent decades, the introduction of ester functional groups into organic compounds has become increasingly common in drug design and development, as ester substitution can greatly improve the solubility, bioavailability, and absorption of drugs [27]. In this context, previous studies on the biological activity of *ent*-kaurene-type diterpenoids and triterpenes reported that the presence of oxygenated groups such as hydroxyls or esters on the C-3 carbon of the *ent*-kaurene core, as well as in triterpenes (whose core is largely constituted by a decahydronaphthalene ring, such as the *ent*-kaurene core with rings A and B), increases biological activity [28-30]. Therefore, in this study, structural modifications were performed on the carbon C-3 of the *ent*-kaurene core of compound **1** (Scheme 1) by introducing different oxygenated groups, especially esters, to investigate the structure–activity relationship (SAR) of the derivatives *ent-kaur-3-acetoxyalkylated* and further enhance their antimicrobial power. The structure of the synthetized derivatives was characterized by IR, ^{1}H NMR, ^{13}C NMR, and GC-MS.

The natural product *ent*-kaur-3-acetoxy-15-ene (**1**) was first subjected to a reduction reaction of the acetate group using $LiAlH_4$, forming the C3-hydroxylated derivative *ent*-kaur-3-hydroxy-15-ene (**2**). FT-IR analysis showed evidence of this transformation by the appearance of a characteristic absorption band of alcohol (3392 cm^{-1}, O-H) and the disappearance of the characteristic absorption bands of the ester group [1735 cm^{-1} (O−C=O) and 1243 cm^{-1} (O−C)] [23]. In the ^{1}H NMR spectrum, the disappearance of the singlet of the methyl protons of the ester group at δ 1.98 ppm and the appearance of a small singlet at δ 3.57 ppm of the proton of a secondary hydroxyl group, as well as the high-field shift of the signal coming from the H_3 methine proton at δ 3.15 ppm is mainly observed compared to compound **1**. The ^{13}C NMR spectrum confirms the presence of 20 carbons, mainly the disappearance of the carbonyl signal of the ester group at δ 171.17 ppm is observed, together with the methyl signal of the same terminal ester at δ 21.47 ppm, both signals belonging to the starting compound (**1**) [23], and the shift to a high field of the methine carbon at δ 79.06 ppm. On the other hand, its mass spectrum showed the molecular ion peak at *m/z* 288 [M+] consistent with their molecular formula with five unsaturations.

The derivative **3**, *ent*-kaur-3-oxo-15-ene, was obtained through an oxidation reaction of the hydroxyl group of compound **2** using SIBX as an oxidizing agent. FT-IR showed the characteristic band of the carbonyl group at 1709 cm^{-1}, which is an absent band compared to the IR spectrum of compound **2**, as well as the disappearance of the hydroxyl group signal. In the ^{1}H NMR spectrum, the multiplet corresponding to the methyne proton H_3 at δ 3.15 ppm disappears compared to the ^{1}H NMR of **2**, as well as the small downfield singlet of the hydroxyl group at δ 3.57 ppm. The low-field shift of the H2 methylene proton signal at δ 2.71 ppm is also notable, which is due to the effective oxidation of the hydroxyl group to carbonyl. The ^{13}C NMR spectrum confirms the presence of 20 carbons, mainly showing the shift to the low field of carbon C_3 at δ 216.86 ppm with respect to compound **2**, as well as an increase in the δ of methylene C_2 at δ 34.37 ppm and the carbon quaternary C_4 at δ 47.58 ppm. The GC-MS study showed the molecular ion peak at *m/z* 286 [M+] consistent with their molecular formula with six unsaturations.

Acetoxy-alkylated derivatives (**4**-**8**) were obtained by Steglich Esterification between compound **2** and variation of different carboxylic acids (Scheme 1), using DCC as reaction activator and DMAP as catalyst. In view of the FT-IR spectra, the absence of the peak corresponding to -OH and the appearance of two characteristic absorption bands in the range of 1730-1740 cm^{-1} (-O-C=O) and 1300-1000 cm^{-1} (C-O) indicated that compound **2** (alcohol) had been converted into products (esters). Specifically, derivative **4** shows a smaller absorption shift compared to the carbonyl band (1702 cm^{-1}, CO-C=C-) due to being conjugated, and a more intense band corresponding to the alkene group stands out (1633 cm^{-1}, C=C). For derivative **5**, in addition to the presence of bands of the ester group at 1733 cm^{-1} (-O-C=O) and 1255 cm^{-1} (C-O), a band at 1136 cm^{-1} characteristic of the halogen-aromatic ring bond (Br-Ar) is also shown, and at 804 cm^{-1} the band indicates the *para* disposition of this halogen on the aromatic ring. In derivative **6**, the presence of a carboxyl group is confirmed by observing a characteristic broad absorption band of the O-H bond at 3368 cm^{-1} and a characteristic carbonyl band at 1710 cm^{-1}, as well as the presence of ester group bands at 1719 cm^{-1} (-O-C=O), 1378 cm^{-1} (C-O, tension vibration) and 1082 cm^{-1} (C-O, bending vibration). Compound **7** showed bands of the ester group at 1731 cm^{-1} (-O-C=O) and 1133 cm^{-1} (C-O); and in derivative **8**, the presence of characteristic bands of the anhydride group at 1789 cm^{-1} (>C=O, asymmetric vibration), 1729 cm^{-1} (>C=O, symmetric vibration) and 1378 cm^{-1} (C-O of anhydride) stands out, confirming the formation of this functional group in the structure.

Concerning the ^{1}H NMR spectrum, the downfield shift of the H_3 proton in compounds **4**-**8** in contrast to compound **2** confirms the transformation of the OH group. Derivative **4** showed the H_3 signal at δ 4.66 ppm, two singlets at δ 5.22 ppm and 5.21 ppm attributed to the methylene protons $H_{1''}$ and $H_{1'''}$, respectively, and two doublets at δ 6.26 ppm and δ 7.56 ppm corresponding to the methynic protons $H_{2'}$ and $H_{3'}$, respectively, which lie on the conjugated C=C double bond; a set of signals corresponding to the aromatic rings of the 3,4-bibenzyloxy-caffeoyl system are also observed. Compound **5** showed the methyne H_3 signal at δ 4.51 ppm, a singlet signal at δ 3.59 ppm attributed to the methylene protons $H_{2'}$, and two doublets are observed at δ 7.20 ppm and δ 7.45 ppm at a low field, attributed to the phenyl protons $H_{4'}$ and $H_{5'}$, respectively. Compound **6** exhibited the methyne H_3 signal at δ 4.47 ppm, and two strong triplets at δ 2.57 ppm and δ 2.61 ppm attributed to the methylene protons $H_{2'}$ and $H_{3'}$, respectively, corresponding to the alkyl part of succinic acid. The derivative **7** displayed the methyne H_3 signal at δ 4.47 ppm, and an intense singlet at δ 2.57 ppm attributed to the methylene protons $H_{2''}$ and $H_{2'''}$, which exhibit a single signal due to molecular symmetry. The proton spectrum of compound **8** differs mainly from compound **2** in the following: the H_{13} methine proton is at δ 2.64 ppm; H_3 proton is a doublet and is at δ 2.19 ppm; the vinyl proton H_{15} singlet disappears at δ 5.06 ppm and is now found at δ 2.05 ppm as a multiplet; the signal of the H_{19} methyl protons disappears; H_{17} protons are diasterotopic and show two singlets at δa_{17} 4.74 ppm and δb_{17} 4.80 ppm. These results reveal that the esterification reaction between compound **2** and *ent*-kaurenic acid did not take place; in contrast, the ^{1}H NMR spectrum of this product is consistent with the ^{1}H NMR spectrum of *ent*-kaurenic acid [31].

Table 1. Antibacterial activity of compound **1** and its synthetic derivatives (**2-8**) expressed as diameters of the inhibition halo (mm) [#].

Bacterial strains	Compounds								Positive control				
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**OX**	**VA**	**TO**	**AZ**	**CF**
Staphylococcus aureus	IA	10	IA	16	IA	IA	11	IA	*23	–	–	–	–
Enterococcus faecalis	IA	10	12	12	8	10	10	IA	–	*19	–	–	–
Escherichia coli	8	IA	10	13	8	IA	10	IA	–	–	*26	–	–
Klebsiella pneumoniae	10	8	10	10	8	8	10	8	–	–	–	*34	–
Pseudomonas aeruginosa	8	10	8	8	10	10	10	10	–	–	–	–	*32

[#] IA: inactive; OX: Oxacillin®; VA: Vancomycin®; TO: Tobramycin®; AZ: Aztreonam®; CF: Cefepime®.
* Halo of inhibition in mm for the positive control groups [24,25].

Table 2. Antifungal activity of compound **1** and its synthetic derivatives (**2-8**) expressed as inhibition halo diameters (mm) [#].

Fungal strains	Compounds								Positive control
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**FLU (100 µg)**
Candida krusei	8	10	IA	12	IA	10	8	IA	*15

[#] IA: inactive; FLU: Fluconazole®.
* Halo of inhibition in mm for the control groups [26].

The assignment of ^{13}C NMR spectra of carbon atoms presented in these acetoxy-alkylated derivatives showed the following. Compound **4** contains 43 carbons, highlighting the presence of two signals at δ 71.49 ppm and δ 71.11 ppm corresponding to the carbons of the methylenes $C_{1''}$ and $C_{1'''}$, respectively; two signals at δ 144.12 ppm and δ 117.12 ppm corresponding to the carbons that make up the conjugated double bond $C_{3'}$ and $C_{2'}$, respectively; ester formation is confirmed when the signal corresponding to the quaternary carbon $C_{1'}$ (δ 167.15 ppm, >C=O) is observed in the low field; and a set of signals that are consistent with the carbons that make up the aromatic rings of the 3,4-bibenzyloxy-caffeoyl system. The mass spectrum confirms the structure by showing two abundant ions at *m/z* 341.2 (M^+-$C_{20}H_{18}O_2$) and *m/z* 289.1 (M^+-$C_{26}H_{41}O_2$), which correspond to the breaking of the molecule in half. In derivative **5**, the presence of 28 carbons is confirmed, emphasizing the presence of two signals at δ 121.13 ppm and δ 133.53 ppm corresponding to the quaternary carbons $C_{3'}$ and $C_{6'}$, respectively, and two signals at δ 131, 17 ppm and δ 131.73 ppm corresponding to the $C_{4'}$ and $C_{5'}$ carbons, respectively; which make up the aromatic ring from *p*-bromo-phenylacetic acid; in addition, ester formation is confirmed by observing the signal corresponding to the $C_{1'}$ carbonyl carbon (δ 170.89 ppm, >C=O) in the low field. The mass spectrum confirms the structure by showing an abundant ion at *m/z* 405.2 (M^+-Br).

The presence of 24 carbons is confirmed in compound **6**; the presence in a high field of two signals at δ 29.46 ppm and δ 29.15 ppm stands out with respect to $C_{2'}$ and $C_{3'}$ methylenes, respectively; and the two signals at δ 171.96 ppm and δ 177.94 ppm attributed to the $C_{1'}$ carbonyl carbon of the ester group and the $C_{4'}$ carboxylic carbon, respectively, confirming the presence of both functional groups in the structure of this derivative. The mass spectrum confirms the structure showing the molecular ion peak at *m/z* 388.2 [M+] consistent with their molecular formula with seven unsaturations. Compound **7** highlights 22 carbon signs, showing the presence of a signal at δ 29.80 ppm at a high field corresponding to methylenes $C_{2'}$, and at a low field, a signal at δ 172.16 ppm corresponding to the carbonyl carbons C1' of the ester group. The mass spectrum demonstrates the formation of a dimeric structure by showing three abundant ions at *m/z* 468 (M^+-$C_{14}H_{21}$, 34.6), 426.2 (M^+-$C_{17}H_{27}$, 35.5), and 342.2 (M^+-$C_{21}H_{30}O_2$, 40.7), consistent with their molecular formula with twelve unsaturation. Compound **8** presents quantifiable signals for 19 carbons, highlighting: C_3 signal at δ 33.00 ppm, C_{15} carbon at δ 48.92 ppm, C_{16} at δ 155.77 ppm, C_{17} at δ 103.10 ppm, and C_{19} at δ 173.55 ppm, which is found at δ 184.72 ppm in ^{13}C NMR spectrum of *ent*-kaurenic acid [30]. The similarity of these data with those reported in the bibliography for ^{13}C NMR of *ent*-kaurenic acid [32], and also the displacement obtained from the C_{19} signal indicate that this carboxylic acid reacted with itself, forming a symmetrical anhydride (dimer). This is confirmed with the mass spectrum, where three abundant ions are shown at *m/z* 256.2 (M^+-$C_{21}H_{30}O_3$, 37.6), *m/z* 241.2 (M^+- $C_{22}H_{33}O_3$, 39.9), and *m/z* 157.0 (M^+- $C_{28}H_{45}O_3$, 10.6), consistent with their molecular formula with twelve unsaturation. The formation of this collateral product could be explained by the steric hindrance exerted by the bulky structure of *ent*-kaurenic acid on the alkoxide (also bulky) formed from compound 2, which is a weaker nucleophile than the carboxylate formed from the same acid. Therefore, the formation of the anhydride occurs. According to Steglich *et al*., when poor nucleophiles are used in Steglich esterification, side product formation prevails [33].

3.2. Antimicrobial activity

In this study, the antibacterial activity of the synthetic derivatives of compound **1** (**2**-**8**) was evaluated using the disk diffusion method (Kirby-Bauer). The results obtained are summarized in Table 1 and are expressed as the diameters of the inhibition halo reported in millimeters.

The synthesized compounds evaluated showed specific antibacterial activity against some bacteria used. Compounds **4** and **7** showed antibacterial activity by generating inhibition halos against all tested, compound **4** being the one generating the highest inhibition halos (*Staphylococcus aureus*: 16 mm; *Enterococcus faecalis*: 12 mm; *Escherichia coli*: 13 mm; *Klebsiella pneumoniae*: 10 mm and *Pseudomonas aeruginosa*: 8 mm). Compound **2** was found to be inactive only against *Escherichia coli*, while compounds **3**, **5**, **6**, and **8** were found to be inactive only against *Staphylococcus aureus*. Finally, compound **6** was found to be active against *Enterococcus faecalis* (10 mm), *Klebsiella pneumoniae* (8 mm), and *Pseudomonas aeruginosa* (10 mm). However, the antifungal activity of the synthetic derivatives was also evaluated through the well diffusion method on Müller-Hinton agar (Kirby-Bauer). The results obtained are summarized in Table 2 and are expressed as the diameters of the inhibition halo reported in millimeters.

Some of the synthetic compounds evaluated showed specific antifungal activity against *Candida krusei*. Compounds **2**: 10 mm, **4**: 12 mm, **6**: 10 mm, and **7**: 8 mm were active, compound **4** being the most active of all (12 mm). On the contrary, compounds **3**, **5**, and **8** did not present inhibition halos.

These results of antimicrobial activity reveal a notable relationship between the structural modification of the C_3 carbon of the *ent*-kaurene core and the activity, which, in general terms, is an improvement. Previous studies on the structure-activity relationship of *ent*-kaurenes have identified that the pharmacological action of these compounds is based on the perhydrophenanthrene unit (rings A, B, and C) fused with a cyclopentane unit (ring D) formed by a bridge of two carbons between C_8 and C_{13} (Figure 1), this skeleton confers lipophilic properties that are essential to cross membranes and occupy

hydrophobic pockets in the target cell [34]. In addition, various authors describe some specific structural requirements for the biological activity of *ent*-kaurenes and terpenes based on a decalin ring: 1) a substituted decalin system, capable of inserting itself into a lipophilic region of the cell membrane, and 2) a hydrophilic or relatively hydrophilic fragment that contains a hydrogen bond donor or acceptor group capable of interacting with acceptor or donor groups in the membrane (mostly phosphorylated groups), as an "anchoring" group; this way, these compounds will have the ability to cross the phospholipid membrane and cause cell damage to the microorganism [35,36].

In this regard, the synthetic *ent*-kaurenes tested in this work present structural characteristics very similar to those described above: the substituted perhydrophenanthrene unit is fused with a cyclopentane unit, which makes them possess an essential lipophilic character to cross the cell membrane. However, each compound possesses distinctive characteristics: compounds **4**, **5**, **6**, and **7** have ester groups in C_3 whose hydrophilicity is low; however, they present a well-defined negative dipole moment in the carbonyl oxygen atom that allows them to accept hydrogen bonds (anchor group) with the hydrophilic part of the membrane phospholipids. In addition, each of these compounds has acidic protons or another reactive system that also allow them to interact with the hydrophilic portion of membrane phospholipids. Compound **4**, aside from having five acidic protons (H_3, $H_{2'}$, $H_{3'}$, $H_{1''}$, and $H_{1'''}$), also shows an *α,β*-unsaturated system whose reactivity is very high and causes electron donor or acceptor dipole moments that are highly defined, making the compound more susceptible to form hydrogen bonds with the phosphorylated groups of the cell membrane. This could be the reason why this compound turned out to be the most active against all of the microorganisms tested.

Unlike the others, compound **2** has a hydroxyl group in C_3, which is a hydrophilic group that donates hydrogen bonds responsible for "anchoring" with the phosphorylated hydrogen bond acceptor groups of the cell membrane. On the contrary, compound **3** has a ketone in C_3, which is a non-polar functional group that confers lipophilicity to the molecule; however, it has a negative dipole moment defined by the oxygen atom, which allows it to be an acceptor of hydrogen bonds with the hydrophilic part of the phospholipids, and it anchors itself to the cell membrane and exerts antibacterial activity. Finally, compound **8** turned out to be slightly active against bacteria and inactive against the fungus, possibly due to the high lipophilicity of the molecule conferred by the two *ent*-kaurene core. According to Urzua *et al.*, the high addition of alkyl moieties to the decalinic system causes a decrease in biological activity due to excess lipophilicity that hinders molecular anchorage with the hydrophilic part of phospholipids [33].

4. Conclusion

A series of seven new *ent*-kaurene-type diterpenes was synthesized from the new natural product *ent*-kaur-3-acetoxy-15-ene (**1**). Structural elucidation was performed using FT-IR, ^{1}H NMR, ^{13}C NMR, and GC-MS spectra. The synthesized compounds were evaluated for their antibacterial and antifungal activities and showed specific activity against some bacterial and fungal strains. *Ent*-kaur-3-*O*-(6',7'-bibenzyl-oxy-caffeoyl)-15-ene (**4**) exhibited the higher antibacterial activity against all microorganisms tested: *Staphylococcus aureus* (16 mm), *Enterococcus faecalis* (12 mm), *Escherichia coli* (13 mm), *Klebsiella pneumoniae* (10 mm), *Pseudomonas aeruginosa* (8 mm) and *Candida krusei* (10 mm). These results reveal a remarkable structure-activity relationship on the C_3 carbon of the *ent*-kaurene core, where the presence of oxygenated groups such as hydroxyl or alkyl esters enhances activity. However, this activity will depend (aside from the fundamental decalin system) on the type and number of hydrophilic or relatively hydrophilic fragments on C_3 containing a hydrogen bond donor or acceptor group capable of interacting with acceptor or donor groups in the phospholipid cell membrane, as an "anchor" group, traversing and causing cell damage to the microorganism.

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CRediT authorship contribution statement

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