Cytotoxic and Antimicrobial Activities of Bioactive Monoterpenophenols

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Compounds 1 - 12 were tested for their growth inhibitory effects against tumor cell lines using two different 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) and sulforhodamine B protein (SRB) assays and antimicrobial activity. The cytotoxic activity of methyl-4-[{(2*E*)-3,7-dimethyl-2,6-octadienyl}oxy]-3-methoxy benzoate (1) exhibit more active than that of 5-fluorouracil (11) on human oral epithelioid carcinoma (KB, ATCC No. OCL 17) cell lines. But this compound (1) on human skin melanoma (SK-MEL-3, HBT 69) cell lines shows less active than that of adriamycin (12). However, compound 9 showed the antimicrobial activity against *S. epidermidis* (MIC, 15.625 ㎍/㎖), *S. aureus, C. albicans* (MIC, 31.25 ㎍/㎖), *S. mutans, S. typhimurium, P. putida* (MIC, 125 ㎍/㎖) and *P. aeruginosa* (MIC, 500 ㎍/㎖)

Key words : Growth inhibitory effects, MTT, SRB, KB, SK-MEL-3

Introduction

Plants of the genus *Trichocolea* (Trichocolaceae) are a treasury of biologically active monoterpenoids. The potent cytotoxicity of *Trichocolea* monoterpenoids against BSC cell has been reported. In series of studies on the cytotoxic monoterpenoids of the genus *Trichocolea*, Perry et al. isolated cytotoxic monoterpenoids of the isoprenyl phenyl ether type from *T. mollissima, T. tomentella* and *T. lanata,* and reported cytotoxic and antifungal activities.[1] *Trichocolea* (family Trichocolaceae) are a treasury of isoprenyl phenyl ethers. Extraction of the common New Zealand Trichocolaceae, *T. lanata, T. mollissima,* and *T. hatcheri* may establish a chemotaxonomic pattern based on the presence of the 4-isoprenyl ethers. These discoveries prompted us to investigate another New Zealand's *Trichocolea* species, *T. hatcheri* Hodgs. This species, which grows throughout New Zealand, is distinguished from *T. mollissima* by its smaller size, dark green colour and prostrate habit.[2] An extract of *Trichocolea hatcheri* showed cytotoxic effects against monkey kidney (BSC) cell lines. Reversed-phase flash chromatography concentrated the cytotoxic activity in fractions eluted with CH_3CN - H_2O 3 : 1 and 9 : 1. Si-gel column chromatography spread cytotoxic activity across ethyl acetate - cyclohexane 3 : 97 and 5 : 95 fraction containing methyl-4-[{(2*E*)-3,7-dimethyl -2,6-octadienyl}oxy]-3-methoxybenzoate (1, deoxytomentellin).[3] This compound was obtained pure in quantities too small for biological assays, however synthesis and biological activity are reported here. Cannabidiol (2) has been reported to possess potentially important pharmacological properties as an anti-epileptic,[4] anxiolytic,[5] and as an antidyskinetic agent.[6] Cannabidiol (2) is structually related to olivetol (9), a known inhibitory effect,[7] inhibited the least effective growth-inhibitory activity against the tested cancer cell lines. Most of the cannabinoids were found to be allergenically cross-reactive. The actions of the cannabinoids on membrane associated enzymes are complex and in a dose-dependent. Although these actions are possibly associated with the ability of the cannabinoids to act as anticancer agents,[8] specific structural alterations may be critical in determining enzyme targets. Eisohly et al.[9] have reported that cannabigerol type compounds having a methyl side chain in most cases exhibit an increased antifungal and antibacterial activities.

In the present study, the growth-inhibitory activity against human tumor cell lines and antimicrobial activities were investigated.

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Materials and Methods

1. Instruments

Melting points were determined on a Kofler hot stage and were uncorrected. ^1H and ^{13}C-NMR spectra were recorded using Varian Gemini-200 and Varian VXR-300 spectrometers. When $CDCl_3$ was used as a solvent, $CHCl_3$ (1H, δ H, 7.27) or $CDCl_3$ (^{13}C, δ C, 77.08) was used as internal reference. IR spectra were recorded on a Perkin-Elmer 1600 FT-IR spectrophotometer. Mass spectra were recorded on a Varian Mat CH-5 mass spectrometer. TLC was carried out on Si gel 60 F254 precoated 0.20 mm aluminium sheet (Merck 5562). Developed plates were visualised by UV light and staining with a 5% solution of anisaldehyde in ethanol. Flash chromatography was carried out with Si gel 230 - 400 mesh. Medium pressure liquid chromatography was performed on an ALTEX glass column, 1 meter long, diameter 9 mm internal using an FMI pump and silica gel 60 (230-400 mesh) purchased from Merck. Fractions were collected with LKB 2070 or LKB 7000 fraction collectors at a rate of 2-10 ㎖/min. Cells were counted by Hemocytometer. KB and SK-MEL-3 cell lines were cultured in RPMI-1640 medium (Gibco Laboratories) containing 10% fetal bovine serum. Exponentially growing tumor cells (5×10^4) were cultured for 48 hrs at 37 ℃ in a humidified 5% CO_2 incubator. Absorbance was read on ELISA reader (SPECTRA max 250, Molecular Devices, U.S.A.).

2. Chemicals

5-Fluorouracil, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazoliumbromide, fetal bovine serum (FBS), sulforhodamine B protein, streptomycin, adriamycin, and penicillin were obtained from Sigma Chemical Co., Ltd. (St. Louis, U.S.A.). Olivetol, geranyl bromide, sodium hydride and dimethyl sulphate were purchased from Aldrich Chemical Co., Ltd. (Milwaukee, U.S.A.). 3-Methyl-4-hydroxybenzoic acid was obtained from Merck Chemical Co. (Germany). Cannabidiol (CBD, 2)[10] was kindly provided by Professor Raphael Mechoulam in the Department of Medicinal Chemistry and Natural Products at School of Pharmacy, Hebrew University, Israel. All other chemicals were of reagent grade.

3. Tumor cell lines and culture conditions

Tumor cells were obtained from Korean Cell Line Bank in the Seoul National University. In vitro RPMI-1640 medium was supplemented with 10% FBS, 0.1% ㎎/㎖ streptomycin and 100 units/㎖ penicillin at 37 ℃ in 5% carbon dioxide. Cells were dissociated with 0.25% trypsin just before transferring for the experiment and were counted by Hemocytometer. The cytotoxic activities of methyl-4-[{(2*E*)-3,7-dimethyl-2,6-octadienyl}oxy]-3-methoxybenzoate (1), cannabidiol (2), methyl-4-[{(2*E*)-3,7-dimethyl-2,6-octadienyl}oxy]-3-hydroxybenzoate (3), methyl-3,4-di[{(2*E*)-3,7-dimethyl-2,6-octadienyl}oxy]benzoate (4), 3-methyl-4-hydroxybenzoic acid (5), methyl-4-methoxy-3-hydroxybenzoate (6), methyl-3,4-dihydroxybenzoate (7), geraniol (8), olivetol (9), cannabigerol (10), 5-fluorouracil (11) and adriamycin (12) against human oral epithelioid carcinoma (KB) cell lines, human skin melanoma (SK-MEL-3) cell lines and NIH 3T3 fibroblasts were measured by MTT and SRB methods.

4. *Preparation of cannabigerol (10)*

Cannabigerol (10) was identified by comparison of its spectral data (TLC, MS, NMR and IR) with those published or by direct comparison with an authentic sample.[11,12]

5. Preparation of cannabidiol (2).

Cannabidiol (2) was kindly provided by Professor Raphael Mechoulam in the Department of Medicinal Chemistry and Natural Products at School of Pharmacy, Hebrew University, Israel.

6. Preparation of methylbenzoates (6, 7).[13]

3-Methyl-4-hydroxybenzoic acid (5, 3.08 g, 20.0 mmol) in dry acetone (10 ㎖)was mixed with anhydrous sodium hydrogen carbonate (2.52 g, 30.0 mmol) and dimethyl sulfate (2.78 g, 22.0 ㎖) and was heated gently under reflux. On completion of the reaction, as shown by TLC, the solvent was removed under reduced pressure, the reside stirred with cold water and chloroform, the separated ester was filtered, washed and dried. The residue was chromatographed on flash chromatography (20% EtOAC / Hex. to afford methyl-4-methoxy-3-hydroxybenzoate (6, 2.88 g, 39.6%); TLC R_f 0.44 (UV, 40% EtOAC / Hex. 2 times); IR (film), 3409, 2952, 1719, 1700, 1592, 1514, 1413, 1285, 1221, 1128, 1025, 989, 891, 763 cm^{-1}.; ^1H-NMR ($CDCl_3$), δ 7.54 (1H, ddd, J=6.6 Hz, H-6), 7.49 (1H, dd, J=7.2 Hz, H-2), 6.82 (1H, dd, J=19.8 Hz, H-5), 3.82 (3H, s, CH_3), 3.81 (3H, s, CH_3); ^{13}C-NMR ($CDCl_3$), δ 51.7 (C-8), 55.7 (C-9), 109.9 (C-5), 115.5 (C-2), 122.5 (C-1), 122.8 (C-6), 145.1 (C-3), 150.6 (C-4), 166.9 (C-7). Methyl-3,4-dihydroxybenzoate (7, 3.69 g); Yield (55%); TLC Rf 0.25 (UV, 40% EtOAC / Hex. 2 times), mp. 124-126℃, IR (Nujol), 3466, 1684, 1610, 1533, 1456, 1377, 11294, 1185, 1090, 984, 910, 764, 721 cm^{-1}.; ^1H-NMR ($CDCl_3$), δ 7.61 (1H, brs, H-6), 7.57 (1H, d, J=2 Hz, H-2), 6.92 (1H, d, J=8 Hz, H-5), 5.88 (1H, brs, 4-OH), 5.78 (1H, brs, 3-OH), 3.89 (3H, s, CH3); ^{13}C-NMR ($CDCl_3$), δ 52.1 (C-8), 114.8 (C-5), 116.5 (C-2), 123.8 (C-1), 124.6 (C-6), 140.4 (C-4), 148.6 (C-3), 167.1 (C-7).

7. Preparation of methyl-4-[{(2*E*)-3,7-dimethyl-2,6-octadienyl} oxy]-3-hydroxybenzoate (3) and methyl-3,4-di-[{(2*E*)-3,7- dimethyl -2,6-octadienyl}oxy]benzoate (4)

Methyl-3,4-dihydroxybenzoate (7, 278 ㎎, 1.65 mmol); geranyl bromide (434 ㎎, 2.00 mmol); NaH (60%, 66 ㎎, 1.65 mmol) in dry DMF (2 ㎖); 0℃; 17 hr; flash chromatography (5% EtOAC-hexane) gave 3 (220 ㎎, 44%). Also methyl-3,4-di [{(2*E*)-3,7-dimethyl-2,6-octadienyl}oxy]benzoate (4) (130 ㎎, 18%) was isolated. Compounds (3, 4) were identified by comparing its spectral data (TLC, MS, NMR and IR) with those published or by directly comparing it with an anthentic sample.[3]

8. Preparation of methyl-3-methoxy-4-hydroxybenzoate (13).

3-Methyl-4-hydroxybenzoic acid (7, 10.610 g, 63 mmol), and sodium bicarbonate (8.100 g, 96 mmol) in acetone (50 ㎖) was heated under reflux with dimethyl sulphate (8.731 g, 69 mmol) for 48 hrs. On completion of the reaction, as shown by TLC, solvent was removed under reduced pressure, to give a brown oil. Ice cold water (50 ㎖) was added and vigorously stirred for two hours to give a pale solid (9.7 g, 85%) which was collected by filtration. Found; TLC Rf 0.75 (50% ether /hexane); mp 60-64℃. methyl-3-methoxy-4-hydroxybenzoate (methyl vanillate, 13) obtained was identified by comparison of its spectral data (TLC, ^{1}H-NMR and ^{13}C-NMR) with those published or by direct comparison with an authentic sample.[13,14]

9. Preparation of methyl-4-[{(2E)-3,7-dimethyl-2,6-octadienyl} oxy]-3-methoxybenzoate (1).

Geranyl bromide (14, 295 ㎎, 2.0 mmol) dissolved in dry DMF (2.0 ㎖) was added dropwise, with stirring, to a flame dried flask containing methyl vanillate (13, 300 ㎎, 1.65 mmol) and sodium hydride (66 ㎎, 1.65 mmol) under nitrogen. The mixture was stirred at 25 ℃ for 26 hrs, following which the reaction mixture was partitioned between water (50 ㎖) and CH_2Cl_2 (50 ㎖). The water fraction was re-extracted with CH_2Cl_2 (50 ㎖), the organic fractions combined, dried with $MgSO_4$, and the solvent removed under reduced pressure. The residue was chromatographed on flash chromatography (5% EtOAc/Hex) to afford methyl-4-[{(2*E*)-3.7-dimethyl-2.6-octadienyl} oxy]-3-methoxybenzoate (1, 380 ㎎, 60%):[14] yellow crystals ; TLC Rf 0.31 (grey with vanillin/H_2SO_4) : mp 39 - 40 ℃: calculated for $C_{19}H_{26}O_4$, C 71.70%, H 8.18%, found C 71.79%, H 8.31% ; methyl-4-[{(2*E*)-3.7-dimethyl-2,6-octadienyl}oxy]-3-methoxybenzoate (1) was shown to be identical with authentic sample on the basis of comparison of the spectral data (^{1}H and ^{13}C-NMR).[2]

10. Evaluation of cytotoxic activity

The antitumor activity of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 was determined by the modification of the literature methods.[15-17]

11. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay

The assay is dependent on the cellular reduction of water-soluble MTT (Sigma Chemical Co. St. Louis, M.O.) by mitochondrial dehydrogenase of vial cells to a blue water -insoluble formazan crystal product which can be measured spectrophoto- metrically.[15,16,18] KB and SK-MEL-3 cell lines were cultured in RPMI-1640 medium (Gibco Laboratories) containing 10% fetal bovine serum. Exponentially growing tumor cells (5 × 10^4) were cultured for 48 hrs at 37 ℃ in a humidified 5% CO_2 incubator in the presence or absence of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12.

12. Sulforhodamine B protein (SRB) assay

The SRB assay was performed essentially according to the method of Skehan et al.16 The methods of plating and incubation of cells were identical to those cells of the MTT assay.

13. Microorganisms

The microorganisms used included: *Streptococcus aureus* (ATCC 29213), *Streptococcus mutans* (JC-2), *Staphylococcus epidermidis* (ATCC 12228), *Pseudomonas aeruginosa* (KCTC 1636). *Pseudomonas putida* (KCTC 8729), *Candida albicans* (KCTC 1940).

14. Screening for antimicrobial activities

Sample was dissolved in 10% aqueous dimethyl sulfoxide (DMSO) to a final concentration of 2,000 ㎍/㎖ and sterilized by filtration through a 0.45 ㎛ membrane filter. Antimicrobial tests were then carried out by the agar serial dilution method.[19,20] Each of several concentrations of a tube of molten agar, was then mixed and poured into a the petri plates, and allowed to solidify. The organisms containing 10^6 bacterial cells/㎖ or 10^8 yeast cells/㎖ were inoculated in to the petri plates. After the plates have been incubated for 24 hrs at 37 ℃ for bacteria and for 5-7 days at 22 ℃ for fungi, the lowest concentration of methanolic extracts that inhibits grows of the organisms, was determined by the MIC of the antimicrobial agents. Ampicillin was served as positive controls for *S. aureus, S. mutans,* and *S. epidermidis* whereas ketoconazole was served as a negative control for *P. aeruginosa. S. typhimurium* and *P. putida.* Each test was carried out in triplicate experiments.

15. Statistical Analysis

All values, expressed as mean ± S.D., were statistically

analyzed through the analysis of Student's t-test. The P value less than 0.05 was considered to be significant.

Results and Discussion

The present study also compares the *in vitro* growth inhibitory activities of 1, 2, 3, 4, 8, 9, 10, 11, and 12 against KB and SK-MEL-3 cell lines. These compounds (1, 2, 3, 4, 8, 9, 10, 11, and 12) were evaluated for antitumor efficacy against KB and SK-MEL-3 cell lines. The antitumor activity of these compounds (1, 2, 3, 4, 8, 9, 10, 11, and 12) against KB and SK-MEL-3 cell lines is given in Tables 1 and 2.[21,22] Tables 1, and 2 show the *in vitro* growth inhibitory activities of 1, 2, 3, 4, 8, 9, 10, 11, and 12 as reference against KB and SK-MEL-3 cell lines. In general, the antitumor activities of these compounds (1, 2, 3, 4, 8, 9, 10, 11, and 12) were in a dose-dependent over the micromolar concentration range 1 μM to 100 μM, and the susceptibilities of KB and SK-MEL-3 cell lines to these compounds were quite different. The comparison of IC_{50} values of these compounds in KB cell lines shows that their susceptibilities to these compounds decrease in the following order: 1 > 2 > 8 > 11 > 10 > 2 > 8 by MTT assay and 1 = 2 > 11 > 10 > 8 > 9 > 2 by SRB assay (Table 1).

Table 1. The cytotoxic activities of 1, 2, 3, 4, 8, 9, 10, and 11 on KB cell lines by SRB and MTT assays

Compounds[a]	IC_{50} (μ M)[b]	
	MTT assay	SRB assay
1	5.55	41.42
2	19.22	41.42
3	160.68	554.12
4	837.54	9946.20
8	482.74	221.73
9	36.21	89.80
10	45.55	69.40
11	44.36	45.20

a) Each compound was examined in four concentrations in four sets of experiments. b) IC_{50} represents the concentration of a compound required for 50% inhibition of cell growth.

Methyl-4-[{(2*E*)-3,7-dimethyl-2,6-octadienyl}oxy]-3-methoxybenzoate (1, DTM) was evaluated for antitumor efficacy against KB cell lines. The *in vitro* growth inhibitory activity of methyl-4-[{(2*E*)-3,7-dimethyl-2,6-octadienyl}oxy]-3-methoxybenzoate (1) against the KB cell lines is given in Table 1. However, methyl-4-[{(2*E*)-3,7-dimethyl-2,6-octadienyl}oxy]-3-methoxybenzoate (1) was the most effective growth inhibitor of KB cell lines, producing an IC_{50} of about 5.6 μM by MTT assay and 41 μM by SRB assay. The sulforhodamine B protein stain assay was compared with the tetrazolium (MTT) colorimetric assay for in vitro chemosensitivity testing of KB cell lines. The MTT assay appeared to be more sensitive than the SRB assay, with a better linearity with cell number and higher reproducibility. Olivetol (8), the simplest compound of this series of phenolics, was more potent than cannabigerol. This compound is structurally related to cannabigerol (10). However, the antitumor activity of methyl-4-[{(2*E*)-3,7-dimethyl-2,6-octadienyl}oxy]-3-methoxybenzoate (1) exhibit to be more active than that of 5-fluorouracil (11) on KB cell lines (Table 1). As shown in table 1, the antitumor activity of 5-fluorouracil (11) shows the effective growth inhibitor of KB cell lines as a reference.

The comparison of IC_{50} values of these compounds on SK-MEL-3 cell lines show that their susceptibilities to these compounds decrease in the following order: 10 > 12 > 2 > 1 > 9 > 3 > 8 by MTT assay and 12 > 1 > 10 > 2 > 9 > 8 > 3 by SRB assay (Table 2).

Table 2. The cytotoxic activities of 1, 2, 3, 4, 8, 9, 10, and 12 on human skin melanoma (SK-MEL-3) cell lines by SRB and MTT assays

Compounds[a]	IC_{50} (μ M)[b]	
	MTT assay	SRB assay
1	42.48	49.99
2	25.22	84.90
3	111.63	445.61
4	1261.03	1760.79
8	199.96	219.17
9	105.10	116.69
10	9.13	51.93
12	20.12	38.63

a) Each compound was examined in four concentrations in four sets of experiments. b) IC_{50} represents the concentration of a compound required for 50% inhibition of cell growth.

Methyl-4-[{(2*E*)-3,7-dimethyl-2,6-octadienyl}oxy]-3-methoxybenzoate (1, DTM) was evaluated for antitumor efficacy against SK-MEL-3 cell lines. The antitumor activity of methyl-4-[{(2*E*)-3,7-dimethyl-2,6-octadienyl}oxy]-3-methoxybenzoate (1) against the SK-MEL-3 cell lines is given in Table 2. However, methyl-4-[{(2*E*)-3,7-dimethyl-2,6-octadienyl}oxy]-3-methoxybenzoate (1) was less effective than the growth inhibitor of SK-MEL-3 cell lines, producing an IC_{50} of about 42 μM by MTT assay and 50 μM by SRB assay. The sulforhodamine B protein stain assay was compared with the tetrazolium (MTT) colorimetric assay for in vitro chemosensitivity testing of human skin melanoma cell lines. The MTT assay appeared to be more sensitive than the SRB assay, with a better linearity with cell number and higher reproducibility. As shown in table 2, the antitumor activity of adriamycin (12) shows the most effective growth inhibitor of human skin melanoma cell lines as a reference. Table 3 shows in vitro growth inhibitory activities of 1, 2, 3, 4, 8, 9, 10, 11, and 12 against NIH 3T3 fibroblasts. In general, in vitro growth inhibitory activities of these compounds (1, 2, 3, 4, 8, 9, 10, 11, and 12) were in a dose-dependent manner over the concentration range of 1 μM to 100 μM, and the susceptibility of NIH 3T3 fibroblasts to these compounds was quite different. As a reference the comparison of IC_{50} for 1, 2,

3, 4, 8, 9, 10, 11, and 12 were tested on NIH 3T3 fibroblasts by SRB and MTT assays. The comparison of IC_{50} values of these compounds in NIH 3T3 fibroblasts show that their susceptibilities to these compounds decrease in the following order; 12 > 2 > 11 > 1 > 10 > 8 > 3 > 9 by MTT assay, 12 > 2 > 11 > 10 > 1 > 8 > 9 > 3 by SRB assay. Cannabidiol (2) had the most cytotoxic effect of NIH 3T3 fibroblasts, producing a IC_{50} of about 36 μM by MTT assay and 60 μM by SRB assay. The comparison of IC_{50} for 1, 2, 3, 4, 8, 9, 10, 11, and 12 were tested on NIH 3T3 fibroblasts by SRB and MTT assays. As shown in table 3, the growth inhibitory activity of adriamycin (12) shows the most cytotoxic effect of NIH 3T3 fibroblasts, producing a IC_{50} of about 23 μM by MTT assay and 47 μM by SRB assay as a reference. However, olivetol (9) had the least cytotoxic effect of NIH 3T3 fibroblasts, producing a CD_{50} of about 163 μM by MTT assay and 318 μM by SRB assay. The growth inhibitory activity of 1 *in vitro*, exhibits more active than that of 11 on KB cell lines. But this compound (1) on human skin melanoma cell lines shows less active than that of adriamycin (12). This compound (1) has been selected as a lead compound on KB cell lines for further examination.

Table 3. The cytotoxic activities of 1, 2, 3, 4, 8, 9, 10, 11, and 12 on NIH 3T3 fibroblasts by SRB and MTT assays.

Compounds[a]	IC_{50} (μ M)[b]	
	MTT assay	SRB assay
1	56.92	102.70
2	36.27	60.25
3	113.71	447.55
4	837.54	946.20
8	101.07	181.26
9	162.82	317.66
10	60.46	82.98
11	41.27	75.90
12	23.26	46.94

a) Each compound was examined in four concentrations in four sets of experiments. b) IC_{50} represents the concentration of a compound required for 50% inhibition of cell growth.

Compounds 1 - 9, ampicillin, and ketoconazole were evaluated for antimicrobial activity against the Gram-positive bacteria; *S. mutans, S. epidermidis,* and *S. aureus,* and the Gram-negative bacteria; *S. typhimurim, P. aeruginosa, P. putida,* and fungus; *C. albicans*. Minimum inhibitory concentrations (MICs) of these compounds are summarized in Table 4. These compounds indicate that starting materials (8 and 9) having benzoate moieties have potent activity and isoprenyl phenyl ethers (1, 3 and 4) possessing a geranyl group reduces the antimicrobial activity. Also cannabinoids (2 and 10) decreased the antimicrobial activity. However, compounds 8 and 9 had higher inhibitory activity than the other compounds against Gram-positive bacteria, Gram-negative bacteria, and fungus.

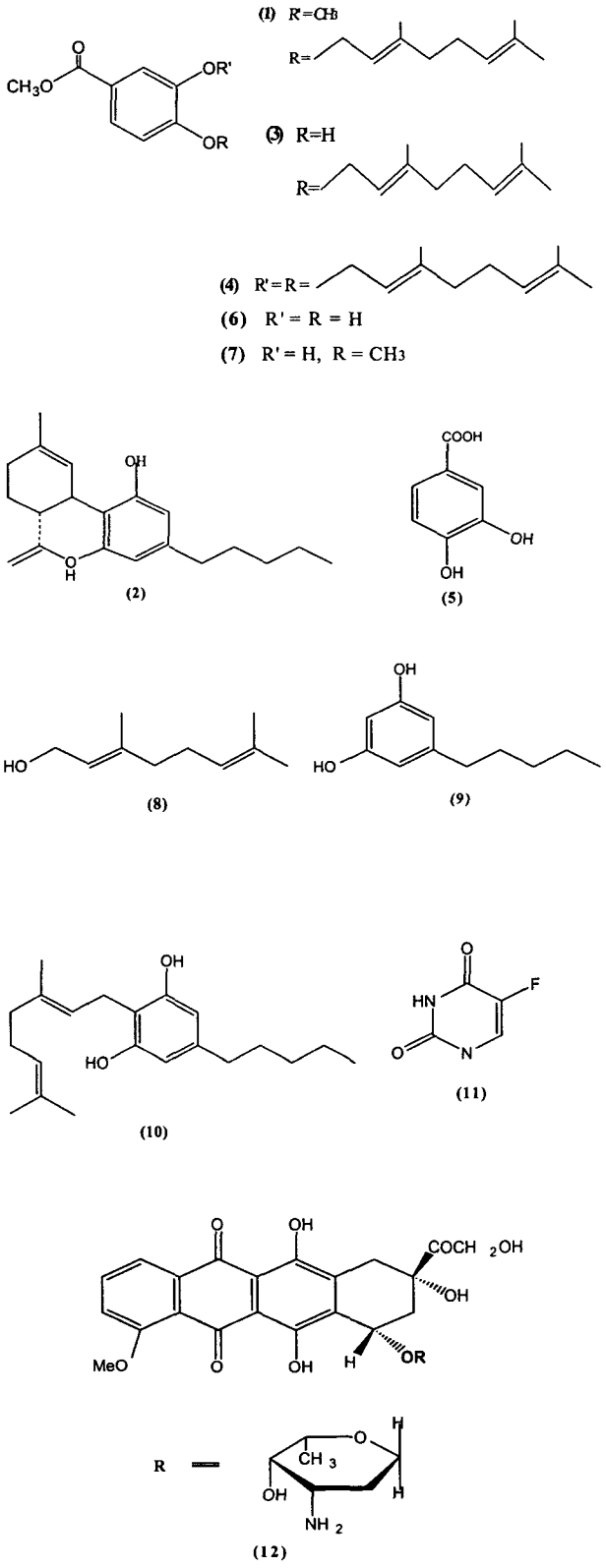

Fig. 1. The structures of methyl-4-[{(2E)-3,7-dimethyl-2,6-octadienyl}oxy]-3-methoxybenzoate (1), cannabidiol (2), methyl-4-[{ (2E)-3,7-dimethyl-2,6-octadienyl}oxy]-3-hydroxybenzoate (3), methyl-3,4-di[{(2E)-3,7-dimethyl-2,6-octadienyl}oxy]benzoate (4) 3-methyl-4-hydroxybenzoic acid (5), methyl-4-methoxy-3-hydr roxybenzoate (6), methyl-3,4-dihydroxybenzoate (7), geraniol (8), olivetol (9), cannabigerol (10), 5-fluorouracil (11) and adriamycin (12).

Table 4. Minimum inhibitory concentrations (MICs) of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 ampicillin and ketoconazole for the reference strains.a

Compounds	MICs (μg/ml) Strains tested		
	S. mutants	*S. epidermidis*	*S. aureus*
1	>1,000	>1,000	>1,000
2	1,000	>1,000	>1,000
3	>1,000	1,000	>1,000
4	>1,000	>1,000	>1,000
5	>1,000	>1,000	>1,000
6	>1,000	>1,000	>1,000
7	>1,000	>1,000	>1,000
8	500	500	1,000
9	125	15.625	31.25
10	>1,000	>1,000	>1,000
AP	3.125	50	3.125
KT	50	50	100

Compounds	MICs (μg/ml) Strains tested			
	P. aeruginosa	*P. putida*	*C. albicans*	*S. typhimurium*
1	>1,000	>1,000	>1,000	>1,000
2	>1,000	>1,000	250	>1,000
3	>1,000	>1,000	>1,000	-
4	>1,000	>1,000	>1,000	>1,000
5	1,000	1,000	>1,000	500
6	>1,000	>1,000	500	>1,000
7	>1,000	1,000	>1,000	1,000
8	1,000	-	250	-
9	500	125	31.25	125
10	>1,000	>1,000	>1,000	>1,000
AP	50	>200	>200	>200
KT	200	50	25	25

MICs were examined in triplicate experiments (mean ± standard deviation, n=3). AP: Ampicillin, KT: Ketoconazole.

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