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Antibacterial constituents from *Melodinus suaveolens*

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[ABSTRACT] To investigate the non-alkaloidal chemical constituents of the stems and leaves of *Melodinus suaveolens* and their antibacterial activities. Compounds were isolated and purified by repeated silica gel, Sephadex LH-20, RP_{18}, and preparative HPLC. Their structures were elucidated by comparison with published spectroscopic data, as well as on the basis of extensive spectroscopic analysis. The antibacterial screening assays were performed by the dilution method. Fourteen compounds were isolated, and identified as lycopersene (**1**), betulinic aldehyde (**2**), 3*β*-acetoxy-22,23,24,25,26,27-hexanordammaran-20-one (**3**), 3a-acetyl-2, 3, 5-trimethyl-7a-hydroxy-5-(4,8,12-trimethyl-tridecanyl)-1,3a,5,6,7,7a-hexahydro-4-oxainden-1-one (**4**), 3*β*-hydroxy-28-norlup-20(29)-ene-17*β*-hydroperoxide (**5**), 3*β*-hydroxy- 28-norlup-20(29)-ene-17*α*-hydroperoxide (**6**), *β*-sitosterol (**7**), 28-nor-urs-12-ene-3*β*, 17*β*-diol (**8**), *α*-amyrin (**9**), ergosta-4,6,8(14),22-tetraen-3-one (**10**), 3*β*-hydroxy-urs-11-en-28,13*β*-olide (**11**), betulin (**12**), obtusalin (**13**), and ursolic acid (**14**). Among the isolates, compounds **1**, **2**, **6**, **8**, **10**, and **14** showed potent antibacterial activities against the four bacteria. This is the first report of the antibacterial activity of the constituents of *Melodinus suaveolens*.

[KEY WORDS] *Melodinus suaveolens*; Apocynaceae; Non-alkaloidal chemical constituents; Meningitis; Antibacterial activity

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Introduction

The genus *Melodinus* (Apocynaceae) is woody lianas or sometimes low shrubs, which is mainly distributed in tropical and subtropical Asia [1]. Previous pharmacological investigations of the crude extracts and purified alkaloids from some *Melodinus* plants have demonstrated promising antitumor [2-3] and antibacterial [4] activities. The fruit of *Melodinus suaveolens* (Hance) Champ. ex. Benth. has been used in Chinese folk medicine for the treatment of meningitis in children and rheumatic heart diseases [5]. Meningitis is an inflammation of the thin tissue that surrounds the brain and spinal cord, called the meninges. A retrospective study of 547 cases of meningitis reported that 236 were bacterial in etiology [6]. The traditional use of the plant stimulated the investigation of the antibacterial constituents from this plant. Phytochemical investigations of *M. suaveolens* mostly afforded alkaloids [7-9], while the non-alkaloidal constituents were seldom reported. Previous work in this laboratory reported the isolation and cytotoxic activities of melodinines M−U from *M. suaveolens* [10]. In a continuation of this research, the non-alkaloidal chemical constituents of *M. suaveolens* were studied. As a result, fourteen compounds were isolated, and all of the isolates were evaluated for their antibacterial activities. This is the first report of the antibacterial activity of the constituents of this species. Here, the isolation, structure determination, and antibacterial activities of compounds from *M. suaveolens* are reported.

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All the authors have no conflict of interest to declare.

Experimental

Apparatus and reagents

NMR spectra were obtaibed on Bruker DRX-500/600

and AV-400 spectrometers (Bruker Co., Bremerhaven, Germany) with TMS as internal standard. Chemical shifts (δ) are expressed in ppm with reference to the solvent signals. Mass spectra were recorded on a VG Autospec-3000 spectrometer (VG instruments, Manchester, UK) or Xevo TQ-S spectrometer (Waters Co., Manchester, UK). Column chromatography (CC) was performed on silica gel (200−300 mesh, Qingdao Marine Chemical Ltd., Qingdao, China), RP_{18} gel (20−45 μm, Fuji Silysia Chemical Ltd., Kasugai, Japan), MCI gel (75−150 μm, Mitsubishi Chemical Co., Ltd., Tokyo, Japan), Sephadex LH-20 (Pharmacia Fine Chemical Co., Ltd., Uppsala, Sweden), and preparative HPLC (Agilent Technologies Co., Ltd., California, USA). Fractions were monitored by TLC (GF_{254}, Qingdao Marine Chemical Co., Ltd. Qingdao, China), and spots were visualized by 10% H_2SO_4 in EtOH.

Plant material

The stems and leaves of *M. suaveolens* were collected from Luchun County, Yunnan Province, China on October 26th (2009), and identified by Dr. ZENG Chun-Xia,, Kunming Institute of Botany. A voucher specimen (No. Zeng 20091026) has been deposited at Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China.

Extraction and isolation

The air-dried stems and leaves (16 kg) of *M. suaveolens* were powdered, and then extracted with 90% MeOH (4 × 24 h). The extract was dissolved in 0.5% HCl solution and partitioned with EtOAc. The acidic water-soluble material, adjusted to pH 9−10 with 10% ammonia solution, was extracted with EtOAc to give non-alkaloidal extract. The non-alkaloidal extract (300 g) was subjected to gradient silica gel column chromatography ($CHCl_3$−Me_2CO, 1 : 0−1 : 1) to afford fractions I−VI. Fraction I (100 g) was separated by silica gel CC (petroleum ether−EtOAc, 200 : 1) to afford compound **1** (48.9 mg). Fraction II (32.5 g) was applied to MPLC with RP-18 CC (MeOH−H_2O, 70 : 30−100 : 0), followed by silica gel CC (petroleum ether−Me_2CO, 50 : 1−20 : 1) to yield compounds **2** (62.2 mg) and **3** (88.8 mg). Fraction III (17.5 g) was subjected to MPLC with RP_{18} CC (MeOH−H_2O, 60 : 40−90 : 10), followed by silica gel CC (petroleum ether−EtOAc, 20 : 1−15 : 1), Sephadex LH-20 ($CHCl_3$−MeOH, 1 : 4) and preparative HPLC to give compounds **4** (29 mg), **5** (36.8 mg), **6** (16.6 mg), **7** (1.5 g), and **8** (8.2 mg). Fraction IV (11.4 g) was subjected to MPLC with RP_{18} CC (MeOH−H_2O, 60 : 40−80 : 20) to give subfractions IV-a and IV-b. Subfraction IV-a was purified by Sephadex LH-20 ($CHCl_3$−MeOH, 1 : 3), and then further separated by silica gel CC ($CHCl_3$−Me_2CO, 15 : 1) to yield compounds **9** (97 mg) and **10** (5.3 mg). Subfraction IV-b was subjected to preparative HPLC to give compound **11** (3.4 mg). Fraction V (5.2 g) was subjected to MCI gel (MeOH−H_2O, 60 : 40−100 : 0), followed by Sephadex LH-20 (MeOH) and then preparative HPLC to afford compound **12** (263 mg). Fraction VI (27.8 g) was eluted by MCI gel (MeOH−H_2O, 60 : 40−80 : 20), and silica gel CC ($CHCl_3$−Me_2CO, 10 : 1) to afford compounds **13** (29.5 mg) and **14** (219.3 mg).

Antibacterial Assays

The antibacterial assay of compounds **1**–**14** were evaluated against *Enterococcus faecalis* ATCC 10541, *Providensia smartii* ATCC 29916, *Staphylococcus aureus* ATCC 25922, and *Escherichia coli* ATCC 8739. All of the bacteria were obtained from the American Type Culture Collection (Rockville, MD, USA). The antibacterial assay was carried out as described in the literature [11]. The preparation of bacterial inocula was done by using 18 h-old overnight bacterialcultures prepared in Nutrient Agar. A few colonies of bacteria were collected aseptically with a sterile loop and introduced into sterile 0.90% saline solution (10 mL). The concentration of the suspension was then standardized by adjusting the

Table 1 Minimum inhibitory concentration (MIC, μg·mL^{-1}) of compounds 1–14 against four bacterial strains

	Enterococcus faecalis ATCC 10541	*Providensia smartii* ATCC 29916	*Staphylococcus aureus* ATCC 25922	*Escherichia coli* ATCC 8739
1	6.25	3.12	6.25	6.25
2	3.12	12.5	12.5	3.12
3	50	100	25	25
4	NA	50	NA	100
5	25	50	6.25	50
6	6.25	6.25	1.56	12.5
7	NA	50	NA	NA
8	1.56	12.5	1.56	12.5
9	NA	NA	NA	NA
10	1.56	1.56	0.78	0.78
11	25	NA	NA	50
12	NA	50	100	50
13	50	100	50	100
14	0.78	3.12	1.56	3.12
Gentamycin	1.56	0.19	0.19	1.56

NA: No active (MIC >100 μg·mL^{-1})

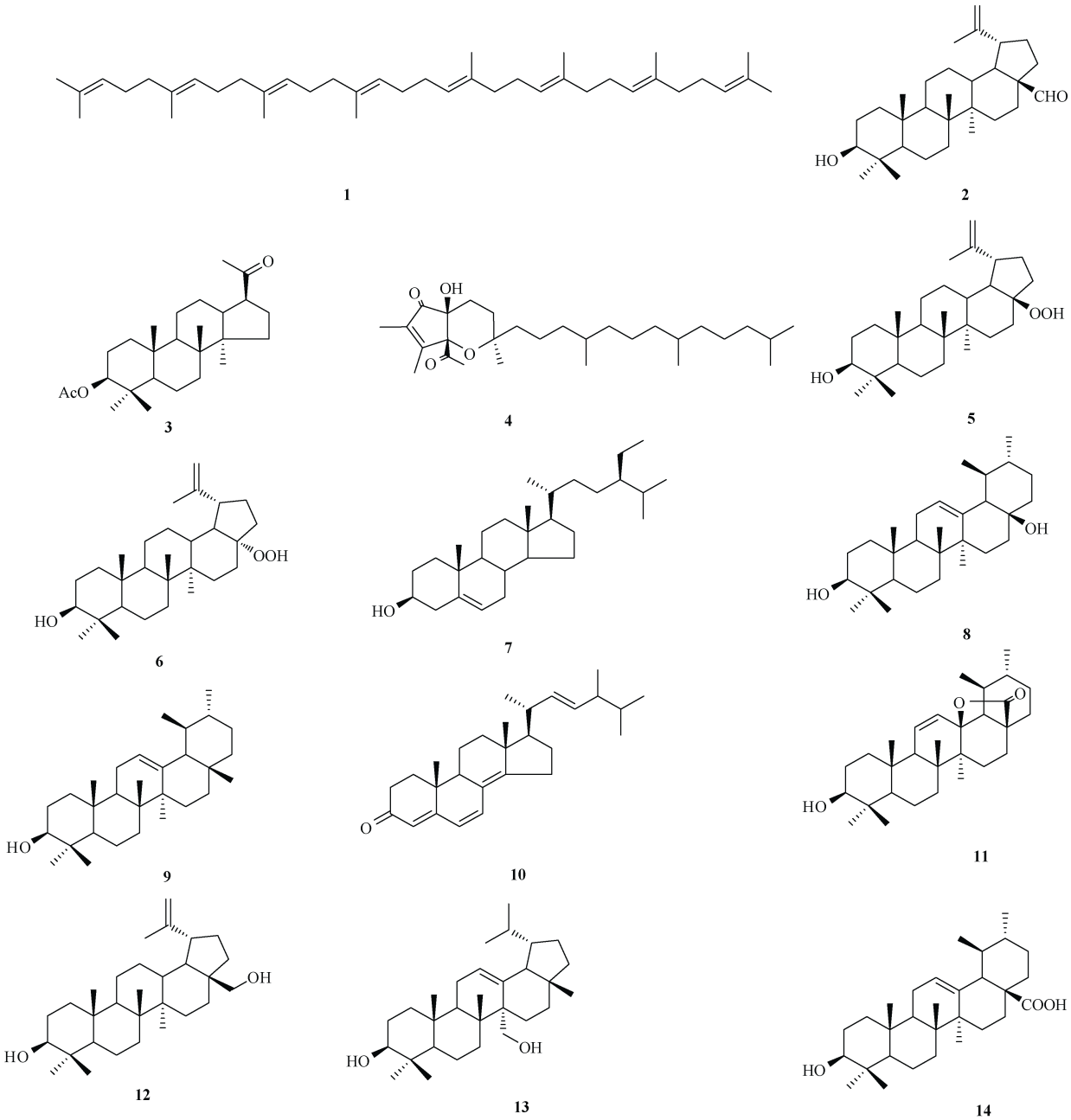

Fig. 1 Structures of compounds 1–14 from *M. suaveolens*

optical density to 0.10 at 600 nm, corresponding to bacterial cell suspension of 10^8 colony-forming units·mL^{-1} (CFU·mL^{-1}) [12]. This cell suspension was diluted 100 times to obtain 10^6 CFU·mL^{-1} before use. The compounds were dissolved in DMSO and serial two-fold dilutions from 200 μg·mL^{-1} were performed in 96-well microtiter plates. Each well contained 100 μL of sample of each concentration at a final DMSO concentration of 5% (*V*/*V*) or less. Into each well was then introduced the bacterial suspension (100 μL). The final concentration range of the test compounds was 100−0.781 μg·mL^{-1}, and the plates were incubated at 37 °C for 24 h. After incubation, the wells were examined for growth of microorganisms and the MICs were determined on addition of INT (4-iodonitrotetrazolium chloride) (50 μL). Viable bacteria turn the yellow die of INT pink. Each experiment was repeated three times and gentamycin was used as a positive control. The dilution solution, 5% DMSO, did not show inhibitory effects on the growth of the bacteria. The MIC is defined as the lowest concentration of the compound at which the bacterium does not demonstrate visible growth (no color change) and MIC > 100 μg·mL^{-1} was considered to be inactive.

Results and Discussion

Compounds were isolated and purified by silica gel, Sephadex LH-20, RP-18, and preparative HPLC. Their structures

were elucidated as lycopersene (**1**) [13], betulinic aldehyde (**2**) [14], 3β-acetoxy-22,23,24,25,26,27-hexanordammaran-20-one (**3**) [15], 3a-acetyl-2,3,5-trimethyl-7a-hydroxy-5-(4,8,12-trimethyl-tridecanyl)-1,3a,5,6,7,7a-hexhydro-4-oxiainden-1-one (**4**) [16], 3β-hydroxy-28-norlup-20(29)-ene-17β-hydroperoxide (**5**) [17], 3β-hydroxy-28-norlup-20(29)-ene-17α-hydroperoxide (**6**) [17], β-sitosterol (**7**) [18], deformylcladocalol (**8**) [19], α-amyrin (**9**) [20], ergosta-4,6,8(14),22-tetraen-3-one (**10**) [21], 3β-hydroxy-urs-11-en-28,13β-olide (**11**) [22], betulin (**12**) [23], obtusalin (**13**) [24], ursolic acid (**14**) [25], by spectroscopic methods and comparison of spectral data with literature values. Among the isolated compounds, ten compounds were reported for the first time from the genus *Melodinus*.

From the bioassay results, compounds **1**, **2**, **6**, **8**, **10**, and **14** showed potent antibacterial activities against the four bacteria, which might support, somewhat, the traditional use of this plant. It is worthwhile to note that *Enterococcus faecalis* is a microorganism commonly detected in a variety of nosocomial infections, of which urinary tract infections are the most common. These infections can be exceptionally difficult to treat because of drug resistance of many *E. faecalis* isolates [26]. The isolated compounds **8**, **10**, and **14** showed strong inhibitory activities, with MIC values of 1.56, 1.56, and 0.87 $\mu g \cdot mL^{-1}$, respectively. These activities were comparable to, or better than that of the reference antibiotic.

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References

[1] International Symposium of Plant Diversity and Conservation in China. *Flora of China* [M]. Science Press, 1977: 17-30.

[2] Yan KX, Hong SL, Feng XZ. Demethyltenuicausine, a new bisindole alkaloid from *Melodinus hemsleyanus* [J]. *Acta Pharm Sin*, 1998, **33**(8): 597–599.

[3] He X, Zhou YL, Huang ZH. Study on the alkaloids of *Melodinus fusiformis* [J]. *Acta Chim Sin*, 1992, **50**(1): 96-101.

[4] Au KS, Gray DE. New antibiotic [J]. *Biochem Pharmacol*, 1969, **18**(11): 2673.

[5] International Symposium of Plant Diversity and Conservation in China. *Flora of China* [M]. Science Press, 1977: 22.

[6] Nadol JB. Hearing loss as a sequel of meningitis [J]. *Laryngoscope*, 1978, **88**(5): 739-755.

[7] [7] Lai MC, Au KS, Gray DE. Alkaloids of *Melodinus suaveolens* and their excretion as a common end-product in the rat [J]. *Biochem Pharmacol*, 1969, **18**(7): 1553-1557.

[8] Ye JH, Zhou YL, Huang ZH, *et al*. Alkaloids from *Melodinus suaveolens* [J]. *Phytochemistry*, 1991, **30**(9): 3168-3170.

[9] Zhang TT, Liu ZW, Wang WJ, *et al*. Alkaloids from *Melodinus suaveolens* [J]. *Heterocycles*, 2013, **87**(10): 2047-2052.

[10] Liu YP, Li Y, Cai XH, *et al*. Melodinines M-U, Cytotoxic alkaloids from *Melodinus suaveolens* [J]. *J Nat Prod*, 2012, **75** (2): 220-224.

[11] Newton SM, Lau C, Gurcha S, *et al*. The evaluation of forty-three plant species for *in vitro* antimycobacterial activities; isolation of active constituents from *Psoralea corylifolia* and *Sanguinaria canadensis* [J]. *J Ethnopharmacol*, 2002, **79**(1): 57-67.

[12] Tereshuck ML, Riera MV, Castro GR, *et al*. Antimicrobial activity of flavonoid from leaves of *Tagetes minuta* [J]. *J Ethnopharmacol*, 1997, **56**: 227-232.

[13] Yamada Y, Seo CW, Okada H. Oxidation of acyclic terpenoids by *Corynebacterium* sp. [J]. *Appl Environ Microbiol,* 1985, **49** (4): 960-963.

[14] Cho JY, Kim CM, Lee HJ, *et al*. Caffeoyl triterpenes from pear (*Pyrus pyrifolia* Nakai) fruit peels and their antioxidative activities against oxidation of rat blood plasma [J]. *J Agric Food Chem*, 2013, **61**(19): 4563-4569.

[15] Kitajima J, Kimizuka K, Tanaka Y. New dammarane-type acetylated triterpenoids and their related compounds of *Ficus pumila* fruit [J]. *Chem Pharm Bull*, 1999, **47**(8): 1138-1140.

[16] Kitajima J, Kimizuka K, Arai M, *et al*. Constituents of *Ficus pumila* leaves [J]. *Chem Pharm Bull*, 1998, **46**(10): 1647-1649.

[17] Abdel Bar FM, Zaghloul AM, Bachawal SV, *et al*. Antiproliferative triterpenes from *Melaleuca ericifolia* [J]. *J Nat Prod*, 2008, **71**(10): 1787-1790.

[18] Lee TH, Chiou JL, Lee CK, *et al*. Separation and determination of chemical constituents in the roots of *Rhus javanica* L. var. *roxburghiana* [J]. *J Chin Chem Soc*, 2005, **52**(4): 833-841.

[19] Benyahia S, Benayache S, Benayache F, *et al*. Cladocalol, a pentacyclic 28-*nor*-triterpene from *Eucalyptus cladocalyx* with cytotoxic activity [J]. *Phytochemistry*, 2005, **66**(6): 627-632.

[20] Seo S, Tomita Y, Tori K. Carbon-13 NMR spectra of urs-12-enes and application to structural assignments of components of *Isodon japonicus* Hara tissue cultures [J]. *Tetrahedron Lett*, 1975, **16**(1): 7-10.

[21] Quang DN, Bach DD. Ergosta-4,6,8(14),22-tetraen-3-one from Vietnamese *Xylaria* sp. possessing inhibitory activity of nitric oxide production [J]. *Nat Prod Res*, 2008, **22**(10): 901-906.

[22] Topcu G, Yapar G, Türkmen Z, *et al*. Ovarian antiproliferative activity directed isolation of triterpenoids from fruits of *Eucalyptus camaldulensis* Dehnh [J]. *Phytochem Lett*, 2011, **4**(4): 421-425.

[23] Ikuta A, Itokawa H. Triterpenoids of *Paeonia japonica* callus tissue [J]. *Phytochemistry*, 1988, **27**(9): 2813-2815.

[24] Siddiqui S, Siddiqui BS, Naeed A, *et al*. Pentacyclic triterpenoids from the leaves of *Plumeria obtusa* [J]. *Phytochemistry*, 1989, **28**(11): 3143-3147.

[25] Seebacher W, Simic N, Weis R, *et al*. Complete assignments of ^{1}H and ^{13}C NMR resonances of oleanolic acid, 18α-oleanolic acid, ursolic acid and their 11-oxo derivatives [J]. *Magn Reson Chem,* 2003, **41**(8): 636-638.

[26] Andrew LK, Steven MM, William L, *et al*. *Enterococcus faecalis* tropism for the kidneys in the urinary tract of C57BL/6J mice [J]. *Infect Immun*, 2005, **70**(3): 2461-2468.

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