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FURTHER CHEMICAL CONSTITUENTS OF *SCOPARIA DULCIS* L. (SCROPHULARIACEAE) ORIGINATING IN VIETNAM

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ABSTRACT

*Palmitic acid (**1**), β-sitosterol (**2**), glutinol (**3**), and a mixture of β-amyrin (**4**) and isomultiflorenol (**5**), whose structures were determined by using spectroscopic methods, were isolated from the aerial parts of Scoparia dulcis L. (Scrophulariaceae). The volatile fraction of S. dulcis was analyzed by GC-MS to show the accumulation of methyl esters of fatty acids. The useful compound methoxybenzoxazolinone (MBOA) was found to be localized in the CH_2Cl_2-soluble fraction of the MeOH extract. For the direct qualitative and quantitative evaluation of MBOA in the CH_2Cl_2-soluble fraction ^{1}H-NMR method was applied in this study. Antimicrobial test of the soluble fractions and MBOA showed inhibition on Escherichia coli, Pseudomonas aeruginosa, and Saccharomyces cerevisiae.*

Keywords: *Scoparia dulcis*; Scrophulariaceae; triterpenoid; methoxybenzoxazolinone (MBOA).

I - INTRODUCTION

The production of secondary metabolites in plants is recognized as an important source for pharmacologically useful compounds. Therefore the full knowledge of chemical profiles of medicinal plants should be the scientific basis for understanding safety and efficacy of many plant-derived pharmaceutical products. In addition, the chemical profiles of many plants are known to be dependent on growth conditions [1]. One of the aims of our study program on Vietnamese medicinal plants is to have comprehensive chemical profiles of the plants; as a result of the studies a variety of apolar and polar compounds have already been found and reported [2a-e]. *Scoparia dulcis* L. (Scrophulariaceae) is a medicinal herb of Vietnam [3] whose chemical profile was unclear until our recent investigation [2d]. Interesting scopadulane-type diterpenoids together with lignans, triterpenoids, and flavonoids were found, however, further study was performed to target volatile compounds and other main compounds present in non-polar organic solvent extracts of *S. dulcis* originating in Vietnam.

II - EXPERIMENTAL

1. General procedure

Infrared (IR) spectra were recorded on an Impact-410 Nicolet FT-IR spectrophotometer. Electron-impact (EI) mass spectra (70 eV) were measured on a Hewlett-Packard 5989B mass spectrometer. ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) with DEPT program spectra were obtained on a Bruker Avance 500 NMR spectrometer. Tetramethylsilane (TMS) was used as zero reference. Analytical high performance liquid chromatography (HPLC) was performed on a Shimadzu HPLC system equipped with a photodiode array detector (PDA) and a HPLC analytical column Supelco

C18 (250 mm × 4.6 mm i.d., particle size 5 mm); mobile phase was 70% MeOH-H_2O in isocratic mode or 20-100% MeOH-H_2O in gradient mode. A gas chromatography - mass spectrometry (GC-MS) Thermo Finnigan system fitted with a capillary column SPB-5MS (30 m × 0.32 mm i.d., film thickness 0.25 mm) and splitless injection was used; the oven temperature was programmed from 40 to 250ºC at 10ºC/min; injector temperature and ion source temperature were at 250 and 200ºC, respectively; MS full scan was from 50-650 ms; He was used as carrier gas. Silica gel 60 (63 - 200 μm, Merck, Darmstadt, Germany) was used for open column chromatography (CC). TLC was performed on precoated DC Alufolien 60 F_{254} sheets (Merck, Darmstadt, Germany) and detected by spraying with 1% vanillin in conc. H_2SO_4.

2. Plant material

The aerial parts of *S. dulcis* were collected in Hanoi, Vietnam in February 2006. The material was dried in the shade, oven-dried at 45-50ºC for two hours and then powdered.

3. Extraction and isolation

The powdered aerial parts (2 kg) was extracted with MeOH at room temperature (6 times, each for 3 days). The MeOH extract was filtered and evaporated under reduced pressure to obtain a MeOH extract. The extract was sequentially partitioned between H_2O and *n*-hexane, CH_2Cl_2, and EtOAc to give the soluble fractions, *n*-hexane- (22.2 g), CH_2Cl_2- (2 g), and EtOAc- (1.5 g) soluble fractions. Part of the *n*-hexane-soluble fraction (10 g) was fractionated by silica gel CC (94 g) and eluted with *n*-hexane and *n*-hexane-EtOAc 6:1 and 3:1 and EtOAc to give the following fractions, **sd1** (*n*-hexane elution), **sd2**-**sd5** (*n*-hexane-EtOAc 6:1 elution), **sd6**-**sd9** (*n*-hexane-EtOAc 3:1 elution), and **sd10** (EtOAc elution) based on the volume of eluents and TLC analysis. Palmitic acid (**1**) (0.86 g) was purified by washing with *n*-hexane from **sd2** (1.27 g). Similar treatment gave *β*-sitosterol (**2**) (20 mg) from **sd5** (0.92 g). Fraction **sd4** (3 g) was futher chromatographed on gradient silica gel CC using *n*-hexane and *n*-hexane-EtOAc (6:1) to yield four subfractions, **sd4.1** (0.97 g), **sd4.2** (0.8 g), **sd4.3** (0.2 g), and **sd4.4** (0.4 g). **2** (20 mg) was recrystallized from **sd4.4**. Subfraction **sd4.2** was subjected to gradient silica gel CC and eluted with *n*-hexane and *n*-hexane-EtOAc (10:1); recrystallization of the chromatographic fractions from *n*-hexane gave **2** (15 mg), glutinol (**3**) (10 mg), and a mixture of *β*-amyrin (**4**) and isomultiflorenol (**5**) (6 mg). Silica gel column chromatography of part of the CH_2Cl_2-soluble fraction (1 g) using *n*-hexane-EtOAc 3:1 and further treatment with *n*-hexane gave 6-methoxybenzoxazolinone (**6**) (10 mg).

Palmitic acid (1): Amorphous powder. R_f = 0.42 (silica gel TLC, *n*-hexane-EtOAc 6:1). IR (KBr): ν_{max} cm^{-1} 3428, 1708, 1457, 1379, 1030. EI-MS: *m/z* 256 ($C_{16}H_{32}O_2$, $M^{+\cdot}$). ^{1}H-NMR ($CDCl_3$): δ (ppm) 0.88 (3H, t, *J* = 6.5 Hz, H_3-16), 1.21-1.29 (24H, brs, $2H_4 \rightarrow 2H_{15}$), 1.63 (2H, quintet, *J* = 7.5 Hz, 2H-3), 2.34 (2H, t, *J* = 7.5 Hz, 2H-2). ^{13}C NMR ($CDCl_3$): δ (ppm) 14.1 (q, C-16), 22.7 (t, C-15), 24.7 (t, C-3), 29.1, 29.3, 29.4, 29.5, 29.6, 29.69, 29.72 (all t, C-4 → C-13), 31.95 (t, C-14), 34.1 (t, C-2), 179.8 (s, C-1).

β-Sitosterol (2): White needles, m.p. 135 - 136ºC. R_f = 0.35 (silica gel TLC, *n*-hexane-EtOAc 10:1). IR (KBr): ν_{max} cm^{-1} 3427, 1637, 1461, 1379, 1056.

Glutinol (3): Amorphous powder. R_f = 0.64 (silica gel TLC, *n*-hexane-EtOAc 10:1). IR (KBr): ν_{max} cm^{-1} 3443, 1649, 1454, 1383, 1030. EI-MS: *m/z* 426 ($C_{30}H_{50}O$, $M^{+\cdot}$). ^{1}H-NMR ($CDCl_3$): δ (ppm) 0.85 (3H, s, H_3-25), 0.95 (3H, s, H_3-29), 0.99 (3H, s, H_3-30), 1.01 (3H, s, H_3-27), 1.04 (3H, s, H_3-23), 1.09 (3H, s, H_3-26), 1.14 (3H, s, H_3-24), 1.16 (3H, s, H_3-28), 3.47 (1H, dd, *J* = 3.0 Hz, 2.5 Hz, H-3), 5.63 (1H, brd, *J* = 6.0 Hz, H-6). ^{13}C-NMR ($CDCl_3$): δ (ppm) 15.6 (q, C-25), 18.2 (t, C-1), 18.4 (q, C-27), 19.6 (q, C-26), 23.7 (t, C-7), 25.5 (q, C-23), 27.8 (t, C-2), 28.3 (s, C-20), 28.9 (q, C-24), 30.1 (s, C-17), 30.4 (t, C-12), 32.0 (q, C-28), 32.1 (t, C-15), 32.4 (q, C-30), 33.1 (t, C-21), 34.5 (q, C-29), 34.6 (t, C-11), 34.9 (s, C-9), 35.1 (t, C-19), 36.0 (t, C-16), 37.9 (s, C-14), 38.8 (t, C-22), 39.3 (s, C-13), 40.8 (s, C-4), 43.1 (d, C-18), 47.5 (d, C-

8), 49.7 (d, C-10), 76.4 (d, C-3), 122.1 (d, C-6), 141.6 (s, C-5).

Mixture of β-amyrin (4) and isomultiflorenol (5): Amorphous powder. R_f = 0.46 (silica gel TLC, *n*-hexane-EtOAc 10:1). EI-MS: *m/z* 426 ($C_{30}H_{50}O$, $M^{+\cdot}$).

β-Amyrin (4): ^{1}H-NMR ($CDCl_3$): δ (ppm) 0.79 (3H, s, H_3-24), 0.83 (3H, s, H_3-28), 0.87 (6H, s, H_3-29, H_3-30), 0.94 (3H, s, H_3-25), 0.97 (3H, s, H_3-26), 1.0 (3H, s, H_3-23), 1.14 (3H, s, H_3-27), 3.23 (1H, dd, *J* = 12.0 Hz, 4.5 Hz, H-3), 5.18 (1H, t, *J* = 3.5 Hz, H-12). ^{13}C-NMR ($CDCl_3$): δ (ppm) 15.5 (q, C-24), 15.6 (q, C-25), 16.8 (q, C-26), 18.4 (t, C-6), 23.6 (t, C-11), 23.7 (q, C-30), 26.0 (q, C-27), 26.2 (t, C-15), 27.0 (t, C-2), 27.3 (t, C-16), 28.1 (q, C-23), 28.3 (q, C-28), 31.1 (s, C-20), 32.5 (s, C-17), 32.7 (t, C-7), 33.3 (q, C-29), 34.8 (t, C-21), 37.0 (s, C-10), 37.2 (t, C-22), 38.6 (t, C-1), 38.8 (s, C-4), 39.8 (s, C-8), 41.8 (s, C-14), 46.9 (t, C-19), 47.3 (d, C-18), 47.7 (d, C-9), 55.2 (d, C-5), 79.0 (d, C-3), 121.8 (d, C-12), 145.2 (s, C-13).

Isomultiflorenol (5): ^{1}H-NMR ($CDCl_3$): δ (ppm) 0.8 (6H, s, H_3-23, H_3-24), 0.96 (3H, s, H_3-25), 0.97 (3H, s, H_3-27), 0.99 (3H, s, H_3-29), 0.998 (3H, s, H_3-30), 1.06 (3H, s, H_3-26), 1.07 (3H, s, H_3-28), 3.23 (1H, m, H-3). ^{13}C-NMR ($CDCl_3$): δ (ppm) 15.6 (q, C-24), 18.9 (q, C-26), 19.3 (t, C-6), 19.8 (q, C-25), 20.8 (t, C-11), 24.7 (t, C-2), 24.7 (q, C-27), 26.3 (t, C-15), 27.5 (t, C-7), 28.1 (q, C-23), 28.4 (s, C-20), 30.7 (t, C-12), 30.8 (s, C-17), 31.5 (q, C-28), 33.3 (q, C-30), 34.2 (t, C-1), 34.4 (t, C-19), 34.5 (q, C-29), 35.1 (t, C-21), 36.7 (t, C-16), 36.8 (t, C-22), 37.3 (s, C-13), 37.6 (s, C-10), 38.8 (s, C-4), 41.0 (s, C-14), 44.1 (d, C-18), 50.7 (d, C-5), 76.8 (d, C-3), 133.5 (s, C-9), 135.1 (s, C-8).

6-Methoxybenzoxazolinone (6): Amorphous powder. R_f = 0.53 (silica gel TLC, *n*-hexane-EtOAc 3:1). ^{1}H-NMR (CD_3OD): δ (ppm) 3.8 (3H, s, 6-OCH_3), 6.76 (1H, dd, *J* = 8.5 Hz, 2.5 Hz, H-5), 6.9 (1H, d, *J* = 2.5 Hz, H-7), 6.98 (1H, d, *J* = 8.5 Hz, H-4).^{13}C-NMR (CD_3OD): δ (ppm) 56.4 (q, 6-OCH_3), 98.1 (d, C-7), 110.7 (d, C-4), 111.0 (d, C-5), 125.0 (s, C-9), 146.1 (s, C-8), 157.6 (s, C-6), 157.7 (s, C-2).

*Qualitative and quantitative evaluation of **6** using ^{1}H-NMR method*

^{1}H-NMR spectra (500 MHz) of the CH_2Cl_2-soluble fraction (10 mg) and reference compound, maleic acid (1.3 mg) were recorded on a Bruker Avance 500 spectrometer in CD_3OD (0.6 ml) using 5 mm NMR tube. The quantitative conditions were: receiver gain 90.5, spectral width 10.000 Hz, acquisition time 3.27 s, relaxation delay 1s, and measurement temperature 300 K; FID was Fourier transformed with LB = 0.3, GB = 0.0, and PC = 1.0. For quantitative analyses integration lines were made using original and final points of each peak.

III - RESULTS AND DISCUSSION

The *n*-hexane-soluble fraction of the MeOH extract from the aerial parts of *S. dulcis* was separated on gradient silica gel open column chromatography (CC) to give a mixture of volatiles compounds and constituents **1**-**5** as the main constituents of the fraction. Silica gel CC of the CH_2Cl_2-soluble fraction afforded the major compound **6**.

Volatile compounds of the aerial parts of Scoparia dulcis

Silica gel CC of the *n*-hexane-soluble fraction gave chromatographic fraction **sd1** (8.6% isolation yield, w/w of the dry material) which was eluted with *n*-hexane. This fraction was found to contain the volatile compounds of the aerial parts of *S. dulcis* and was analyzed by GC-MS. The identification of the compounds was made by comparing the EI-MS spectra with those of the Wiley GC-MS Library. Eight compounds consisting of 77.8% of **sd1** were identified as methyl esters of fatty acids (Table 1); methyl palmitate (43.8%) dominated in the volatile **sd1** fraction.

*Structure determination of **1**-**6***

Compounds **1** and **2** were determined to be palmitic acid and β-sitosterol by comparing their chromatographic and spectroscopic data with those of our authentic samples.

Glutinol (**3**) showed IR absorptions of hydroxyl group (ν_{max} 3443 cm^{-1}) and double bond (ν_{max} 1649 cm^{-1}). The triterpenoid structure of **3** was suggested from the following spectral data: a) the molecular formula $C_{30}H_{50}O$

Table 1: Volatile compounds of the aerial parts of *Scoparia dulcis* L.

No	Retention time (min.)	Compound	Content (% [a)])
1	35.93	Methyl hexadecanoate (methyl palmitate)	43.8
2	37.74	Methyl heptadecanoate (methyl margarate)	1.4
3	39.14	Methyl 9*Z*-octadecenoate (methyl oleate)	17.5
4	39.61	Methyl octadecanoate (methyl stearate)	10.8
5	43.03	Methyl eicosanoate (methyl arachidate)	2.3
6	46.20	Methyl docosanoate (methyl behenate)	1.1
7	49.58	Methyl tetracosanoate (methyl lignocerate)	0.6

[a] % of compounds (TIC) were calculated using the SPB-5MS column.

(*m*/*z* 426) in the EI-MS spectrum; b) 30 carbon signals [eight quartets (8 q), ten methylenes (10 t), five methines (5 d), and seven quaternary carbons (7 s)] in ^{13}C-NMR and DEPT spectra; and c) eight tertiary methyl signals [δ_H 0.85 (3H, s), 0.95 (3H, s), 0.99 (3H, s), 1.01 (3H, s), 1.04 (3H, s), 1.09 (3H, s), 1.14 (3H, s), and 1.16 (3H, s); δ_C 15.6 (q), 18.4 (q), 19.6 (q), 25.5 (q), 28.9 (q), 32.0 (q), 32.4 (q), and 34.5 (q)] in ^{1}H- and ^{13}C-NMR spectra. An olefinic proton [δ_H 5.63 (1H, brd, J = 6.0 Hz); δ_C 122.1 (d)] and an oxymethine [δ_H 3.47 (dd, J = 3.0 Hz, 2.5 Hz); δ_C 76.4 (d)] were also present in the structure of **3**. The MS and NMR spectroscopic data were in good agreement with those of glutinol [4]. The compound showed anti-inflammatory activity [5] and was isolated in the acetate form from the whole plant of *Euphorbia maculata* [4]. The β-orientation of the hydroxyl group at C-3 was determined from the splitting pattern of H-3 (dd, J = 3.0 Hz, 2.5 Hz) (3β-acetoxy: t, J = 2.9 Hz [4]). *Retro*-Diels-Alder fragment from ring B at *m*/*z* 274 in the EI-MS spectrum of **3** was characteristic of a C5-C6 double bond in the glutinane skeleton.

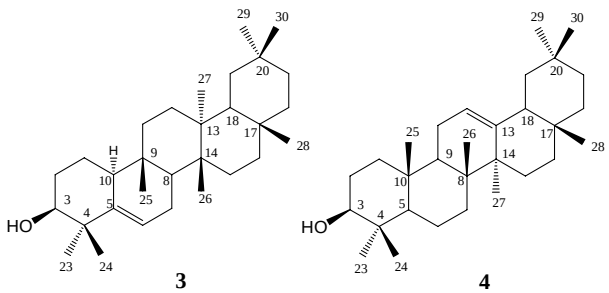
A mixture of β-amyrin (**4**) and isomultiflorenol (**5**) (^{1}H-NMR ratio 1:1) showed 60 carbon signals in ^{13}C-NMR and DEPT spectra and molecular ion peak at *m*/*z* 426 ($C_{30}H_{50}O$) in the EI-MS spectrum of the mixture. Thus, triterpenoid skeletons were suggested for both **4** and **5**.

A trisubstituted double bond [δ_H 5.18 (1H, t, J = 3.5 Hz); δ_C 121.8 (d) and 145.2 (s)], an oxymethine [δ_H 3.23, dd (J = 12.0 Hz, 4.5 Hz); δ_C 79.0 (d)], and eight tertiary methyl groups [δ_H 0.79 (3H, s), 0.83 (3H, s), 0.87 (6H, s), 0.94 (3H, s), 0.97 (3H, s), 1.0 (3H, s), and 1.14 (3H, s); δ_C 15.5 (q), 15.6 (q), 16.8 (q), 23.7 (q), 26.0 (q), 28.1 (q), 28.3 (q), and 33.3 (q)] were resolved for **4** in the ^{1}H-, ^{13}C-NMR, and DEPT spectra of the mixture. Since the MS and NMR spectroscopic data were suggestive for an olean-12-ene skeleton and the placement of the hydroxyl group was suggested at C-3 on the basis of biogenetic considerations **4** was determined to be β-amyrin [4, 6]. The 3-OH group was assigned as β-orientated (equatorial) on the basis of the coupling constant of H-3 ($J_{2a\text{-}3a}$ = 12.0 Hz) and the the chemical shift of C-3 ($\delta_{C\text{-}3}$ 79.0). *Retro*-Diels-Alder fragmentation of the ring C gave peak at *m*/*z* 218 in the EI-MS spectrum of **4** which was in agreement with a C12-C13 double bond.

The ^{1}H-, ^{13}C-NMR, and DEPT spectra for **5** exhibited the presence of a tetrasubstituted double bond [δ_C 133.5 (s) và 135.1 (s)], an oxymethine group [δ_H 3.23 (1H, m); δ_C 76.8 (d)], and eight tertiary methyl groups [δ_H 0.8 (6H, s), 0.96 (3H, s), 0.97 (3H, s), 0.99 (3H, s), 0.998 (3H, s), 1.06 (3H, s), and 1.07 (3H, s); δ_C 15.6 (q), 18.9 (q), 19.8 (q), 24.7 (q), 28.1 (q), 31.5 (q), 33.3 (q), and 34.5 (q)]. Thus, a pentacyclic triterpenoid structure possessing a double bond

which was deduced to be situated between two fused rings of the skeleton. Comparison of the chemical shifts of the methyl groups with those of model triterpenoid compounds revealed the multiflorane skeleton of **3**. The ^{1}H- and ^{13}C-NMR spectroscopic data of **5** determined the structure of **5** to be isomultiflorenol (5α-multiflor-8-en-3β-ol) [7]. The orientation of the hydroxyl group at C-3 was determined to be β on the basis of the large coupling constant $J_{2a\text{-}3a}$ (~ 12.0 Hz).

6-Methoxybenzoxazolinone (**6**) exhibited a Dragendorff-Munier color reaction. The ^{1}H- and ^{13}C-NMR spectroscopic data of **6** showed the presence of a methoxyl group [δ_H 3.8 (s); δ_C 56.4)], a 1,2,4-trisubstituted benzene ring [δ_H 6.98 (d, J = 8.5 Hz), 6.9 (d, J = 2.5 Hz), and 6.76 (dd, J = 8.5 Hz, 2.5 Hz); δ_C 98.1, 110.7, and 111.0], three aromatic carbons bearing nitrogen (δ_C 125.0) and oxygen (δ_C 146.1 and 157.6), and an amide group (δ_C 157.7). Thus, the NMR spectroscopic data of **6** were in good accordance with a benzoxazolinone skeleton. Literature search for benzoxazolinone compounds determined **6** as 6-methoxybenzoxazolin-2(3*H*)-one (MBOA) which was found in the plants of families Gramineae [8], Acanthaceae [9], Scrophulariaceae (*Scoparia dulcis*) [9], and Ranunculaceae [10].



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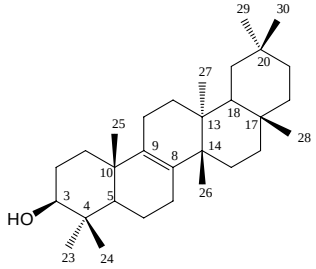

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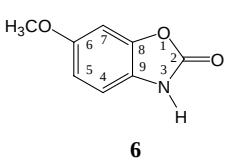

6

*Qualitative and quantitative evaluation of **6** in the CH_2Cl_2-soluble fraction using ^{1}H-NMR method*

Benzoxazolinones were identified as thermal degradation products of cyclic hydroxamic acids (1,4-benzoxazin-3-ones) in aqueous or aprotic organic solutions. 1,4-Benzoxazin-3-ones are the natural chemical resistance factors against bacteria and insects; these compounds were liberated from the corresponding stable β-glucosides by enzymatic hydrolysis reactions when plants were disturbed and cells were ruptured. MBOA was the major nitrogen-containing compound in the *S. dulcis* and may be formed similarly from 2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (DIMBOA) β-D-glucoside, therefore, the collection, processing, and extraction procedures have great influence on the content of MBOA obtained. Consequently, a rapid, simultaneous, and non-destructive qualitative and quantitative analysis of **6** in the CH_2Cl_2-soluble fraction by using ^{1}H-NMR method [11, 12] was developed in this study. The ^{1}H-NMR spectrum of the fraction was recorded in CD_3OD and showed clearly resolved aromatic signals of the main compound **6** [$\delta_{H\text{-}4}$ 6,99 (d, J = 8.5 Hz), $\delta_{H\text{-}7}$ 6.91 (d, J = 2.5 Hz), $\delta_{H\text{-}5}$ 6.77 (d, J = 8.5 Hz, 2.5 Hz), and $\delta_{6\text{-}OMe}$ 3.80 (s)] from the crowded aliphatic and oxygenated region (δ_H 0,8-4.2). Due to very high intensities of all resonances of **6** in comparison with those of the other compounds of the mixture the complete assignment of all protons signals of **6** was an easy task making clear confirmation of the presence of **6** by the ^{1}H-NMR method. Quantification was performed by calculating the relative ratio of the peak areas of selected proton signals of the target compound **6** to the known amount of the internal standard, maleic acid. The signal due to H-7 of **6** (δ_H 6.91, 1H, intensity 0.722) was resolved from other minor aromatic signals of **6** and was selected as the target signal for the quantification of **6**. The signal of maleic acid appeared at δ_H 6.32 ppm (s, 2H, intensity 1.656). The content of MBOA was

calculated to be 16.1% (w/w) in the CH_2Cl_2-soluble fraction and 0.016% (w/w) in the dried aerial parts of *S. dulcis*.

Evaluation on antibacterial and antifungal activity of the three soluble fractions and MBOA

Antimicrobial activity of the three soluble fractions and MBOA (**6**) was tested on a 96-well plate using the method described by Vander Berghe and Vlietinck [13]. The laboratory strains were two Gram (–) bacteria strains, *Escherichia coli* and *Pseudomonas aeruginosa,* two Gram (+) bacteria strains, *Bacillus subtilis* and *Staphylococcus aureus,* and four fungi/yeasts *Aspergillus niger*, *Fusarium oxysporum, Candida albicans*, and *Saccharomyces cerevisiae*. The results were summarized in table 2. **6** was active against *E. coli, P. aeruginosa*, and *S. cerevisiae* at the same MIC at 50 mg/ml. The increased activity of **6** against *E. coli* in comparison with that of the CH_2Cl_2-soluble fraction (MIC at 200 mg/ml) was noticeable. The weak activity of the EtOAc-soluble fraction against *P. aeruginosa* (MIC at 200 mg/ml) and *S. cerevisiae* (MIC at 200 mg/ml) was also detected.

Table 2: Antimicrobial activity of the soluble fractions and MBOA

No.	Soluble fraction/ Compound	Minimum Inhibitory Concentration (MIC, mg/ml)							
		E. coli	*P. aeruginosa*	*B. subtillis*	*S. aureus*	*A. niger*	*F. oxysporum*	*S. cerevisiae*	*C. albicans*
1	Hexane-	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
2	CH_2Cl_2-	200	(-)	(-)	(-)	(-)	(-)	(-)	(-)
3	EtOAc-	(-)	200	(-)	(-)	(-)	(-)	200	(-)
4	MBOA	50	50	(-)	(-)	(-)	(-)	50	(-)

(-): *no inhibition on microorganism growth.*

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REFERENCES

1. S. Y. Yang, H. K. Kim, A. W. M. Lefeber, C. Erkelens, N. Angelova, Y. H. Choi, R. Verpoorte. Planta Med., **72**, 364 - 369 (2006).
2. (a) Phan Tong Son, Phan Minh Giang, W. C. Taylor, Aust. J. Chem., **53**, 1003 - 1005 (2000); (b) Phan Minh Giang, Phan Tong Son, H. Otsuka. Chem. Pharm. Bull., **53**, 232 - 234 (2005); (c) Phan Minh Giang, Phan Tong Son, K. Matsunami, H. Otsuka. Chem. Pharm. Bull., **53**, 1600 - 1603 (2005); (d) Phan Minh Giang, Phan Tong Son, K. Matsunami, H. Otsuka. Chem. Pharm. Bull., **54**, 546 - 549 (2006); (e) Phan Minh Giang, Phan Tong Son, K. Matsunami, H. Otsuka. Heterocycles, **74**, 977 - 981 (2007).
3. V. C. Vo. The Dictionary of Vietnamese Medicinal Plants, p. 1234, Publishing House Medicine, Ho Chi Minh city (1999).
4. S. Matsunaga, R. Tanaka, M. Akagi. Phytochemistry, **27**, 535 - 537 (1988).
5. S. M. Frere, L. M. Torres, N. F. Roque, C. Souccar, A. J. Lapa. Mem. Inst. Oswaldo Cruz., **86** (Suppl. 2), 149 - 151 (1991).
6. M. Ali, A. Heaton, D. Leach. J. Nat. Prod., **60**, 1150 - 1151 (1997).
7. T. Itoh, T. Shigemoto, N. Shimizu, T.

Tamura, T. Matsumoto. Phytochemistry, **21**, 2414 - 2415 (1982).

8. A. Gierl, K. Frey. Planta, **213**, 493 - 498 (2001).
9. K. Pratt, P. Kumar, W. S. Chilton. Biochem. Syst. Ecol., **23**, 781 - 785 (1995).
10. S. Özden, T. Özden, I. Attila, Küçükislamoglu, A. Okatan. J. Chromatog., **609**, 402 - 406 (1992).
11. U. Holzgrabe, B. W. K. Diehl, I. Wawer. J. Pharm. Biomed. Anal., **17**, 557 - 616 (1998).
12. G. F. Pauli. Phytochem. Anal., **12**, 28 - 42 (2001).
13. D. A. Vanden Bergher, A. J. Vlietlink in Methods in Plant Biochemistry, ed. Hostettman K., **Vol. 6**, p. 47 - 68, Academic Press, London (1991).