Chemical Constituents and Biological Activities from the Aerial Parts of *Sphaeranthus indicus*

องค์ประกอบทางเคมีและฤทธิ์ทางชีวิภาพจากส่วนเหนือดินของต้นมะต่อมเสื้อ

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ABSTRACT

The goal of this study was to isolate and determine the chemical constituents of *Sphaeranthus indicus* prior to bioactivity tests, with the hope of finding active principles. Seven known compounds (**1-7**), were isolated from the aerial parts of *S. indicus*. The structures of these compounds were established on the basis of their 1D and 2D NMR spectroscopic data. Compound **1** showed cytotoxicity against cancer cell lines KB and MCF-7 with IC_{50} values 147.37 and 93.1 μM, respectively. In addition, compound **7** showed antimalarial activity against *Plasmodium falciparum* (IC_{50} 4.50 μM).

บทคัดย่อ

ในการศึกษานี้มีวัตถุประสงค์คือแยกองค์กอบทางเคมีและศึกษาฤทธิ์ทางชีวภาพจากต้นมะต่อมเสื้อ ซึ่งสามารถแยกสารได้ทั้งหมด 7 ชนิด (**1-7**) จากส่วนเหนือดินของต้นมะต่อมเสื้อ โครงสร้างสารเหล่านี้พิสูจน์เอกลักษณ์ด้วยเทคนิคทางสเปกโทรสโกปี (1D NMR และ 2D NMR) จากการทดสอบฤทธิ์ทางชีวภาพ พบว่า สาร **1** มีฤทธิ์ยับยั้งเซลล์มะเร็งในช่องปาก (KB) และมะเร็งเต้านม (MCF-7) มีค่า IC_{50} 147.37 และ 93.1 μM ตามลำดับ นอกจากนี้ สาร **7** มีฤทธิ์ยับยั้งเชื้อมาลาเลีย *Plasmodium falciparum* (IC_{50} 4.50 μM)

Keywords: Asteraceae, Eudesmanolide, Antimalarial

คำสำคัญ: แอสเทอราซิอี้ ยูเดสมาโนไลด์ ยับยั้งเชื้อมาลาเรีย

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Introduction

Sphaerantus indicus (Asteraceae) is a sprawling annual and strongly aromatic herb, distributed mainly in the tropical and subtropical areas of Africa, Asia and Australia. It grows in rice fields and in cultivated lands of Thailand. *S. indicus* is known locally as Matomsuea and used as a folk medicine for the treatment of cough, fever, skin diseases and nervous depression (Marknoi, Chanwit, 2012; Galani et al., 2010). The previous physiochemical and chemical constituent reports have demonstrated the presence of bioactive sesquiterpene lactones, eudesmanolides, glycosides, eudesmanoids, flavonoids, and essential oils in several *Sphaerantus* species (Shekhani et al., 1991; Yadava, Kumar, 1999; Rojatkar, Nagasampagi, 1992; Rojatkar et al., 1994; Sohoni et al., 1998; Pujar et al., 2000; Kaul et al., 2005; Jadhav et al., 2007; Mishra et al, 2007). The aerial parts of *S. indicus*, showed cytotoxicity against cancer cells, NCI-H187 (IC_{50} 11.64 µg/mL) and MCF-7 (IC_{50} 13.49 µg/mL) in the EtOAc crude extract. We report herein our recent, characterization and biological activities of the isolated compounds from the aerial parts of *S. indicus*.

Result and Discussion

Seven known compounds (Figure 1) were identified by physical and spectroscopic methods (1D and 2D NMR) including comparison with spectral data reported for known and related compounds as 3α-hydroxy-eudesm-4-en-12,6β-olide (**1**) (Oksuz, Topcu, 1992), ilicic acid (**2**) (Guilhon, Müller, 1998), 5α-hydroxy-4α,15-dihydrocostic acid (**3**) (Hegazy et al., 2014), 2,5-dimethoxy-*p*-cymene (**4**) (Kaul et al., 2005), pangelin (**5**) (Thanh et al., 2004), luteolin 4'-methyl ether (**6**) (Zhi et al., 2012), and 3,5-di-*O*-caffeoylquinic acid methyl ester (**7**) (Hu et al., 2014). It should be noticed that this is the first report of compounds **1**, **3**, **5**, **6** and **7** isolated for the first time from *Sphaeranthus* species.

Compounds **1-7** were tested for bioactivities and results are shown in table 1. Compound **1** showed cytotoxicity against MCF-7 and KB cell lines with IC_{50} values of 93.1 and 147.37 µM, respectively, while compound **7** showed antimalarial activity against *P. falciparum* with IC_{50} 4.5 µM.

Material and Methods

Melting points were determined using an Electrothermal IA9200 digital melting point apparatus. Optical rotations were measured on a JASCODIP-1000 digital polarimeter and UV spectra were recorded using an Agilent 8453 UV-visible spectrophotometer. IR spectra were obtained using a Bruker Tenser 27 spectrophotometer. NMR spectra were recorded on a Varian Mercury Plus 400 spectrometer using $CDCl_3$, CD_3OD and DMSO-d_6 as solvents. The internal standards were referenced from the residue of those solvents. The HR-ESI-TOF-MS were recorded on a Bruker micrOTOF mass spectrometer. Column chromatography was carried out on MERCK silica gel 60 (230–400 mesh). Thin-layer chromatography was carried out with pre-coated MERCK silica gel 60 PF254; the spots were visualised under UV light (254 and 365 nm) and further by spraying with anisaldehyde and then heating until charred.

Extraction and Isolation

Air-dried aerial parts of *S. indicus* (2.0 kg) were ground to powder and then extracted successively with EtOAc and MeOH three times each (4L x 3),

respectively. Removal of solvents under reduced pressure gave crude EtOAc (150.0 g, 7.5 %) and MeOH (300.0 g, 15.0 %) extracts. The crude EtOAc extract (150.0 g) was subjected initially to silica gel CC, eluted with gradient systems of EtOAc-hexane and MeOH-EtOAc to give seventeen fractions, EF_1-EF_{17}. Fraction EF_2 was separated on silica gel FCC, eluting with hexane to give six subfractions, $EF_{2.1}$-$EF_{2.6}$. Subfraction $EF_{2.3}$ was purified on silica gel FCC, eluted with EtOAc-hexane to yield a white solid of **6** (61.2 mg). Fraction EF_5 was separated by FCC, eluted with a gradient system of EtOAc-hexane to give six subfractions, $EF_{5.1}$-$EF_{5.6}$. Subfraction $EF_{5.5}$ was further separated by FCC, eluted with an isocratic system of EtOAc-hexane (1:5) to give a white powder of **1** (7.8 mg). Fraction EF_7 was chromatographed on silica gel FCC, eluted with EtOAc-CH_2Cl_2-hexane (1:4:5) to give three subfractions, $EF_{7.1}$-$EF_{7.3}$. Subfraction $EF_{7.2}$ was separated by FCC, eluted with an isocratic system of EtOAc-hexane (3:7) to give seven subfractions, $EF_{7.2.1}$-$EF_{7.2.7}$. Subfraction $EF_{7.2.2}$ was recrystallized from CH_2Cl_2 to give a yellow solid of **4** (10 mg). Subfraction $EF_{7.3}$ was separated by silica gel FCC, eluted with an isocratic system of EtOAc-hexane (1:1) to give six subfractions, $EF_{7.3.1}$-$EF_{7.3.6}$. Subfraction $EF_{7.3.6}$ was separated by siliga gel FCC, eluted with an isocratic system of EtOAc-hexane (1:1) to yield a brown oil of **3** (6.5 mg). Fraction EF_8 was subjected to silica gel FCC, eluted with an isocratic system of EtOAc-CH_2Cl_2 (1:9) to yield a yellow solid of **2** (4.0 mg). Fraction EF_{14} was separated by FCC, eluted with a gradient system of MeOH-CH_2Cl_2 to give an orange gum of **5** (8.0 mg). The MeOH extract (300.0 g) was separated on silica gel FCC, eluted with gradient systems of hexane-EtOAc and EtOAc-MeOH to give six fractions, MF_1-MF_6. Fraction MF_4 was separated by FCC, eluted with an isocratic system of EtOAc-hexane (4:1) to give six subfractions, $MF_{4.1}$-$MF_{4.6}$. Subfraction $MF_{4.4}$ was further subjected to chromatography using a silica gel FCC, eluted with an isocratic system of EtOAc-hexane (7:3) to give seven subfractions, $MF_{4.6.1}$- $MF_{4.6.7}$. Subfractions $MF_{4.6.2}$ gave a yellow gum of **7** (100.0 mg).

3α-hydroxy-eudesm-4-en-12,6β-olide (**1**)

White powder, mp 141-142 ºC, 7.8 mg (0.0004%). R_f = 0.39 (70% EtOAc:hexane); IR (neat) ν_{max} cm^{-1}: 3289, 2925, 1754, 1267, 1148, 950; ^{1}H NMR ($CDCl_3$, 400 MHz): [6.17, 5.60 (each 1H, s, H-13)], 5.22 (1H, d, J = 6.0 Hz, H-6), 4.01 (1H, d, J = 4.0 Hz, H-3), 2.98 (1H, m, H-7), [2.07, 1.78 (each 1H, m, H-2)], 1.92 (3H, s, H-15), 1.69 (2H, m, H-8), [1.64, 1.32 (each 1H, m, H-1)], [1.52, 1.33 (each 1H, m, H-9)], 1.06 (3H, s, H-14); ^{13}C NMR ($CDCl_3$, 100 MHz): 170.6 (C-12), 141.7 (C-11), 138.1 (C-4), 132.9 (C-5), 120.6 (C-13), 75.3 (C-6), 69.5 (C-3), 41.1 (C-7), 37.6 (C-9), 33.7 (C-1), 33.3 (C-10), 27.5 (C-2), 24.9 (C-8), 24.1 (C-14), 17.5 (C-15).

ilicic acid (**2**)

Yellow solid, mp 179-180 ºC, 4.0 mg (0.0002%). R_f = 0.41 (70% EtOAc:hexane); IR (neat) ν_{max} cm^{-1}: 3429, 2929, 1742, 1647, 1452, 1404, 1376, 1285, 1226, 1164, 1108, 1079, 1051, 941, 864, 818,759, 731, 679, 650; ^{1}H NMR ($CDCl_3$, 400 MHz): [6.24, 5.80 (each 1H, s, H-13)], 2.48 (1H, t, J = 12.0 Hz, H-7), [1.97, 1.14 (each 1H, m, H-8)], 1.80 (2H, d, J = 1. 3.2 Hz, H-3), [1.57, 1.38 (each 1H, m, H-1)], 1.57 (2H, m, H-6), 1.55 (2H, m, H-2), [1.44, 1.22 (each 1H, m, H-9)], 1.32 (1H, m, H-5), 1.09 (3H, s, H-15), 0.89 (3H, s, H-14); ^{13}C NMR ($CDCl_3$, 100 MHz): 171.2 (C-12),

145.2 (C-11), 124.0 (C-13), 72.7 (C-4), 54.9 (C-5), 44.5 (C-9), 43.3 (C-3), 40.9 (C-1), 40.0 (C-7), 26.9 (C-6), 26.9 (C-8), 34.7 (C-10), 22.2 (C-15), 20.1 (C-2), 18.8 (C-14).

5α-hydroxy-4α,15-dihydrocostic acid (**3**)

Brown oil, 6.5 mg (0.0003%). R_f = 0.47 (70% EtOAc:hexane); IR (neat) ν_{max} cm^{-1}: 3390, 3018, 2962, 2933, 2874, 1744, 1678, 1638, 1422, 1247, 1216, 1176, 1093, 1037, 799, 750, 667; ^{1}H NMR ($CDCl_3$, 400 MHz): [6.30, 5.67 (each 1H, s, H-13)], 3.09 (1H, m, H-7), [2.06, 1.33 (each 1H, m, H-6)], [1.88, 1.33 (each 1H, m, H-1)], [1.88, 1.02 (each 1H, m, H-9)], [1.64, 1.06 (each 1H, m, H-3)], 1.63 (1H, m, H-4), 1.60 (2H, m, H-2), 1.43 (2H, m, H-8), 1.10 (3H, s, H-14), 1.02 (3H, d, J = 8 Hz, H-15); ^{13}C NMR ($CDCl_3$, 100 MHz): 171.3 (C-12), 145.1 (C-11), 124.8 (C-13), 75.8 (C-5), 41.1 (C-4), 38.1 (C-1), 37.9 (C-9), 36.8 (C-10), 34.7 (C-3), 34.4 (C-7), 28.0 (C-6), 26.3 (C-2), 21.7 (C-14), 17.0 (C-8), 16.7 (C-15).

2,5-dimethoxy-*p*-cymene (**4**)

Yellow oil, 61.2 mg (0.0031%). R_f = 0.56 (5% EtOAc:hexane); IR (neat) ν_{max} cm^{-1}: 2957, 2931, 2868, 1502, 1463, 1398, 1342, 1205, 1046, 859, 809, 638; ^{1}H NMR ($CDCl_3$, 400 MHz): 6.75 (1H, s, H-2), 6.71 (1H, s, H-5), 3.81 (3H, s, 3-OCH_3), 3.80 (3H, s, 6-OCH_3), 3.33 (1H, h, J = 6.8 Hz, H-7), 2.21 (3H, s, H-10), 1.22 (3H, d, J = 6.8 Hz, H-8), 1.22 (3H, d, J = 6.8 Hz, H-9); ^{13}C NMR ($CDCl_3$, 100 MHz): 151.9 (C-3), 150.6 (C-6), 135.1 (C-4), 124.2 (C-1), 114.2 (C-2), 109.3 (C-5), 56.3 (3-OCH_3), 56.2 (6-OCH_3), 26.8 (C-7), 22.9 (C-8), 22.9 (C-9), 16.0 (C-10).

pangelin (**5**)

Orange gum, 8.0 mg (0.0004%). R_f = 0.45 (55% EtOAc:hexane); IR (neat) ν_{max} cm^{-1}: 3436, 2926, 2857, 1726, 1620, 1620, 1456, 1347, 1134; ^{1}H NMR ($CDCl_3$, 400 MHz): 8.19 (1H, d, J = 10.0 Hz, H-4), 7.61 (1H, d, J = 2.4 Hz. H-2'), 7.18 (1H, s, H-8), 6.97 (1H, d, J = 2.4 Hz, H-3'), 6.30 (1H, d, J = 10.0 Hz, H-3), [5.20, 5.07 (each 1H, s H-5")], 4.55 (1H, dd, J = 7.6, 3.6 Hz, H-3"), [4.47 (1H, dd, J = 10.0, 3.6 Hz, H-2"), 4.40 (1H, dd, J = 9.6, 7.6 Hz, H-2")], 1.83 (3H, s, H-6"); ^{13}C NMR ($CDCl_3$, 100 MHz): 161.1 (C-2), 158.1 (C-7), 152.6 (C-8a), 148.5 (C-5), 145.2 (C-2'), 143.3 (C-4"), 139.1 (C-4), 113.4 (C-5"), 114.2 (C-6), 113.0 (C-3), 107.4 (C-4a), 104.7 (C-3'), 94.8 (C-8), 75.6 (C-2"), 74.2 (C-3"), 18.7 (C-6").

luteolin 4'-methyl ether (**6**)

Brown solid, mp 206-207 ºC, 9.0 mg (0.0005%). R_f = 0.57 (70% EtOAc:hexane); IR (neat) ν_{max} cm^{-1}: 3383, 2929, 1657, 1599, 1502, 1439, 1341, 1260, 1202, 1159, 840; ^{1}H NMR (CD_3OD, 400 MHz): 7.43 (1H, dd, J = 6.4, 2.0 Hz, H-2'), 7.34 (1H, d, J = 2.0 Hz, H-6'), 6.93 (1H, d, J = 6.4 Hz, H-3'), 6.53 (1H, s, H-3), 6.50 (1H, d, J = 2.0 Hz, H-8), 6.31 (1H, d, J = 2.0 Hz, H-6), 3.94 (3H, s, 4'-OCH_3), 3.86 (3H, s, 7-OCH_3); ^{13}C NMR (CD_3OD, 100 MHz): 182.6 (C-4), 165.7 (C-7), 164.9 (C-2), 161.4 (C-5), 157.8 (C-8a), 150.5 (C-5'), 147.9 (C-4'), 122.4 (C-1'), 120.7 (C-2'), 116.6 (C-3'), 109.2 (C-6'), 105.2 (C-4a), 103.6 (C-3), 98.2 (C-6), 92.6 (C-8), 55.9 (4'-OCH_3), 55.6 (7-OCH_3).

3,5-di-*O*-caffeoylquinic acid methyl ester (**7**)

Yellow gum, 100.0 mg (0.0050%). R_f = 0.52 (80% EtOAc:hexane); IR (neat) ν_{max} cm^{-1}: 3370, 2966, 1689, 1602, 1519, 1444, 1364, 1261, 1159, 1037, 980; ^{1}H NMR (CD_3OD, 400 MHz): 7.54 (1H, d, J = 15.6

Hz, H-7''), 7.49 (d, *J* = 15.6 Hz, H-7'), 7.02 (1H, s, H-2'), 7.02 (1H, s, H-2''), 6.86 (1H, t, *J* = 8.0 Hz, H-6'), 6.86 (1H, t, *J* = 8.0 Hz, H-6''), 6.76 (1H, t, *J* = 8.8 Hz, H-5'), 6.76 (1H, t, *J* = 8.8 Hz, H-5''), 6.27 (1H, d, *J* = 16.4 Hz, H-8''), 6.15 (1H, d, *J* = 16.4 Hz, H-8'), 5.43 (1H, m, H-3), 5.37 (1H, m, H-5), 3.98 (1H, m, H-4), 3.98 (3H, s, 7-OCH_3), 2.28-2.16 (2H, m, H-2), 2.28-2.16 (2H, m, H-6); ^{13}C NMR (CD_3OD, 100 MHz): 174.7 (C-7), 167.8 (C-9''), 167.4 (C-9'), 147.9 (C-4''), 147.7 (C-4'), 146.7 (C-7''), 146.6 (C-7'), 145.0 (C-3''), 144.9 (C-3'), 126.8 (C-1''), 126.4 (C-1'), 122.3 (C-6''), 122.2 (C-6'), 115.5 (C-5'), 115.5 (C-5''), 114.3 (C-2''), 114.3 (C-8''), 114.2 (C-2'), 113.7 (C-8'), 73.8 (C-1), 71.0 (C-5), 70.6 (C-3), 69.7 (C-4), 52.7 (7-OCH_3), 36.5 (C-2), 34.8 (C-6).

Bioassays: Antimalarial activity was evaluated against the parasite *Plasmodium falciparum* (K1, multidrug resistant strain), using the method of Trager and Jensen (Targar, Jensen, 1978). Quantitative assessment of malarial activity *in vitro* was determined by means of the microculture radio isotope technique based up on the method described by Desjardins et al. (Desjardins et al., 1979). The inhibitory concentration (IC_{50}) represents the concentration which causes 50% reduction in parasite growth as indicated by the *in vitro* incorporation of [^{3}H]-hypoxanthine by *P. falciparum*. The standard compound was dihydroartemisinin (Table 1). The anti-mycobacterial activity was assessed against *Mycrobacterium tuberculosis* H37Ra using the Microplate Alamar Blue Assay (MABA) (Collins, Franzblau, 1997). Isoniazid was used as the reference (Table 1). The compounds were also evaluated against human epidermoid carcinoma (KB) and human small cell lung cancer (NCI-H187). The standard was ellipticine. Human breast cancer assays were performed on cell lines (MCF-7) employing the colorimetric method as described by Skehan et al. (Skehan et al., 1990). The reference substances were tamoxifen and doxorubicin (Table 1).

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Table 1 Biological activities of the isolated compounds.

compound	antimalarial	antimycobacterial	cytotoxicity (IC_{50}, µM)		
	(IC_{50}, µM)	(MIC, µM)	KB[a]	NCI-H187[b]	MCF-7[c]
1	inactive	nd[d]	147.37	nd[d]	93.10
2	inactive	inactive	inactive	inactive	inactive
3	inactive	nd[d]	inactive	nd[d]	inactive
4	inactive	inactive	inactive	inactive	inactive
5	inactive	nd[d]	inactive	nd[d]	inactive
6	inactive	nd[d]	inactive	nd[d]	inactive
7	4.50	nd[d]	inactive	nd[d]	inactive
dihydroartemisinin	0.007				
isoniazid		0.34			
ellipticine			4.91	3.80	
tamoxifen					18.84
doxorubicin			1.57	0.10	14.53

[a]Humanepidermoid carcinoma in the mouth, [b]Human lung cancer cell, [c]Human breast cancer cell, [d]Not determined.

Inactive = >50 µg/mL

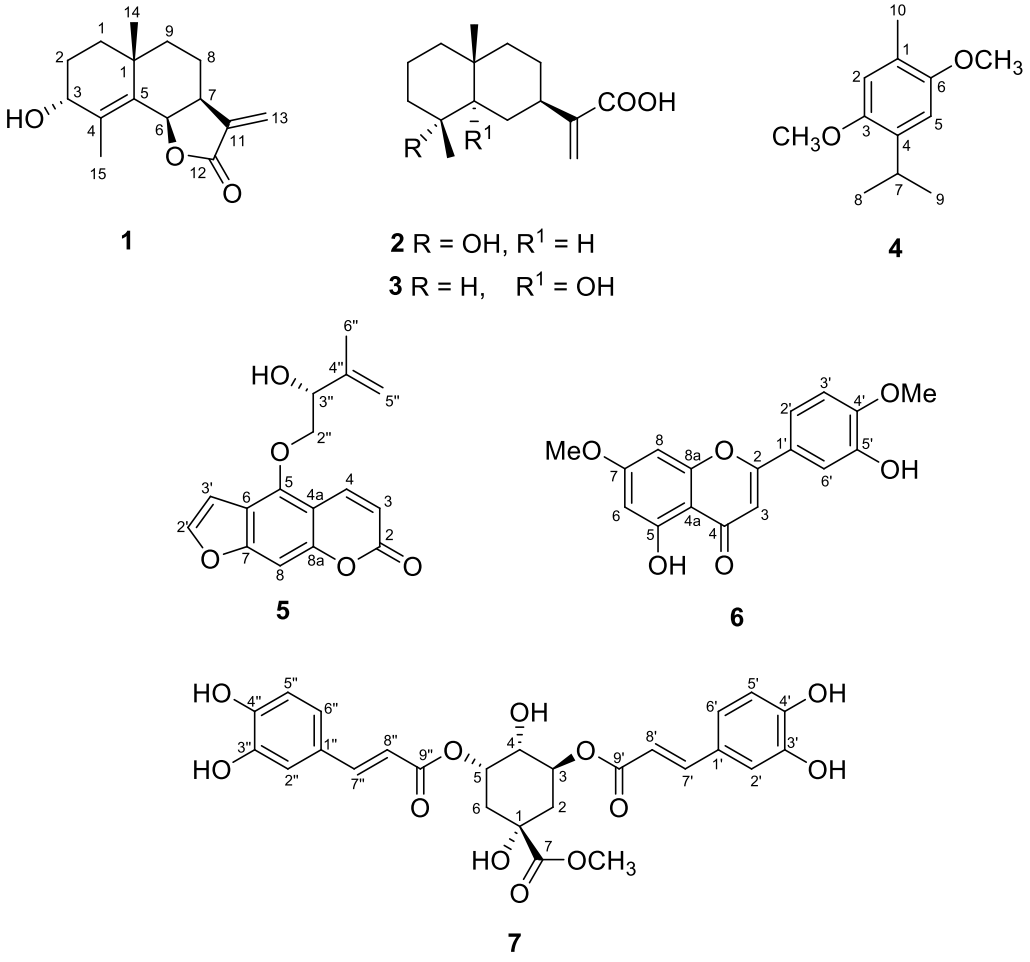

Figure 1 Structures of compounds **1**-**7**.

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