The components from aerial parts of *Sarcosperma affinis* Gagnep. and their antibacterial activities

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The components from aerial parts of *Sarcosperma affinis* Gagnep. and their antibacterial activities

Hoai Thi Nguyen¹, Duc Viet Ho¹, Hung Quoc Vo¹, Trang Huyen Xuan Hoang¹, Takeshi Kodama², Takuya Ito², Hiroyuki Morita² and Ain Raal³*

**Abstract:** Sixteen compounds were isolated from the aerial parts of *Sarcosperma affinis* Gagnep. including (E)-phytol (1), heptadecan-1-ol (2), lupeol (3), oleanolic acid (4), 3β-hexadecanoyloleanolic acid (5), pomolic acid (6), euscaphic acid (7), myrianthic acid (8), (+)-pinoresinol (9), (+)-medioresinol (10), (+)-syringaresinol (11), quercetin-3-O-β-D-galactopyranoside (12), epicatechin (13), bis-(2-ethylhexyl)phthalate (14), 3-prenyl-4-O-β-D-glucopyranosyloxy-4-hydroxybenzoic acid (15), icariside E₅ (16). Their structures were elucidated by the combination of spectroscopy including 1D, 2D NMR, HR-ESI-MS and in comparison with the reported NMR data in the literature. Among those, compounds 4 and 6 showed potent antibacterial activity against *Mycobacterium smegmatis* with MIC values of 5.48 and 2.65 μM, respectively. The chemical composition and biological activity of constituents isolated from aerial parts of *S. affinis* was studied for the first time.

**Subjects:** Biochemistry; Microbiology; Pharmaceutical Science; Pharmacology

**Keywords:** *Sarcosperma affinis*; antibacterial activity; oleanolic acid; pomolic acid; *Mycobacterium smegmatis*; chemical composition

**ABOUT THE AUTHOR**

Hoai Thi Nguyen, PhD, is a lecturer in Pharmacognosy, and is currently associate professor, dean of the Faculty of Pharmacy, Hue University of Medicine and Pharmacy, Vietnam. Hoai graduated from Hanoi University of Pharmacy with BS, MS, and PhD degree, then had postdoctoral training at Japan. Her research interest focuses on natural products chemistry including isolation, structural elucidation of phytochemical compounds, and bioactivity test of extracts and isolated compounds such as antibacterial, anticancer, and antidiabetic activities. Most of her research focuses on discovering bioactive compounds from the medicinal plants in Central Vietnam. These plants were collected based on the two main criteria: one is whether the plants are used for treatment of several diseases under Vietnamese traditional experience. Other is whether the plants are rare even in the natural forest of Vietnam. Indeed, a large number of plants collected under the later criteria have never been researched.

**PUBLIC INTEREST STATEMENT**

Medicinal plants can improve human health, their biological activity depends on chemical composition of plant material. Sixteen compounds were isolated and detected from aerial parts of the tropical plant *Sarcosperma affinis* with unknown chemical composition. *S. affinis* is an endemic plant and known in ethnomedicine of Vietnam; it grows in different forest types from sea level up to 1,300 m. The antimicrobial activity against 6 bacterial strains of these 16 compounds was studied. It was found that oleanolic acid and pomolic acid from *S. affinis* showed potent antimicrobial activity against bacteria *Mycobacterium smegmatis* which is generally considered as a non-pathogenic micro-organism. The chemical composition of this plant was studied for the first time.
1. Introduction

*Sarcosperma* Hook. f. is a genus of perennial shrub or small timber in Sapotaceae family. This genus includes more than 11 species distributed in tropical regions such as India, Indonesia, Malaysia, Thailand, China, Philippine, and Vietnam. These species grow in different forest types from sea level up to 1,300 m altitude. Among five species of *Sarcosperma* genus found in Vietnam, the *Sarcosperma affinis* Gagnep. is an endemic plant and known in local ethnomedicine (Chai & Yii, 2002; Ho, 2003; Lee & Pennington, 1996).

As part of our ongoing research on finding bioactive compounds, 207 Vietnamese plants species samples were collected and their methanol extracts were screened for antibacterial activity. The results showed that *S. affinis* was one of the most active plants. However, there is no report on chemical constituents of *Sarcosperma* genus as well as the compounds being responsible for the activity of *S. affinis* so far.

Therefore, the current study was conducted to isolate those compounds and to evaluate their antibacterial activity *in vitro*.

2. Results and discussion

2.1. Isolation and identification of constituents

The present chemical investigation led to the isolation and the structural elucidation of 16 known compounds including 2 long-chain alcohol (1, 2), 6 triterpenoids (3–8), 4 lignans (9–11, 16), 2 flavonoids (12, 13), 1 phthalic acid ester (14), and 1 prenylated benzoic acid derivative (15). Their chemical structures were determined to be (E)-phytol (1) (Bhattacharya & Rana, 2013), heptadecan-1-ol (2) (Gu et al., 2016), lupeol (3) (Mouffok, Haba, Lavaud, Long, & Benkhaled, 2012), oleanolic acid (4) (Mahato & Kundu, 1994), 3β-hexadecanoyloleic acid (5) (Carvalho, Carvalho, Ferreira, Faria, & Braz-Filho, 2001), pomolic acid (6) (Lee, Juang, Hsu, & Wu, 2005), euscaphic acid (7) (Chen, Li, Wu, Ren, & Zhang, 2008), myrianthic acid (8) (Lee et al., 2005), (+)-pinoresinol (9) (Abe & Yamauchi, 1988), (+)-medioresinol (10) (Abe & Yamauchi, 1988), (+)-syringaresinol (11) (Abe & Yamauchi, 1988), quercetin-3-O-β-D-galactopyranoside (12) (Pereira, Barreto Júnior, Kuster, Simas, & Sakuragui, 2012), epicatechin (13) (Cren-Olivé, Wieruszeski, Maes, & Rolando, 2002), bis-(2-ethylhexyl) phthalate (14) (Lyutskanova et al., 2009), 3-prenyl-4-O-β-D-glucopyranosylxy-4-hydroxylbenzoic acid (15) (Sang, Lapsley, Rosen, Ho, & Agricult, 2002), and icarisideE (16) (Miyase, Ueno, Takizawa, Kobayashi, & Oguchi, 1989) (Figure 1). The spectral data of the known compounds were confirmed with the reported data. By our best knowledge, the chemical composition of *Sarcosperma* genus plants was studied for the first time, at least on modern level.

2.2. Antimicrobial activities of isolated compounds

The inhibitory effect of 16 isolated compounds was investigated toward 6 bacterial strains, including three Gr(+) and three Gr(−) strains. However, our isolated compounds did not display inhibitory activity on Gr(−) strains (*Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa*). The results of three Gr(+) bacterial strains including *Staphylococcus aureus*, *Bacillus subtilis*, and *Mycobacterium smegmatis* were significant, the minimal inhibitory concentration (MIC) values of

| Compounds | MIC (μM) |
|-----------|----------|
|           | *Staphylococcus aureus* | *Bacillus subtilis* | *Mycobacterium smegmatis* |
| 1         | >67.57   | >67.57   | 67.57   |
| 4         | 21.93    | >21.93   | 5.48    |
| 6         | 10.59    | 21.19    | 2.65    |
| 7         | >40.98   | >40.98   | 40.98   |
| Ampicillin| 14.33    | 28.65    | 28.65   |
active compounds are provided in Table 1. The results showed that compounds 1, 4, 6, and 7 exhibited remarkable antibacterial activity toward tested bacterial strains. Among those, compounds 4 and 6 possessed potent effect toward *M. smegmatis* with MIC values of 5.48 and 2.65 μM, respectively, whereas compounds 1 and 7 displayed moderate effect with MIC values of 67.57 and 40.98 μM, respectively. Compounds 4 and 6 also exhibited significant activity toward *S. aureus* with MIC values of 21.93 and 10.59 μM, respectively. Moreover, only compound 6 demonstrated moderate effect against *B. subtilis* with MIC value of 21.19 μM.

Several previous works discovered the significant activity of 4 against *Enterococcus faecium*, *Streptococcus pneumoniae*, *S. aureus* (Horiuchi et al., 2007), *Listeria monocytogenes* (Hee, 2015), *Streptococcus downei* (Park & Kim, 2011), *Streptococcus mutans* and *S. sobrinus* (Kim et al., 2010), *Enterococcus faecalis*, *Propionibacterium acnes* (Jo et al., 2014). It is known that oleandolic acid (4) has antimicrobial effect against Gr(+) bacteria but not Gr(−) bacteria (Kook & Park, 2013). Compound 6 showed 100% growth inhibition in 10 Gr(−) multidrug-resistant phenotypes with the MICs ranging from 32 to 256 μg/mL (Seukep, Sandjo, Ngadjui, & Kuete, 2016) as well as *P. aeruginosa* strain (Sidjui et al., 2016). Pomolic acid (6) inhibited significantly the growth of some Gr(−) bacteria also (Mbosso et al., 2008).

These results indicate that aerial parts of *S. affinis* could be useful in the future development of internal antimicrobial preparation. The positive experience of folk medicine may be sometimes conceptional, for example the needles of *Pinus sylvestris* used against cancer in Estonian ethnomedicine (Sak, Jürisoo, & Raal, 2014) showed cytotoxic effect to breast cancer cells (Hoai, Duc, Thao, Orav, & Raal, 2015).
3. Conclusion
The chemical composition and biological activity of constituents isolated from aerial parts of S. affinis was studied for the first time. Among the 16 constituents tested, oleanolic acid (4) and pomolic acid (6) showed potent antibacterial activity against M. smegmatis.

4. Experimental

4.1. General
Melting points were determined on a Yanaco Micro MP apparatus. Optical rotations were measured on a JASCO P-2100 polarimeter (Hachioji, Tokyo, Japan). Infrared spectra were recorded on Jasco FT/IR-460 Plus spectrometer. 1D and 2D NMR were carried out using BrukerAvance 500 spectrometer with tetramethylsilane as an internal standard. ESI-MS data including high resolution mass spectrum were measured on Shimadzu LCMS-IT-TOF spectrometer (Kyoto, Japan). Column chromatography was performed using silica gel (Kanto, 40–50 μm), Cosmosil 75C18-OPN (NacalaiTesque Inc., Kyoto, Japan) and Sephadex LH-20 (Dowex® 50WX2–100, Sigma-Aldrich). Analytical TLC was performed on pre-coated silica gel 60F254 and RP-18 F254 plates (0.25 or 0.50 mm thickness, Merck). Cosmosil 5C18-AR-II (NacalaiTesque) was used for analytical and semi-preparative HPLC (250 × 4.6 mm for analytical HPLC, and 250 × 10.0 mm for semi preparative HPLC).

4.2. Plant material
The aerial parts of S. affinis Gagbep. were collected in Bach Ma mountain, Thua Thien Hue province, Vietnam in June, 2014 and were identified by Dr Nguyen The Cuong, Institute of Ecology and Biological Resources, VAST, Vietnam. A voucher specimen (NTG01) is deposited at the Faculty of Pharmacy, Hue University of Medicine and Pharmacy, Vietnam.

4.3. Extraction and isolation of constituents
The aerial parts of S. affinis (2.5 kg) were extracted with hot MeOH (3 × 3 L, 3 h each, 60°C) to yield 70.0 g of a dark solid extract. This extract was then suspended in water and successively partitioned with chloroform (CHCl3), ethyl acetate (EtOAc) to obtain the CHCl3 (SC, 28.0 g), the EtOAc (SE, 23.0 g) and the water (SW, 19.0 g) layers after removal of solvent

The SC extract was chromatographed on a silica gel column eluted with n-hexane–EtOAc gradient system (100:0, 90:10, 70:10, 50:10 and 10:10, v/v, each 1.0 L) and 100% MeOH (1.0 L) to obtain six fractions, SC1–SC6. Fraction SC1 (1.6 g) was applied to a silica gel column eluted with n-hexane–CHCl3–EtOAc (10:1:1, v/v) to obtain four sub-fractions (SC1A-SC1D). Fraction SC4 (2.5 g) was chromatographed on a silica gel column using a step gradient of CHCl3 (0.5 L), CHCl3–EtOAc(10:1, v/v, 0.5 L), CHCl3–EtOAc(5:1, v/v, 0.5 L), CHCl3–EtOAc (1:1, v/v, 0.5 L) to obtain four fractions, SC4A–SC4D, respectively. Fraction SC4C (750 mg) was chromatographed on a silica gel column eluted with CHCl3–acetone (15:1, v/v) to afford 3 (7.5 mg) and 5 (12.1 mg). Further purification of fraction SC4D (930 mg) using reverse-phase RP-18 silica gel column eluted with MeOH–water system (40:1, v/v) led to the isolation of compounds 4 (11.0 mg), 6 (6.5 mg), 7 (19.0 mg), and 8 (5.7 mg) (Figure 2(B)).

The SE extract was subjected to silica gel column eluted with CHCl3–MeOH(30:1, v/v) to get eight smaller fractions, SE1–SE8. Fraction SE4 (940 mg) was chromatographed on a Sephadex LH-20 column eluted with MeOH to yield 9 (7.5 mg) and three sub-fractions, SE4A–SE4C. Fraction SE4B was then subjected to a reverse-phase RP-18 silica gel column eluted with acetone–MeOH–water (5:3:1, v/v) to afford 10 (6.6 mg) and 11 (4.8 mg). Fraction SE4C was further purified by preparative TLC using CHCl3–MeOH(20:1, v/v) as the mobile phase to get 12 (3.5 mg) and 13 (5.0 mg) (Figure 2(C)).

The water-soluble fraction SW was subjected to a Diaion HP-20 column and eluted with stepwise increases of MeOH in water (0, 25, 50, 75, and 100%, each 1.0 L) to obtain five fractions, SW1–SW5. Fraction SW5 (1.9 g) was subjected to a silica gel column eluted with CHCl3–MeOH(5:1, v/v) to obtain
four sub-fractions, SW5A–SW5D. Fraction SW5A (770 mg) was further purified by RP-18 column eluted with MeOH–water (2:1, v/v) to give 16 (7.0 mg) and three smaller fractions, SW5A1–SW5A3. Fraction SW5A2 was finally chromatographed on a Sephadex LH-20 column eluted with MeOH–water (8:2, v/v) to yield 14 (6.7 mg) and 15 (8.2 mg) (Figure 2(D)).

4.4. Bioassay

Antimicrobial activity was performed using the dilution method according to a published procedure with slight modifications. The experiment was conducted on six bacterial strains, including three Gr (+) and three Gr(−) strains: three Gr(+) strains were *S. aureus* (NBRC 100910), *Bacillus subtilis* (NBRC 13719), *M. smegmatis* (NBRC 13167), and three Gr(−) strains were *Klebsiella pneumoniae* (NBRC 14940), *E. coli* (NBRC 102203), and *P. aeruginosa* (NBRC 106052). These strains were tested using microdilution assays, and MIC values were determined. Bacterial strains were inoculated on YP agar plates [1% polypeptone (Nihon Pharmaceutical, Japan), 0.2% yeast extract (Difco, USA), 0.1% MgSO₄·7H₂O, and 2% agar (Nacalai Tesque, Japan)] and incubated at 30°C for 12 h. A stock solution of samples was prepared at 1 mg/mL in DMSO and further diluted to varying concentrations in 96-well plates that contained microbial strains incubated in YP medium for the bacterial strains. The plate was further incubated at 37°C for 12 h. Ampicillin and kanamycin (Nacalai Tesque, Japan) were used as the reference reagents for bacterial strains (Eloff, 1998).
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