ฤทธิ์ยับยั้งแบคทีเรียและฤทธิ์ยับยั้งเชื้อราของสารที่แยกได้จากจากต้นสำมะงา

Anti-bacterial and Anti-fungal Activities of Isolated Compounds from *Clerodendrum inerme*

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บทคัดย่อ

ประเมินฤทธิ์ยับยั้งแบคทีเรียของสารที่ได้จากใบและรากต้นสำมะงา (วงศ์ผกากรอง) โดยนำพืชตากแห้งมาสกัดด้วยไดคลอโรมีเทน จากนั้นนำกากพืชมาสกัดต่อด้วยเมทานอล นำสิ่งสกัดทั้งหมดมาทดสอบฤทธิ์ยับยั้งแบคทีเรียด้วยวิธี broth microdilution susceptibility พบว่าสิ่งสกัดไดคลอโรมีเทนจากใบและรากมีฤทธิ์ยับยั้งแบคทีเรียแกรมบวก (*Staphylococcus aureus* และ *Bacillus subtilis*) และแบคทีเรียแกรมลบ (*Pseudomonas aeruginosa* และ *Escherichia coli*) ได้สูงกว่าสิ่งสกัดเมทานอล นำสิ่งสกัดไดคลอโรมีเทนจากใบและรากมาแยกสารออกฤทธิ์ต่อไป ได้สารบริสุทธิ์จำนวน 7 สาร และวิเคราะห์โครงสร้างทางเคมีด้วยข้อมูลจากเทคนิคเอ็น เอ็ม อาร์และแมสสเปกโทรสโกปี สารบริสุทธิ์ที่ได้คือ (3β, 22*E*, 24*S*)-stigmasterol-5,22,25-trien-3-ol (1), pectolinarigenin (2), acacetin (3), (3β, 22*E*, 24*S*)-stigmasterol-5,22,25-triene-3-yl-β-D-glucopyranoside (4), stigmasterol (5), lupeol laurate (6) และ betulinic acid (7) สาร 1 มีฤทธิ์ยับยั้งการเจริญของ *S. aureus* สูงที่สุด ขณะที่สาร 1, 6 และ 7 มีฤทธิ์ยับยั้งการเจริญของ *P. aeruginosa* สูง

คำสำคัญ: ต้นสำมะงา วงศ์ผกากรอง ฤทธิ์ยับยั้งแบคทีเรีย ฤทธิ์ยับยั้งเชื้อรา

Abstract

Evaluation of anti-microbial activity of phytochemicals from leaves and roots of *Clerodendrum inerme* (Vebenaceae) was conducted. Air dried materials were extracted with CH_2Cl_2 and residues were consequently extracted with MeOH. All extracts were determined for anti-microbial activity by broth microdilution susceptibility testing. The CH_2Cl_2 extracts of leaves and roots exhibited anti-microbial activity toward Gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) higher than MeOH extracts. The CH_2Cl_2 extracts of leaves and roots were further isolated for their active compounds. Seven compounds were obtained and further characterized for their chemical structures by NMR and Mass Spectroscopy data as (3β, 22*E*, 24*S*)-stigmasterol-5,22,25-trien-3-ol (1), pectolinarigenin (2), acacetin (3), (3β, 22*E*, 24*S*)-stigmasterol-5,22,25-triene-3-yl-β-D-glucopyranoside (4), stigmasterol (5), lupeol laurate (6) and betulinic acid (7). Compound 1 exhibited the highest inhibition on *S. aureus* growth whereas compounds 1, 6 and 7 exhibited high inhibition on *P. aeruginosa* growth.

Keywords: *Clerodendrum inerme*, Vebenaceae, anti-bacterial activity, anti-fungal activity

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Introduction

Clerodendrum inerme L. is a mangrove plant in Verbenaceae family and its common name is scrambling clerodendrum. This plant usually grows along the beach forest in many places such as Western Australia, North East Queensland and the Pacific Islands. The extracts of *C. inerme* have been reported to possess a variety of biological properties such as anti-oxidation[1-2], anti-inflammatory[3-5], anti-tumor[6], anti-virus[7] and growth inhibition of insecticide activities[8-9]. This extract has also been noted to possess anti-microbial activity. Isoamyl alcoholic extract of *C. inerme* can inhibit *Bacillus subtilis* and *Staphylococcus aureus*[10]. Moreover, the *C. inerme* extracts have the anti-fungal activity on human pathogen fungi (*Epidermophyton floccosum* and *Trichophyton tonsurans*) and plant pathogen fungi (*Aspergillus flavus* and *Aspergillus niger*)[11]. One of the constituents isolated from *C. inerme*, (5*S*,6*R*,8αR)-5,6,8α-trimethyl-5-[2-(3-oxo-cyclobutyl)-ethyl] 3,4,4α, 5,6,7,8,8α-octahydro-naphthalene-1-carboxylic acid methyl ester, showed inhibition on *B. pumilis*, *E. coli* and *A. flavus*[12].

Although *C. inerme* extracts were extensively reported to have anti-microbial potential, it was a rare study that revealed the active constituents responsible for those activities. Thus, in this study, extracts of *C. inerme* were investigated for activity against Gram-positive bacteria (*S. aureus* and *B. subtilis*) and Gram-negative bacteria (*P. aeruginosa* and *E. coli*). The constituents of this plant were isolated and tested for anti-bacterial potency on *S. aureus* and *P. aeruginosa*, including anti-fungal potency on *Candida albicans*.

Materials and Methods

Plant Material Preparation

Roots and leaves of *C. inerme* were collected in Rayong province, Thailand, on May 2012. A voucher specimen (BCU 013514) has been deposited at the Department of Botany, Faculty of Science, Chulalongkorn University, Thailand.

Extraction and Isolation

Air dried leaves (3.8 kg) were macerated twice with CH_2Cl_2. After filtration and solvent removal, 57.4 g of CH_2Cl_2 (L-CH_2Cl_2) extract was obtained. Leaf residue was subsequently extracted twice with MeOH and 95.2 g of MeOH extract was given after evaporating solvent.

A portion of L-CH_2Cl_2 extract (57.4 g) was fractionated by Si-gel CC and eluted with an increasing polarity of mobile phase *n*-hexane:EtOAc, followed by EtOAc:MeOH, to afford seven fractions (C1-C7). Fraction C2 was further separated by Si-gel CC eluted with *n*-hexane:EtOAc (100:0 → 10:90) to afford three fraction C2.1 to C2.3. Fraction C2.1 was purified by Si-gel CC eluting with *n*-hexane:EtOAc (9:1) to obtain compound **1** (1.1 g). Fraction C3 was separated by Si-gel CC eluted with a gradient of *n*-hexane:EtOAc to afford two fractions, and after repeat separation on fraction C3.1 by Si-gel CC eluted with a gradient of *n*-hexane:CH_2Cl_2, compound **2** (46.5 mg) was obtained. Compound **3** (25.0 mg) was gained from fraction C3.2 by Sephadex LH-20 CC eluted with *n*-hexane:CH_2Cl_2:MeOH (7:2.5:0.5), then by Sephadex LH-20 CC eluted with *n*-hexane:CH_2Cl_2 (1:1). Fraction C5 was separated by Si-gel CC eluted with *n*-hexane:EtOAc:MeOH (100:0:0 → 0:0:100) to afford two fractions C5.1-C5.2. Fraction C5.1 was separated by Si-gel CC eluted with CH_2Cl_2:MeOH (9:1) to afford compound **4** (9.0 mg).

The CH_2Cl_2 (R-CH_2Cl_2, 69.9 g) and MeOH (446.5 g) extracts from roots were obtained by extracting the roots of *C. inerme* (4.5 kg) in the same manner as leaves. The R-CH_2Cl_2 extract (60.5 g) was firstly fractionated by Si-gel CC eluted with *n*-hexane:EtOAc:MeOH (100:0:0 → 0:10:90) to afford nine fractions R1-R9. Fraction R2 was separated by Si-gel CC eluted with *n*-hexane:EtOAc (100:0 → 10:90) to obtain three fractions R2.1-R2.3. and compound **5** (42 mg) was isolated at solvent ratio of 9:1 from this separation. Fraction R2.2 was separated by Si-gel CC eluted with *n*-hexane:EtOAc (100:0 → 10:90), followed by Si-gel CC eluted with *n*-hexane:CH_2Cl_2 (9:1) to yield compound **6** (6 mg). Fraction R4 was separated by Si-gel CC eluted with *n*-hexane:EtOAc (100:0 → 10:90) to afford two fractions R4.1-R4.2. Fraction R4.1 was then purified by Si-gel CC eluted with *n*-hexane:EtOAc (8:2) to yield compound **7** (30 mg).

(3β, 22*E*, 24*S*)-stigmasterol-5,22,25-trien-3-ol (**1**): white powder; mp. 121-125°C; ^{1}H-NMR ($CDCl_3$,400 MHz) δ 3.54 (1H, *m*, H-3), 5.36 (1H, *brd*, *J*=4.8, H-6),

0.72 (3H, *brs*, H-18), 1.03 (3H, *s*, H-19), 1.04 (3H, *s*, H-21), 5.28 (1H, *dd*, *J*=15.6, 8.0, H-22), 5.21 (1H, *dd*, *J*=15.2, 7.2, H-23), 4.72 (2H, *m*, H-26), 1.67 (3H, *brs*, H-27), 0.86 (3H, *t*, *J*=7.4, H-27); ^{13}C-NMR ($CDCl_3$, 100 MHz) 37.3 (C-1), 31.9 (C-2), 71.8 (C-3), 42.3 (C-4), 140.8 (C-5), 121.7 (C-6), 31.9 (C-7), 31.7 (C-8), 50.2 (C-9), 36.5 (C-10), 21.1 (C-11), 39.7 (C-12), 42.3 (C-13), 56.9 (C-14), 24.3 (C-15), 28.7 (C-16), 55.9 (C-17), 12.1 (C-18), 19.4 (C-19), 40.1 (C-20), 20.8 (C-21), 137.2 (C-22), 130.1 (C-23), 52.0 (C-24), 148.6 (C-25), 109.5 (C-26), 20.2 (C-27), 25.7 (C-28), 12.1 (C-29).

Pectolinarigenin (**2**): yellow needle; mp. 210-211°C; ^{1}H-NMR (acetone-*d6*, 400 MHz) δ 6.48 (1H, *s*, H-3), 6.53 (1H, *s*, H-8), 7.87 (1H, *d*, *J*=8.8, H-2′, 6′), 6.96 (1H, *d*, *J*=8.8, H-3′, 5′), 3.76 (3H, *s*, 6-OMe), 3.72 (3H, *s*, 4'-OMe), 13.02 (1H, *s*, 5-OH), 9.31 (1H, *s*, 7-OH) ; ^{13}C-NMR (acetone-*d6*, 100 MHz) 165.0 (C-2), 104.0 (C-3), 183.6 (C-4), 154.0 (C-5), 132.3 (C-6), 157.8 (C-7), 94.8 (C-8), 154.0 (C-9), 105.8 (C-10), 124.4 (C-1′), 115.4 (C-3′, 5′), 129.1 (C-2′, 6′), 163.0 (C-4′), 60.7 (6-OMe), 56.0 (4′-OMe).

Acacetin (**3**): yellow needle; mp. 284-289°C; ^{1}H-NMR (acetone-*d6*, 400 MHz) δ 6.59 (1H, *s*, H-3), 6.17 (1H, *d*, *J*=1.6, H-6), 6.46 (1H, *d*, *J*=1.6, H-8), 7.94 (2H, *d*, *J*=8.8, H-2′, 6′), 7.05 (2H, *d*, *J*=8.8, H-3′,5′), 3.93 (3H, *s*, 4′-OMe), 12.87 (1H, *s*, 5-OH); ^{13}C-NMR (acetone-*d6*, 100 MHz) 165.1 (C-2), 104.5 (C-3), 183.1 (C-4), 163.7 (C-5), 99.8 (C-6), 164.8 (C-7), 94.7 (C-8), 158.9 (C-9), 104.6 (C-10), 124.3 (C-1′), 129.1 (C-2′,6′), 115.4 (C-3′,5′), 163.3 (C-4′), 56.0 (C4′-OMe)

(3β, 22*E*, 24*S*)-Stigmasterol-5,22,25-triene-3-yl-β-D-glucopyranoside (**4**): white powder; mp. 259-261°C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 3.51 (1H, *m*, H-3), 5.29 (1H, *brd*, *J*=4.4, H-6), 5.12 (1H, *m*, H-22), 5.12 (1H, *m*, H-23), 1.59 (1H, *s*, H-26), 4.63 (1H, *brs*, H-27), 4.35 (1H, *d*, *J*=7.6, H-1′), 3.76 (1H, *m*, H-2′), 3.76 (1H, *m*, H-3′), 3.40 (1H, *m*, H-4′), 3.33 (1H, *m*, H-5′), 3.40 (2H, *m*, H-6′); ^{13}C-NMR ($CDCl_3$, 100 MHz) 38.7 (C-1), 29.6 (C-2), 76.3 (C-3), 42.2 (C-4), 140.2 (C-5), 122.1 (C-6), 31.8 (C-7), 36.7 (C-8), 50.1 (C-9), 37.2 (C-10), 21.0 (C-11), 39.6 (C-12), 48.5 (C-13), 56.8 (C-14), 24.2 (C-15), 28.6 (C-16), 55.8 (C-17), 12.0 (C-18), 20.7 (C-19), 40.1 (C-20), 21.0 (C-21), 137.1 (C-22), 130.0 (C-23), 51.9 (C-24), 148.6 (C-25), 19.2 (C-26), 109.4 (C-27), 25.7 (C-28), 12.0 (C-29), 101.0 (C-1′), 73.5 (C-2′), 79.2 (C-3′), 69.9 (C-4′), 75.6 (C-5′), 61.7 (C-6′).

Stigmasterol (**5**): white powder; mp. 147-151°C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.25 (1H, *m*, H-1α), 1.99 (1H, *m*, H-1β), 1.52 (1H, *m*, H-2α), 1.83 (1H, *m*, H-2β), 3.52 (1H, *m*, H-3), 2.27 (1H, *m*, H-4α, H-4β), 5.34 (1H, *m*, H-6), 1.60 (1H, *brs*, H-7α), 1.83 (1H, *m*, H-7β), 1.52 (1H, *m*, H-8), 1.03 (1H, *brs*, H-9), 1.52 (1H, *m*, H-11α), 1.60 (1H, *brs*, H-11β), 1.25 (1H, *m*, H-12α), 1.99 (1H, *m*, H-12β), 1.25 (1H, *m*, H-14), 1.03 (1H, *brs*, H-15α), 1.60 (1H, *brs*, H-15β), 1.25 (1H, *m*, H-16α), 1.69 (1H, *m*, H-16β), 1.25 (1H, *m*, H-17), 0.70 (3H, *brs*, H-18), 1.01 (3H, *brs*, H-19), 1.99 (1H, *m*, H-20), 1.01 (3H, *brs*, H-21), 5.15 (1H, *m*, H-22), 5.03 (1H, *dd*, *J*=8.4, 15.2, H-23), 1.52 (1H, *m*, H-24), 1.52 (1H, *m*, H-25), 0.80 (3H, *brs*, H-26), 0.84 (3H, *brs*, H-27), 1.25 (1H, *m*, H-28α), 1.52 (1H, *m*, H-28β), 0.79 (3H, *brs*, H-29); ^{13}C-NMR ($CDCl_3$, 100 MHz) 39.7 (C-1), 31.7 (C-2), 71.8 (C-3), 42.3 (C-4), 140.8 (C-5), 121.7 (C-6), 31.9 (C-7), 31.9 (C-8), 51.2 (C-9), 37.3 (C-10), 21.2 (C-11), 36.5 (C-12), 42.2 (C-13), 56.9 (C-14), 25.4 (C-15), 29.7 (C-16), 56.0 (C-17), 12.0 (C-18), 21.0 (C-19), 40.4 (C-20), 24.4 (C-21), 138.3 (C-22), 129.3 (C-23), 50.2 (C-24), 31.9 (C-25), 21.1 (C-26), 19.4 (C-27), 28.9 (C-28), 12.2 (C-29).

Lupeol laurate (**6**): white powder; mp. 214-219°C; ESI-MS *m/z* 631.54 $[M+Na]^+$; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 4.39 (1H, *dd*, *J*=5.6, 10.4, H-3), 2.30 (1H, *m*, H-19), 0.79 (3H, *s*, H-23), 0.96 (3H, *s*, H-24), 0.79 (3H, *s*, H-25), 0.76 (3H, *s*, H-26), 0.76 (3H, *s*, H-27), 0.71 (3H, *s*, H-28), 4.61 (1H, *m*, H-29α), 4.57 (1H, *m*, H-29β), 1.61 (3H, *s*, H-30), 2.21 (2H, *t*, *J*=7.6, H-2′), 1.25 (2H, *m*, H-3′-11′), 0.87 (3H, *m*, H-12′); ^{13}C-NMR ($CDCl_3$, 100 MHz) 39.9 (C-1), 23.6 (C-2), 80.5 (C-3), 38.3 (C-4), 55.3 (C-5), 18.1 (C-6), 34.1 (C-7), 42.9 (C-8), 50.2 (C-9), 37.7 (C-10), 22.5 (C-11), 25.0 (C-12), 37.9 (C-13), 42.7 (C-14), 29.7 (C-15), 34.7 (C-16), 40.7 (C-17), 48.2 (C-18), 47.8 (C-19), 151.0 (C-20), 27.8 (C-21), 37.0 (C-22), 29.5 (C-23), 17.9 (C-24), 16.4 (C-25), 16.0 (C-26), 14.4 (C-27), 15.9 (C-28), 109.2 (C-29), 20.8 (C-30), 173.5 (C-1′), 35.5 (C-2′), 23.0-29.5-(C-3′-C9′), 31.8 (C-10′), 19.1 (C-11′), 13.9 (C-12′).

Betulinic acid (**7**): white powder; mp. 219-239°C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.91 (1H, *m*, H-1α), 1.69 (1H, *brs*, H-1β), 1.69 (2H, *m*, H-2), 3.00 (1H, *m*, H-3), 0.75 (1H, *m*, H-5), 1.69 (1H, *m*, H-6α), 1.41 (1H, *m*, H-6β), 1.41 (1H, *m*, H-7α), 1.38 (1H, *m*, H-7β), 1.38 (1H, *m*, H-9), 1.38 (1H, *m*, H-11α), 1.25 (1H, *m*, H-11β), 1.25 (1H, *m*, H-12α), 2.23 (1H, *m*, H-12β), 2.23 (1H, *m*, H-13), 1.25 (1H, *m*, H-15α), 2.23 (1H, *m*, H-15β), 1.69 (1H, *m*, H-16α), 1.69 (1H, *brs*, H-18), 3.18 (1H, *m*, H-19), 1.53 (1H, *m*, H-21α), 1.53 (1H, *m*, H-22α), 1.98 (1H, *m*, H-22β), 1.21 (1H, *m*, H-23), 0.94 (3H, *s*, H-24), 0.83 (3H, *s*, H-25), 0.97(3H, *s*, H-26), 0.98 (3H, *s*, H-27), 4.74 (1H, *s*, H-29α), 4.61 (1H, *s*, H-29β), 1.69 (3H, *brs*, H-30); ^{13}C-NMR ($CDCl_3$, 100 MHz) 38.8 (C-1), 27.4 (C-2), 79.0 (C-3), 39.0 (C-4), 55.4 (C-5), 18.3 (C-6), 34.4 (C-7), 40.7 (C-8), 50.6 (C-9), 37.0 (C-10), 20.9 (C-11), 25.5 (C-12), 38.4 (C-13), 42.5 (C-14), 29.7 (C-15), 32.2 (C-16), 56.3 (C-17), 49.3 (C-18), 46.9 (C-19), 150.4 (C-20), 30.6 (C-21), 37.2 (C-22), 28.0 (C-23), 16.1 (C-24), 15.3 (C-25), 16.0 (C-26), 14.7 (C-27), 179.9 (C-28), 109.7 (C-29), 19.4 (C-30).

Antimicrobial Activity Testing

Bacterial inhibitory activity was tested against Gram positive bacteria (*S. aureus* ATCC25923 and *B. subtilis* ATCC6633) and Gram negative bacteria (*P. aeruginosa* ATCC27853 and *E. coli* ATCC25922) by broth dilution susceptibility testing according to Clinical and Laboratory Standards Institute (2011)[13]. *S. aureus*, *P. aeruginosa* and *E. coli* were maintained in ATCC® medium 18 (trypticase soy medium) at 37°C, *B. subtilis* was in ATCC® medium 44 (brain heart infusion medium) at 30°C and *C. albicans* was in ATCC® medium 200 (YM medium) at 24-26°C in ATCC® medium 200 (YM medium). A single colony of bacteria was isolated by cross streaking on medium and incubated at 37°C for 24 hr. It was further cultured in Müller-Hinton broth and the turbidity was adiusted with Mcfarland standard No. 0.5 (1×10^8 CFU/mL). Culture suspension 100 µL was mixed with the serial dilution of extract suspension in Müller-Hinton broth at the concentration ranging from 50-300 µg/mL in sterile microplate. They were then incubated at 37°C for 16-18 hr. Minimum inhibitory concentration (MIC) was defined as the lowest concentrations of samples that resulted in no bacterial growth. In this study, clavulanic acid was used as a standard and all experiments were done in triplicate. The isolated compounds (5-50 µg/mL) were tested using the same method as mentioned and amphotericin B was used as a standard for antifungul activity.

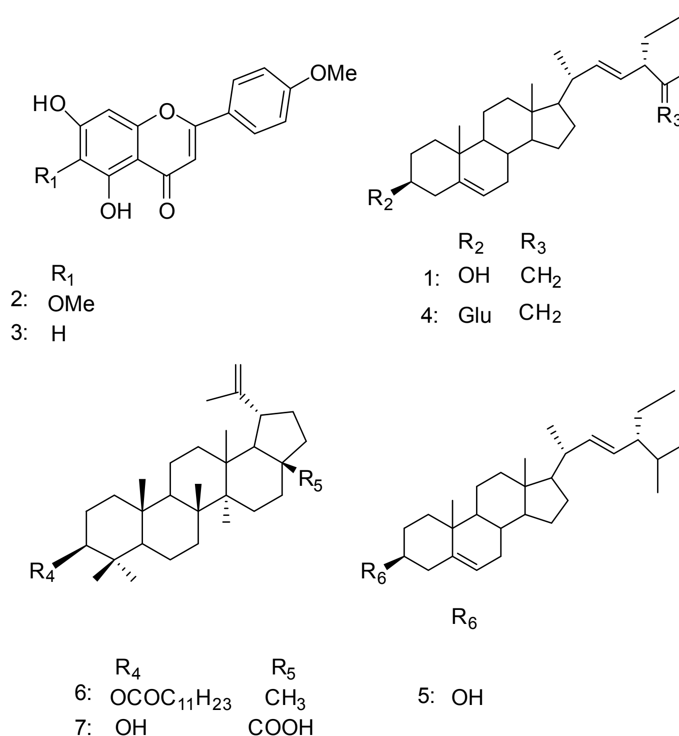

Figure 1 Chemical structures of the constituents isolated from *C. inerme*

Results and Discussions

The CH_2Cl_2 and MeOH extracts of *C. inerme* were evaluated the antibacterial activity on Gram positive bacteria (*S. aureus* and *B. subtilis*) and Gram negative bacteria (*P. aeruginosa* and *E. coli*) at a range of concentration of 50-300 µg/mL (Table 1). All extracts have the potential to inhibit bacteria with the concentration of 50 and 100 µg/mL. This result was found to agree with a previous report in which the methanolic extract of *C. inerme* inhibited these four bacteria[14]. However, in this study the CH_2Cl_2 extract from leaves and roots were subjected to fractionation to obtain bioactive compounds. Phytochemicals separation of *C. inerme* led to the isolation of seven compounds. The chemical structures of all isolated compounds were identified based on the NMR data and compared to those in the previous reports. The names of these isolated compounds were (3β, 22*E*, 24*S*)-stigmasterol-5,22,25-triene-3-ol (**1**)[15], pectolinarigenin (**2**)[16], acacetin (**3**)[17], and (3β, 22*E*, 24*S*)-stigmasterol-5,22,25-trien-3-yl-D-glucopyranoside (**4**)[18], stigmasterol (**5**)[19], lupeol laurate (**6**)[20] and betulinic acid (**7**)[21]. Their chemical structures were shown in Figure. 1. Compounds **2**, **4** and **6** were reported for the first time from this species.

Table 1 Minimum inhibitory concentration (MIC) of extracts from *C. inerme* against *S. aureus*, *B. subtilis, P. aeruginosa* and *E. coli*

Part Of Plant	Solvent	MIC (µg/mL)			
		Gram Positive Bacteria		Gram negative Bacteria	
		S. aureus ATCC25923	***B. subtilis*** ATCC6633	***P. aeruginosa*** ATCC27853	***E. coli*** ATCC25922
Leaves	CH_2Cl_2	50	50	100	50
	MeOH	50	100	50	100
Roots	CH_2Cl_2	100	100	100	100
	MeOH	100	100	50	100

These isolated compounds were tested for anti-microbial activity by microdilution broth susceptibility method at a range of concentration of 5-50 µg/mL (Table 2).

Table 2 Minimum inhibitory concentration (MIC) of phytochemical compounds from *C. inerme* against *S. aureus, P. aeruginosa* and *C. albicans*

Compound	Name	MIC (µg/mL) ± SE			
		Part of Plant	*S.* **aureus** ATCC25923	**P. aeruginosa** ATCC27853	**C. albicans** ATCC10231
1	(3β, 22*E*, 24*S*)-stigmasta-5,22,25-trien-3-ol	Leaves	20.0±0.00	45.0±0.00	45.0±0.00
2	Pectolinarigenin	Leaves	45.0±0.00	> 50.0±0.00	40.0±0.00
3	Acacetin	Leaves	41.7±0.96	> 50.0±0.00	36.7±0.96
4	(3β, 22*E*, 24*S*)-stigmasta-5,22,25-triene-3-yl-β-D-glucopyranoside	Leaves	> 50.0±0.00	> 50.0±0.00	> 50.0±3.84
5	Stigmasterol	Roots	33.3±0.96	46.7±0.96	> 50.0±0.00
6	Lupeol laurate	Roots	> 50.0±0.00	45.0±0.00	> 50.0±0.00
7	Betulinic acid	Roots	45.0±0.00	45.0±0.00	> 50.0±0.00
8	Clavulanic acid	-	1.35±0.0.96	2.45±0.00	-
9	Amphotericin B	-	-	-	2.51±0.00

Compound **1** showed the highest inhibition on *S. aureus* growth with the MIC value of 20 µg/mL while compounds **1**, **6** and **7** showed the highest inhibition on *P. aeruginosa* with the same MIC value (45 µg/mL). The best *C. albicans* inhibitor was compound **3**. This study is the first report of compounds **1** and **6** activities against *S. aureus* and *P. aeruginosa*. The glucoside moiety on compound **1** was found to significantly reduce the anti-bacterial and also anti-fungal activities (compound **1** *vs.* compound **4**). This phenomenon was previously found in the saponin skeleton reported by Avato *et al*.[22] Flavanoids **2** and **3** exerted the inhibition on *S. aureus* and *C. albicans* which might be related to the hydroxyl groups at C-5 and C-7 of ring A[23].

Conclusion

The *C. inerme* extracts were shown to possess anti-bacterial activity with the MIC values of 50-100 µg/mL. The isolation led to obtain 4 compounds from leaf extract and 3 compounds from root extract. Compound **1** showed inhibition on *S. aureus*, *P. aeruginosa* and *C. albicans* with the concentration lower than 50 µg/mL while compound **4** had no inhibitory potency at this concentration against three microbial isolates. Although the structure activity relationship of the isolated compounds could not be determined, this study confirmed their antimicrobial activity.

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