Evaluation of in-vitro antimicrobial activity of Artemisia apiacea H. and Scutellaria baicalensis G. extracts

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Abstract

Purpose. In traditional Korean medicine, Artemisia apiacea H. (ART) and Scutellaria baicalensis G. (SCU) are combined for the treatment of malaria and other malaria-like diseases. Because SCU is well-known as an antibacterial agent, the antimicrobial effect of a mixture of ART and SCU was investigated.

Methodology. Plant samples were purchased from Kyungdong mart and extracted with 70 % ethanol. The in vitro antimicrobial activity of ART and SCU against pathogenic fungi (Aspergillus niger, Aspergillus oryzae, Candida albicans, Candida tropicalis and Candida glabrata), Gram-negative bacteria (Escherichia coli and Pseudomonas aeruginosa) and Gram-positive bacteria (Bacillus subtilis and Staphylococcus aureus) was evaluated using a broth microdilution assay, modified-disc diffusion and agar dilution methods with further CH2Cl2-fractionated ART, SCU and a mixture of ART/SCU (at a ratio of 3 : 5) (THAN-1).

Results. ART and SCU were effective against A. niger, C. albicans, B. subtilis and S. aureus. The range of minimum inhibitory concentration (MIC) values was 0.03125 to 4 mg ml−1 in the ART and SCU treatments. ART exhibited stronger activity than SCU. Interestingly, a 3 : 5 ratio mixture of ART and SCU (THAN-1) showed stronger antimicrobial activity than ART or SCU used individually.

Conclusion. A treatment using a mixture of herbs such as THAN-1 would be useful in the suppression of the growth of pathogenic bacterial and fungal strains.

INTRODUCTION

Antimicrobial herbal extracts have recently been receiving more attention because of increasing concern regarding infections by harmful micro-organisms, the development of antibiotic resistance and the potential side-effects of synthetics [1, 2].

Herbal plants, along with their active compounds, have enormous significance in modern scientific research [3]. There are many benefits of using herbal extracts. Because they have optimal absorption properties, extracts affect the body quicker than other methods of taking herbs. In addition, herbal extracts allow for higher concentrations, longer preservation and reduced cytotoxicity. Many different herbs can be combined to make a customized formula for specific beneficial effects and they can be easily used in water, juice or tea [4].

Aspergillus oryzae, Candida albicans, Candida tropicalis and Candida glabrata), Gram-negative bacteria (Escherichia coli and Pseudomonas aeruginosa) and Gram-positive bacteria (Bacillus subtilis and Staphylococcus aureus) was evaluated using a broth microdilution assay, modified-disc diffusion and agar dilution methods with further CH2Cl2-fractionated ART, SCU and a mixture of ART/SCU (at a ratio of 3 : 5) (THAN-1).

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Artemisia apiacea H. (ART), an annual grass, is a type of weed and is 1 of over 350 species in the genus Artemisia [5]. This herb is distributed widely in eastern Asia, including Korea, Japan, China and Vietnam [6], and has been used in oriental medicine to treat fever, malaria, jaundice, eczema and dyspeptic symptoms [7]. ART has specifically been reported to reduce inflammation [7] and increase hair growth [8]. Recent studies with other Artemisia species showed various biological properties, including antimalarial, antiviral, antitumour, antipyretic, antihaemorrhagic, antioxidant, antihepatitis and anticomplementary activities [9, 10].

Scutellaria baicalensis G. (SCU) is a perennial herbaceous plant that is widely distributed in oriental areas [11] and belongs to the family Lamiaceae, which is represented by 45 genera and 574 species, with 256 endemic species [12]. Three- or 4-year-old roots of SCU have been used as effective medical herbs [13]. SCU is already known to have antibacterial, antifungal, anti-inflammatory and antiviral activities [14]. In addition, SCU has demonstrated anti-inflammatory and anti-arthritic effects [15]. SCU also has potential for use in the treatment of diabetes and cancer [16].

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antibacterial effects [14, 15] and is used to prevent atherosclerosis, prostate cancer and breast cancer, and to treat hypertension, anxiety and headache caused by flu [16].

In this study, ART, SCU and a mixture of ART/SCU were used to investigate antimicrobial activity against pathogenic fungi (Aspergillus niger, Aspergillus oryzae, Candida albicans, Candida tropicalis and Candida glabrata), Gram-negative bacteria (Escherichia coli and Pseudomonas aeruginosa) and Gram-positive bacteria (Bacillus subtilis and Staphylococcus aureus).

METHODS

Plant sample preparation

The dried parts of six Korean medicinal plants, including ART (all above-ground parts), Chrysanthemum indicum L, Caesalpinia sappan L, Forsythia suspensa V, Morus alba L and SCU (root) were purchased from Kyungdong mart in Seoul, Republic of Korea. The samples were extracted three times in 70% ethanol, filtered and concentrated into powdered form by vacuum evaporation at 40°C in order to initially screen for antimicrobial activity. ART (all above-ground parts) and SCU (root) were mixed in ratios of 1:1, 1:2, 2:1, 3:5 and 5:3. A broth microdilution assay was carried out with the highest concentration of 20 mg ml⁻¹, while modified-disc diffusion and agar dilution assays were performed with 1 mg ml⁻¹ of extracts.

Chemicals and micro-organism strains

The chemicals were purchased from Sigma Chemicals, USA. All media were purchased from Oxoid Ltd., United Kingdom. B. subtilis (KACC 14394), S. aureus (KCTC 3881), E. coli (CCARM 0237), P. aeruginosa (KACC 14021), C. albicans (KACC 30071, KCTC 7965 and KCTC 7270), C. tropicalis (KCTC 7212), C. glabrata (KCTC 7219), A. niger (KACC 44333, KACC 41018, KACC 40280, KACC 41858 and KACC 43547) and A. oryzae (KACC 40242, KACC 44969, KACC 44823 and KACC 40232) were purchased from the Korean Agricultural Culture Collection (KACC), the Korean Collection for Type Cultures (KCTC) and the Culture Collection of Antimicrobial Resistant Microbes (CCARM) and used for antimicrobial activity assays.

Micro-organisms were cultured in Luria–Bertani media (LA; for E. coli), nutrient media (NA; for other bacteria) and yeast media (YM; for Candida strains) plates, and then transferred into broth medium maintained at 32°C overnight. The Aspergillus strains were cultured on potato dextrose media (PDA) for 5 days before the assay.

Broth microdilution assay

The minimum inhibitory concentration (MIC) values were determined for antimicrobial activity. The MIC values of the herbal fractions were tested using the method described by Andrews [17]. The test extract was dissolved in 10% dimethyl sulfoxide (DMSO) and used to obtain the required concentration by sequential two-fold dilution with 0.1 ml media. We transferred 0.1 ml suspensions of the micro-organisms to each tube and incubated these at 32°C for 24-48-72h. The concentrations of the micro-organisms were calculated at an OD₆₀₀ of 1.0 and are listed as follows: B. subtilis [OD₆₀₀ of 1.0=0.4×10⁶ colony-forming units (c.f.u.) ml⁻¹], S. aureus (1.5×10⁶ c.f.u. ml⁻¹), E. coli (8×10⁸ c.f.u. ml⁻¹), P. aeruginosa (10⁶ c.f.u. ml⁻¹), C. albicans (18.4×10⁶ c.f.u. ml⁻¹), C. tropicalis (20×10⁶ c.f.u. ml⁻¹), C. glabrata (19.3×10⁶ c.f.u. ml⁻¹), A. niger (60.4×10⁶ c.f.u. ml⁻¹) and A. oryzae (61.2×10⁶ c.f.u. ml⁻¹). Tubes containing only the micro-organism were used as negative controls. The MIC was determined to be the concentration of the test samples at which no visual growth of the test organisms was observed. The positive controls were amphotericin B and miconazole for fungi, and tetracycline for bacteria. Each experiment was repeated at least five times.

Modified-disc diffusion assay

The standard disc diffusion method was performed as described by Serrano et al. [18]. Sterilized petri dishes were prepared using Muller–Hinton agar supplemented with ART, SCU, THAN-1 and positive controls. Negative controls were prepared using a disk supplemented with 10% DMSO. Standardized inoculum (10 μl) containing spores adjusted to 0.5 McFarland units was loaded onto Whatman no. 1 sterile filter paper discs (8 mm diameter) and allowed to dry for 5 min. The plates were then incubated at 32°C for 24–48 h. Anti-Aspergillus sp. activity was evaluated by measuring the radius of the inhibition zone (RIZ) against the tested fungi [12]. The RIZ values indicated weak activity (0 and <0.5 cm), moderate activity (>0.5 and <1.0 cm) and high activity (≥1.0 cm).

Agar dilution assay

Starter cultures were diluted with media to reach an OD₆₀₀ of 0.8, as determined by an UV/vis spectrophotometer (Mecasys Co., Ltd.), using the method described by Hanson and Martin [19] with small modifications. Standardized inoculum (5 μl) containing bacterial suspensions was loaded onto agar medium and incubated for 24 h at 32°C.

LC/MS analysis

The active components of the effective samples (ART and SCU) were determined by an LC/MS system. The CH₂Cl₂ fraction at 10 mg ml⁻¹ was analysed. Standard compounds of scoparin, isofraxidin, baicalin, wogonin and oroxylin A were prepared by diluting the stock solution with 50% methanol to concentrations ranging from 3.125 μg ml⁻¹ to 100 μg ml⁻¹. Chromatographic analysis of the fractions was performed using an Acquity UPLC system (Waters, Milford, MA, USA) and separation was achieved using an Acclaim 120 C18 (150×2.1 mm, 3 μm particle size). Chromatographic separation was performed using a gradient elution with 0.1% acetic acid (v/v) in water for eluent A and 0.1% acetic acid (v/v) in acetonitrile for eluent B. The concentration of B was 30% at 0–3 min, 50% at 3–10 min and 90% at 10–30 min. The flow
rate was 0.2 ml min\(^{-1}\) and the injection volume was 2 µl. A photodiode array detector was used at 280 nm. Mass spectrometry analysis was carried out using a Waters Acquity SQ quadrupole mass spectrometer (Waters, Manchester, UK). The electrospray ionization source was operated with the sample in the positive mode. For MS detection, the ionization source parameters were as follows: the capillary voltage was set at 3.5 kV; the source temperature was 150 \(^{\circ}\)C and the desolvation temperature was 400 \(^{\circ}\)C; the cone gas (nitrogen) and desolvation gas were set at flow rates of 50 and 550 l h\(^{-1}\), respectively [20]. Data acquisition was operated using Masslynx 4.1 software with Quanlynx programs (Waters, Milford, MA, USA).

**Statistical analysis**

The data are expressed as mean±SD. Statistical significance was calculated between the control and plant extract-treated groups by using Student’s \(t\)-test. \(P<0.05\) was regarded to be statistically significant.

**RESULTS**

**Antifungal activity using MIC values**

The MIC values for the fungal strains tested were between 0.0625 and 4 mg ml\(^{-1}\). The presence of fungi in the negative control was confirmed by using 10 % DMSO. In the positive control, miconazole and amphotericin B showed high antifungal activity, with MIC values of 12.5 and 50 µg ml\(^{-1}\), respectively. There were some differences in antifungal activity among *Aspergillus* and *Candida* species (Table 1). In the ART treatment, the MIC values for all strains of *A. niger* were the same at 0.0625 mg ml\(^{-1}\). However, the MIC values for *A. niger* were between 0.25–1 mg ml\(^{-1}\) SCU and 0.125–1 mg ml\(^{-1}\) in the THAN-1 treatment. *A. oryzae* growth was completely inhibited by 4 mg ml\(^{-1}\) of SCU. Similarly, 4 mg ml\(^{-1}\) of SCU treatment was effective against *C. albicans* KACC 30071 and *C. glabrata* KCTC 7219. Although THAN-1 did not show higher potential antifungal activity than ART, it was more effective than the SCU treatment.

**Anti-*Aspergillus* activity using a modified-disc diffusion assay**

The *in vitro* antifungal activity (anti-*Aspergillus* sp.) was evaluated by comparing the RIZ values between the control and compound-containing media conditions. The concentrations of the compounds, amphotericin B and miconazole were 1, 50 and 12.5 µg ml\(^{-1}\), respectively. The anti-*Aspergillus* sp. activity, as shown by the RIZ of ART, SCU and THAN-1, was clear, with a range of 0.6–1.6 cm (Fig. 1). The two positive controls, amphotericin B and miconazole, showed high anti-*A. niger* activity, with an RIZ between 1.4–1.5 cm. Interestingly, THAN-1 also exhibited anti-*A. niger* activity. Although ART did not possess anti-*A. niger* activity and SCU showed activity with 0.6 cm of RIZ, THAN-1 achieved up to 1.6 cm of RIZ, showing higher antifungal activity than the two positive controls.

Table 1. Antimicrobial activity of ART, SCU and THAN-1

<table>
<thead>
<tr>
<th>Herbal extract</th>
<th>ART</th>
<th>SCU</th>
<th>THAN-1</th>
<th>Miconazole</th>
<th>Amphotericin B</th>
<th>Tetracycline</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. niger</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>44333*</td>
<td>0.0625</td>
<td>1</td>
<td>0.25</td>
<td>0.00625</td>
<td>0.05</td>
<td>ND</td>
</tr>
<tr>
<td>40108*</td>
<td>0.0625</td>
<td>0.5</td>
<td>0.125</td>
<td>0.00625</td>
<td>&gt;0.05</td>
<td>ND</td>
</tr>
<tr>
<td>40280*</td>
<td>0.0625</td>
<td>1</td>
<td>0.125</td>
<td>0.003125</td>
<td>0.05</td>
<td>ND</td>
</tr>
<tr>
<td>41858*</td>
<td>0.0625</td>
<td>1</td>
<td>1</td>
<td>0.0125</td>
<td>&gt;0.05</td>
<td>ND</td>
</tr>
<tr>
<td>43547*</td>
<td>0.0625</td>
<td>0.25</td>
<td>0.125</td>
<td>0.003125</td>
<td>0.05</td>
<td>ND</td>
</tr>
<tr>
<td><em>A. oryzae</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40242*</td>
<td>0.125</td>
<td>4</td>
<td>0.25</td>
<td>0.00078</td>
<td>&gt;0.05</td>
<td>ND</td>
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<tr>
<td>44969*</td>
<td>0.125</td>
<td>4</td>
<td>0.25</td>
<td>0.00625</td>
<td>&gt;0.05</td>
<td>ND</td>
</tr>
<tr>
<td>44823*</td>
<td>0.125</td>
<td>4</td>
<td>0.125</td>
<td>0.00625</td>
<td>&gt;0.05</td>
<td>ND</td>
</tr>
<tr>
<td>40232*</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>0.0125</td>
<td>&gt;0.05</td>
<td>ND</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30071*</td>
<td>0.5</td>
<td>4</td>
<td>0.5</td>
<td>0.0125</td>
<td>&gt;0.05</td>
<td>ND</td>
</tr>
<tr>
<td>7965†</td>
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<td>1</td>
<td>1</td>
<td>0.0125</td>
<td>&gt;0.05</td>
<td>ND</td>
</tr>
<tr>
<td>7270†</td>
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<td>1</td>
<td>0.5</td>
<td>0.00625</td>
<td>&gt;0.05</td>
<td>ND</td>
</tr>
<tr>
<td><em>C. tropicalis</em> 7212†</td>
<td>4</td>
<td>1</td>
<td>0.5</td>
<td>0.0125</td>
<td>&gt;0.05</td>
<td>ND</td>
</tr>
<tr>
<td><em>C. glabrata</em> 7219†</td>
<td>0.25</td>
<td>4</td>
<td>0.25</td>
<td>0.00625</td>
<td>&gt;0.05</td>
<td>ND</td>
</tr>
<tr>
<td><em>B. subtilis</em> 14394*</td>
<td>0.25</td>
<td>1</td>
<td>0.125</td>
<td>ND</td>
<td>ND</td>
<td>0.05</td>
</tr>
<tr>
<td><em>S. aureus</em> 3881†</td>
<td>0.25</td>
<td>0.03125</td>
<td>0.0625</td>
<td>ND</td>
<td>ND</td>
<td>0.025</td>
</tr>
</tbody>
</table>

The concentration unit for the extracts and compounds was mg ml\(^{-1}\). ND, not determined.

*KACC.*

†KCTC.
Anti-Candida activity using the agar dilution assay

The MICs of the positive controls (amphotericin B and miconazole), ART and SCU were about 50, 12.5, 1 and 1 mg ml\(^{-1}\), respectively. Here, 1 mg ml\(^{-1}\) of ART treatment showed moderate activity against the *C. albicans* strain, while 1 mg ml\(^{-1}\) of SCU treatment showed higher activity against the *C. albicans* strain than the ART treatment. THAN-1 showed greater antimicrobial activity compared to treatment with ART and SCU separately. The synergistic activity of THAN-1 was better than that of the positive controls (Fig. 2a).

Antibacterial activity using MIC values

The MIC values for the bacterial strains tested were in the range of 0.0625 and 1 mg ml\(^{-1}\). The presence of bacteria in the negative control was confirmed by using 10% DMSO. The positive control, tetracycline, showed antibacterial activity at 50 µg ml\(^{-1}\) (Table 1). THAN-1 showed greater antibacterial activity against *B. subtilis*, with an MIC value of 0.125 mg ml\(^{-1}\), and *S. aureus*, with an MIC value of 0.0625 mg ml\(^{-1}\) (Table 1). ART and SCU also exhibited moderate antimicrobial activity, with MIC values ranged between 0.03125 and 1 mg ml\(^{-1}\) against *B. subtilis* and *S. aureus*. All three samples (ART, SCU and THAN-1) were not effective against the two Gram-negative bacteria (*E. coli* and *P. aeruginosa*).

Antibacterial activity using agar dilution assay

The MIC of the positive control, tetracycline, was determined to be 50 µg ml\(^{-1}\). We found that 1 mg ml\(^{-1}\) of ART treatment showed moderate activity against *E. coli*, high activity against *P. aeruginosa* and very high activity against the two Gram-positive bacteria, *B. subtilis* and *S. aureus* (Fig. 2b), while 1 mg ml\(^{-1}\) of SCU treatment showed high activity against *E. coli* and very high activity against *B. subtilis*, *P. aeruginosa* and *S. aureus*. Interestingly, THAN-1 showed the highest antimicrobial activity compared to treatment with ART and SCU separately. THAN-1 treatment demonstrated synergistic activity against *S. aureus* and *P. aeruginosa*. However, THAN-1 did not show a synergistic effect against *E. coli* and *B. subtilis*. The anti-*B. subtilis* activity of THAN-1 was the same as the activity of SCU alone. The antibacterial activity of THAN-1 against *E. coli* was lower than that of the SCU treatment.

Analysis of ART and SCU

For ART, the ESI mass spectra showed the [M+H]\(^+\) ion throughout *m/z* 207.2 [M+H]\(^+\) and 223.2 [M+H]\(^+\) as peak 1 (retention time, RT: 10.6) (dimethylesculetin) and peak 2 (RT: 11.2) (isofraxidin), respectively. For SCU, the ESI mass spectra showed the [M+H]\(^+\) ion throughout *m/z* 271.2 [M+H]\(^+\), 270.2 [M+H]\(^+\) and 270.2 [M+H]\(^+\) as peak 3 (RT: 19.7) (baicalin), peak 4 (RT: 22.4) (wogonin) and peak 5 (RT: 23.4) (oroxinyl A), respectively (Fig. 3). Thus, the active components of the CH\(_2\)Cl\(_2\) fraction were identified as three purified flavone derivatives (baicalin, wogonin and oroxynyl A) and two phytoalexins (dimethylesculetin and isofraxidin). Baicalin was identified as a major active component in the crude ethanol extract of SCU, as well as in THAN-1. The proportion of baicalin, wogonin, oroxynyl A, dimethylesculetin (scoparin, 6,7-dimethoxycoumarin) and isofraxidin in THAN-1 was 62.0, 26.0, 5.0, 0.34 and 0.04 %, respectively.

DISCUSSION

Based on the MIC results, ART showed moderate antimicrobial activity against *Aspergillus* sp. (except for *A. oryzae* KACC 40232), *C. albicans* sp., *B. subtilis* and *S. aureus*, weak activity against *C. tropicalis* and *C. glabrata*, and no activity against *E. coli* and *P. aeruginosa*. The activity of SCU was weak against *A. niger*, *A. oryzae*, *C. albicans*, *C. tropicalis*, *C. glabrata*, *B. subtilis* and *S. aureus*, and it showed no activity against *E. coli* and *P. aeruginosa*. MIC breakpoints were not detected by ART and SCU treatment against *E. coli* and *P. aeruginosa*. However, the agar dilution data showed the antibacterial effect of ART and SCU against these two pathogens. The MIC values indicate the lowest concentration of an antimicrobial agent that inhibits cell growth. In the MIC method, slow microbial growth could be
Fig. 2. (a) Comparison of the inhibition of ART and SCU to the *Candida albicans* growth. (b) Comparison of the inhibition of ART and SCU to bacteria growth.
distinguished from normal growth because invisible growth points were taken. So, errors could possibly occur in each test, in addition to inconsistency in comparison with larger scales. On the other hand, the agar dilution method was practical qualitatively but lacked an automatic test. In addition, drug stability could be different between liquid and solid states. Thus, the MIC, modified-disc diffusion and agar dilution methods were performed simultaneously to guarantee the accuracy of the experimental results. Based on the modified-disc diffusion and agar dilution results, the antimicrobial activity of ART against *A. niger* was weak and moderate against *C. albicans* and *E. coli*, but higher than that of the negative control against *P. aeruginosa*, *B. subtilis* and *S. aureus*. The antimicrobial activity of SCU against *A. niger* was moderate, and higher than that of the negative control against *C. albicans*, *E. coli*, *P. aeruginosa*, *B. subtilis* and *S. aureus*. 

Antibacterial activity of the *S. baicalensis* G. extract has been reported against *Streptococcus mutans* [21] and shown against *S. aureus*, *B. cereus*, *E. coli* and others [22]. The antimicrobial activity of *S. baicalensis* G. was 0.312 to 4 mg ml⁻¹ against all tested pathogens in the present study. In contrast to *S. baicalensis* G., *A. apiacea* H. has not been examined for antimicrobial effects. However, it has been used to treat fever, malaria, jaundice, eczema and dyspeptic symptoms in oriental medicine. ART exhibited higher antifungal activity than SCU.

Many medicinal plant mixtures with synergistic antimicrobial activity have enormous significance in modern scientific research [3]. Medicinal plant mixtures of ART and SCU could likewise be beneficial for various reasons: (i) to achieve broad-spectrum antimicrobial activity; (ii) to minimize drug toxicity using the lowest possible doses of two or more agents that individually have side-effects; and (iii) to reduce the mode of resistance action. The mixture of *A. apiacea* H. and *S. baicalensis* G. extracts demonstrated synergistic antimicrobial activity, as shown in our data. THAN-1, a 3 : 5 mixture of ART and SCU, presented with significantly higher activity than each individual component.

For active compound analysis, LC/MS identified scoparin, isofraxidin, baicalin, wogonin and oroxylin A as five compounds of ART and SCU. Johann et al. [23] showed that the antimicrobial activity of *Citrus* sp. extracts is mainly due to 6,7-dimethoxycoumarin (scoparin). In *Artemisia capillaris* and *Artemisia iwayomogi*, isofraxidin was identified as a minor active component against *B. cereus*, *B. subtilis*, *S. aureus*, *E. coli*, *Pseudomonas fluorescens* and *Saccharomyces cerevisiae* [24]. Baicalin, which is a principal antibacterial ingredient in *S. baicalensis* G., is known to inhibit *S. aureus* growth by reducing bacterial membrane penetrability and protein synthesis [11, 25]. Cai et al. demonstrated the synergistic effect of baicalin and cefotaxime against *K. pneumoniae* strains [26]. Wogonin has shown efficacy against *Flavobacterium columnare* at an MIC of 0.3 mg ml⁻¹ [27]. Oroxylin A derivatives have been synthesized and evaluated for antibacterial activity [28]. Therefore, the presence and combination of these active components may contribute to the higher antimicrobial activity in THAN-1.

To date, there are only a few patented and approved medicinal plant antimicrobials in use. For example, Lepilleur [29] patented *Cassia tora* and *Cassia obtusifolia* for use in personal care, health care, household, institutional and medical applications.
industrial products. Green tea or olive leaf extracts have long been used in many antimicrobial products. In Italy, Eurol BT, extracted from the olive leaves, is used for skin and sun care because of its photoprotective properties. In New Zealand and Australia, LEMA oil is a famous antimicrobial and anti-inflammatory blend of manuka and tea tree oils that is used in hand sanitizers, antiseptics, acne treatments and antibacterial personal care products. Therefore, ART, SCU and THAN-1 could have potential as a novel medicinal plant extract for use in cosmetic products such as shampoo, conditioner, toothpaste, body wash, soap and infant care.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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