Antimicrobial and Antimalarial Activities of Secondary Metabolites from Some Turkish Verbascum Species

I. İrem TATLI*, Zeliha Ş. AKDEMİR*

Summary
Antimicrobial and antimalarial activities of 22 secondary metabolites isolated from the methanolic extracts of Verbascum cilicicum Boiss., Verbascum lasianthum Boiss. ex Bentham and Verbascum pterocalycinum var. mutense Hub.-Mor. (Scrophulariaceae) were investigated. Growth inhibition, using microdilution method, was determined against Candida albicans ATCC 90028, Cryptococcus neoformans ATCC 90113, Staphylococcus aureus ATCC 29213, methicillin-resistant S. aureus ATCC 43300, Pseudomonas aeruginosa ATCC 27853, Aspergillus fumigatus ATCC 90906 and Mycobacterium intracellulare ATCC 23068. Amphotericin B, ciprofloxacin and rifampin were used as controls. It was found that ilwensisaponin A and C showed antimicrobial activity against Aspergillus fumigatus ATCC 90906, but no activity was seen against Gram (+) and Gram (-) bacteria and the yeasts used in this study. None of the other compounds had antimicrobial activities. The antimicrobial activities of the compounds were also tested to Plasmodium falciparum clone [Sierra Leone D6 and W2 (chloroquine-sensitive)]. The antimalarial agents chloroquine and artemisinin were used as controls. None of the compounds showed antimalarial activities.

Key Words: Ilwensisaponin A, ilwensisaponin C, Verbascum cilicicum, Verbascum lasianthum, Verbascum pterocalycinum var. mutense, Scrophulariaceae, antimicrobial activity, antimalarial activity.

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INTRODUCTION
The incidence of microbial infections has increased dramatically in the past 20 years because of the increase in the number of people whose immune systems are compromised by acquired immunodeficiency syndrome (AIDS), aging, organ transplantation, and cancer therapy. Accordingly, increases in the rates of morbidity and mortality because of microbial infections have been regarded as a major problem. Most

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of the current antimicrobial drugs simply reduce the level of growth of bacteria or fungi, and some of them are very toxic to the kidney and the hematopoietic and central nervous systems. The development of resistant bacteria and fungal strains in response to the widespread use of current antimicrobial drugs will cause serious problems in the future. The recent emergence of microbial infections and resistant strains has stimulated the development of novel antimicrobial drugs\(^1\).

There is also wide variation in the availability and efficacy of drugs for the therapy and prophylaxis of parasitic diseases, in both humans and domestic animals. Malaria remains one of the most important diseases in humans in terms of both mortality and morbidity, with \textit{Plasmodium falciparum} being the most important infecting agent. Despite considerable therapeutic success with the antimalarial quinolines, there are serious doubts about the future of this class of drugs as well as other established antimalarial drugs\(^2\).

\textit{Verbascum} species have been used as diuretic, sudorific, mucolytic, expectorant, sedative and constipate in traditional Turkish medicine\(^3\). Additionally, some of these species are known as piscicide, antiseptic, astringent, demulcent, emollient, antimalarial, and as a treatment for tumors, inflammations, migraine, asthma and spasmodic coughs in Europe, Asia and North America\(^4\).

Antimicrobial effects of \textit{V. bombyciferum}, \textit{V. olympicum}, \textit{V. prusianum}, \textit{V. macrurum} and \textit{V. qulebrium} have been previously investigated\(^5-8\). Furthermore, aqueous extracts of \textit{V. fruticulosum} demonstrated strong growth inhibition on the malaria parasite\(^9\).

In our previous studies, ethylacetate and methanol extracts of five \textit{Verbascum} species, \textit{V. chionophyllum}, \textit{V. ciliicum}, \textit{V. pterocalycinum} var. mutense, \textit{V. pycnostachyum} and \textit{V. splendidum} were tested \textit{in vitro} against Gram (+), Gram (-) bacteria and the yeasts using microdilution method. \textit{V. ciliicum} and \textit{V. chionophyllum} had shown strong activity against \textit{Staphylococcus aureus} ATCC 29213, methicillin-resistant \textit{S. aureus} ATCC 43300, and \textit{Pseudomonas aeruginosa} ATCC 27853, while \textit{V. pycnostachyum} was found to be moderately active\(^10\).

Fractionation and isolation on \textit{Verbascum} species have revealed the presence of one monoterpen and 17 iridoid glycosides, two oleanane type triterpene saponins and two phenylethanoid glycosides from \textit{Verbascum ciliicum} Boiss., \textit{Verbascum lasianthum} Boiss. ex Bentham\(^11\), \textit{Verbascum lasianthum} Boiss. ex Bentham\(^12\), \textit{Verbascum pterocalycinum} var. \textit{mutense} Hub.-Mor. in our previous studies\(^14\).

Iridoids are glycosides that contain cyclopentane and pyrane rings. Iridoid glycosides possess a number of biological activities including choleretic, purgative, liver protective, vasoconstrictor, sedative, antimicrobial, antidiabetic, analgesic, antitumor, anticancer, immunostimulant and anti-inflammatory activities\(^15-17\).

The functions of triterpene saponins in plants, such as antimicrobial, fungicidal, antibacterial, antiviral, analgesic, anti-inflammatory, antitumor, cytotoxic, immunostimulant, piscicidal, molluscicidal, antihelminthic, expectorant and antitussive activities, have been known for many years\(^18\).

Phenylethanoids are glycosides of phenylethanol esterified by a cinnamic acid (e.g. caffeic, ferulic and \(\beta\)-coumaric acid) molecule. Phenylethanoid glycosides are known to possess antibacterial, antifungal and antiviral activities. The immunosuppressive, antioxidant, analgesic and anti-hepatotoxic activities of phenylethanoids have also been shown\(^19\).

The biological activities of monoterpenoids (non-steam form) have not been investigated to a great extent, although the activity studies of essential oils have increased continuously\(^20\).

In these contexts, natural compounds are receiving increasing attention. They can be an alternative to the use of synthetic compounds in pharmaceutical technology or serve as lead compounds for the development of new drugs with the prospect of improving the treatment of various disorders. As a part of our ongoing research on pharmacological activities of
Verbascum species, we investigated antimicrobial and antimalarial activities of 22 secondary metabolites from Verbascum species growing in Turkey against Candida albicans ATCC 90028, Cryptococcus neoformans ATCC 90113, Staphylococcus aureus ATCC 29213, methicillin-resistant S. aureus ATCC 43300, Pseudomonas aeruginosa ATCC 27853, Aspergillus fumigatus ATCC 90906 and Mycobacterium intracellulare ATCC 23068 as well as the malarial parasite P. falciparum. This is the first report on antimicrobial and antimalarial properties of isolated compounds from Verbascum species using microdilution method.

MATERIALS AND METHODS

Plant Material

Extraction and Isolation
Air-dried and powdered flowers of Verbascum pterocalycinum var. mutense (485.6 g) were extracted with MeOH (2x2 L) under reflux. The MeOH extract was evaporated to dryness in vacuo to yield 43.5 g of crude extract. The methanolic extract was fractionated by open column chromatography on silica gel (500 g) employing hexane, ethylacetate, chloroform, acetone and methanol (each, 4 L), respectively, to yield five fractions (Frs. A-E). Fraction E (11.5 g) eluted with methanol was subjected to vacuum liquid chromatography (VLC) using reversed-phase material (Sepralyte 40 µm, 750 g), employing MeOH-H₂O mixtures (0-100 %) to give ilwensisaponin A (= mimengoside A, 19, 283.1 mg) and ilwensisaponin C (20, 260.1 mg).

The extraction procedures of V. cilicicum, V. lasianthum and V. pterocalycinum var. mutense, as well as the isolation and the structure elucidation of all compounds, were given in detail in our previous studies11-14.

Microorganism Information and Storage
All organisms were obtained from the American Type Culture Collection (Rockville, MD). They included the fungi Candida albicans ATCC 90028, Cryptococcus neoformans ATCC 90113 and Aspergillus fumigatus ATCC 90906 and the bacteria Staphylococcus aureus ATCC 29213, methicillin-resistant S. aureus ATCC 43300 (MRS), Pseudomonas aeruginosa ATCC 27853, and Mycobacterium intracellulare ATCC 23068. Temporary cultures (for immediate use in assays) of all organisms were stored on either agar slants or plates at 4°C until needed. Long-term storage of strains was accomplished via freezing cells in 10% glycerol/broth at -70°C [Sabouraud Dextrose broth (Difco, Detroit) for C. albicans and C. neoformans; YM broth (Difco) for A. fumigatus; Eugon broth (Difco) for S. aureus, MRS and P. aeruginosa; and Middlebrook 7H9 broth (Difco) with OADC enrichment (BBL, Maryland) for M. intracellulare]. Fresh slants and plates were prepared every 3-4 weeks by streaking agar with suspensions of frozen stocks and incubating them as below:

Preparation of Agar Slants or Plates
C. albicans on Sabouraud Dextrose agar (Difco) plates for 18-24h at 37°C
C. neoformans on Sabouraud Dextrose agar plates for 72h at 30ºC
S. aureus, MRS, and P. aeruginosa on Eugon agar (Difco) plates for 18-24h at 37°C
M. intracellulare on Lowenstein-Jensen agar slants (BBL) for 1 week at 37°C

Antimicrobial Assay
Susceptibility testing was performed using a modified version of the National Committee for Clinical Laboratory Standards (NCCLS) methods21-24. Seventy-two to 96h prior to the assay, the M. intracellulare subculture was prepared by resuspending cells from the surface of the slant in Middlebrook 7H9 broth with OADC enrichment and incubating at 37°C. On
the day of the assay, prepared samples (dissolved in DMSO), are serially-diluted using 0.9% saline and transferred in duplicate to 96 well microtiter plates (flat bottom plates for C. albicans, C. neoformans, S. aureus, MRS, P. aeruginosa, and M. intracellulare and round bottom plates for A. fumigatus). C. albicans, C. neoformans, S. aureus, MRS, and P. aeruginosa inocula were prepared by picking a single colony from agar plates and resuspending in ~ 3 ml 0.9% saline. The absorption of 100 ml of the saline suspensions and the M. intracellulare subculture at 630 nm using the EL-340 Biokinetics Reader (Bio Tek Instruments, Vermont) was compared to the 0.5 McFarland standard. The microorganisms were diluted in broth [Sabouraud Dextrose and cation-adjusted Mueller-Hinton (Difco) for the fungi and bacteria, respectively, and 5% Alamar Blue (BioSource International, Camarillo, CA) in Middlebrook 7H9 broth with OADC enrichment for M. intracellulare to afford final target inocula of: C. albicans: 2.5 x 10^3, C. neoformans and M. intracellulare: 1.5 x 10^5, and S. aureus, MRS, and P. aeruginosa: 3.0 x 10^5 CFU/ml after addition to the samples. The A. fumigatus inoculum was prepared by gently removing spores from a slant and transferring to ~ 3 ml 0.9% saline. This suspension was filtered through Miracloth (Calbiochem, La Jolla, CA) and diluted appropriately (via comparison to a standard curve) in YM broth to afford a final target inocula of 4.5 x 10^4 CFU/ml. The microbial inocula were re-added to the samples to achieve a final volume of 200 µl and final sample concentrations starting with 50 µg/ml for pure compounds. Growth (saline only), solvent and blank (media only) controls were included on each test plate. Drug controls [Ciprofloxacin (ICN Biomedicals, Ohio) for bacteria and Amphotericin B (ICN Biomedicals, Ohio) for fungi] were included in each assay. Except for A. fumigatus, which was inspected visually, all other organisms were read at either 630 nm or 570 nm (M. intracellulare) prior to and after incubation: C. albicans, S. aureus, MRS, and P. aeruginosa at 37°C for 18-24h, C. neoformans and A. fumigatus at 30°C for 72h, and M. intracellulare at 37°C for 48-72h. For organisms read on the plate reader, percent growth was calculated and plotted versus test concentration to afford the IC_{50} (sample concentration that affords 50% growth of the organism) and, for pure compounds only, the minimum inhibitory concentration (MIC) and minimum fungicidal or bactericidal concentrations (MFC/MBCs). Except for A. fumigatus and M. intracellulare, the MIC is defined as the lowest test concentration that significantly inhibits growth. Minimum fungicidal and bactericidal concentrations were determined by removing 5 µl from each clear well, transferring to agar and incubating as previously mentioned. The MFC/MBC is defined as the lowest test concentration that kills 100% of the organism (allows no growth on agar).

### Antimalarial/Parasite Lactate Dehydrogenase (pLDH) Assay

The *in vitro* antimalarial assay procedure utilized at the NCNPR, University of Mississippi, is an adaptation of the pLDH assay developed by Makler et al., using microdilution method protocol with a *P. falciparum* clone [Sierra Leone D6 (chloroquine-sensitive)]. The antimalarial agents chloroquine and artemisinin were used as controls, while DMSO was the solvent control^{25-27}. The procedure is described as follows:

Prepare a suspension of red blood cells with a 2% parasitemia and 2% hematocrit in malaria complete medium (approximately 20 ml per 96-well plate). Dispense 200 µl aliquots of this suspension into each well of a 96-well, flat-bottomed microtiter plate. Next, add 10 µl volumes of the drugs to be tested in duplicate to the appropriate wells. Place the plates into the humidified chamber and flush the cultures with gas mixture 90% N\textsubscript{2}, 5% O\textsubscript{2}, 5% CO\textsubscript{2}. Place chamber containing the plates into a 37°C incubator for approximately 48h. After 48h, add 100 µl aliquots of the Malstat\textsuperscript{m} reagent to each well of a new 96-well microtiter plate. Resuspend the cultures from the assay plate by mixing each well up and down several times. Remove 20 µl from each well of the resuspended culture and add to the plate containing the Malstat reagent. Incubate the plates at room temperature for 30 min. After 30 min. add to each well 20 µl of the 1:1 mixture of the NBT/PE solution (2 mg/ml and 0.1 mg/ml, respectively). Incubate plates in the dark for 1h. At the end of the 1 h incubation, the reaction is stopped by the addition of approximately 100 µl of a 5% acetic acid solution. The plate is then read at an endpoint of approximately 650 nm.
RESULTS AND DISCUSSION

According to the antimicrobial activity procedure, 21-24, 22 secondary metabolites from *Verbascum* species were tested at three different concentrations, 50, 10 and 2 µg/ml, on a primary assay, and then the active compounds were screened at 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, 0.19, 0.098, 0.05, 0.024 µg/ml concentrations in a secondary assay to evaluate MIC values.

The antimicrobial activities of the compounds (1-22) against Gram-positive and Gram-negative bacteria and yeast-like fungi as % growth inhibitions and MIC values were summarized and the results of primary screening are given in Table 1. The compounds showing 50% growth inhibition (IC50) ≤ 15 µg/ml were considered to be active and these samples were screened in a secondary assay. Ilwensisaponin A (19) and C (20) showed antimicrobial activity against *Aspergillus fumigatus* ATCC 90906, but no significant activity against Gram (+) and Gram (-) bacteria and other tested fungi in primary assay (Table 1). Saponins were retested against *Aspergillus fumigatus* ATCC 90906 for secondary assay. IC50 values which were ≤ 15 µg/ml of the compounds presented an activity in the secondary assay of the antimicrobial screening.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Plant Source</th>
<th>*IC50 (µg/ml)</th>
<th>**MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ca</td>
<td>Cn</td>
</tr>
<tr>
<td>Aucubin (1)</td>
<td><em>V. lasianthum</em></td>
<td>&gt; 50</td>
<td>&gt; 50</td>
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<tr>
<td>Unduloside III (2)</td>
<td><em>V. lasianthum</em></td>
<td>&gt; 50</td>
<td>&gt; 50</td>
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<tr>
<td>Catalpol (3)</td>
<td><em>V. ellicicum</em></td>
<td>&gt; 50</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>Sinatol (4)</td>
<td><em>V. lasianthum</em></td>
<td>&gt; 50</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>Verbaspinoside (5)</td>
<td><em>V. ellicicum</em></td>
<td>&gt; 50</td>
<td>&gt; 50</td>
</tr>
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<td>6-O-(3'-O-trans-cinnamoyl)-α-L-rhamnopyranosylcatalpol (6)</td>
<td><em>V. ellicicum</em></td>
<td>&gt; 50</td>
<td>&gt; 50</td>
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<td>6-O-(4'-O-trans-cinnamoyl)-α-L-rhamnopyranosylcatalpol (7)</td>
<td><em>V. ellicicum</em></td>
<td>&gt; 50</td>
<td>&gt; 50</td>
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<tr>
<td>Sacatoside (8)</td>
<td><em>V. ellicicum</em></td>
<td>&gt; 50</td>
<td>&gt; 50</td>
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<td>6-O-(3'-O-trans-p-coumaroyl)-α-L-rhamnopyranosylcatalpol (9)</td>
<td><em>V. ellicicum</em></td>
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<td>Verbascoside A (10)</td>
<td><em>V. lasianthum</em></td>
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<td>Pulverulentoside I (11)</td>
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<td>&gt; 50</td>
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<td>Buddlejoside A1 (12)</td>
<td><em>V. lasianthum</em></td>
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<td>&gt; 50</td>
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<tr>
<td>Picroside IV (13)</td>
<td><em>V. pterocalycinum</em> var. mutense</td>
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<td>&gt; 50</td>
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<td>Ajugol (14)</td>
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<td>6-O-Vanilyloajugol (15)</td>
<td><em>V. lasianthum</em></td>
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<tr>
<td>8-O-Acetylharpagide (16)</td>
<td><em>V. lasianthum</em></td>
<td>&gt; 50</td>
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<td>Harpagoside (17)</td>
<td><em>V. lasianthum</em></td>
<td>&gt; 50</td>
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<td>1-(β-D-glucopyranosyl)-8-hydroxy-7, 7-dimethyl-occ-2(E), 6(E)-dienoate (18)</td>
<td><em>V. pterocalycinum</em> var. mutense</td>
<td>&gt; 50</td>
<td>&gt; 50</td>
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<tr>
<td>Ilwensisaponin A (19)</td>
<td><em>V. pterocalycinum</em> var. mutense</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Ilwensisaponin C (20)</td>
<td><em>V. pterocalycinum</em> var. mutense</td>
<td>&gt; 50</td>
<td>40</td>
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<td>Verbascoside (21)</td>
<td><em>V. lasianthum</em></td>
<td>&gt; 50</td>
<td>&gt; 50</td>
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<tr>
<td>Polymouside (22)</td>
<td><em>V. lasianthum</em></td>
<td>&gt; 50</td>
<td>&gt; 50</td>
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<tr>
<td>Amphoterin B</td>
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<td>0.10</td>
<td>0.35</td>
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<tr>
<td>Ciprolfoaxin</td>
<td></td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Rifampin</td>
<td></td>
<td>1.56***</td>
<td></td>
</tr>
</tbody>
</table>

*Ca: Candida albicans ATCC 90028. Cn: Cryptococcus neoformans ATCC 90113. Sa: Staphyloccocus aureus ATCC 29213. MRS: Meticillin-resistant S. Aureus ATCC 43300. Pa: Pseudomonas aeruginosa ATCC 27853. Af: Aspergillus fumigatus ATCC 90906. Mi: Mycobacterium intracellulare ATCC 23068. *The concentration (µg/ml) that affords 50% inhibition of growth. **MIC (minimum inhibitory concentration) is the lowest test concentration (µg/ml) of the compounds that allows no detectable growth. ***The active concentration is the lowest test concentration (µg/ml) that significantly inhibits growth.*

Table 1. Antimicrobial activities of the compounds (1-22) from *Verbascum* species (primary assay)
program. None of the compounds was found to be active in the secondary assays (Table 2). Antimicrobial activity against each strain was recorded in comparison with the results of amphotericin B, ciprofloxacin and rifampin, as controls.

The compounds from *Verbascum* species were also tested for antimalarial activity\(^{25-27}\). The primary screening involved determination of pLDH inhibition (%) of each sample tested at 4760, 1587 and 528.8 ng/ml concentrations. The tested compounds did not exhibit any activity in the antimalarial assay at the tested concentrations.

**CONCLUSION**

The antimicrobial activity of two oleanane type triterpenoid saponins was detected with potent growth inhibition against *Aspergillus fumigatus* ATCC 90906. However, the other tested compounds did not show any antimicrobial activity.

The *in vitro* antifungal activities of saponins from *Tribulus terrestris* L., against *Candida albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *Cryptococcus neoformans* were studied using microbroth dilution assay and the reported saponins have significant antifungal activity, weakening the virulence of *C. albicans* and killing fungi through destroying the cell membrane\(^ {29}\). The results showed that *Verbascum* species contain potentially bioactive saponins and supported the further study of antimicrobial and antimalarial activities of triterpenoid saponins with different assays.

The antimicrobial activity of triterpene saponins was found to be mainly dependent on their olefinic structures and the number of sugars, depending on the type, their linkage to each other, and location within the molecule. The availability of a longer sugar chain and the number of hydroxyl groups lead to an increase in activity. On the other hand, methylation of at least one of the hydroxyl groups may abolish the activity as evidenced by the observation that methoxylated derivative ilwensisaponin C may lose its activity. In addition, monodesmosidic saponins, in which the sugar units are attached to the aglycone at the C-3 position, are more potent in this antimicrobial system\(^ {18}\). Favel et al. (1994) also showed that bidesmosides saponins were devoid of any growth inhibitory activity, even in concentrations as high as 200 mg/ml. Growth inhibition was influenced by the number and kinds of sugar residues\(^ {30}\).

According to literature data, some saponins and phenylethanoid glycosides possess a dose-dependent antimicrobial activity against several bacteria and fungi. Mandal *et al*. (2005) investigated antimicrobial activity of two triterpene saponins isolated from the funicles of *Acacia auriculiformis*. Complete inhibition of conidial germination of *Aspergillus ochraceous*

### Table 2. Antimicrobial activities of the compound 19-20 (secondary assay) with MFC/MBC

<table>
<thead>
<tr>
<th>Compound</th>
<th>Plant Source</th>
<th>*MIC (µg/ml)</th>
<th>***MFC/MBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ilwensisaponin A (19)</td>
<td><em>V. pterocalyicum</em> var. mutense</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Ilwensisaponin C (20)</td>
<td><em>V. pterocalyicum</em> var. mutense</td>
<td>&gt;50</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td></td>
<td>1.25**</td>
<td>2.50****</td>
</tr>
</tbody>
</table>

Af: *Aspergillus fumigatus* ATCC 90906. *MIC (minimum inhibitory concentration) is the lowest test concentration (µg/ml) of the compounds that allows no detectable growth. **The active concentration is the lowest test concentration (µg/ml) that significantly inhibits growth. ***Minimum fungicidal/bactericidal concentration. ****The lowest test concentration (µg/ml) of the compounds that kills 100% of the organism.
Curvularia lunata was recorded at 300 µg/ml or less, whereas to inhibit the growth of Bacillus megaterium, Salmonella typhimurium and Pseudomonas aeruginosa, 700 µg/ml or higher concentrations of the mixture were required. The results suggest strongly that the saponins act differently to inhibit bacterial and fungal growth under cultural condition with higher doses.

Antimicrobial activities of some phenylethanoid glycosides isolated from Scrophularia scopolii were tested using microdilution method. The data indicated that angoroside A, B and verbascoside showed antimicrobial activity against Staphylococcus aureus ATCC 25923 and Streptococcus faecalis MN 10541 at 50 µg/ml dose. In the other study, verbascoside showed no activity against Pseudomonas stutzeri; however, it was a very potent inhibitor of Bacillus subtilis and exhibited at 2.5 Mg/spot.

As a result, it was determined that the application doses of 50, 10 and 2 µg/ml in this study were less than the doses which were reported for saponins and verbascoside by Mandal et al. (2005) and Calis et al. (1988). Therefore, the conclusion suggested that potent antimicrobial activity could be provided by a higher dose. Furthermore, these compounds might be studied with different strains for the definite evaluation.

In a reference survey, there have been no other reports about the antimicrobial and antimalarial activities of any isolated compounds using microdilution method protocol. This is the first demonstration that ilwensisosaponin A and C were shown to possess a significant antimicrobial activity as compared with other tested compounds in this study. However, correlated results to compare antimicrobial activities in both plant extracts and the isolated compounds could not be achieved. It could be said that there was an antagonist effect. In order to correlate the obtained data in the field of antimicrobial activities of effective compounds and plant extracts, further examinations in different assays need to be evaluated.

Acknowledgements

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