ANTIBACTERIAL AND ANTI-BIOFILM ACTIVITY OF FLAVONOIDS AND TRITERPENES ISOLATED FROM THE EXTRACTS OF FICUS SANSBARICA WAR. SUBSP. SANSBARICA (MORACEAE) EXTRACTS

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Abstract

Background: Ficus species are used in African traditional medicine in the treatment of a wide variety of ailments and diseases such as convulsive disorder, wound healing, gonorrhea, tuberculosis, diabetes, diarrhoeal infections, dysentery, malaria and HIV. The aim of this study was to isolate the phytochemical constituents in the plant and test them for their antibacterial activity.

Materials and methods: The fruits, leaves and stem bark were extracted with organic solvents and the compounds in the extracts separated and purified by column chromatography before being identified by NMR spectroscopy and by comparison of the NMR data against values reported in the literature. The antibacterial activity of the pure compounds and extracts were tested using the disk diffusion method.

Results: Three triterpenes and three flavonoids: lupeol acetate (1); cyclooct-23-ene-3,25-diol (2); β-sitosterol (3); 5,7,4’-trihydroxyflavan-3-ol (4); epicatechin (5) and isovitexin (6) were isolated in this study. Antimicrobial activity was observed at 8 mg mL−1 for Staphylococcus aureus ATCC 29213 with four of the six isolated compounds, with no activity being observed at 1 – 4 mg mL−1 against Escherichia coli ATCC 35218 and S. aureus ATCC 43300. Epicatechin (5) was found to decrease adhesion of E. coli ATCC 29222 and S. aureus ATCC 29213. Decreased adhesion of S. aureus ATCC 29213 was also observed with 5,7,4’-trihydroxyflavan-3-ol (4) and isovitexin (6).

Conclusions: The results of this study provide baseline information on F. sansbarica’s potential validity in the treatment of infections associated with Gram-positive microorganisms.

Key words: Moraceae, Ficus sansbarica, antimicrobial, anti-adhesion properties, flavonoids, triterpenoids.

Introduction

Primary healthcare in Africa still remain inaccessible to the vast majority of its populace due to inadequate transport systems to and from health facilities and the cost of drugs and the high rates charged for in-hospital treatment. In most cases, ~80% of the world’s population, especially those living in the rural areas of African countries, rely on traditional medicine for the healing and treatment of various ailments from coughs and colds to HIV, malaria and tuberculosis (WHO, 1996; Street and Prinsloo, 2013). Research into pharmacologically active African medicinal plants is being carried out with the aim of providing scientific evidence for the use of the plants in traditional medicine, commercialization and pharmaceutical application of the active components. This has primarily centered on antimicrobial activity but is now being targeted towards microbial virulence mechanisms such as biofilm formation and/or quorum sensing. Microbial biofilm formation of microorganisms is associated with persistent tissue and foreign body infections which are resistant to treatment with antimicrobial agents. Up to 80% of human bacterial infections are biofilm-associated; with infections most frequently being caused by Staphylococcus aureus, E. epidermidis, Pseudomonas aeruginosa, and Escherichia coli (Romling and Balsalobre, 2012).

In tropical and subtropical regions of the world, the genus Ficus (Moraceae), of the Mulberry family constitutes one of the largest genera of angiosperms with over 900 species consisting of trees, shrubs and epiphytes (Lansky and Paavilainen, 2011). Among these, about 110 Ficus species are indigenous to Africa (Berg and Wiebes, 1992; Singh et al., 2011). Many Ficus species have been used in African traditional medicine either in the treatment or management of a variety of ailments and diseases such as convulsive disorder, wound healing, gonorrhea, tuberculosis, diabetes, diarrhoeal infections, dysentery, malaria and HIV (Wakeel et al., 2004; Yadav et al., 2006; Parekh and Chanda, 2007; Annan and Houghton, 2008; Abdul et al., 2010; Lamorde et al., 2010; Ndembea et al., 2011). Ficus species have also been used in Indian ayurvedic and traditional medicine (Joseph and Justin Raj, 2010). Extracts of Ficus species have also been documented to have antioxidant, anti-diabetes, antibacterial, antifungal, antiviral, anti-protozoal, anticancer, cytotoxic, anti-ulcer, anti-inflammatory, anti-hyperglycemic, anti-diarrhoea, hepato-protective, muco-protective and gastro-protective activity (Kuete et al., 2009). Phytochemical studies on species from the genus Ficus have shown these plants to be rich sources of flavonoids, lignans, terpenoids, alkaloids, coumarins steroids, and cerebroside (Ragas et al., 1999; Kuete et al., 2008; Rakam et al., 2009; Ueda et al., 2009; Chen et al., 2010; Kim et al., 2011; Naressi et al., 2012). However, although there are many phytochemical and pharmacological reports on species within the genus Ficus, there are many species that have not been studied and whose ethno-botanical relevance is yet to be investigated. Ficus sansbarica Warb. subsp. sansbarica is a large tree with a spreading crown which is up to 20 × 30 m and is commonly known as the Zanzibar fig, Angola fig or knobbly fig. It is usually without aerial roots and contains milky latex. The knobby fig is distributed in South Africa (Mpumalanga and Northern Province), Swaziland, Mozambique, Zimbabwe, Zambia, Malawi, Tanzania and Kenya. It has also been visually recorded in Zululand (Burrows and Burrows, 2003). In Botswana, both adults and child eat the fresh ripe fruits (Daniel and Topo, 2012). The ripe fruits are also consumed by birds, fruit bats, baboons, monkeys and other animals as well as insectivorous birds which feed upon the wasps that pollinate the figs. The leaves are browsed by nyala, kudu, elephant and giraffe (Venter and Venter, 1996; Boon, 2010). The only biological study reported so far was the analysis of the fresh ripe fruits for its nutrients, total phenols and antioxidant activity (Daniel and Topo, 2012). This prompted us to investigate the phytochemical composition of Ficus sansbarica which to the best of our knowledge has not been studied previously, and to test the isolated compounds for antimicrobial and anti-biofilm activity with the aim of providing a scientific rationale for the use of the plant in the treatment of some bacterial infections.

Materials and Methods

Collection of plant materials

The fruits, leaves and stem bark of Ficus sansbarica were collected in 2011, in KwaZulu-Natal, South Africa. The plant was identified and a voucher specimen (G. V Awolola & H. Baij Nath 1), was deposited in the Ward herbarium at the University of KwaZulu-Natal.
General experimental procedure

The $^1$H, $^{13}$C and 2D NMR spectroscopy were recorded at room temperature either using deuterated chloroform (CDCl$_3$), dimethyl sulfoxide (DMSO), or methanol (MeOH), on a Bruker Avance$^{11}$ 400 and 600 MHz spectrometer with tetramethylsilane (TMS) as internal standard. All GC-MS analyses of samples were recorded on an Agilent GC-MSD apparatus equipped with DB-5MSL MS (30 m × 0.25 mm i.d., 0.25 µm film thickness), fused-silica capillary column. The carrier gas was Helium (2ml min$^{-1}$). The MS was operated in the EI mode at 70 eV. The LC-MS sample analyses were recorded on an Agilent LC-MSD apparatus equipped with a UV detector using a mobile phase of 95% acetonitrile, 10% water, and both containing 1.1% formic acid at a flow rate of 1ml min$^{-1}$. Infrared (IR), spectral data was obtained using a Perkin Elmer Spectrum 100 Fourier transform infrared spectroscopy (FT-IR), spectrometer with universal attenuated total reflection (ATR) sampling accessory. All UV spectra were obtained using a Varian Cary UV-VIS spectrophotometer. Optical rotations were measured at room temperature using a 10 cm flow tube in a PerkinElmer$^{TM}$, Model 341 Polarimeter. Melting points were obtained using an Ernst Leitz Wetzlar micro-hot stage melting point apparatus. For column chromatography, Merck silica gel 60 (0.040-0.063 mm), was used while Merck 20 cm × 20 cm silica gel 60 F$_{254}$, aluminum sheets were used for TLC. The TLC plates were analyzed under UV (254 and 366 nm) before being visualized by spraying the plate with a 10% sulphuric acid in MeOH solution followed by heating.

Extraction and Isolation

Freshly crushed *F. sansibarica* fruits (516 g) were subjected to cold extraction using MeOH, while air-dried and ground leaves (520 g), and stem bark (1.5 kg), were subjected to exhaustive extraction using solvents of increasing polarity: hexane, dichloromethane (DCM), ethyl acetate (EtOAc), and MeOH by maceration with continuous shaking on an orbital shaker for 48 hrs, each time at room temperature. The different solvent extracts were concentrated using a rotary evaporator and the obtained crude extracts in each case were stored for further analysis. Using suitably sized columns the crude extracts were subjected to column chromatography for fractionation and the fractions collected were monitored using TLC. Similar fractions were combined and concentrated using a rotary evaporator.

The MeOH extract from the fruits was partitioned with EtOAc and the EtOAc portion concentrated yielding 1 g of extract. This was separated on a 1.5 cm diameter column over silica gel (0.040-0.063 mm), using a gradient elution of n-hexane:EtOAc (with 10% increments of EtOAc), and finally with 100% MeOH, collecting 20 mL at each stage. The fractions were combined on the basis of TLC to afford six main fractions (I-VI). Fraction IV was purified using CC over Sephadex LH-20 (MeOH), to afford isovitexin (25 mg), a dark yellow solid.

The crude hexane extract of the leaves (10.3 g) was separated using CC in a similar manner with a hexane:EtOAc step gradient (with 10% increments every 100 mL). Compound 2, cycloart-23-ene-3,25-diol (13 mg) was eluted with hexane:EtOAc (9:1) and evaporated to a white powder. Lupeol acetate (25 mg), a dark yellow solid.

![Figure 1](http://dx.doi.org/10.4314/ajtcam.v11i3.19)

**Figure 1:** Chemical structures of compounds isolated from *Ficus sansibarica*. Lupeol acetate (1), cycloart-23-ene-3,25-diol (2), sitosterol (3), 5,7,4′-trihydroxyflavan-3-ol, R = H, (4), epicatechin, R = OH (5), isovitexin (6).
Antimicrobial susceptibility testing

Antimicrobial susceptibility of the crude MeOH leaf and stem bark extracts and compounds 1-6 were determined using the disk-diffusion method. Crude extracts and isolated compounds were dissolved in chloroform or DMSO to a final concentration of 100 mg mL\(^{-1}\). Blank discs (6 mm; MAST, UK), were impregnated with 10 \(\mu\)L (1 mg), 20 \(\mu\)L (2 mg) and 40 \(\mu\)L (4 mg) and allowed to dry.

Bacterial isolates (Gram-negative: Escherichia coli ATCC 25922 and E. coli ATCC 35218; Gram-positive: Staphylococcus aureus ATCC 29213 and S. aureus ATCC 43300), were grown overnight on tryptic soy agar (TSA) plates. Cell suspensions were prepared with sterile de-ionized water and the turbidity adjusted equivalent to that of a 0.5 McFarland standard. These were used to inoculate Mueller-Hinton (MH) agar plates by streaking swabs over the entire agar surface followed by the application of the respective phytochemical extract discs (CLSI, 2007). Plates were then incubated for 24 h at 37 °C. Testing was done in duplicate with tetracycline (TE30); 30 \(\mu\)g mL\(^{-1}\), and ampicillin (AMP10; 10 \(\mu\)g mL\(^{-1}\)) discs being used as standard antimicrobial agent controls, in addition to DMSO and chloroform.

Following the preliminary disk-diffusion assays, the agar-well diffusion assay was also used for S. aureus ATCC 29213, in order to accommodate a greater concentration of the isolated compounds. Instead of using impregnated discs, agar wells with a diameter of ~6 mm were cut into MH agar swabs with S. aureus ATCC 29213. Wells were filled with 80 \(\mu\)L of 100 mg mL\(^{-1}\) stock solutions of compounds 1, 2, 4 and 5 (8 mg mL\(^{-1}\)) and 80 \(\mu\)L of 20 mg mL\(^{-1}\) stock solution of compound 6 (1.6 mg mL\(^{-1}\)) (Table 2). Plates were then incubated for 24 hrs at 37 °C. Testing was done in duplicate and tetracycline (TE30) and ampicillin (AMP10) discs were used as standard antimicrobial agent controls, in addition to DMSO and chloroform as negative controls. Zone diameters were determined and averaged. The following zone diameter criteria were used to assign susceptibility or resistance to the phytochemicals tested: Susceptible (S) \(\geq 15\) mm, Intermediate (I) = 11 – 14 mm, and Resistant (R) \(\leq 10\) mm (Okoth et al., 2013).

Criteria for assigning susceptibility or resistance to AMP10 was as follows: (S) \(\geq 17\) mm, (I) = 14 – 16 mm, (R) \(\leq 13\) mm, while those for TE30 were: (S) \(\geq 19\) mm, (I) 15 – 18 mm, (R) \(\leq 14\) mm (CLSI, 2007).

Biofilm microtitre plate assays

In order to ascertain the anti-biofilm potential of the isolated compounds, E. coli ATCC 29922, E. coli ATCC 35218, S. aureus ATCC 29213 and S. aureus ATCC 43300 were exposed to lupeol acetate (100 mg mL\(^{-1}\) stock; 2, 5, 10 and 15 mg mL\(^{-1}\)); 5,7,4'-trihydroxyflavan-3-ol 4 (100 mg mL\(^{-1}\) stock; 1 and 2.5 mg mL\(^{-1}\)); epicatechin 5 (100 mg mL\(^{-1}\) stock; 1, 2.5, 5 and 7.5 mg mL\(^{-1}\)); and isovitexin 6 (20 mg mL\(^{-1}\) stock; 0.2 and 0.5 mg mL\(^{-1}\)) using microtitre plate assays (Basson et al., 2008). The purified cycloart-23-ene-3,25-diol 2 could not be tested due to unavailability of sufficient material. All isolates were cultured overnight in tryptic soy broth (TSB), and centrifuged for 2 min at 12000 rpm. Cell pellets were washed and re-suspended to a turbidity equivalent to a 0.5 McFarland standard. Each well of the sterile 96-well U-bottomed polystyrene microtitre plates contained 90 \(\mu\)L of TSB medium and 10 \(\mu\)L of culture after standardization. To investigate the effect on adherence, respective volumes of extracts were added to the TSB medium at the time of inoculation in the micro-titre plate assays and volumes were standardized to 200 \(\mu\)L with sterile distilled water. Positive control wells contained TSB broth and respective cultures without the tested compounds whilst the negative control wells contained un-inoculated TSB broth only to test for sterility and non-specific binding of media. Plates were incubated aerobically for 24 h with agitation on an Orbit P4 microtitre plate shaker (Labnet), at 37 °C to allow cell attachment and biofilm formation (Basson et al., 2008).

Following incubation, the crystal violet assay was performed. The contents of each well were aspirated. Micro-titre plate assays were washed three times with sterile de-ionized water to remove loosely attached cells. The remaining cells were fixed with 200 \(\mu\)L of MeOH for 15 min and air-dried. Following drying, the wells were stained with 150 \(\mu\)L of 10% Hucker's crystal violet for 5 min. Then, wells were rinsed gently with water, air-dried and 150 \(\mu\)L of 33% (v/v) of glacial acetic acid was added for re-solubilization of dye bound to adherent cells. Plates were then read using the GloMax®-Multi+ Detection System (Promega) at an optical density of 600 nm. Tests were done in triplicate on two separate occasions and the results were averaged (Basson et al., 2008). OD\(_{600}\) values of treated cells were compared to untreated controls to investigate the increase or decrease in adhesion as a result of phytochemical exposure. Treated and untreated samples were compared statistically using Paired t-tests (SigmaStat V3.5, Systat Software, Inc).

Results and Discussion

The phytochemical investigation of the leaves, stem bark and fruits of F. sasibarica yielded six compounds: three triterpenoids and three flavonoids. The non-polar hexane extract of both leaves (1 and 2) and stem bark (3) contained triterpenoids while the flavonoids were isolated from the more polar EtOAc extract of both the stem bark (4 and 5), and the fruits (6). The isolated compounds were identified based on their \(^1\)H and \(^13\)C NMR spectra and 2D NMR spectra and compared with literature values. Thus, they were identified as lupeol acetate (1) (Mahato et al., 2008; Escobedo-Martínez et al., 2012; Khan et al., 2006), sitosterol (2) (Chaturvedula and Prakash, 2012), 5,7,4'-trihydroxyflavan-3-ol 4 (Dafalla, 2005; Iida et al., 2007), epicatechin 5 (Hubert et al., 2011; Markham and Ternai, 1976) and isovitexin 6 (Lin et al., 2009).

Epicatechin 5 has been isolated from several Ficus species like F. microcarpa and F. spragueana (Kiem et al., 2011; Ragab et al., 2013). In contrast, isovitexin 6 was only reportedly isolated from F. microcarpa (Kiem et al., 2011), while 574'-trihydroxyflavan-3-ol 4 was isolated from F. capensis and F. spragueana (Owolabi et al., 2009; Ragab et al., 2013). Lupeol acetate 1 and sitosterol 3 are ubiquitous to plants. To the best of our knowledge, this is the first report of cycloart-23-ene-3,25-diol 2, a cycloartane type triterpenoid with molecular formula C\(_{30}\)H\(_{40}\)O\(_2\) in the genus Ficus. Furthermore, a comparison of the NMR data obtained for 2 with that published in the literature (Abdel-Monem et al., 2008; Escobedo-Martinez et al., 2012, Khan et al., 2006) matched the NMR data of the cycloartane backbone only and there were discrepancies in the NMR data of the side chain, which we have unambiguously assigned from HMBC data. The cis isomer of 2 is also reported (Gherraf et al., 2010), and is also included here for comparison. We speculate that the reports of 2 in Abdel-Monem et al., 2008, Escobedo-Martinez et al. (2012), and Khan et al., 2006, are also that of the cis isomer as the H-23 and H-24 resonances were always reported as overlapping multiplets at 5.40 and 5.80 ppm. In our spectra, we were able to clearly define the trans nature of the \(\delta\) double bond by clear coupling constants. A full NMR structural elucidation is given below and a comparison with that in the literature given in Table 1.

The \(^1\)H-NMR spectrum of compound 2 displayed two pairs of doublets with resonances at \(\delta\) 0.55 (1H, \(\text{H}-19\)) and at 0.30 (1H, d, \(J = 3.4\) Hz, \(\text{H}-19\)). In addition, there were the presence of seven methyl groups at \(\delta\) 0.82 (s, \(\text{CH}_3\)-29), 0.89 (d, \(J = 6.5\) Hz, \(\text{CH}_2\)-21), 0.90 (s, \(\text{CH}_3\)-28), 0.99 (s, \(\text{CH}_3\)-30, \(\text{CH}_3\)-18), 1.36 (s, \(\text{CH}_2\)-26/27), the typical H-3 resonance for triterpenoids at 5.26, a doublet of doublet of doublets (ddd) at \(\delta\) 5.71 (1H, \(J = 15.7, 8.5, 6.1\) Hz, H-23), a doublet at \(\delta\) 5.53 (1H, \(J = 15.7\) Hz, H-24) and one proton of the H-22 methylene group at \(\delta\) 2.26 which could clearly be distinguished. The \(^13\)C NMR spectrum was able to be
resolved into 7 methyl, 10 methylene, 7 methine and 6 singlet carbon resonances, including two olefinic methine resonances for the double bond in the side chain, an oxygenated singlet resonance at δ 82.3 and an oxygenated methine resonance at δ 78.8, indicating two hydroxy groups in the molecule.

Table 1: $^1$H and $^{13}$C NMR data of cycloart-23-ene-3,25-diol 2 (600 MHz, CDCl$_3$)

<table>
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<th>Pos.</th>
<th>$^1$H</th>
<th>$^{13}$C</th>
<th>$^1$C (2)</th>
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<td>1.40 (m), 1.23 (m)</td>
<td>1.78 (m), 1.58 (m)</td>
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<tr>
<td>3</td>
<td>3.24 (m)</td>
<td>3.10 (m)</td>
<td>3.26 (m)</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>1.30 (m)</td>
<td>0.91 (m)</td>
<td>1.30 (m)</td>
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<td>1.26 (m), 0.45 (dd, 12.5, 8)</td>
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<td>1.51 (m)</td>
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<tr>
<td>9</td>
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<td>18</td>
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<td>0.62 (s, 3H)</td>
<td>0.98 (s, 3H)</td>
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</tbody>
</table>

$^{a}$ Escobedo-Martinez et al. (2012); $^{b}$ Gherraf et al. (2010). C $^{13}$ is isomer; $^{c}$ It is highly likely that the $^1$H NMR spectrum in Gherraf et al. (2010) is offset by approximately δ 0.33; coupling constants are given in parenthesis.

Antimicrobial susceptibility testing

The isolated compounds and extracts were subjected to antimicrobial screening and the results are presented in Table 2. Initial disk-diffusion assays with 1 – 4 mg mL$^{-1}$ of the six isolated compounds, as well as the crude methanolic leaf and stem-bark extracts proved ineffective against the four bacterial strains (Gram-negative: E. coli ATCC 25922 and β-lactam resistant E. coli ATCC 35218; Gram-positive: methicillin-susceptible S. aureus ATCC 29213 and methicillin-resistant S. aureus ATCC 43300) tested, with no zones of inhibition observed (Table 2). However, S. aureus ATCC 29213 demonstrated intermediate susceptibility to 8 mg mL$^{-1}$ for four of the compounds: lupeol acetate, 1-cycloart-ene-3,25-diol 2, sitosterol 3 and epicatechin 5, using the agar-diffusion assay (Table 2). However, the flavonoids, 5,7,4'-trihydroxyflavan-3-ol 4 and isovitexin 6 were not active against the bacteria being tested (Table 2). Most phytochemicals work best against Gram-positive bacteria and there are very limited small-molecule plant-derived antimicrobial compounds with high activity against Gram-negative species (Lewis, 2013).

The limited inhibition observed for the crude MeOH extracts is not surprising. Reports on the antimicrobial activity of extracts from other Ficus species have been variable, with differences related to the Ficus species tested, the part of the plant sampled, the extraction solvent and the method of antimicrobial testing (Salem et al., 2013). Lawal et al. (2012) observed F. exasperata root bark MeOH extract antimicrobial activity against three S. aureus strains at ≥ 12.5 mg mL$^{-1}$, while activity against E. coli strains required ≥ 25 mg mL$^{-1}$. They also noted the potency of the MeOH extract compared to the EtOAc extract. Murti and Kumar (2011) demonstrated that the F. racemosa ethanol root extract

http://dx.doi.org/10.4314/ajtcam.v11i3.19

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epicatechin. The reported weak activity of some isolated cycloartane-type triterpenoids from the genus *S. aureus* (Kuet et al., 2009), while *F. polita* MeOH root extract and euphol-3-O-cinnamate and (E)-3,5,4'-trihydroxystilbene-3,5-O-β-δ-diglucopyranoside MICs ranged from 32 - ≥512 µg mL\(^{-1}\) against Gram-positive and Gram-negative bacteria (Kuet et al., 2011).

The antimicrobial potencies of the two classes (triterpenes and flavonoids), of compounds isolated have previously been established (Cowan, 1999). Akiyama et al. (2001), obtained epicatechin MICs of 8 mg mL\(^{-1}\) with 18 clinical *S. aureus* strains, since it causes limited damage to bacterial plasma membranes, while Mahmoud et al. (2013) observed no antimicrobial effect against methicillin-susceptible and -resistant *S. aureus* strains with 32 – 1024 µg mL\(^{-1}\) of epicatechin. The reported weak activity of some isolated cycloartane-type triterpenoids from the genus *Aphanamixis* against *S. aureus* (Wang et al., 2013) was consistent with the weak activity observed for cycloart-23-ene-3,25-diol (2) in this study.

**Table 2**: Antimicrobial susceptibility profile of *Staphylococcus aureus* ATCC 29213 following exposure to six compounds isolated from *Ficus sansibarica* and two crude methanol extracts

<table>
<thead>
<tr>
<th>Compound/Extract</th>
<th>Concentration mg mL(^{-1})</th>
<th>Zone diameter (mm) 40 µL(^{2})</th>
<th>Zone diameter (mm) 80 µL(^{2})</th>
</tr>
</thead>
<tbody>
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<td>Lupeol acetate (1)</td>
<td>8</td>
<td>13.5 (I)</td>
<td></td>
</tr>
<tr>
<td>Cycloart-23-ene-3,25-diol (2)</td>
<td>8</td>
<td>11.5 (I)</td>
<td></td>
</tr>
<tr>
<td>Sitosterol (3)</td>
<td>8</td>
<td>13 (I)</td>
<td></td>
</tr>
<tr>
<td>5,7,4'-trihydroxylavanan-3-ol (4)</td>
<td>8</td>
<td>0 (R)</td>
<td></td>
</tr>
<tr>
<td>Epicatechin (5)</td>
<td>8</td>
<td>13.5 (I)</td>
<td></td>
</tr>
<tr>
<td>Isovitexin (6)</td>
<td>1.6</td>
<td>0 (R)</td>
<td></td>
</tr>
<tr>
<td>MeOH leaf extract</td>
<td>8</td>
<td>0 (R)</td>
<td></td>
</tr>
<tr>
<td>MeOH stem bark extract</td>
<td>8</td>
<td>9 (R)</td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td>10</td>
<td>25 (S)</td>
<td>25 (S)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>30</td>
<td>28 (S)</td>
<td>28 (S)</td>
</tr>
<tr>
<td>Chloroform</td>
<td>0</td>
<td>0 (R)</td>
<td></td>
</tr>
<tr>
<td>DMSO</td>
<td>0</td>
<td>0 (R)</td>
<td></td>
</tr>
</tbody>
</table>

\(^{1}\) Tested using disk-diffusion assay;  
\(^{2}\) Tested using agar-well diffusion assay; and  
S = susceptible, I = intermediate susceptibility, and R = resistant (CLSI, 2007).  

**Biofilm microtitre plate assays**

In the face of increasing incidence of antimicrobial resistance and recalcitrance of bacteria to current antimicrobial therapy, drug discovery research is focusing on limiting the pathogenicity mechanisms demonstrated by bacteria. Given the focus on biofilms and their role in microbial pathogenicity, screening for the anti-biofilm activity of phytochemicals is imperative in identifying alternative therapeutic options. The anti-biofilm activity of lupeol acetate 1; 5,7,4'-trihydroxyflavan-3-ol 4; epicatechin 5 and isovitexin 6 against *E. coli* ATCC 29922, *E. coli* ATCC 35218, *S. aureus* ATCC 29213 and *S. aureus* ATCC 43300 was variable. Lupeol acetate (triterpene), exposures resulted in statistically significant increased adhesion for all strains (Fig. 2a) at all concentrations tested (2 – 15 mg/ml).

Epicatechin 5 exposures decreased adhesion of antimicrobial-susceptible *E. coli* ATCC 25292 and *S. aureus* ATCC 29213 at all concentrations tested (1 – 7.5 mg mL\(^{-1}\)), with 5 mg mL\(^{-1}\) being the most effective (Fig. 2b) indicating that epicatechin has the ability to interfere with the adhesion ability of these microorganisms to polystyrene surfaces. El-Adawi (2012) obtained a 55-66% decrease in *Streptococcus mutans* biofilm formation upon exposure to 2-15% epicatechin. Previous reports have suggested that epicatechin was unable to inhibit biofilm formation as observed for *Eikenella corrodens*, a periodontopathogenic bacterium (Matsunaga et al., 2010) and for *P. aeruginosa* PAO1 biofilm formation after 18 h of growth (Vandeputte et al., 2010). Similarly, Nyila et al. (2012) observed that epicatechin from *Acacia karroo* did not reduce *Listeria monocytogenes* biofilms. The observed anti-biofilm activity of epicatechin was, however, strain-specific as adhesion of β-lactam resistant *E. coli* ATCC 35218 and methicillin-resistant *S. aureus* ATCC 43300 was increased at all concentrations tested (Fig. 2b). Biofilm formation of *P. aeruginosa* PAO1 was enhanced 3- to 7-fold under the action of epicatechin. Epicatechin caused an up to 5-fold enhancement of the adhesion of *S. aureus* PAO1 biofilm formation when compared with *S. aureus* ATCC 29213 at all concentrations tested (Fig. 2c).

574'-trihydroxyflavan-3-ol 4 (1 – 2.5 mg mL\(^{-1}\)) and isovitexin 6 (0.2 – 0.5 mg mL\(^{-1}\)) exposures also decreased adhesion of methicillin-susceptible *S. aureus* ATCC 29213 (Figs. 2c - 2d). Exposure to 2.5 mg mL\(^{-1}\) of 4 almost completely inhibited adhesion of *S. aureus* ATCC 29213 and decreased adhesion of β-lactam susceptible *E. coli* ATCC 25922 (Fig. 2c). No previous studies have examined the effect of lupeol acetate, 5,7,4'-trihydroxyflavan-3-ol 4 and isovitexin 6 on microbial adhesion. The anti-adhesion activity of 4, 5 and 6 was strain-specific, being most effective against *S. aureus* ATCC 29213, while lupeol acetate (1) increased adhesion of all *E. coli* and *S. aureus* strains tested.
The phytochemical investigation of *Ficus sansibarica* resulted in the isolation of three triterpenes and three flavonoids. The antimicrobial activity of the crude extracts and the isolated compounds were weak with a narrow-spectrum activity against Gram-positive bacteria. Most phytochemicals work best against Gram-positive bacteria and there are very limited small-molecule plant-derived antimicrobial compounds with high activity against Gram-negative species. The anti-biofilm activity demonstrated by the flavonoids 5,7,4′-trihydroxyflavan-3-ol, epicatechin and isovitexin (4-6), is most significant for *S. aureus* ATCC 29213. The results of this study provide baseline information on *F. sansibarica* potential validity in the treatment of Gram-positive biofilm-associated infections, despite its limited antimicrobial potential.

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**References**


