



Antimicrobial flavonoids and diterpenoids from *Dodonaea angustifolia*



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ABSTRACT

The surface exudates of the leaves of *Dodonaea angustifolia* from Ngong forest population (6 km from Nairobi city center, Kenya) demonstrated antimicrobial activity against Gram-negative (*Escherichia coli*), Gram-positive (*Staphylococcus aureus* and *Bacillus pumilus*) bacteria and the fungus *Saccharomyces cerevisiae*. Chromatographic separation of the exudates yielded eight methylated flavonoids; 5-hydroxy-3, 4',7-trimethoxyflavone (**1**), 3,5-dihydroxy-4',7-dimethoxyflavone (**2**), santin (**3**), kumatakenin (**4**), rhamnocitrin (**5**), isokaempferide (**6**), 3,4',5,7, tetrahydroxy-6-methoxyflavone (**7**), pinocembrin (**8**); two clerodanes, dodonic acid (**9**) and 2 β -hydroxyhardwickiic acid (**10**) and one labdane; (ent-3 β ,8 α)-15,16-epoxy-13(16),14-labdadiene-3,8-diol (**11**) diterpenoids. The flavonoid aglycones; **6**, **7** and the clerodane diterpenoids; **9** and **10** and labdane diterpenoid, **11** were isolated for the first time from this plant species. The structures of the isolated compounds were identified using ultraviolet (UV), mass spectroscopy (MS), one dimension (1D) and two dimension (2D) nuclear magnetic resonance (NMR) spectroscopy and by comparison of the spectral data with literature. The quercetin derivative, 3,4',5-trihydroxy-3',7-dimethoxyflavone (**12**) showed broad spectrum antibacterial activities against *E. coli* and *B. pumilus* with minimum inhibition concentration (MIC) values less than 31.25 μ g/well and against *S. aureus* with MIC below 62.5 μ g/well. This compound showed poor antifungal activity against *S. cerevisiae* (MIC < 500 μ g/well). Good antifungal activities were observed for 5,4'-dihydroxy-7-methoxyflavanone (**13**) and hautriwaic acid lactone (**14**) against *S. cerevisiae* with MIC values less than 7.8 μ g/well. The most active antifungal compound was 5,7-dihydro-3,4',6-trimethoxyflavone (**3**, santin) with an MIC value less than 3.9 μ g/well against *S. cerevisiae*. The rest of the compounds exhibited weak to moderate activities. For comprehensive structure activity relationship studies (SAR), hautriwaic acid lactone (**14**), hautriwaic acid (**15**), penduletin (**16**) isolated from the surface exudates of *D. angustifolia* from Voi (200 km from Mombasa city center, Kenya) and **12** and **13** from *Senecio roseiflorus* isolated earlier were included in the bioassays.

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1. Introduction

Dodonaea angustifolia L.f. (Sapindaceae) is a medium-sized shrub or small tree 0.5 to 7.5 m high with characteristic glossy green leaves covered by sticky surface exudates. The new leaves are stickier than the old ones which have characteristic rough, and sand papery texture (Beentje, 1994). *D. angustifolia* is an extremely variable species throughout its natural range; in Australia, Africa, Asia and South America, but many distinctive populations have been described as separate species (Beentje, 1994); in Kenya it is reported to exist along with *Dodonaea viscosa* (Beentje, 1994). *D. angustifolia* is used in traditional medicine to treat a number of ailments including tuberculosis and pneumonia (Watt and Breyer-Brandwijk, 1962; Cano et al., 1980). The leaf surface exudates (up to 13% dry leaf weight) of *D. angustifolia* constitute of mainly

methylated flavonoids in a clerodane and labdane diterpenoid milieu (Ghisalberti, 1998). There is great geographical variability in the composition for this substance both in quality and yields of each component in *Dodonaea* populations as observed from their thin layer chromatography (TLC) profiles. This created the interest to study the phytochemistry of *D. angustifolia* from Ngong forest to compare with the Voi population investigated earlier.

Previous phytochemical investigations have shown that lipophilic flavonoids, with structural features akin to those isolated from *D. angustifolia* surface exudates in this study, display antimicrobial activity due to their ability to penetrate biological membranes (Harborne, 1983). It was also suggested that the hydroxyl groups on flavonoids may interact with biological structures through hydrogen bonding, and that the relative positions of the hydroxyl group on the flavone skeleton is important in determining antimicrobial activity (McClure, 1975). Here the antimicrobial activities of the exudates and constituents from *D. angustifolia* collected from Ngong forest, Kenya is reported.

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2. Materials and methods

2.1. General experimental procedures

Column chromatography was carried out using Merck silica gel 40 (70–230 mesh) and Sephadex LH-20. Analytical TLC and preparative TLC were done using Merck pre-coated 60 F₂₅₄ and Merck 60 PF₂₅₄ respectively. ¹H NMR (500 MHz) and ¹³C NMR (125 MHz) were run on AVANCE-500 (Bruker) machine. Heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond correlation (HMBC) spectra were acquired using standard Bruker software. Electron Ionization Mass Spectroscopy (EIMS) spectra were recorded on 70 eV, on SSQ 710 MAT mass spectrometer. UV values were obtained using SP8 150 ultraviolet visible (UV/VIS) spectrophotometer. Melting points were recorded using a Gallenkamp melting point apparatus with capillary tubes. Inhibition zone diameters were read using a Wezu electronic digital caliper (Messzengge GmbH, Germany).

2.2. Plant collection and identification

The fresh leaves of *D. angustifolia* were collected from Ngong forest (6 km from Nairobi city center) in December, 2010. The plant material was identified by Mr. S.G. Mathenge of the University of Nairobi Herbarium, School of Biological Sciences (SBS), where voucher specimen (Mathenge-012/December, 2010) is deposited.

2.3. Extraction and isolation

Extraction of the surface exudates of the leaves of *D. angustifolia* from Ngong forest (450 g), was done by successive dipping fresh aerial parts into fresh portions of acetone for short periods (less than 15 s) thus avoiding the extraction of the internal tissue components. The extracts obtained were filtered under pressure and concentrated *in vacuo* using a rotary evaporator to yield 52 g of crude extract. A portion of the crude extract (45 g) was dissolved in 2% dichloromethane (CH₂Cl₂) in methanol (MeOH) and adsorbed on silica gel (45 g). The adsorbed silica gel was loaded onto a column packed with silica gel (450 g) under 50% CH₂Cl₂ in normal hexane (*n*-C₆H₁₂). Separation was carried out by stepwise gradient elution with mixtures of CH₂Cl₂ in *n*-C₆H₁₂ and then with CH₂Cl₂ containing increasing amounts of MeOH. The total number of fractions collected in the main column was 20 of 200 ml each which was subsequently combined based on the similarities of their TLC profiles (50% CH₂Cl₂ in *n*-C₆H₁₂ and 5% MeOH in CH₂Cl₂) into only 7 fractions. Yellow amorphous solids of 3,5-dihydroxy-4',7-dimethoxyflavone (**2**, 30 mg) precipitated out of the fraction eluted with 50% CH₂Cl₂ in *n*-C₆H₁₂. The solids were filtered, dried and weighed. The fraction eluted with 60% CH₂Cl₂ in *n*-C₆H₁₂ was purified further by column chromatography using silica gel, eluting with increasing gradient of CH₂Cl₂ in *n*-C₆H₁₂ up to 100% and then MeOH in CH₂Cl₂ up to 5%. The eluants were collected in 100 ml Erlenmeyer flasks leading to 20 fractions. TLC (2% MeOH in CH₂Cl₂) analysis of fraction 6–13 of this minor column showed similar profiles and therefore were combined. Removal of the solvent of the combined fractions and recrystallization (80% CH₂Cl₂ in *n*-C₆H₁₂) afforded yellow needles of 5-hydroxy-3,4',7-trimethoxyflavone (**1**, 204 mg). Santin (**3**, 330 mg) precipitated out of the fraction eluted with 90% CH₂Cl₂ in *n*-C₆H₁₂. The solids were filtered, dried and weighed. Purification of the mother liquor of the above fractions using PTLC (silica gel, 100% CH₂Cl₂ multiple development) afforded (*ent*-3β,8α)-15,16-epoxy-13(16),14-labdadiene-3,8-diol (**11**, 10 mg). The fraction eluting with 1% MeOH in CH₂Cl₂ after column chromatography on Sephadex LH 20 (MeOH in CH₂Cl₂; 1:1) resulted to 2 minor fractions of 100 ml of each. Fraction 1 after filtration yielded white crystals of dodonic acid (**9**, 500 mg), while that eluting with 2% MeOH in CH₂Cl₂ after column chromatography on Sephadex LH 20 (MeOH in CH₂Cl₂; 1:1) gave pinocembrin (**8**, 120 mg) in the third eluant of 100 ml each. The fraction eluted with 3% MeOH in CH₂Cl₂ afforded

kumatakenin (**4**, 200 mg) from fractions 5–9 and rhamnocitrin (**13**, 184 mg) from fractions 12–15 (100 ml each) of a silica gel column eluted with increasing gradient of CH₂Cl₂ in *n*-C₆H₁₂ and then MeOH in CH₂Cl₂. The fraction eluted with 4% MeOH in CH₂Cl₂ after purification using column chromatography on Sephadex LH 20 (MeOH in CH₂Cl₂; 1:1) afforded 2β-hydroxyhardwickiic acid (**10**, 778 mg), rhamnocitrin (**5**, 60 mg) and isokaempferide (**6**, 40 mg) in the first, second and third fractions (100 ml each) respectively. The TLC analysis of the fraction eluted with 5% MeOH in CH₂Cl₂ indicated only two spots, one of which was minor and therefore was subjected to PTLC (silica gel, 100 ml of 2% MeOH in CH₂Cl₂ multiple development) to yield 3,4',5,7-tetrahydroxy-6-methoxyflavone (**7**, 60 mg).

2.4. In vitro antimicrobial assay

Evaluation of antimicrobial activity of extracts and pure compounds was accomplished using the agar well-diffusion method (Bauer et al., 1966). The extracts and pure compounds were tested for activity against three strains of bacteria; *Escherichia coli* (American Type Culture Collection, ATCC25922), *Staphylococcus aureus* (ATCC29737) and *Bacillus pumilus* (local strain) and a local strain of fungus, *Saccharomyces cerevisiae*. The bacterial test organisms were cultured on tryptone soya agar and the fungi on Saboraud's dextrose agar. The nutrient agar was inoculated uniformly with standardized test organisms. Reservoir wells were formed by cutting out cylindrical plugs from the solidified nutrient agar at equidistant points, using a sterile cork borer, to produce wells (diameter 5 mm, depth 2 mm). The wells were each filled with 50 μl of the stock solutions in dimethylsulfoxide (DMSO): 50 mg/ml (2500 μg/well) for plant extracts and 10 mg/ml (500 μg/well) for pure compounds. The standard drugs gentamicin 0.3 mg/ml (15 μg/well), nystatin 0.25 mg/ml (12.5 μg/well) used as the antibacterial and antifungal positive controls respectively, while the solvent, DMSO, used as the negative control were similarly introduced into their respective wells. For determination of minimum inhibition concentration (MIC) of the extract and pure compounds, serial dilution of the stock solution was carried out resulting in concentration range from 625–2500 μg/well for the extract and 3.9 to 500 μg/well for each compound. All determinations were carried out in triplicate. The inoculated petri-dishes with test solutions in wells were allowed to diffuse for 30 min before overnight (18 h) incubation at 37 °C and 25 °C for bacteria and fungi, respectively. The antimicrobial activity was recorded as the diameter (mm) of the clear circular zone of inhibition surrounding the agar well after incubation. The MICs of the test microorganisms was similarly determined by the agar well-diffusion method and is defined as the lowest concentrations of the compounds that visually showed no growth compared with growth in control wells.

3. Results and discussion

3.1. Structure elucidation

Chromatographic separation of surface exudates of the leaves of *D. angustifolia* led to isolation of flavonoids: 5-hydroxy-3,4',7-trimethoxyflavone (**1**) (Dreyer, 1978), 3,5-dihydroxy-4',7-dimethoxyflavone (**2**) (Dreyer, 1978), 5,7-dihydro-3,4',6-trimethoxyflavone (**3**, santin) (Abdel-Mogib et al., 2001), 4',5-dihydroxy-3,7-dimethoxyflavone (**4**, kumatakenin) (Sarmiento da Silva, 2002), 3,4',5-trihydroxy-7-methoxyflavone (**5**, rhamnocitrin) (Valant-Vetschera et al., 2003), 4',5,7-trihydroxy-3-methoxyflavone (**6**, isokaempferide) (Dreyer, 1978), 3,4',5,7-tetrahydroxy-6-methoxyflavone (**7**) (Valant-Vetschera et al., 2003), 5,7-dihydroxyflavanone (**8**, pinocembrin) (Sachdev and Kulshreshtha, 1983) and diterpenoids: dodonic acid (**9**) (Sachdev and Kulshreshtha, 1984), 2β-hydroxyhardwickiic acid (**10**) (Jefferies et al., 1973; Anis et al., 2001) and (*ent*-3β,8α)-15,16-epoxy-13(16),14-labdadiene-3,8-diol (**11**) (Dawson et al., 1966) (Fig. 1). Five of the compounds, two flavonoids (**6**, **7**) and three diterpenoids (**9**, **10** and **11**) are

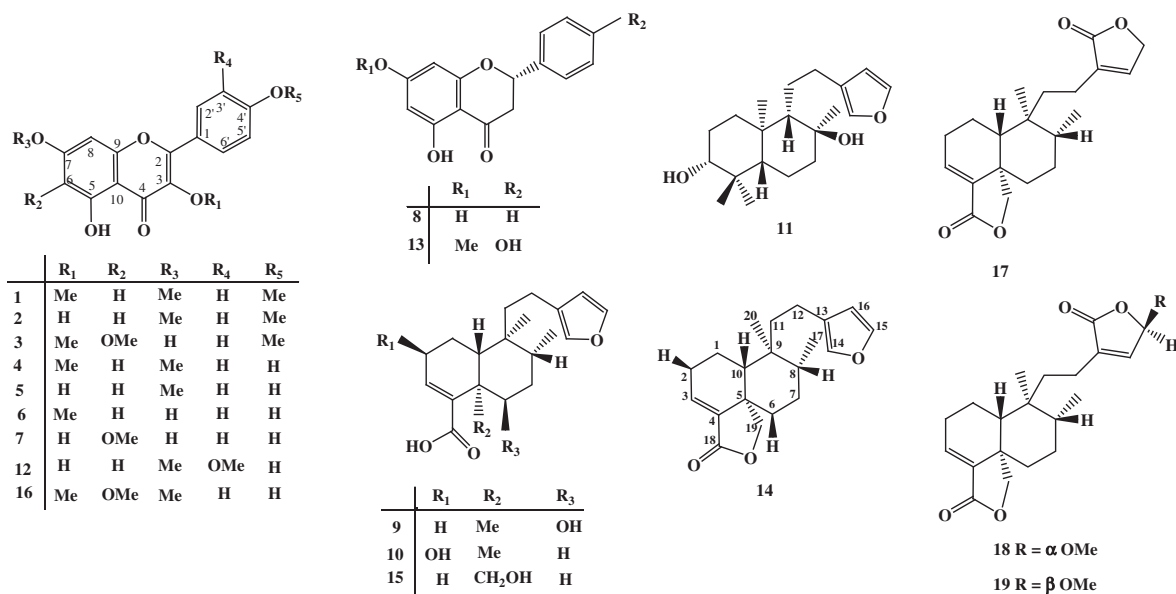


Fig. 1. Surface exudates compounds from *D. angustifolia* and *S. roseiflorus*.

reported for the first time from this species. The structures of these compounds were identified using UV, MS, IR and 2D NMR spectroscopy and by comparison of the spectral data with literature in parenthesis. The identification of the most active compounds is presented below.

The ^1H (δ_{H} 12.13, for chelated OH), ^{13}C (δ_{C} 148.0 for C-2, 136.3 for C-3, 178.2 for C-4) NMR and mass (M^+ m/z 330, $\text{C}_{17}\text{H}_{14}\text{O}_7$) spectral data of compound **12** were consistent with a 5-hydroxyflavone skeleton (Mabry et al., 1970; Agrawal, 1989). The ^1H NMR indicated the presence of two *meta* coupled aromatic protons at δ_{H} 6.72 and 6.33 ($d, J = 2.0$ Hz) assigned to H-6 and H-8 of ring A; whereas an ABX spin system at δ_{H} 7.92 ($d, J = 2.0$ Hz), 7.85 ($dd, J = 2.1$ and 8.7 Hz) and 7.02 ($d, J = 8.7$ Hz) were assigned to H-2', H-6' and H-5', respectively of a C-3' and C-4' oxygenated ring B. Furthermore, the ^1H NMR also displayed peaks for two methoxyl groups (δ_{H} 3.93 and 3.90) which were placed at C-7, C-3' and/or C-4' due to the fact that the ^{13}C NMR resonance value (δ_{C} 55.7), is typical of methoxyl groups which are not sterically crowded (methoxyl at C-3 is expected to appear above δ_{C} 59 due to steric crowding, resulting from *di-ortho* substitution). The placement of one of the methoxyl groups at C-7 was confirmed by the HMBC correlation between the methoxyl group at δ 3.90 and C-7 (δ 165.9). The nuclear overhauser effect (NOE) correlation between the second methoxy group (δ_{C} 3.93) with the signal at δ_{H} 7.92 (H-2') confirmed the placement of the second methoxy group at C-3' and hence the compound was characterized as 3,4',5-trihydroxy-3',-7-dimethoxyflavone (**12**). This compound under trivial name rhamnazin (**12**) has been isolated previously from the aerial parts *Grindelia nana* (Wollenweber et al., 1997) and *Polygonum punctatum* (Marin et al., 2001).

The ^1H and ^{13}C NMR spectra of compound **3** were similar to those of compound **12** except that ring B was mono-substituted as shown by the ^1H NMR spectrum which displayed an AA'XX' system resonating at δ_{H} 8.11 and 7.12 (each 2H, $d, J = 8.8$ Hz). A singlet at δ 6.60 (1H) was assigned to H-8 on the *tri*-substituted ring A. The ^1H NMR further displayed signals for three methoxyls at δ 3.91 3.87 and 3.86, two of which were *di-ortho* substituted (δ 60.0 and 59.6 as shown in the ^{13}C NMR spectrum). The location of one of the methoxyl group was assigned to C-4' due to NOE interaction between the aromatic protons at C-3'/C-5' (δ_{H} 7.12) with the methoxyl group at δ 3.91. The spectral data of this compound is in close agreement with what has been reported in literature (Barbera et al., 1986) for santin (**3**), especially the ^{13}C NMR for C-6, C-8 and C-9 showing identical ring A. Hence, the compound was identified as 5,7-dihydroxy-3,6,4'-trimethoxyflavone (**3**, santin) that was previously isolated from the leaf extract of three *Dodonaea* species

namely; *D. attenuata* var. *linearis* (Anis et al., 2001), *D. viscosa* (Abdel-Mogib et al., 2001) and *D. angustifolia* (Sachdev and Kulshreshtha, 1984).

The UV (λ_{max} 283.0 nm) (Mabry et al., 1970), ^1H [δ 12.02 for OH-5, 5.36 ($dd, J = 3.0$ and 13.0 Hz for H-2), 2.79 ($dd, J = 3.0$ and 17 Hz for H-3 eq) and δ 3.09 ($dd, J = 13.0$ and 17.0 Hz for H-3ax)] and ^{13}C (δ 79.2 for C-2, 43.4 for C-3 and 196.3 for C-4) NMR and mass (M^+ 286, $\text{C}_{16}\text{H}_{14}\text{O}_5$) spectra of compound **13** is consistent with a 5-hydroxyflavanone derivative (Agrawal, 1989). The ^1H NMR indicated the presence of two *meta* coupled aromatic protons at δ 6.07 and δ 6.06 ($d, J = 2.0$ Hz) which were assigned to H-6 and H-8 of a *di*-substituted (at C-5 and C-7) ring A. The ^1H NMR spectrum, showed the presence of an AA'XX' spin system centered at δ 6.89 (H-2'/H-6') and 7.34 (H-3'/H-5') consistent with 4'-substituted ring B. Furthermore, the ^1H NMR also displayed the presence of a methoxyl group at δ 3.81 which was located at C-7 as established from HMBC spectrum, which showed correlations between the methoxyl protons (δ_{H} 3.81) with C-7 (δ 164.4). The compound was therefore identified as 5,4'-dihydroxy-7-methoxyflavanone (**13**), a compound previously isolated from the aerial parts of *D. viscosa* (Mata et al., 1991).

The ^{13}C NMR attached proton test (APT) spectrum of compound **14** (m/z 332, $\text{C}_{20}\text{H}_{28}\text{O}_4$) corroborated the presence of two methyls, seven methylenes and six methines and five quaternary carbon atoms. The fragmentation peaks at m/z 95 and 81 suggested the presence of furan ring with an alkyl chain (Spanevello and Vila, 1994). These results indicated that compound **14** is a diterpene with a furan ring. The ^{13}C NMR spectrum exhibited signals at δ 19.1 and 16.7 due to tertiary and secondary methyl groups at C-9 and C-8, respectively, in agreement with the data of compounds having both of these substituents as alpha (α) on a *trans*-clerodane skeleton (San-Martin et al., 1986; Manabe and Nishino, 1986). The ^1H NMR spectrum of compound **14** displayed broad singlets at δ 6.28, 7.26 and 7.37 attributed to the H-14, H-16 and H-15 protons of the β substituted furan ring. The presence of an α, β -unsaturated γ -lactone moiety is evident in this compound from the ^1H NMR signals at δ 6.63 ($dd, J = 7.4, 2.0$ Hz) the olefinic β -protons, δ 4.30 ($d, J = 8.1$ Hz) and 3.92 ($dd, J = 8.0, 2.0$ Hz) for oxymethylenes at C-19. The corresponding carbons in the ^{13}C NMR for the lactone moiety appeared at δ 169.3 for C=O; δ 71.7 for the oxymethylene and the olefinic carbons resonated at δ 135.8, 138.4. The methylene protons at C-19 had an AB spin system. The *pro*-19S diastereotopic proton of this group (δ 3.92) was also ω -coupled ($^4J = 2.0$ Hz) with the H-6 β proton, indicating an α -axial orientation for C-19 (Bruno et al., 1981; Esquivel et al., 1986; Stapel, 1980). In the ^1H NMR the *pro*-19R proton resonated

at δ 4.30 which is in agreement with the lack of a substituent at C-7 position in this compound (Herz, 1977; Zdero et al., 1989; Esquivel et al., 1988). In addition, a three proton doublet at δ 0.87 ($J = 6.6$ Hz) was attributed to the secondary methyl and a three proton singlet at δ 0.79 attributed to the tertiary methyl group typical of clerodane-type diterpenes. The correlation spectroscopy (COSY) experiment showed coupling between the methyl at δ 0.87 and the H-8 proton at δ 1.63. Furthermore, the COSY experiment showed coupling between the protons at δ 6.28 and δ 2.42 assigned to H-12 and between the proton at δ 7.26 and the H-12 methylene protons at δ 2.42 and δ 2.20. There were also cross peaks from the protons at δ 6.28, 7.26 and 7.34. The structure of **14** was confirmed from the HMBC experiment, with the olefinic proton at δ 6.63 showing correlations to C-4 (δ 138.9), C-5 (43.3), C-2 (δ 27.7). Similarly, the proton at δ 6.28 assigned to H-14 showed cross peak correlations to the C-13 (δ 126.7), C-15 (δ 144.0), C-16 (δ 139.7) and C-12 (δ 18.1*/ δ 19.1*). The relative configuration was established on the basis of nuclear overhauser and exchange spectroscopy (NOESY) cross peaks observed between H-20/H-17 and H-20/H-19 (the two protons). However, there were no cross peaks between H-20/H17/H-19 (the two protons) and H-10. These results can be rationalized only if C-20, C-17, C-19 are on the same face of the molecule and H-10 on another face of the molecule. All the data are in agreement with compound **14** being hautriwaic acid lactone previously isolated from *D. viscosa* (Hsu et al., 1971).

3.2. Bioactivity analysis

The exudates of *D. angustifolia* exhibited antimicrobial activity against Gram-negative (*E. coli*), Gram-positive (*S. aureus* and *B. pumilus*) bacteria and the fungus *S. cerevisiae* (Table 1). The isolated compounds were also tested and showed varied antimicrobial activities against the Gram-positive bacteria *S. aureus*, *B. pumilus* and the fungus *S. cerevisiae* but were inactive against the Gram negative bacteria *E. coli*. Interestingly, the flavonoid 3,4',5-trihydroxy-3',7'-dimethoxyflavone (**12**) isolated from the surface exudates of *Senecio roseiflorus* (Omosa et al., 2013) showed good activity against *E. coli* with MIC < 31.25 $\mu\text{g}/\text{well}$. Rhamnocitrin (**5**) with a similar oxygenation pattern; except for the absence of a methoxy group at 3' position; was inactive, indicating the importance of a 3'-methoxy (OMe) group for activity against *E. coli* in this type of flavonoids. All the 3-methoxyflavones tested; **1**, **3**, **4** and **15** (Omosa et al., 2010) were inactive against *S. aureus* even at the highest concentration tested, 500 $\mu\text{g}/\text{well}$. For good activity against *S. aureus*, hydroxyl group at C-3 position in the flavone skeleton, as encountered in compounds **5** and **12**, which exhibited activity with MIC < 62.5 $\mu\text{g}/\text{well}$, seems to be important. Santin (**3**) was the most active flavone against *S. cerevisiae* with an MIC < 3.9 $\mu\text{g}/\text{well}$ while penduletin (**16**) was less active with an MIC < 125 $\mu\text{g}/\text{well}$. The presence of 5,7-dihydroxy substitution seems to substantially improve activity of flavones against the fungus *S. cerevisiae*. This is evident from the observation that compound **7**, having 5,7-dihydroxy substituents, was more active than compound **16** with 5,4'-dihydroxy substituents. The presence of two hydroxyl groups and a minimum of two methoxy groups, appears to be necessary for good activity while complete methylation, even if C-5 hydroxy is free, negates activity as encountered in 5-hydroxy-3,7,4'-trimethoxyflavone (**1**).

Compared to the antibacterial flavones, the antifungal compounds tend to be more lipophilic (McClure, 1975). The flavanone (**13**) and hautriwaic acid lactone (**14**) showed good antifungal activity against *S. cerevisiae* with an MIC < 7.8 $\mu\text{g}/\text{well}$, almost as active as santin (**3**) the most active compound, probably due to their lipophilic nature. The other diterpenoids, dodonic acid (**9**), 2 β -hydroxyhardwickiic acid (**10**) and hautriwaic acid (**15**) were less active with MIC < 125 $\mu\text{g}/\text{ml}$ and < 62.5 $\mu\text{g}/\text{ml}$, respectively.

The three diterpenoids **9**, **10** and **15** have the same carbon skeleton and one hydroxyl substituent each, but differ in the position of this group around the ring; the hydroxyl group being at C-6, C-2, and C-19

Table 1

MIC in $\mu\text{g}/\text{well}$ and inhibition zones (mm) of the surface exudates and compounds from *D. angustifolia* against Gram-negative (*E. coli*), Gram-positive (*S. aureus*, *B. pumilus*) bacteria and *S. cerevisiae* fungus.

Sample	MIC values $\mu\text{g}/\text{well}$	Inhibition zone			
		<i>E. coli</i>	<i>S. aureus</i>	<i>B. pumilus</i>	<i>S. cerevisiae</i>
Crude extracts					
Surface exudates of <i>D. angustifolia</i> (Ngong forest)	2500	18.86 ^a	20.05 ^a	19.42 ^a	10.79 ^a
	1250	13.56	14.45	13.82	8.07
	625	8.97	9.32	9.27	–
Compounds					
3-Methoxy flavones					
5-Hydroxy-3,7,4'-trimethoxyflavone (1)	500	–	–	–	–
Kumatakenin (4)	500	–	–	–	–
Santin (3)	250	–	–	9.89	11.89
	125	–	–	–	11.60
	62.50	–	–	–	11.22
	31.25	–	–	–	11.15
	15.6	–	–	–	10.88
	7.8	–	–	–	9.94
	3.9	–	–	–	8.95
Penduletin (16)	500	–	–	9.38	11.50
	250	–	–	–	10.99
	125	–	–	–	9.72
Flavonols					
Rhamnocitrin (5)	125	–	11.40	11.73	–
	62.50	–	10.80	–	–
Quercetin-3',7'-dimethyl ether (12)	500	12.76	11.72	10.57	10.8
	250	12.47	9.68	9.72	–
	125	11.97	9.58	9.42	–
	62.50	10.66	9.37	9.20	–
	31.25	9.08	–	8.97	–
Flavanones					
5,4'-Dihydroxy-7-methoxyflavanone (8)	125	–	12.04	10.18	12.04
	62.50	–	11.32	–	11.48
	31.25	–	–	–	10.59
	15.6	–	–	–	9.96
	7.8	–	–	–	9.52
Pinocembrin (14)	62.50	–	9.49	–	11.92
	31.25	–	–	–	9.34
Clerodane Diterpenoids					
Dodonic acid (9)	250	–	11.13	10.71	10.90
	125	–	–	–	10.40
	62.50	–	–	–	10.10
2 β -hydroxyhardwickiic acid (10)	250	–	10.17	10.47	10.82
	125	–	–	–	10.80
Hautriwaic acid lactone (9)	31.25	–	–	–	10.34
	15.6	–	–	–	9.86
	7.8	–	–	–	8.93
Hautriwaic acid (15)	500	–	11.77	12.27	9.65
	250	–	9.93	11.07	–
	125	–	–	9.48	–
Gentamicin	15	12.39	24.74	30.34	–
Nystatin	12.5	–	–	–	25.6

“–” not active.

^a Inhibition zone in mm.

in compounds **9**, **10** and **15**, respectively. The activities of these diterpenoids are dependent on the position of the hydroxyl group, *vis a vis* OH-6 (**9**) > OH-2 (**10**) > OH-19 (**15**). Compound **1** and **4** exhibited no antimicrobial activity against the three strains of bacterial and one fungal strain. Santin (**3**) and hautriwaic acid lactone (**9**) which showed good antifungal activity with MIC values < 3.9 and 7.8 $\mu\text{g}/\text{well}$ respectively exhibited poor antibacterial activity with MIC value > 250 $\mu\text{g}/\text{well}$. The results on antibacterial activities of compounds **1** and **3** are consistent with those reported by Teffo et al. (2010). In general no marked changes in inhibition zones were observed relative to dilution. The compounds that were isolated in small quantities were not tested for activity.

The phytochemistry of the two populations of *D. angustifolia* (Ngong forest and Voi) studied are closely related as they both elaborate the same classes of compounds; flavonoids (mainly kaempferol methyl ethers and clerodane and labdane terpenoids). However, the two populations in Kenya have different sets of these compounds. Only four flavonoids; **1**, **2**, **4** and **5** out of sixteen flavonoids isolated from the two populations are shared between them (Omosa et al., 2010). The diterpenoid profile of *D. angustifolia* (Ngong forest) consists of dodonic acid (**9**), 2 β -hydroxyhardwickiic acid (**10**) and 15,16-epoxy-13(16), 14-labdadiene-3, 8-diol; *ent*-3 β , 8 α form (**11**) while hautriwaic acid (**15**), *neoclerodan*-3,13-dien-16,15: 18,19-diolide (**17**), 15 α -methoxy-*neoclerodan*-3,13-dien-16,15: 18,19-diolide (**18**), 15 β -methoxy-*neoclerodan*-3,13-dien-16,15: 18,19-diolide (**19**) were identified from the Voi population. Compounds **9**, **10** and **11** could serve as markers for *D. angustifolia* from Ngong Forest while hautriwaic acid (**15**) and the three clerodane diterpenes (**17**, **18** and **19**) could also serve as markers for *D. angustifolia* species from Voi. The two populations of *D. angustifolia* could be presenting two different chemotypes, however, high performance TLC and high performance liquid chromatography (HPLC) profiles of these populations and other populations in Kenya need to be determined before a definite conclusion can be made.

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