Phytochemistry 70 (2009) 403–406

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

# Dibenzofuran and pyranone metabolites from *Hypericum revolutum* ssp. *revolutum* and *Hypericum choisianum*

Winnie Ka Po Shiu, Simon Gibbons *

*Centre for Pharmacognosy and Phytotherapy, The School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1N 1AX, UK*

ARTICLE INFO

*Article history:*
Received 3 October 2008
Received in revised form 8 December 2008
Available online 4 February 2009

*Keywords:*
*Hypericum revolutum*
*Hypericum choisianum*
Guttiferae
Dibenzofuran
Pyranone
Pyran-2-one
Antibacterial
MRSA
*Staphylococcus aureus*
MDR

ABSTRACT

In a project to isolate and characterise anti-staphylococcal compounds from members of the genus *Hypericum*, a dibenzofuran and a pyranone were isolated from the dichloromethane and hexane extracts of *Hypericum revolutum* ssp. *revolutum* Vahl (Guttiferae) and *Hypericum choisianum* Wall. ex. N. Robson (Guttiferae), respectively. The structures of these compounds were elucidated by 1- and 2D-NMR spectroscopy and mass spectrometry as 3-hydroxy-1,4,7-trimethoxydibenzofuran (**1**) and 4-(3-*O*-3″)-3″-methylbutenyl-6-phenyl-pyran-2-one (**2**). The metabolites were evaluated against a panel of multi-drug-resistant strains of *Staphylococcus aureus*. Compound **1** exhibited a minimum inhibitory concentration (MIC) of 256 μg/ml, whereas compound **2** was inactive at a concentration of 512 μg/ml.



## 1. Introduction

*Hypericum* belongs to the Guttiferae family (alternative name Clusiaceae) and is a genus of approximately 450 species. The widespread interest in the use of *H. perforatum* (St. John's Wort) in mild to moderate depression has attracted much attention in investigating the bioactive metabolites from other species in this genus. Extracts from various *Hypericum* species have been shown to possess antibacterial (Rocha et al., 1995), anti-staphylococcal (Shiu and Gibbons, 2006), antiviral (Weber et al., 1994), anti-inflammatory (Trovato et al., 2001) and anti-oxidant (Conforti et al., 2002) activities. The most commonly isolated compounds from this genus include acylphloroglucinols (Gibbons et al., 2005; Shiu and Gibbons, 2006), xanthones (Bennett and Lee, 1989), flavonoids (Crockett et al., 2005), tannins (Ploss et al., 2001) and, less frequently, benzopyrans (Décosterd et al., 1986).

The anti-staphylococcal activity of 34 *Hypericum* species was studied previously by our group (Gibbons et al., 2002). In the preliminary study, 33 out of 34 chloroform extracts showed activity in a disk diffusion assay. The chloroform extract of *H. revolutum* ssp. *revolutum* Vahl exhibited a minimum inhibitory concentration (MIC) of 128 μg/ml against the methicillin-resistant *Staphylococcus aureus* (MRSA) strain, XU212. There is very little literature on this species although a paper by Kassu et al. (1999) refers to the use of *H. revolutum* as toothbrush sticks. This prompted us to carry out a large-scale plant collection and bioassay-guided isolation of antibacterial compounds from this plant. A new dibenzofuran (**1**) was isolated from *H. revolutum* ssp. *revolutum*. Previous phytochemistry of *H. choisianum* is limited to the characterisation of its essential oil content (Demirci et al., 2005), and we therefore investigated this species which afforded a new pyranone (**2**). The structural elucidation of these compounds is described in this paper. Extensive literature and internet searches indicate that the medicinal and traditional uses of these two species are unknown.

## 2. Results and discussion

Bioassay-guided fractionation of the dichloromethane (DCM) extract of the aerial parts of *H. revolutum* ssp. *revolutum* led to the isolation of (**1**) as a pale yellow oil. HRESI-MS suggested a molecular formula of $C_{15}H_{14}O_5$ $[M-H]^-$ (273.0763). In the $^{13}C$ NMR spectrum (Table 1), 12 signals in the aromatic region and three methoxyl signals were observed. The pattern of the aromatic carbon signals showed similarity to that of a xanthone nucleus, differing in the absence of a carbonyl signal in this compound. These data suggested that compound **1** was a dibenzofuran substituted with three methoxyl groups (Kokubun et al., 1995, 1995a). The

* Corresponding author. Tel.: +44 207 7535913; fax: +44 207 7535909.
*E-mail address:* simon.gibbons@pharmacy.ac.uk (S. Gibbons).

0031-9422/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2008.12.016

**Table 1**
$^1$H (500 MHz) and $^{13}$C NMR (125 MHz) spectral data and $^1$H–$^{13}$C long-range correlations of **1** recorded in $CDCl_3$.

| Position | $^1$H ($J$, Hz) | $^{13}$C | $^2J$ | $^3J$ |
|---|---|---|---|---|
| 1 | – | 143.9 | – | – |
| 2 | 6.25 s | 94.2 | C-1 | C-4, C-9b |
| 3 | – | 135.7 | – | – |
| 4 | – | 128.5 | – | – |
| 4a | – | 144.0 | – | – |
| 5a | – | 141.2 | – | – |
| 6 | 6.53 d (3) | 102.6 | C-5a, C-7 | C-8, C-9a |
| 7 | – | 155.9 | – | – |
| 8 | 6.47 dd (3, 8.5) | 109.1 | – | C-6, C-9a |
| 9 | 6.89 d (8.5) | 116.9 | C-9a | C-5a, C-7 |
| 9a | – | 135.9 | – | – |
| 9b | – | 126.2 | – | – |
| 1-$OCH_3$ | 3.83 s | 56.5 | – | C-1 |
| 4-$OCH_3$ | 3.92 s | 61.9 | – | C-4 |
| 7-$OCH_3$ | 3.76 s | 55.8 | – | C-7 |
| 3-OH | 5.42 bs | – | – | C-2, C-4 |

**Table 2**
$^1$H (500 MHz) and $^{13}$C NMR (125 MHz) spectral data and $^1$H–$^{13}$C long-range correlations of **2** recorded in $CDCl_3$.

| Position | $^1$H ($J$, Hz) | $^{13}$C | $^2J$ | $^3J$ |
|---|---|---|---|---|
| 1 | – | – | – | – |
| 2 | – | 161.7 | – | – |
| 3 | – | 163.9 | – | – |
| 4 | – | 99.3 | – | – |
| 5 | 6.42 s | 97.8 | C-4, C-6 | C-3, C-1′ |
| 6 | – | 160.1 | – | – |
| 1′ | – | 131.3 | – | – |
| 2′/6′ | 7.78 m | 125.6 | – | C-6, C-4′, C-6′/2′ |
| 3′/5′ | 7.43 m | 128.9 | C-4′ | C-1′, C-5′/3′ |
| 4′ | 7.42 m | 130.9 | – | C-2′/6′ |
| 1″ | 6.45 d (10) | 116.4 | – | C-3, C-3″ |
| 2″ | 5.42 d (10) | 125.5 | C-3″ | C-4 |
| 3″ | – | 80.2 | – | – |
| 4″ | 1.48 s | 28.6 | C-3″ | C-2″, C-5″ |
| 5″ | 1.48 s | 28.6 | C-3″ | C-2″, C-4″ |

$^1$H NMR spectrum (Table 1) showed signals for three aromatic hydrogens in an ABD system with resonances at $\delta_H$ 6.89 (d, $J$ = 8.5 Hz, H-9), $\delta_H$ 6.47 (dd, $J$ = 3, 8.5 Hz, H-8) and $\delta_H$ 6.53 (d, $J$ = 3 Hz, H-6), an aromatic hydrogen singlet ($\delta_H$ 6.25 s, H-2), a broad signal at $\delta_H$ 5.42 corresponding to an hydroxyl group and three methoxyl singlets ($\delta_H$ 3.92 s, 3.83 s, 3.76 s).

In the HMBC spectrum, correlations were seen between the methoxyl groups with resonances at $\delta_H$ 3.76 (C7–$OCH_3$), 3.83 (C1–$OCH_3$) and 3.92 (C4–$OCH_3$) and $\delta_C$ 155.9 (C-7), 143.9 (C-1) and 128.5 (C-4), respectively, confirming the chemical shifts of the carbons to which the methoxyl groups were attached. A cross-peak in the NOESY spectrum was observed between the methoxyl group at C-1 and the aromatic proton singlet (H-2), and the methoxyl group at C-4 and the hydroxyl group, thus placing them next to each other (Fig. 1). The hydrogen of the hydroxyl group showed a $^3J$ correlation with the carbon to which the aromatic hydrogen singlet was attached ($\delta_C$ 94.2, C-2) and to the carbon to which a methoxyl group was attached ($\delta_C$ 128.5, C-4). The hydroxyl group was placed between these groups in the same aromatic ring. The aromatic hydrogen (H-2) also displayed a $^3J$ HMBC correlation (Fig. 1) with C-9b ($\delta_C$ 126.2) but not to the oxygen-bearing carbon at $\delta_C$ 144.0 (C-4a), thus placing it at C-2 and not C-3.

For the other aromatic ring, a cross-peak could be seen between the methoxyl singlet at $\delta_H$ 3.76 (C7–$OCH_3$) and H-6 and H-8 in the NOESY spectrum (Fig. 1). This methoxyl group was therefore placed between the aromatic hydrogens in the ABD system. Since the *meta*- and *ortho*-coupled aromatic proton at $\delta_H$ 6.47 (H-8) showed a strong $^3J$ HMBC correlation with carbons at $\delta_C$ 135.9 (C-9a) but not $\delta_C$ 141.2 (C-10a), it could be implied that the methoxyl group was placed at C-7 and not C-8, which would also yield an ABD system. This completed the NMR assignment of compound **1**, which was characterised as the new natural product 3-hydroxy-1,4,7-trimethoxy-dibenzofuran. This compound was structurally related to a series of dibenzofurans, which are phytolexins isolated from *Mespilus* (Rosaceae) species (Kokubun et al., 1995, 1995a). To our knowledge, compound **1** is the first dibenzofuran to be isolated from the *Hypericum* genus to date.

Compound **2** was isolated as a yellow oil from the hexane extract of the aerial parts of *H. choisianum*. It showed an $[M + H]^+$ peak at $m/z$ 255.1021 in the HRESI-MS, which corresponded to a molecular formula of $C_{16}H_{14}O_3$. The $^1$H NMR spectrum (Table 2) revealed signals for five aromatic hydrogens in a mono-substituted benzene ring with resonances at $\delta_H$ 7.78 (m, 2H, H-2′, H-6′), $\delta_H$ 7.43 (m, 2H, H-3′, H-5′) and $\delta_H$ 7.42 (m, 1H, H-4′), an olefinic singlet ($\delta_H$ 6.42, H-5), two olefinic hydrogens with resonances at $\delta_H$ 6.45 (d, $J$ = 10 Hz, H-1″) and $\delta_H$ 5.42 (d, $J$ = 10 Hz, H-2″), and a methyl singlet integrating for six protons ($\delta_H$ 1.48 s, $H_3$-4″, $H_3$-5″). The $^{13}$C NMR spectrum (Table 2) displayed signals for three highly deshielded aromatic carbons ($\delta_C$ 161.7, C-2; 163.9, C-3; 160.1, C-6), two quaternary aromatic carbons at $\delta_C$ 131.3 (C-1′) and 99.3 (C-4), three aromatic methines belonging to the mono-substituted benzene ring ($\delta_C$ 125.6, C-2′, C-6′; 128.9, C-3′, C-5′; 130.9, C-4′), two olefinic methines ($\delta_C$ 116.4, C-1″; 125.5, C-2″), a further olefinic methine at $\delta_C$ 97.8 (C-5), a deshielded quaternary aliphatic carbon at $\delta_C$ 80.2 (C-3″) and two methyl carbons at $\delta_C$ 28.6 (C-4″, C-5″). The molecular formula determined by mass spectrometry suggested that the deshielded carbons were oxygen-bearing.

In the HMBC spectrum, a cross-peak was seen between the methyl singlet ($\delta_H$ 1.48, 6H, H-4″, H-5″) and a methyl carbon at $\delta_C$ 28.6 (C-4″, C-5″), indicating the presence of a *gem*-dimethyl moiety (Fig. 2). The methyl groups also correlated to the oxygen-bearing quaternary carbon at $\delta_C$ 80.2 (C-3″) and an olefinic methine carbon at $\delta_C$ 125.5 (C-2″). A correlation between the olefinic hydrogen at C-2″ ($\delta_H$ 5.42) and the neighbouring olefinic proton ($\delta_H$ 6.45, H-1″) was seen in the COSY spectrum. The coupling constant between the olefinic hydrogens was 10 Hz, implying that they were in a *cis*-configuration. H-1″ exhibited an HMBC correlation to a highly deshielded oxygen-bearing carbon at $\delta_C$ 163.9 (C-3),

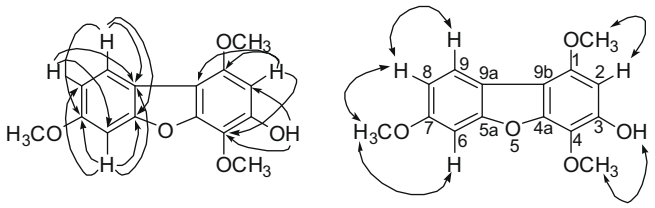


**Fig. 1.** Key HMBC (single headed arrows) and NOESY interactions (double headed arrows) correlations for **1**.

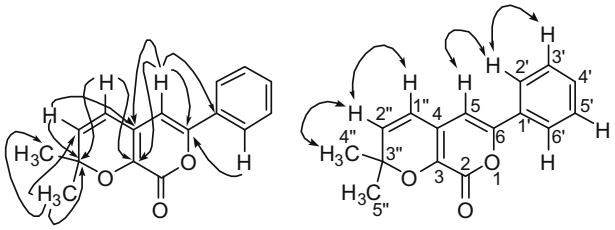


**Fig. 2.** Key HMBC (single headed arrows) and NOESY (double headed arrows) correlations for **2**.

whereas H-2″ showed an HMBC correlation to a quaternary aromatic carbon at $\delta_C$ 99.3 (C-4). These $^1$H and $^{13}$C NMR data were suggestive of the presence of a dimethylpyran ring (Seo et al., 1999).

Further inspection of the HMBC spectrum revealed correlations between the aromatic hydrogen singlet at $\delta_H$ 6.42 (H-5) and C-3, C-4, and two quaternary carbons at $\delta_C$ 160.1 (C-6) and 131.1 (C-1′), thus placing it at C-5 (Fig. 2). The presence of a mono-substituted phenyl group at C-6 was confirmed by investigating the HMBC spectrum. The hydrogen resonance at $\delta_H$ 7.42 (H-4′) was correlated to the equivalent carbons, C-2′ and C-6′, at $\delta_C$ 125.6 *via* three bonds. The two equivalent aromatic hydrogens at $\delta_H$ 7.43 (2H, H-3′, H-5′) showed a $^2J$ correlation to C-4′, and $^3J$ correlations to the corresponding equivalent carbon (C-5′, C-3′) and the substituent-bearing quaternary aromatic carbon ($\delta_C$ 131.3, C-1′). The remaining equivalent signals in the phenyl moiety ($\delta_H$ 7.78, 2H, H-2′, H-6′), showed $^3J$ correlations to the corresponding equivalent carbon ($\delta_C$ 125.6, C-6′, C-2′), C-4′, and to an oxygen-bearing carbon at $\delta_C$ 160.1 (C-6). Finally, the position of the phenyl substituent at C-6 was also confirmed by an NOE correlation between H-5 and H-2′/H-6′ (Fig. 2).

The remaining carbon ($\delta_C$ 160.1), which was not correlated to any hydrogen in the HMBC spectrum, was placed at C-2 to complete the structure of this compound, giving a pyran-2-one nucleus. The chemical shift of C-2 was typical of the carbonyl group in a pyran-2-one ring (Dharmaratne et al., 2002). Compound **2** was therefore identified as the new 4-(3-*O*-3″)-3″-methylbutenyl-6-phenyl-pyran-2-one, and is reported here for the first time.

Compounds **1** and **2** were assessed for antibacterial activity against a panel of *S. aureus* strains in an MIC assay (Table 3). Compound **1** was weakly active against the tested strains with an MIC value of 256 μg/ml against all strains. Unlike the control antibiotics, the antibacterial activity of compound **1** was constant against all the tested strains. This might imply that the mechanism of action of this compound was not affected by the multidrug-resistant mechanisms in these strains. A series of structurally similar dibenzofurans have been isolated from the sapwood of *Mespilus germanica* when challenged by *Nectria cinnabarina*, a coral spot fungus (Kokubun et al., 1995, 1995a). Those authors found that these dibenzofurans acted as the major phytoalexins and showed antifungal activity, with $ED_{50}$s in the range of 12–100 ppm. The antibacterial activity of these compounds has not been investigated, however. Compound **2** was inactive at a concentration of 512 μg/ml.

## 3. Experimental

### 3.1. General experimental procedures

NMR spectra were recorded on a Bruker AVANCE 500 MHz spectrometer. Chemical shifts values ($\delta$) were reported in parts per million (ppm) relative to the appropriate internal solvent standard and coupling constants ($J$ values) were given in Hz. IR spectra were recorded on a Nicolet 360 FT-IR spectrophotometer and UV spectra on a Thermo Electron Corporation Helios spectrophotometer. The specific rotation was measured on a Perkin–Elmer Polarimeter Model 343. Mass spectra were recorded on a Finnigan MAT 95 high resolution, double focusing, magnetic sector mass spectrometer. Accurate mass measurement was achieved using voltage scanning of the accelerating voltage. This was nominally 5 kV and an internal reference of heptacosa was used. Resolution was set between 5000 and 10,000.

**Table 3**
MICs of **1**, **2** and standard antibiotics in μg/ml.

| Strain (MDR efflux protein) | **1** | **2** | Norfloxacin | Tetracycline | Erythromycin | Oxacillin |
|---|---|---|---|---|---|---|
| ATCC 25923 | 256 | >512 | 1 | – | – | – |
| SA-1199B (NorA) | 256 | >512 | 32 | – | – | – |
| XU212 (TetK) | 256 | >512 | – | 128 | – | – |
| RN4220 (MsrA) | 256 | >512 | – | – | 256 | – |
| EMRSA-15 | 256 | >512 | – | – | – | 32 |
| EMRSA-16 | 256 | >512 | - | - | - | 512 |

All MICs were determined in duplicate.

### 3.2. Plant material

The aerial parts of *H. revolutum* ssp. *revolutum* (Accession no. 1972–3163) and *H. choisianum* (Accession no. 1977–4670) were collected from the Royal Botanic Garden at Wakehurst Place in Surrey, England in August 2003 and August 2005, respectively. Voucher specimens were deposited in the herbarium at the Centre for Pharmacognosy and Phytotherapy at the University of London School of Pharmacy.

### 3.3. Extraction and isolation

Air-dried and powdered aerial parts of *Hypericum revolutum* ssp. *revolutum* (495 g) were extracted in a Soxhlet apparatus sequentially with hexane (3 l), dichloromethane (3 l) and methanol (3 l). The DCM extract (1.8 g) was subjected to LH-20 Sephadex chromatography, affording five fractions eluted with chloroform:methanol (1:1) and one fraction with a final methanol wash. Fractions five and six were combined and further separated on a LH-20 Sephadex column, giving eight fractions eluted with chloroform:methanol (1:1) and one fraction eluted with methanol. The fraction eluted with methanol was purified by preparative thin-layer chromatography (silica) using toluene-ethyl acetate-acetic acid (60:38:2), yielding compound **1** (4.5 mg; $R_f$ = 0.58).

1.0 kg of air-dried, powered aerial parts of *H. choisianum* was extracted in a Soxhlet apparatus using sequential extraction as described above. The hexane extract (9.9 g) was subjected to vacuum liquid chromatography (VLC) on silica gel eluting with hexane containing 10% increments of ethyl acetate to yield 12 fractions. Fractions 5–7 showed similar TLC profiles and were combined (1.1 g) and this was further separated using LH-20 Sephadex chromatography. This resulted in seven fractions eluted with chloroform:methanol (1:1) and one fraction with a final methanol wash. Fractions 7 and 8 were combined (332.1 mg) and purified further by preparative reverse phase HPLC (Waters Prep $C_{18}$ column, 19 × 300 mm, 10 μm) using a gradient of 5% acetonitrile in water to 100% acetonitrile over 30 min. The flow rate was 50 ml/min. Compound **2** (5.0 mg) had a retention time of 13.0 min.

VLC fraction 4 was subjected to reverse phase solid phase extraction (SPE; Phenomenex Strata, $C_{18}$, 10 g/60 ml Giga tubes), affording three fractions eluted with methanol and one fraction eluted with acetone. The first SPE fraction was further fractionated using reverse phase SPE, giving five fractions eluted with 80% acetonitrile in water and one fraction eluted with 100% acetone as a final wash. SPE fraction 1 was purified using preparative TLC (silica) with toluene-ethyl acetate-acetic acid (80:18:2), yielding compound **2** (7.2 mg, $R_f$ = 0.59). A total of 12.2 mg of compound **2** was isolated using the two methods.

### 3.4. Bacterial strains

*S. aureus* standard strain ATCC 25923 and tetracycline-resistant strain XU212 which possesses the TetK tetracycline efflux protein were provided by (Gibbons and Udo (2000)). Strain SA-1199B

which overexpresses the norA gene encoding the NorA MDR efflux pump was provided by Kaatz et al. (1993). Strain RN4220 which possess the MsrA macrolide efflux protein was provided by (Ross et al. (1989). The epidemic strains EMRSA-15 and EMRSA-16 were provided by Richardson and Reith (1993) and Cox et al. (1995).

### 3.5. Minimum inhibitory concentration (MIC) assay

All strains were cultured on nutrient agar (Oxoid) and incubated for 24 h at 37 °C prior to MIC determination. Control antibiotics norflorxacin, tetracycline, erythromycin and oxacillin were obtained from Sigma Chemical Co. Mueller–Hinton broth (MHB; Oxoid) was adjusted to contain 20 and 10 mg/l of $Ca^{2+}$ and $Mg^{2+}$, respectively. An inoculum density of $5 \times 10^5$ cfu of each *S. aureus* strain was prepared in normal saline (9 g/l) by comparison with a 0.5 MacFarland turbidity standard. The inoculum (125 μl) was added to all wells and the microtitre plate was incubated at 37 °C for 18 h. For MIC determination, 20 μl of a 5 mg/ml methanolic solution of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT; Sigma) was added to each of the wells and incubated for 20 min. Bacterial growth was indicated by a colour change from yellow to dark blue. The MIC was recorded as the lowest concentration at which no growth was observed (Gibbons and Udo, 2000).

### 3.6. 3-hydroxy-1,4,7-trimethoxydibenzofuran (**1**)

Pale yellow oil; $[a]_D^{22}$ 0° (*c* 0.19, $CHCl_3$); UV ($CHCl_3$) $\lambda_{max}$ (log $\varepsilon$): 240 (3.87) nm; IR $\nu_{max}$ (thin film) $cm^{-1}$: 3412, 1504, 1472, 1264, 1208, 1155; $^1H$ NMR and $^{13}C$ NMR ($CDCl_3$): see Table 1; HR ESI-MS (*m*/*z*): 273.0763 $[M-H]^-$ (calc. for $C_{15}H_{14}O_5$, 273.0768).

### 3.7. 4-(3-O-3″)-3″-methylbutenyl-6-phenyl-pyran-2-one (**2**)

Yellow oil; $[a]_D^{22}$ 0° (*c* 0.24, $CHCl_3$); UV ($CHCl_3$) $\lambda_{max}$ (log $\varepsilon$): 242 (4.03), 255 (3.98), 374 (3.82) nm; IR $\nu_{max}$ (thin film) $cm^{-1}$: 1717, 1700, 1696, 1653, 1616, 1558, 1506; $^1H$ NMR and $^{13}C$ NMR ($CDCl_3$): see Table 2; HR ESI-MS (*m*/*z*): 255.1021 $[M+H]^+$ (calc. for $C_{16}H_{14}O_3$, 255.1016).

## Acknowledgements

We thank the Engineering and Physical Sciences Research Council for funding.

## References

Bennett, G.J., Lee, H.H., 1989. Xanthones from Guttiferae. Phytochemistry 28, 967–998.

Conforti, F., Statti, G.A., Rundis, R., Menichini, F., Houghton, P., 2002. Antioxidant activity of methanolic extract of *Hypericum triquetrifolium* Turra aerial part. Fitoterapia 73, 479–483.

Cox, R.A., Conquest, C., Mallaghan, C., Marples, R.R., 1995. A major outbreak of methicillin-resistant *Staphylococcus aureus* caused by a new phage-type (EMRSA-16). J. Hosp. Infect. 29, 87–106.

Crockett, S.L., Schaneberg, B., Khan, I.A., 2005. Phytochemical profiling of new and old world *Hypericum* (St. John's Wort) species. Phytochem. Anal. 16, 479–485.

Décosterd, L., Stoeckli-Evans, H., Msonthi, J.D., Hostettmann, K., 1986. A new antifungal chromene and a related di-chromene from *Hypericum revolutum*. Planta Med. 52, 429.

Demirci, B., Baser, K.H.C., Crockett, S.L., Khan, I.A., 2005. Analysis of the volatile constituents of Asian *Hypericum* L. (Clusiaceae, Hyperidoideae) species. J. Essent. Oil Res. 17, 659–663.

Dharmaratne, H.R.W., Nanayakkara, N.P.D., Khan, I.A., 2002. Kavalactones from *Piper methysticum*, and their $^{13}C$ NMR spectroscopic analyses. Phytochemistry 59, 429–433.

Gibbons, S., Udo, E.E., 2000. The effect of reserpine, a modulator of multidrug efflux pumps, on the in vitro activity of tetracycline against clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) possessing the Tet(K) determinant. Phytother. Res. 14, 139–140.

Gibbons, S., Ohlendorf, B., Johnsen, I., 2002. The genus Hypericum – a valuable resource of anti-staphylococcal leads. Fitoterapia 73, 300–304.

Gibbons, S., Moser, E., Hausmann, S., Stavri, M., Smith, E., Clennett, C., 2005. An anti-staphylococcal acylphloroglucinol from *Hypericum foliosum*. Phytochemistry 66, 1472–1475.

Kaatz, G.W., Seo, S.M., Ruble, C.A., 1993. Efflux-mediated fluoroquinolone resistance in *Staphylococcus aureus*. Antimicrob. Agents Chemother. 37, 1086–1094.

Kassu, A., Dagne, E., Abate, D., Castro, A., Van Wyk, B.E., 1999. Ethnomedical aspects of the commonly used toothbrush sticks in Ethiopia. East Afr. Med. J. 76, 651–653.

Kokubun, T., Harborne, J.B., Eagles, J., Waterman, P.G., 1995. Dibenzofuran phytoalexins from the sapwood tissue of *Photinia*, *Pyracantha* and *Crataegus* species. Phytochemistry 39, 1033–1037.

Kokubun, T., Harborne, J.B., Eagles, J., Waterman, P.G., 1995a. Four dibenzofuran phytoalexins from the sapwood from *Mespilus germanica*. Phytochemistry 39, 1039–1042.

Ploss, O., Petereit, F., Nahrstedt, A., 2001. Procyanidins from the herb of *Hypericum perforatum*. Pharmazie 56, 509–511.

Richardson, J.F., Reith, S., 1993. Characterization of a strain of methicillin-resistant *Staphylococcus aureus* (EMRSA-15) by conventional and molecular methods. J. Hosp. Infect. 25, 45–52.

Rocha, L., Marston, A., Potterat, O., Kaplan, M.A., Stoeckli-Evans, H., Hostettmann, K., 1995. Antibacterial phloroglucinols and flavonoids from *Hypericum brasiliense*. Phytochemistry 40, 1447–1452.

Ross, J.I., Farrell, A.M., Eady, E.A., Cove, J.H., Cunliffe, W.J., 1989. Characterisation and molecular cloning of the novel macrolide-streptogramin B resistance determinant from *Staphylococcus epidermidis*. J. Antimicrob. Chemother. 24, 851–862.

Seo, E.K., Huang, L., Wall, M.E., Wani, M.C., Navarro, H., Mukherjee, R., Farnsworth, N.R., Kinghorn, A.D., 1999. New biphenyl compounds with DNA strand-scission activity from *Clusia paralicola*. J. Nat. Prod. 62, 1484–1487.

Shiu, W.K.P., Gibbons, S., 2006. Anti-staphylococcal acylphloroglucinols from *Hypericum beanii*. Phytochemistry 67, 2568–2572.

Trovato, A., Raneri, E., Kouladis, M., Tzakou, O., Taviano, M.F., Galati, E.M., 2001. Anti-inflammatory and analgesic activity of *Hypericum empetrifolium* (Guttiferae). Farmaco 56, 455–457.

Weber, N.D., Murray, B.K., North, J.A., Wood, S.G., 1994. The antiviral agent hypericin has *in vitro* activity against HSV-1 through non-specific association with viral and cellular membranes. Antiviral Chem. Chemother. 5, 83–90.