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Diphenylpropanoids from *Quisqualis indica* Linn. and their Anti-staphylococcal Activity

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SUMMARY. **Four diphenylpropanoids- 1-(4-hydroxy-3-methoxyphenyl)-2-(4-allyl-2,6-dimethoxyphenoxy)propan-1-ol (1), 1-(3,4-dimethoxyphenyl)-2-(4-allyl-2,6-dimethoxyphenoxy)propan-1-ol (2), 1-(3,4-dimethoxyphenyl)-2-(4-allyl-2,6-dimethoxyphenoxy)propan-1-ylacetate (3) and 1-(4-hydroxy-3,5-dimethoxyphenyl)-2-(4-allyl-2,6-dimethoxyphenoxy) propan-1-ol (4) were isolated from the chloroform soluble fraction of a methanol extract of *Quisqualis indica*. The structures of these compounds were established unambiguously by MS and a series of 1D and 2D-NMR analyses. All compounds were tested for their anti-staphylococcal activity against a total of five multidrug-resistant (MDR) and methicillin-resistant *Staphylococcus aureus* strains and the minimum inhibitory concentrations (MICs) were in the range of 128-256 μg/ml.**

INTRODUCTION

Quisqualis indica Linn. (Combretaceae), locally known as modhumaloti, is an evergreen creeping shrub attaining a height of 70 feet in tropical climates [1]. It flowers throughout the summer with fragrant blossoms (specially at night) that open white, darken to pink and eventually red. The plant is widely distributed in all districts of Bangladesh [2], in thickets or secondary forests of the Philippines, India and Malaysia [3] and has been introduced in most tropical countries [4]. Fruits of this plant are used to treat ascariasis and oxyuriasis [5], while the decoction of the fruit is useful in toothache and nephritis [6]. The roasted ripe seeds are beneficial in diarrhoea, fever and rickets. Furthermore, seeds macerated in oil can also be applied in parasitic skin troubles [7]. The leaves are also used to treat various kinds of infantile disorders and skin diseases, whereas the roots are used in rheumatism and diarrhoea. Besides, the plant extract has been found to be anticoccidial for veterinary purposes [8]. Previous phytochemical investigation on *Q. indica* revealed the occurrences of flavonoids [9], ellagitannins including quisqualin A and quisqualin B [10] and sterols and terpenes [11]. As part of our continuing research projects focusing on phytochemical investigation on Bangladeshi medicinal plants [12-14], we now report on the isolation of four diphenylpropanoids (**1-4**) from the stem bark of *Q. indica* as well as their anti-staphylococcal activity against a number MDR and methicillin-resistant *Staphylococcus aureus.*

MATERIALS AND METHODS

General

High Resolution Electron Impact Mass Spectrometry (HREIMS) data were recorded on a Jeol JMS-AX505HA double-focusing instrument at 70 eV and HR-FABMS were obtained on a Jeol SX102 spectrometer. NMR spectra (both 1D and 2D) were obtained on a Bruker Avance (500

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MHz for ^1H and 125 MHz for ^{13}C) spectrometer, using the residual solvent peaks as internal standard. Chemical shift values (δ) were reported in parts per million (ppm) relative to appropriate internal solvent standard and coupling constants (*J* values) are given in Hertz. Heteronuclear Multiple Bond Correlation (HMBC) spectra were optimized for a long range J_{H-C} of 7Hz (d_6 = 0.07 s) and the Nuclear Overhauser Enhancement Spectroscopy (NOESY) experiment was carried out with a mixing time of 0.4s. Column chromatography (CC) was carried out using Merck Si gel (Kieselgel 60, mesh 70-230). Preparative TLC was carried out using Merck Si gel 60 PF_{254} on glass plates (20 cm X 20 cm) at a thickness of 0.5 mm. Spots on TLC and PTLC plates were visualized under UV light (254 and 366 nm) and spraying with 1% vanillin-H_2SO_4 followed by heating at 110ºC for 5-10 min.

Plant Material

The stem bark of *Quisqualis indica* was collected from Dhaka, Bangladesh, in August 2005. A voucher specimen (DACB-312338) of this collection has been deposited in the Bangladesh National Herbarium, Mirpur, Dhaka.

Extraction and Isolation

The sun-dried and powdered plant material (750 g) was macerated with methanol (1000 ml) followed by filtration. The filtrates were concentrated with a rotary evaporator at low temperature (40-45 °C) and reduced pressure.

A portion (5 g) of the concentrated methanol extract was fractionated by the modified Kupchan partitioning method [15] into petroleum ether, carbon tetrachloride and chloroform. Based on TLC analysis the chloroform soluble fraction was chromatographed for the isolation of compounds. The chloroform soluble fraction (500 mg) was further fractionated by column chromatography over Si gel 60H (20 g) using *n*-hexane- EtOAc and EtOAc-MeOH mixtures of increasing polarity. The eluates were combined together on the basis of TLC analysis. Preparative TLC (Si Gel; 1% MeOH in $CHCl_3$ plus 2-3 drops of AcOH) of the column fraction eluted with 25% EtOAc in $CHCl_3$ afforded **1** (2.9 mg), **2** (4.0 mg), **3** (3.1 mg) and **4** (3.2 mg) as colorless gum.

Bacterial strains

The antibacterial assay was performed against a panel of multi-drug and methicillin-resistant strains of *Staphylococcus aureus*. *S. aureus* standard strain ATCC 25923 and tetracycline-resistant strain XU212 which possesses the TetK tetracycline efflux protein were provided by Dr Edet Udo [16]. Strain SA-1199B which overexpresses the norA gene encoding the NorA MDR efflux pump was provided by Professor Glenn Kaatz [17]. Strain RN4220 which possess the MsrA macrolide efflux protein was provided by Dr Jon Cove [18]. EMRSA-15 [19] was the generous gift of Dr Paul Stapleton.

Minimum inhibitory concentration (MIC) assay

The Minimum inhibitory concentrations (MICs) of the compounds (**1-4**) were determined according to the method described before [20].

RESULTS AND DISCUSSION

The stem bark of *Q. indica* was macerated with methanol followed by sequential solvent-solvent partitioning with *n*-hexane, carbon tetrachloride and chloroform. Column chromatography (CC) on the chloroform soluble fraction followed by preparative thin-layer chromatography yielded compounds **1-4** (Fig. 1).

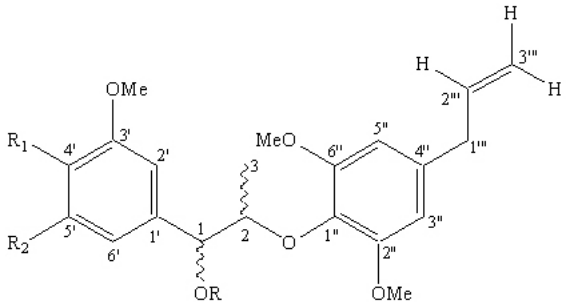

Figure 1. Structure of compounds isolated from the stem bark of *Q. Indica*. **1**: R =R_2 = H, R_1 = OH; **2**: R =R_2 = H, R_1 = OMe; **3**: R = $COCH_3$, R_1 = OMe, R_2 = H; **4**: R = H, R_1 = OH, R_2 = OMe.

The HREIMS of **1** established its molecular formula as $C_{21}H_{26}O_6$. The ^1H NMR spectrum (500 MHz, $CDCl_3$, Table 1) of 1 exhibited signals for a methoxyl (δ 3.90), two superimposed methoxyl groups (δ 3.88) integrating for six hydrogens, two aromatic protons as broad singlet (δ 6.46) and an ABX aromatic splitting pattern (6.68, dd, *J* = 8.0, 2.0 Hz; 6.84, d, *J* = 8.0; 6.98, d, *J* = 2.0 Hz suggesting the presence of a biphenyl derivative. The signals at δ 5.14 (1H, d, *J* = 17.0 Hz), 5.12 (1H, d, *J* = 10.0 Hz), 5.99 (1H, ddd, *J* =17.0, 10.0, 6.5 Hz), and 3.38 (2H, d, *J* = 6.5 Hz) in the ^1H NMR spectrum were indicative of a prop-2-enyl (allyl) side chain in the molecule.

Protons	**1**	**2**	**3**	**4**
H-1	4.80, *d, J* = 3.5 Hz	4.80, *d, J* = 3.5 Hz	4.44, *q, J* = 3.5 Hz	4.78, *d, J* = 3.5 Hz
H-2	4.34, *q, J* = 3.5 Hz	4.35, *q, J* = 3.5 Hz	5.87, *d, J* = 3.5 Hz	4.33, *q, J* = 3.5 Hz
H-2'	6.98, *d, J* = 2.0 Hz	6.96, *d, J* = 2.0 Hz	6.90, *d, J* = 2.0 Hz	6.53, *d, J* = 2.0 Hz
H-5'	6.84, *d, J* = 8.0 Hz	6.81, *d, J* = 8.0 Hz	6.81, *d, J* = 8.0 Hz	-
H-6'	6.68, *dd, J* = 8.0, 2.0 Hz	6.78, *dd, J* = 8.0, 2.0 Hz	6.86, *dd, J* = 8.0, 2.0 Hz	6.53, *d, J* = 2.0 Hz
H-3'',5''	6.46, br s	6.47, br s	6.40, br s	6.46, br s
H-1'''	3.38, *d, J* = 6.5 Hz	3.38, *d, J* = 6.5 Hz	3.35, *d, J* = 6.5 Hz	3.36, *d, J* = 6.5 Hz
H-2'''	5.99, *ddd, J* = 17.0, 10.0, 6.5 Hz	6.00, *ddd, J* = 17.5, 10.0, 6.5 Hz	5.98, *ddd, J* = 17.5, 10.0, 6.5 Hz	5.97, *ddd, J* = 17.0, 10.0, 6.5 Hz
H-3'''*cis*	5.12, *d, J* = 10.0 Hz	5.12, *d, J* = 10.0 Hz	5.10, *d, J* = 10.0 Hz	5.10, *d, J* = 10.0 Hz
H-3'''*trans*	5.14, *d, J* = 17.0 Hz	5.14, *d, J* = 17.5 Hz	5.11, *d, J* = 17.5 Hz	5.11, *d, J* = 17.0 Hz
Me-3	1.13, *d, J* = 6.5 Hz	1.14, *d, J* = 6.5 Hz	1.30, *d, J* = 6.5 Hz	1.13, *d, J* = 6.5 Hz
MeO-3'	3.90, s	3.89, s	3.85, s	3.90, s
MeO-4'	-	3.86, s	3.86, s	-
MeO-5'	-	-	-	3.88, s
MeO-2'',6''	3.88, s	3.88, s	3.78, s	3.88, s
AcO-1	-	-	2.18, s	-

Table 1. ^{1}H NMR data (500 MHz, $CDCl_3$) of compounds **1 – 4**.

Further, the presence of a methyl group (δ 1.13, d, *J* = 6.5 Hz) and two oxymethine protons (δ 4.34, q, *J* =3.5 Hz; 4.80, d, *J* =3.5 Hz) indicated the presence of a oxypropan-1-ol moiety in the molecule.

The ^{13}C and DEPT135 NMR spectra (125 MHz, $CDCl_3$, Table 2) revealed signals for the methyl, methoxyls, methylenes (one aliphatic and one vinylic), aromatic methines, oxygenated aliphatic methines and quaternary carbons including oxygen bearing ones. The assignment of carbons and the placement of the methoxyl groups, allyl group and oxypropan-1-ol bridge within the molecule were achieved by 2D experiments. In the HMBC experiment, a common ^{3}J correlation by methoxyl protons at 3.90 (δ_C 56.2 from HMQC) and ABX pattern H-5' (δ_H 6.84; δ_C 114.1 from HMQC) to an oxygenated quaternary carbon at 146.6 confirmed its assignment as C-3' and placement of this methoxyl at this carbon. The ABX pattern H-2' (δ_H 6.98; δ_H 108.7 from HMQC) and H-6' (δ_H 6.68; δ_C 119.0 from HMQC) exhibited a common ^{3}J correlation to a quaternary carbon at 144.6 (C-4') and a benzylic oxymethine at 73.0 (C-1; δ_H 4.80 from HMQC). The carbon was also connected to the methyl protons (δ_H 1.13; δ_C 13.0 from HMQC) and another oxymethine proton (δ_H 4.34; δ_C 82.5 from HMQC) by ^{3}J and ^{2}J, respectively. These HMBC correlations suggested the linkage of 3-methoxyphenyl nucleus to oxypropan-1-ol

Carbons	**1**	**2**	**3**	**4**
C-1	73.0	73.0	76.9	73.0
C-2	82.5	82.5	80.3	82.5
C-1'	132.4	132.9	130.8	132.3
C-2'	108.7	109.4	110.5	108.5
C-3'	146.6	149.0	148.7	146.6
C-4'	144.6	148.1	148.9	144.6
C-5'	114.1	111.0	110.0	145.4
C-6'	119.0	118.3	119.5	108.5
C-1''	136.4	136.4	136.0	136.4
C-2'', C-6''	153.7	153.7	153.6	153.7
C-3'', C-5''	105.7	105.7	105.8	105.7
C-4''	133.1	133.2	134.0	133.1
C-1'''	40.8	40.8	40.7	40.8
C-2'''	137.3	137.3	137.5	137.3
C-3'''	116.4	116.4	116.2	116.4
Me-2'''	13.0	13.0	14.7	13.0
MeO-3'	56.2	56.1	56.3	56.2
MeO-4'	-	56.0	56.0	-
MeO-5'	-	-	-	56.1
MeO-2'',6''	56.3	56.3	56.2	56.3
$\underline{C}H_3CO$-2'''	-	-	21.5	-
$CH_3\underline{C}O$-2'''	-	-	170.4	-

Table 2. ^{13}C NMR data (125 MHz, $CDCl_3$) of compounds **1 – 4.**

Bacteria	1	2	3	4	Norfloxacin	Tetracycline
SA 1199B (NorA)	128	256	256	128	32	0.25
RN4220 (MsrA)	256	128	256	256	0.5	0.125
EMRSA-15 (mecA)	256	128	256	128	0.25	0.125
ATCC 5923	256	128	256	256	0.5	0.125
XU212 (TetK)/(mecA)	128	128	256	256	8	64

Table 3. MICs of compounds **1–4** and standard antibiotics in µg/ml.

moiety through C-1' (δ_C 132.4; ^3J by H-5'). The signal at 6.46 integrated for two protons showed both direct and ^3J correlation at 105.7. This mean that these protons (H-3'',5'') are meta to each other in another benzene ring. These protons (H-3'',5'') also showed ^2J correlations to quaternary carbons at 153.7 (C-2'',6'') and 133.1 (C-4'') and ^3J correlations to an oxygenated quaternary (136.4; C-1'') and a methylene (40.8; C-1''; δ_H 3.38 from HMQC) carbons confirming the attachment of prop-2-enyl (allyl) side chain via C-4''. A ^3J correlation by two superimposed methoxyl groups (δ 3.88; δ_C 56.3 from HMQC) integrating for six hydrogens to the oxygenated quaternary at 153.7 (C-2'',6'' having a peak height of almost double of C-3') confirmed the placement of these identical methoxyls at C-2'',6''. These methoxyls also revealed NOE interaction with H-3'',5'' while another NOE interaction between H-2' and methoxyl hydrogens at 3.90 also existed. On this basis, compound **1** was identified as 1-(4-hydroxy-3-methoxyphenyl)-2-(4-allyl-2,6-dimethoxyphenoxy) propan-1-ol. The spectral data are in good agreement to those published in literature [21,22].

The HR-FABMS of compound **2** showed $[M+Na]^+$ peak at m/z 411.1776 (411.1783 calculated for $C_{22}H_{28}O_6Na$). and thereby, established its molecular formula as $C_{22}H_{28}O_6$ -14 amu more than **1**. The ^1H (Table 1) and ^{13}C NMR data (Table 2) of **2** were identical to those of **1** except C-4' and the resonances for the methoxyl groups. In addition to the superimposed methoxyls (δ_H 3.88; δ_C 56.3 from HMQC) integrated for six hydrogens, the ^1H and ^{13}C NMR data revealed the presence of two methoxyl signals resonating at 3.86 (δ_C 56.0 from HMQC) and 3.89 (δ_C 56.1 from HMQC). In the HMBC experiment, a common ^3J correlation by methoxyl protons at 3.89 and H-5' to an oxygenated quaternary carbon at 149.0 confirmed its assignment as C-3' and placement of this methoxyl at this carbon. H-2', H-6' and the other methoxyl at 3.86 connected *via* ^3J to another oxygen bearing quaternary carbon at 148.1 (C-4') and thereby confirmed the linkage of this methoxyl through C-4'. A NOE interaction between methoxyl at 3.85 and 3.86 was also revealed. Accordingly, **2** was identified as 1-(3,4-dimethoxyphenyl)-2-(4-allyl-2,6-dimethoxyphenoxy)propan-1-ol [23].

The HR-FABMS of compound **3** showed [M+Na]+ peak at m/z 453.1896 (453.1889 calculated for $C_{24}H_{30}O_7Na$) and thereby, established its molecular formula as $C_{24}H_{30}O_7$ which was 42 amu more than **2**. The ^1H (Table 1) and 13C NMR (Table 2) data of **3** were almost identical to those of **2** except the resonances were for C-1, C-2 and the presence of an acetyl group in the molecule. The ^1H NMR spectrum (500 MHz, $CDCl_3$ and Table 1) of **3** revealed the presence of an extra methyl signal at 2.18 (δ_C 21.5 from HMQC) which showed HMBC connectivity to a carbonyl at 170.4. The latter carbon exhibited ^3J correlation to H-1 (δ_H 4.44; δ_C 80.3 from HMQC). This indicated the presence of a acetyl group connected at C-1 as an ester. Thus, **3** was identified as 1-(3,4-dimethoxyphenyl)-2-(4-allyl-2,6-dimethoxyphenoxy)propan-1-ylacetate. The spectral data are in good agreement to those published in literature [22].

The molecular formula of **4** was established as $C_{22}H_{28}O_7$ from the HREIMS. The ^1H (Table 1) and ^{13}C NMR (Table 2) data of 4 were almost identical to those of 1 except the resonances were for C-5' and the presence of another methoxyl group in the same benzene ring of the molecule. In stead of ABX pattern aromatic system for this ring, the ^1H NMR spectrum (500 MHz, $CDCl_3$ and Table 1) revealed meta coupled (J =2.0 Hz) superimposed protons at 6.53 which showed both direct and ^3J correlation to a methane carbon at 108.5 ppm. The spectrum showed the presence of another methoxyl (δ_H

3.88; δ_C 56.1 from HMQC) connected to C-5' (δ_C 145.4) by ^3J. Thus, **4** was identified as 1-(4-hydroxy-3,5-dimethoxyphenyl)-2-(4-allyl-2,6-dimethoxyphenoxy)propan-1-ol [21]. This the first time report of isolation of compounds 1-4 from this plant.

All compounds (**1-4**) were tested for their anti-staphylococcal activity against a total of five MDR and methicillin-resistant *Staphylococcus aureus* strains and revealed weak activity having minimum inhibitory concentrations (MICs) in the range of 128-256 μg/ml (Table 3).

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