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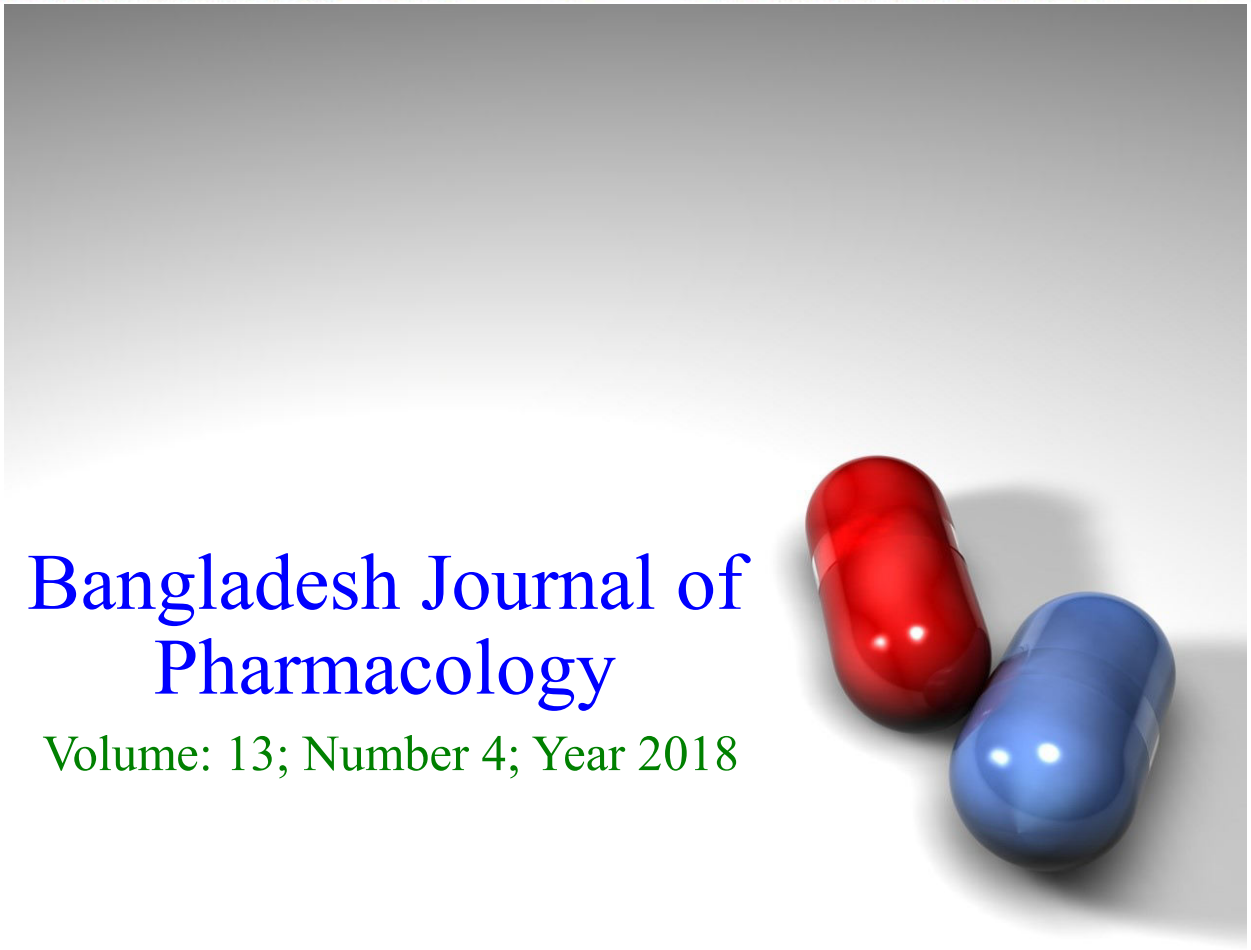

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Letter to the Editor

Antimycobacterial and anti-oxidant potential of the bioactive metabolite isolated from the endophytic fungus *Daldinia eschscholtzii*

Sir,

Endophytic fungi from the plant are considered as one of the predominant resources to produce novel bioactive metabolites with many therapeutic potentials, used since the production of taxol (an anti-cancer drug) obtained from the endophytic fungus *Taxomyces andreanae* (Gouda et al., 2016).

In this study, endophytic fungi *Daldinia eschscholtzii* was isolated from the ornamental plant *Mussaenda luteola* (Family: Rubiaceae) since this plant was reported with many therapeutic properties like cytotoxicity, anti-inflammatory, antimicrobial and many others with predominant phytochemicals such as iridoids, triterpenes and phenols (Shylaja and Sathiavelu, 2017). *Daldinia* sp. belongs to the class ascomycetes and order xylariales constantly accompany with certain genera of host plants as an endophyte but also appear on other woody substrates. This genus is considered as a warehouse of bioactive metabolites with major therapeutic potentials. The immune suppressive compound dalesconol A and B polyketides, daeschol A, helicascolide A, dalesconol C and dalmanol A are some of the compounds obtained from *D. eschscholtzii* (Yuyama et al., 2013). Some other metabolites isolated from this species include selesconol, isoindolinones, dihydroisocoumarins, benzofuran lactones with therapeutic properties like immunosuppressive, antioxidant, cytotoxic and anti-HIV respectively. This fungus had been investigated as an endophyte from the plant Pogostemoncablin and identified bioactive metabolites such as eschscholin A, 3,5-dihydroxy-2-methyl-4H-chromen-4-one etc. (Liu et al., 2017). The aim of the present study was to isolate and characterize the secondary metabolite from the endophytic fungus *D. eschscholtzii* and evaluation of anti-oxidant and antimycobacterial potential.

The endophytic fungus was isolated from the leaf segment of the plant *M. luteola* (Petrini, 1986) and it was identified as *D. eschscholtzii* by 18S rRNA sequencing (Guo et al., 2000). The 25 days grown culture was filtered and extracted with ethyl acetate and the filtrate (2 L) was dried to obtain a crude extract (620 mg). It was subjected to a modified solvent-solvent fractionation method (Mtunzi et al., 2017; Shylaja et al., 2018) for isolation of secondary metabolites. Initially, the crude extract was partitioned with petroleum ether (26 mg), chloroform (242 mg) and methanol (147 mg). The chloroform fraction was dried and purified further by the column chromatography using chloroform solvent alone. Then the resultant fraction was washed again with petroleum ether, chloroform and methanol in the ratio of 3:3:1 to obtain a soluble fraction and insoluble precipitate. The precipitate was dissolved in chloroform and eluted in the column chromatography to yield compound **1** (46 mg). The structure elucidation of the compound **1** was analyzed based on the spectral data obtained from FT-IR, MS and ^{1}H and ^{13}C NMR.

The compound **1** was isolated as oily yellow substance, and the FT-IR spectrum shows the absorption maxima ν_{max} at 3292, 2926, 2854 which revealed the presence of OH and CH stretch, 1689 showed C=O, the peak at 1195 -1331 showed the presence of C-O (aromatic ester). The ν_{max} from 503-947 revealed the presence of C-H. The molecular ion peak at m/z 503.992 $[M+H]^+$ by HRESIMS indicated the molecular formula of compound **1** was $C_{33}H_{58}O_3$ (Calcd. 502.824 g/mol). The ^{13}C and ^{1}H NMR spectra of the compound **1** showed signals for a tetrasubstituted benzene. The peaks at δ_C 160.09 and δ_C 156.96 were due to the presence of two conjugated ketone (C=O) at C-1 and C-4 positions of the benzene ring respectively. A methoxy group (CH_3O-) at the C-5 position and free methyl group at C-2 position showed peaks at δ_C 22.69 and δ_C 21.48 respectively. A long linear alkyl chain (pentacosyl, C-25) at a C-3 position of benzene rings exhibited peak range from δ_C 24.73 to δ_C 45.58 and free methyl group from the long alky chain (C-31 position) exhibited signal at δ_C 21.37. The olefinic carbons in the aromatic benzene ring at C-2, C-3, C-5 and C-6 represented peaks ranging from δ_C 117.06 to δ_C 123.99. In ^{1}H NMR, the free methyl (–CH_3) group at positions C-31(3H) and C-32 (3H) showed signals at δ_H 0.842 and δ_H 0.872 respectively. The proton signal at δ_H 2.196 was due to the methoxy group (CH_3O) at position C-5 (3H). The methylene group (-CH_2) from linear alkyl chain at position C-8 to C-30 (2H) exhibited signal range from δ_H 0.948 to δ_H 2.883. The olefinic proton at C-6 (1H) of aromatic ring showed signal from δ_H2.926. Based on the spectral data obtained

and comparing with the previous report that the compound **1** was characterized as 5-methoxy-2-methyl-3-pentacosyl-1,4 benzoquinone (Qin and Liu, 2004) and the structure is given in Figure 1.

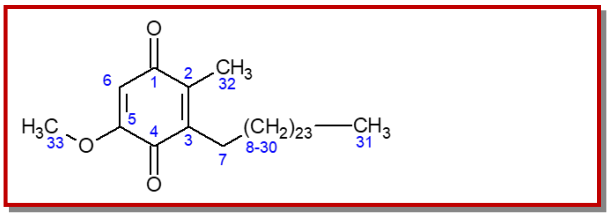

Figure 1: Structure of the compound 1 (5- methoxy-2-methyl-3-pentacosyl-1,4 benzoquinone)

Earlier studies show that three new homologous series metabolites of 3-alkyl-5-methoxy-2-methyl-1,4-benzo-quinones, with chain lengths varies of C21 to C23 and many other benzoquinones metabolites were isolated from the fungus *D. concentrica* (Stadler et al., 2014; Qin and Liu, 2004). The antimycobacterial potential of the compound **1** was determined using resazurin-based microtitre assay (REMA) which is used to study the metabolism and sustainability of microorganisms (Martin et al., 2005). Resazurin dye is a blue color oxido -reduction reagent which gets oxidized and turns to pink color due to the metabolism of growing microorganisms (Rivoire et al., 2007). The various concentration of isolated compound **1** was tested for antimycobacterial potential against *Mycobacterium tuberculosis* H37Rv strain. The results revealed that compound **1** showed the significant inhibition compared to the standard drugs and compound **1** had the MIC value of 25 µg/mL (Figure 2). The compound **1** was also screened for its anti-oxidant potential using the DPPH free radical scavenging assay (Yadav et al., 2014). The result showed maximum inhibition of 80.2 ± 1.3% at 100 µg/mL of concentration (Table I).

This is the first report on the isolation of *Daldinia* sp. from the plant *M. luteola*. The results of the present study conclude that compound **1** is 5-methoxy-2-methyl

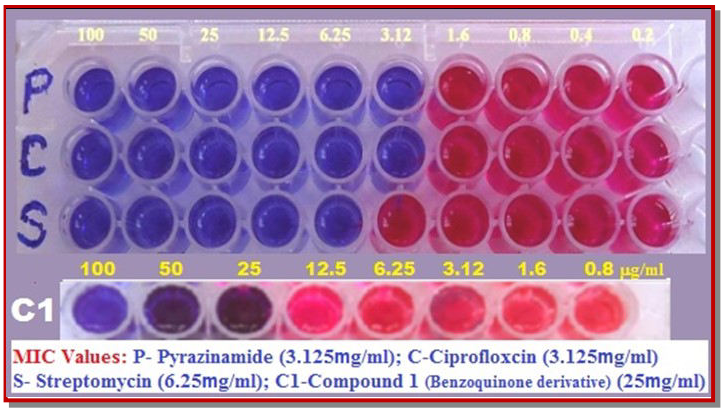

Figure 2: Antimycobacterial potential of endophytic fungal metabolite C1 from *D. eschscholtzii*

Table I

DPPH free radical scavenging potential of the compound 1

Concentration (µg/mL)	% Inhibition of DPPH free radical	
	Compound 1	Standard
25	68.7 ± 0.6	76.5 ± 1.8
50	72.5 ± 0.4	81.6 ± 2.2
75	75.0 ± 0.4	85.6 ± 2.0
100	80.2 ± 1.3	90.0 ± 1.6

-3-pentacosyl-1,4 benzoquinone isolated from the fungus *D. eschscholtzii* which may be a prominent resource of the pharmacologically active lead molecule for the development of antimicrobial drug.

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