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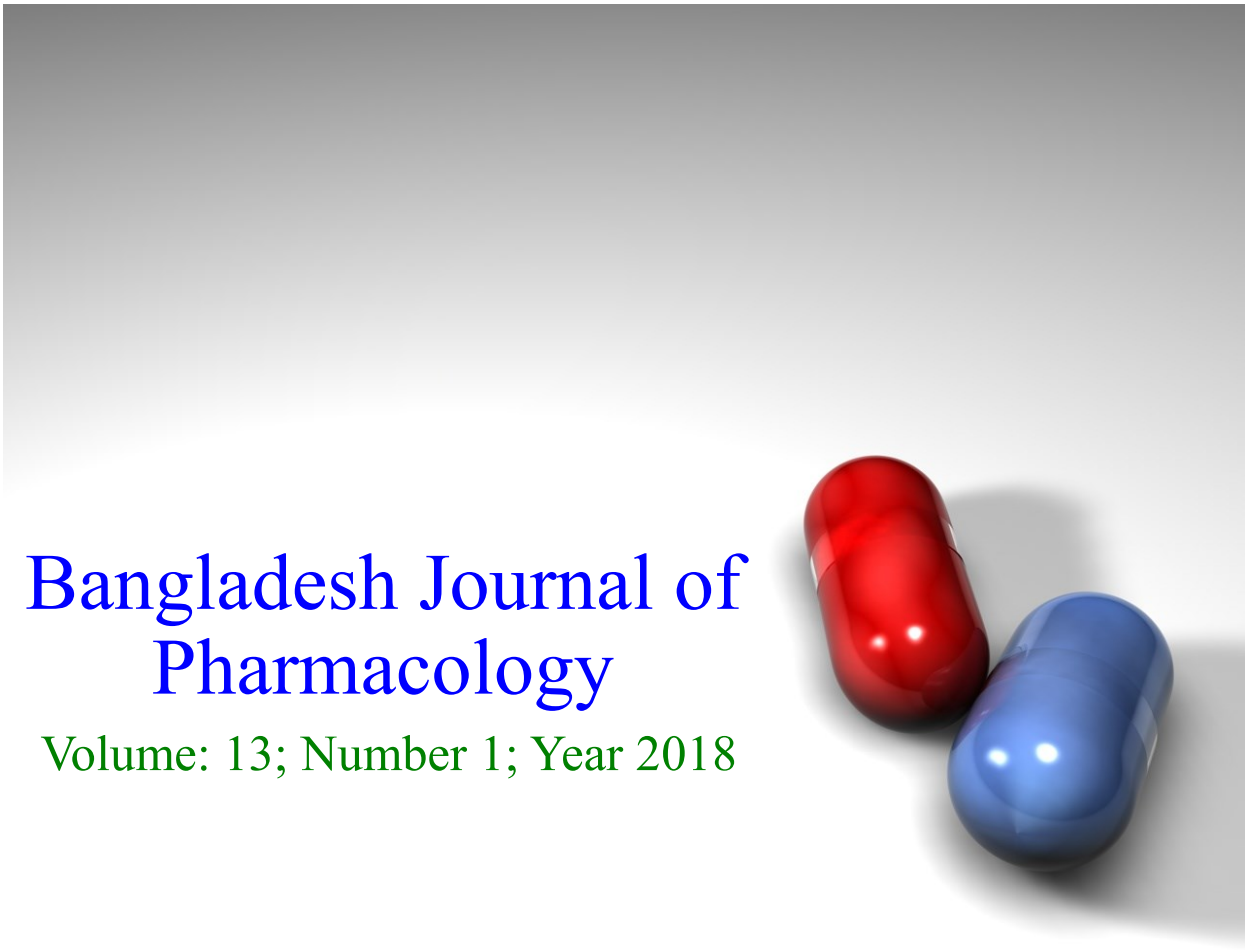

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Letter to the Editor

Isolation of isosativenetriol from endophytic fungus *Cochliobolus* spp. of *Aerva lanata*

Sir,

Endophytic fungi produce variety of secondary metabolites with novel structures and some of these bioactive compounds possess interesting biological activities. Consequently, there has been growing research interest in the plant–associated microorganisms and marine-associated microorganisms (sponges and algae). Novel secondary metabolites synthesized from the endophytic microorganisms were recognized as potential sources for exploitation in the field of medicine, agriculture and industry (Guo et al., 2008). Endophytic fungus was isolated from the medicinal plant *Aerva lanata* (family: Amaranthaceae) and identified as *Cochliobolous* spp. The genus *Cochliobolus* and its anamorphic spp. include around 55 species occurring all over the world and many *Cochliobolus* and its anamorphic species (*Bipolaris, Curvularia*) are pathogens of weeds and can successfully be applied as weed herbi -cides because the weeds and pathogens have co-evolved over a long period (Strobel et al., 1991). Secondary metabolites like Curvularides, cochlioquinones, anthraquinones, helminthosporol, helminthosporal, pre -helminthosporol and many other related metabolites have been reported from *Cochliobolus* strains. These compounds may have important pharmacological potentials, such as antifungal, antioxidant, antimicrobial properties (Chomcheon et al., 2010). The whole plant of *A. lanata* was used for the treatment of hepatitis, anticalculus, diuretic, demulcent, anthelmintic and antidiarrheal (Shoba and Mythili, 2017). Some of the phytochemicals from *A. lanata* (aervitrin, aervolanine, aervoside, amyrin, betulin, campesterol, canthin-6-one, 10-hydroxycanthin-6-one, chrysin, daucosterol, hentriacontane, narcissin, β-sitosterol, and syringic acid) were isolated and reported with biological activities (Vetrichelvan and Jegadeesan, 2002).

The aim of this research was to isolate the secondary metabolite from the endophytic fungus *Cochliobolus* spp. and evaluation of antimycobacterial potential.

The fungus used in this study was isolated from the leaves of *A. lanata* (Shoba and Mythili, 2017) and identified as *Cochliobolus* spp. (KY800380) on the basis of the rDNA internal transcribed spacer gene sequence. Three weeks old grown fungal cultured broth was filtered to remove fungal mycelium (matt). Methanol (250 mL) was added to a flask containing fungal mycelium and kept it overnight in a shaker. The matt extracted methanol was collected, concentrated, dried and stored at 4°C for further analysis (Shylaja and Sathiavelu, 2017). Isolation of bioactive metabolites from the matt methanolic extract was done using modified solvent–solvent fractionation (Sasikumar and Ghosh, 2017). The dried methanolic matt extract was sequentially partitioned with petroleum ether to produce a petroleum ether-soluble fraction (pink color). The obtained petroleum ether-soluble fraction was dried and washed with ethyl acetate followed by chloroform, acetone and methanol respectively. The ethyl acetate, chloroform, acetone and methanol-soluble fraction were dried and all dried fractions were washed with acetone. All the acetone soluble fractions were combined, dried and further purified with silica column (60-120 mesh) using an isocratic solvent of acetone (100%). The acetone eluted fraction was completely evaporated and subjected for spectral analysis.

Isosativenetriol (**1**) was isolated as light pink color crystal (Figure 1) (yield: 50 mg) and the molecular formula was proposed as $C_{15}H_{24}O_3$ based on HR-EIMS showing an ion at m/z 253.4900 (calculated for 252.1725). The FT-IR spectrum of compound (**1**) revealed a broad peak situated at 3292 cm^{-1} which was assigned to O-H stretching vibration. The peaks at 2926, 2854 cm^{-1} were assigned to C-H stretching vibrations of methyl group, respectively to asymmetric and symmetric stretching vibrations of methylene groups. At 1381cm^{-1} it was identified the C-H scissoring vibration, while at 947 cm^{-1} it was found the C-O stretching vibration. The rocking vibration of methyl group was easily detectable in the spectrum at 742 cm^{-1}. ^{13}C NMR (100 MHz, DMSO) spectrum revealed the presence of 15 carbons and among that showed three methyl groups. δ34.93 (C-1), δ31.36 (C-2), δ29.47 (C-3), δ34.12 (C-4), δ29.16 (C-5), δ69.75 (C-6), δ173.46 (C-7), δ51.61 (C-8), δ65.94 (C-9), δ33.94 (C-10), δ69.21 (C-11), δ24.95 (C-12), δ24.90 (C-13), δ63.08 (C-14) and δ22.55 (C -15). ^1H NMR (DMSO, 500MHz): δ 1.492 (m, 2H, H-2), 1.508 (m, 2H, H-3), 2.179 (s, 1H, H-4), 3.432 (brs, 1H, H-5), 2.267 (dd, J = 8.9, 5.2 Hz, H-8), 2.280 (m, 1H, H-9), 2.299 (m, 1H, H-10), 0.083 (d, J = 5.6 Hz, H3-10), 0.955 (s, 3H, H-12), 0.890 (s, 3H, H-13), 1.335 (s, 2H, H-14), 0.868

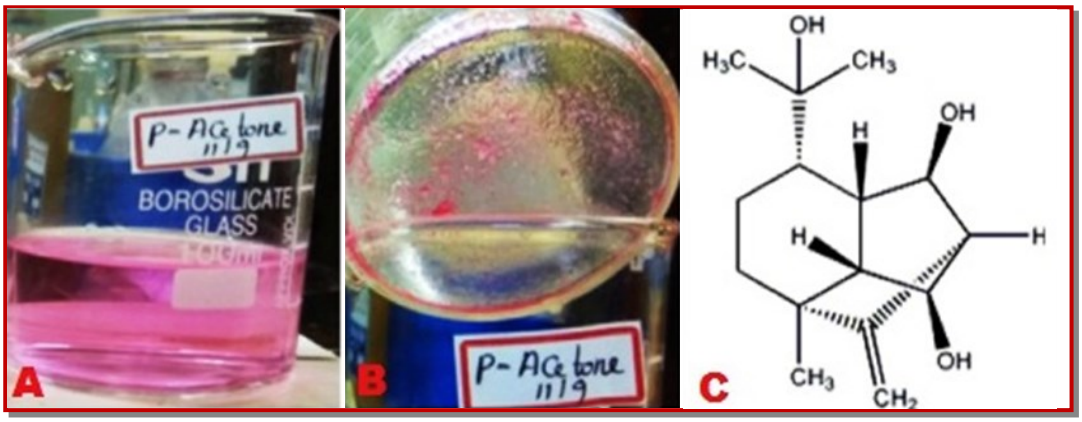

Figure 1: Isolation of metabolite from *Cochliobolus* spp. A) Acetone soluble fraction; B) Dried acetone fraction (pink oily); C) Structure of isosativenetriol (1)

(s, 3H, H-15), 4.013 (s, 1H, OH-6), 4.051 (s, 1H, OH-9) and 4.041 (s, 1H, OH-10). The FT-IR and NMR data of compound **1** was identical to the published data of the isosativenetriol (sesquiterpenoids types) (Osterhage et al., 2002; Dorn and Arigoni, 1975) and the structure was shown in Figure 1.

Isosativenetriol was isolated along with a number of similar structure metabolites from the *Helminthosporium sativum* (Dorn and Arigoni, 1975) and also been isolated from a marine algicolous isolate of *Drechslera dematioidea*, along with sesquiterpenoids derivatives and other metabolites (Osterhage et al., 2002). Isosativenetriol exhibits significant antimycobacterial potential with MIC of 25 µg/mL against *M. tuberculosis* $H_{37}R_v$ using microplate alamar blue assay (Sharma et al., 2014). The results were depicted in Table I.

Table I

Antimycobacterial activity of isosativenetriol from *Cochliobolus* sp.

Samples	Minimum inhibition concentration (µg/mL) *M. tuberculosis* $H_{37}R_V$
Isosativenetriol	25
Pyrazinamide	3.125
Ciprofloxacin	3.125
Streptomycin	6.25

From the findings of the present study, it revealed that the isosativenetriol isolated from endophytic fungus *Cochliobolus* spp. will be a promising lead molecule for anti-microbial and antitubercular drug development.

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