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A new bioactive curvularin macrolide from an endolichenic fungus, *Curvularia trifolii*, isolated from *Usnea sp.*, in Sri Lanka

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The search for new bioactive natural products is still the main way of discovering new drug leads. Although fungi are well-known producers of secondary metabolites with new structurally diverse bioactive compounds, isolation of bioactive compounds from endolichenic fungi still remain underexplored as potential source of natural products. Endolichenic fungi are microorganisms living in the thalli of lichens that are analogous to the plant endophytic fungi inhabiting the intercellular spaces of the host. *Curvularia trifolii* was isolated from the lichen, *Usnea* sp., available in Hakgala botanical garden, Sri Lanka. Identification of the fungus was confirmed using morphological characters followed by the molecular identification (DNA sequencing). *In vitro* large scale cultures of *C. trifolii* were prepared in 60 large Petri dishes containing potato dextrose agar medium and after 10 days secondary metabolites were extracted into ethyl acetate. The crude extract was tested using two types of bio assays, for the investigation of antioxidant and anti-inflammatory activities. Antioxidant activity of the crude ethyl acetate extract was explored by carrying out DPPH free radical scavenging assay and Anti-inflammatory activity was evaluated using Human Red Blood Cell Membrane Stability (HRBC) assay. Since the crude extract showed high activity in both the assays, it was partitioned into hexane, chloroform (*CHCl₃*) and 60% *aq. MeOH* and identified the bioactive fractions. The activity of *CHCl₃* fraction was confirmed and it was further fractionated using bioassay guided column chromatography (silica, sephadex) and preparative TLC to isolate pure compounds. One florescent active pure bioactive compound (C₂₃H₃₀O₆) was isolated from the *CHCl₃* fraction and identified as using 1D, 2D NMR, MS, IR and UV spectral data. The structure of new compound was thus established as 5-methoxy-4,8,15-trimethyl-3,7-dioxo-1,3,7,8,9,10,11,12,13,14,15,15α-dodecahydrocyclooctadecal[de]isochromene-15-carboxylic acid and the bioassay results revealed that it is a new curvularin macrolide with IC₅₀ values, 68.6±4.3 μg/mL and 310±48.2 μg/mL for DPPH antioxidant assay and anti-inflammatory assay respectively. Hence it is revealed that derivative isolated from *C. trifolii* can be developed as an important antioxidant and anti-inflammatory drug.

**Keywords:** Anti-inflammatory activity, Antioxidant activity, Endolichenic fungus, Curvularin, *curvularia* sp.

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