

## RESEARCH ARTICLE

# Two new bioactive polyketides from *Curvularia trifolii*, an endolichenic fungus isolated from *Usnea* sp., in Sri Lanka

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**Abstract:** Two new polyketides (compounds 1 and 2) were isolated from the EtOAc extract of an endolichenic fungus, *Curvularia trifolii* obtained from *Usnea* sp. in Sri Lanka. The structures of these compounds were elucidated on the basis of spectroscopic methods (UV, IR, MS, 1D NMR and 2D NMR). The bioactivity of the compounds 1 and 2 were studied using DPPH antioxidant assays and they showed radical scavenging activity with IC<sub>50</sub> values of 4.0 ± 2.6 and 1.3 ± 0.2 mg/mL, respectively. The radical scavenging activity of compound 2 was higher than that of compound 1 and it was comparable to BHT. Moreover, the new compounds 1 and 2 were evaluated for their anti-inflammatory activity, and only compound 2 exhibited a significant activity comparable to the standard anti-inflammatory drug, aspirin. Compound 1 was evaluated for the inhibition of cell proliferation in a panel of five cancer cell lines NCI-H460, MCF-7, SF-268, PC-3M and MIA Pa Ca-2, and exhibited > 90 % inhibitory activity at 5 µg/mL with all of the above cell lines.

**Keywords:** Anticancer activity, anti-inflammatory activity, antioxidant activity, *Curvularia* sp., endolichenic fungus.

## INTRODUCTION

Endolichenic fungi are considered as a new source of bioactive fungal metabolites (Paranagama *et al.*, 2007). Only a few investigations have been reported on the isolation and characterisation of secondary metabolites of endolichenic fungi, but they have shown great potential to be a new source for structurally diverse and biologically active natural products (Paranagama *et al.*, 2007; Ding *et al.*, 2009; Zhang *et al.*, 2009; Wang *et al.*, 2010). The endolichenic fungi available in Sri Lanka are still an untapped source of bioactive natural products since their

identity and the chemistry of their secondary metabolites have not been explored thoroughly. The first report on isolation and identification of endolichenic fungi from the lichens in Sri Lanka was published by a research team from the University of Kelaniya (Kannangara *et al.*, 2009). New bioactive compounds from endolichenic fungi from Sri Lanka have been isolated and identified (Kannangara *et al.*, 2009; Kulasekera *et al.*, 2013; Pary *et al.*, 2013; Samanthi *et al.*, 2013a; 2013b; 2014; 2015). In the ongoing study on isolation of bioactive secondary metabolites from endolichenic fungi, the fungal strain US/US/06 was found inhabiting the lichen, *Usnea* sp. collected from the Hakgala Botanical Garden, Central province, Sri Lanka in December 2010. This study was aimed at determining the bioactive compounds present in the ethyl acetate (EtOAc) extract of US/US/06 cultured on potato dextrose agar (PDA) and their potential for the production of bioactive secondary metabolites. This led to the isolation of two new polyketides (compounds 1 and 2). The details of isolation, structure elucidation, and bioactivity screening of compounds 1 and 2 are described here.

## METHODS AND MATERIALS

### Isolation of the fungal strain

*Usnea* sp. was collected from the Hakgala Botanical Garden in Sri Lanka. Sterilised polythene bags were used to store the lichen samples for transport to the laboratory of the Department of Chemistry, University of Kelaniya. Surface sterilisation method was used to isolate the fungi from the lichen (Kannangara *et al.*,

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2009). Samples of *Usnea* sp. were cleaned in tap water and surface sterilised by consecutive immersion for 10 s in 95 % ethanol, 3 min in 0.5 % sodium hypochloride and 30 s in 75 % ethanol. Sterile filter papers were used to dry the cleaned lichens, which were cut aseptically into small segments (1 mm × 1 mm). Twenty pieces of the lichen were placed on 2 % malt extract agar (MEA) supplemented with 0.01 % streptomycin. The plates were sealed with parafilm and incubated up to 14 d at room temperature under ambient light. Pure cultures were prepared using fungi growing from each lichen particle. The identification of fungi was initially carried out using the sticky tape method (Felgel, 1980) and identification keys (Barron, 1988). The pure culture of the fungus was photographed and deposited as a living voucher at the Department of Chemistry, University of Kelaniya, Sri Lanka under the accession number US/US/06.

### Molecular identification of isolated endolichenic fungus

Molecular identification of the isolated pure strain of the endolichenic fungus was performed using a molecular biological protocol by genomic DNA extraction, amplification and sequencing as described by Samanthi *et al.* (2015). The extraction of fungal DNA was carried out using Promega Wizard® Genomic DNA Purification Kit, and the fungal strain was identified by analysis of the nuclear internal transcribed spacer (ITS) region of the extracted DNA and selective amplification by polymerase chain reaction (PCR) using ITS 1 and ITS 4 primers (Arnold & Lutzone, 2007). Before being subjected to sequencing, excess nucleotides and the remaining primers and enzymes in the PCR product were removed according to a gel purification protocol. Agarose gel electrophoresis was carried out to separate the desired amplified DNA bands from non specific bands. To isolate the DNA from the gel, Promega Wizard® SV Gel and PCR Clean-Up System were used (Betz & Strader, 2002). The amplified and purified DNA was subjected to DNA sequencing and the obtained sequences were submitted to the GenBank as a nucleotide query and compared with already existing DNA sequences using NCBI BLAST®. (<http://www.ncbi.nlm.nih.gov/blast/>)

### Extraction and isolation

A culture of *C. trifolii* was grown in PDA for ten days. The secondary metabolites were extracted with EtOAc (6 × 500 mL) and the solvent was evaporated under reduced pressure. This afforded a dark brown solid (1.3 g), which was found to be active in antioxidant assay using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method.

A portion (1.2 g) of the EtOAc extract was partitioned between hexane and 80 % MeOH in water and the bioactive aqueous MeOH fraction was diluted to 50 % aqueous MeOH by the addition of water, and extracted with  $\text{CHCl}_3$ . The evaporation of solvents under reduced pressure yielded hexane (441.3 mg),  $\text{CHCl}_3$  (628.1 mg) and 50 % aqueous MeOH (365.3 mg) fractions. A portion (600 mg) of the bioactive  $\text{CHCl}_3$  fraction was subjected to column chromatography on silica gel (20.0 g) by elution with  $\text{CH}_2\text{Cl}_2$  followed by increasing amounts of MeOH in  $\text{CH}_2\text{Cl}_2$ , and it afforded 44 fractions (20 mL each). These fractions were combined on the basis of their TLC profiles to obtain  $F_1$  (21.5 mg),  $F_2$  (40.8 mg),  $F_3$  (416.2 mg),  $F_4$  (32.9 mg) and  $F_5$  (20.5 mg) of which the fraction  $F_3$  found to contain the pure major compound (416.2 mg), (compound 1). A portion (20.0 mg) of the fraction  $F_1$  was further fractionated over a column of silica gel (1.0 g) made up in  $\text{CH}_2\text{Cl}_2$ , and eluted with  $\text{CH}_2\text{Cl}_2$  containing increasing amounts of MeOH and finally 100 % MeOH. Sixty two fractions (10 mL each) were collected and combined on the basis of their TLC profiles to obtain four fractions of which the fraction  $F_{1B}$  was found to be containing a fluorescent active compound. Further purification of the fraction  $F_{1B}$  (5.0 mg) using normal phase preparative TLC yielded a new pure compound (4.2 mg), (compound 2).

### Scavenging ability on DPPH

In this method, a microplate reader (Microplate Reader, Biotek, USA) and a 96-well plate were used to carry out the determination of the spectral absorption values. This assay is based on the classic method developed by Chatatikun & Chiabchalard (2013). In this method, methanolic DPPH solutions (0.25 mM, 40  $\mu\text{L}$ ) were added to different concentrations of butylated hydroxytoluene (BHT) (3.13, 6.25, 12.50, 25.00, 50.00, 100.00  $\mu\text{g}/\text{mL}$ ) in the 96-well plate. These solutions were gently mixed and incubated in the dark for 30 min at room temperature. Then the absorbances of the resulting solutions were measured at 517 nm with the microplate reader (Biotek, USA). For preparation of the standard curve, different concentrations of DPPH in methanol solutions were used and the BHT concentration ( $\mu\text{g}/\text{mL}$ ) in the reaction medium was calculated from the calibration curve. The inhibition rate was calculated and plotted *versus* test concentrations to determine the  $\text{IC}_{50}$  (Sakat *et al.*, 2010).

### Anti-inflammatory activity using HRBC membrane stability assay

This assay was carried out according to the method of Sakat *et al.* (2010) with modifications. Blood was collected from healthy human volunteers who had not

taken any anti-inflammatory drugs for 2 months prior to the experiment. The blood was transferred to heparinised centrifuge tubes and centrifuged at 3,000 rpm followed by washing three times with an equal volume of normal saline and reconstituted as 10 % v/v suspension with normal saline. The reaction mixture (5.5 mL) consisted of 5 mL of the test solution and 0.5 mL of 10 % RBCs suspension. Saline was also added to the control test tube instead of the sample. Aspirin was taken as the standard drug. All the centrifuge tubes containing the reaction mixtures were incubated in a water bath at 56 °C for 30 min and cooled under running tap water. The reaction mixtures were centrifuged at 3000 rpm for 10 min and the absorbances of the supernatants were taken at 560 nm. The test was performed in triplicate. Percentage membrane stabilisation activity was calculated (Sakat *et al.*, 2010) and plotted against test concentrations to determine the  $IC_{50}$ .

### Cytotoxicity assay

The *in vitro* assay of cytotoxicity to human nonsmall cell lung cancer (NCI-H460), CNS glioma (SF-268), breast cancer (MCF-7), human metastatic breast adenocarcinoma (MDAMB-231), prostate adenocarcinoma (PC-3), metastatic prostate adenocarcinoma (PC-3M) and pancreatic cancer (MIAPaCa-2), and normal human primary fibroblast cells (WI-38) was carried out using the resazurin-based colourimetric (AlamarBlue) assay (Wang *et al.*, 2011). The cancer cells were all cultured under standard culture conditions and the test compound or vehicle control (DMSO) was added to appropriate wells, and the cells were incubated for 72 h. Then 20  $\mu$ L/well of AlamarBlue solution was added into the assay plates for a final assay volume of 200  $\mu$ L/well, yielding a final concentration of 10 % AlamarBlue. After they were shaken for 10 s, the plates were returned to the incubator and kept for 4 h. The plates were then exposed to an excitation wavelength of 560 nm, and the fluorescence emitted at 590 nm was read. The percentage viability was expressed as fluorescence counts in the presence of the test compound as a percentage of that in the vehicle control. Doxorubicin and DMSO were used as positive and negative controls, respectively.

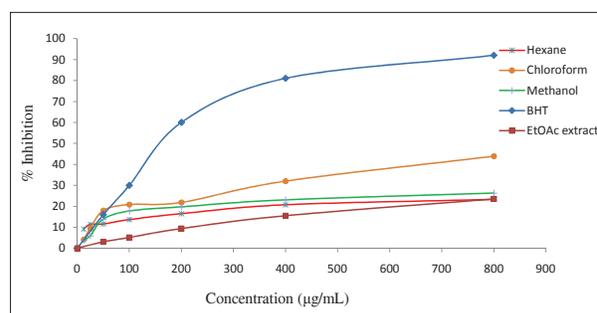
$^1H$ - and  $^{13}C$ -NMR spectra were recorded on Bruker Avance III 400 instrument using  $CDCl_3$  as a solvent. Shimadzu LCMS-QP8000 $\alpha$  was used for MS analysis. Analytical and preparative thin layer chromatography (TLC) were performed on precoated 0.25 mm thick plates of silica gel 60 F254 sprayed with a solution of anisaldehyde in EtOH, followed by heating to visualise the compounds on analytical TLC. The melting point

of compound 1 was determined using a melting point apparatus (MEL-TEMP<sup>®</sup>, USA).

## RESULTS AND DISCUSSION

The fungus, US/US/06 was identified based on the morphological and molecular characteristics. The ITS sequence matched with *C. trifolii* sequences from GeneBank with a sequence identity of 99 %. This endolichenic fungus was identified as *Curvularia trifolii*.

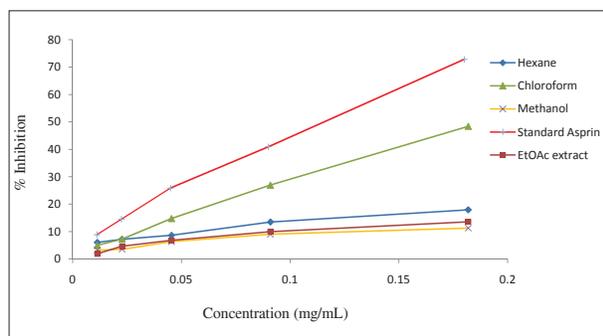
Antioxidant, anti-inflammatory and anticancer bioassays were used to detect the bioactive components in the EtOAc extract. Total antioxidant activity of the EtOAc extract of *C. trifolii* (US/US/06) was determined by DPPH assay and compared with BHT. The positive control, BHT displayed the best results (~60 %) at 200  $\mu$ g/mL concentration and the results are presented in Figure 1. Activity of the EtOAc extract increased in a



**Figure 1:** DPPH radical scavenging activity of the EtOAc extract, hexane, chloroform and methanol fractions of *C. trifolii*. The results were compared with the positive control, BHT after 15 minutes of reaction. Each value is expressed as mean  $\pm$  SD (n = 3)

concentration dependent manner. At the concentration of 200  $\mu$ g/mL the inhibitory effect of the EtOAc extract of *C. trifolii* was found to be 9 % for DPPH (Figure 1).

Total anti-inflammatory activity of the EtOAc extract of *C. trifolii* (US/US/06), was evaluated by the Human Red Blood Cell Membrane (HRBCM) stability assay (Sakat *et al.*, 2010) and compared with the positive control aspirin. Aspirin showed 72 % inhibition at the concentration of 182  $\mu$ g/mL. The anti-inflammatory activity of the EtOAc extract increased in a concentration dependent manner compared to aspirin. At 182  $\mu$ g/mL, effect of the EtOAc extract of *C. trifolii* was found to be 14 % for HRBCM stability (Figure 2).



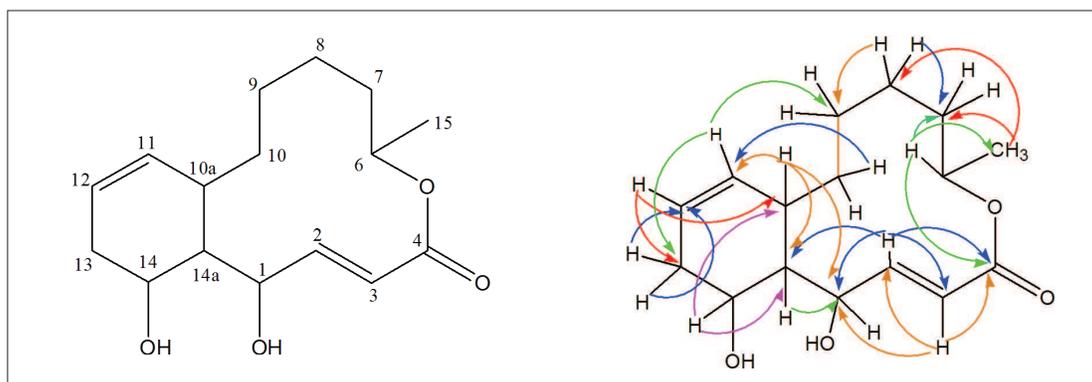
**Figure 2:** Anti-inflammatory activity of the crude EtOAc extract, hexane, chloroform and methanol fractions of *C. trifolii*. The results were compared with the positive control, aspirin. Each value is expressed as mean  $\pm$  SD (n = 3)

Antioxidant active EtOAc extract of the solid culture of *C. trifolii* was partitioned with hexane,  $\text{CHCl}_3$  and 50 % MeOH, and the results revealed that the  $\text{CHCl}_3$  fraction had the highest activity (Figure 1). Bioassay-guided fractionation of the antioxidant active  $\text{CHCl}_3$  fraction of *C. trifolii* using normal-phase column chromatography and preparative TLC indicated two pure compounds 1 and 2. The pure compound 1 was the major compound present in the crude extract (27.8 %) and was obtained as a white crystalline solid with a melting point of 194 – 196 °C and a molecular formula  $\text{C}_{16}\text{H}_{24}\text{O}_4$  and indicated a 5 degree of unsaturation. The structure of compound 1 was confirmed by a combination of  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, HSQC, HMBC, DQF-COSY, DEPT and FABMS spectra. Its IR spectrum had absorption bands at 3425 and 1674  $\text{cm}^{-1}$  suggesting the presence of hydroxyl and carbonyl groups, and the UV  $\lambda_{\text{max}}$  at 240 nm indicated the presence of an  $\alpha,\beta$ -unsaturated lactone chromophore.

The  $^1\text{H}$  NMR spectrum of compound 1 indicated the occurrence of one 3H singlet due to  $\text{CH}_3$  groups ( $\delta_{\text{H}}$  1.2) and three 1H double doublets at  $\delta_{\text{H}}$  7.33, 5.80 and 5.22

due to three olefinic protons attached to C2, C3, C11, and the olefinic C attached to C12 showed a multiplet as it was coupled with the two protons attached to C13 at  $\delta_{\text{C}}$  130.2. DEPT spectrum of compound 1 indicated the occurrence of five  $\text{CH}_2$  ( $\delta_{\text{C}}$  34, 31.8, 26.7, 41.0 and 42.9) groups accounting for 17 protons in compound 1 (Table 1). The  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of compound 1 indicated the presence of one continuous spin system:  $\text{CH}(14\text{a})\text{-CH}(1)\text{-CH}(2)\text{-CH}(3)$  to  $\text{CH}(10\text{a})$ .

The  $^{13}\text{C}$  NMR and DEPT spectra of compound 1 exhibited 16 carbon signals, including one ester carbonyl (C4,  $\delta_{\text{C}}$  167), one methyl carbon (C15,  $\delta_{\text{C}}$  21.0), five methylene carbons (C9, C8, C7, C10 and C13 with  $\delta_{\text{C}}$  26.7, 31.8, 34.0, 41.0 and 42.9, respectively), four olefinic carbons (C2, C11, C12 and C3 with  $\delta_{\text{C}}$  152.8, 136.8, 130.2 and 117.0, respectively) and five methine carbons (C10a, 14a, C6, C14 and C1 with  $\delta_{\text{C}}$  44.3, 51.8, 72.0, 72.1 and 75.6, respectively). The proton at  $\delta_{\text{H}}$  4.8 of compound 1 showed HMBC correlations with carbonyl carbon at C-4 ( $\delta_{\text{C}}$  167), C-7 ( $\delta_{\text{C}}$  35), C-8 ( $\delta_{\text{C}}$  32), and C-9 ( $\delta_{\text{C}}$  27) suggesting that this proton is attached to the twelve membered ring at C-6 (Figure 1). The olefinic proton at H-3 ( $\delta_{\text{H}}$  5.8) of compound 1 showed HMBC correlations with carbonyl carbon C-4 ( $\delta_{\text{C}}$  167), C-2 ( $\delta_{\text{C}}$  153), C-14a ( $\delta_{\text{C}}$  53) and C-1 ( $\delta_{\text{C}}$  80) confirming the presence of the proton ( $\delta_{\text{H}}$  5.8) attached to C-3 ( $\delta_{\text{C}}$  117). The methyl proton at  $\delta_{\text{H}}$  1.3 showed HMBC correlations with C-4 ( $\delta_{\text{C}}$  167), C-7 ( $\delta_{\text{C}}$  35), and C-8 ( $\delta_{\text{C}}$  32) assigning it to C-15 and the carbonyl carbon to C-4. The structure of the new compound 1 was thus established as 1,14-dihydroxy-6-methyl-6,7,8,9,10,10a,14,14a-octahydro-1H-benzo[f][1]oxacyclododecin-4(13H)-one. Compound 1 was found to be active in the anticancer assay at 5  $\mu\text{g}/\text{mL}$  with (NCIH-460), (MCF-7), (SF-268), (PC3M), and (MDA-MB-231) cell lines showing > 90 % inhibitory activity (Table 3). However compound 1 did not show any significant activity against the antioxidant and anti-inflammatory assays (Figure 3).



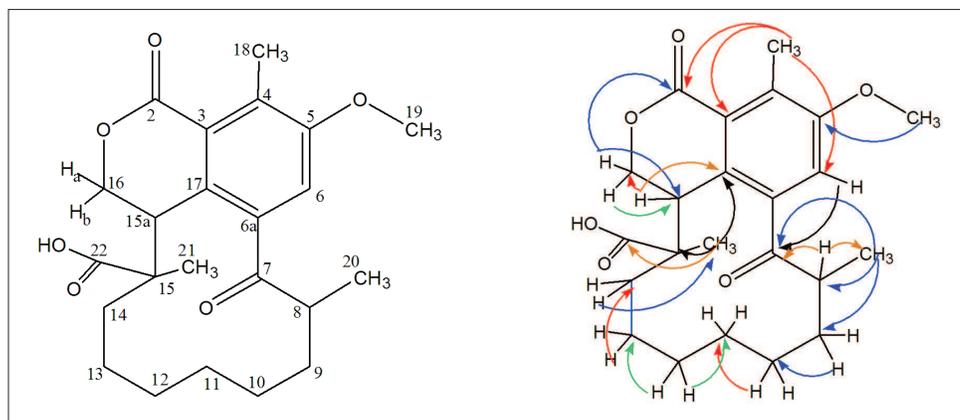
**Figure 3:** Selected HMBC correlations for compound 1

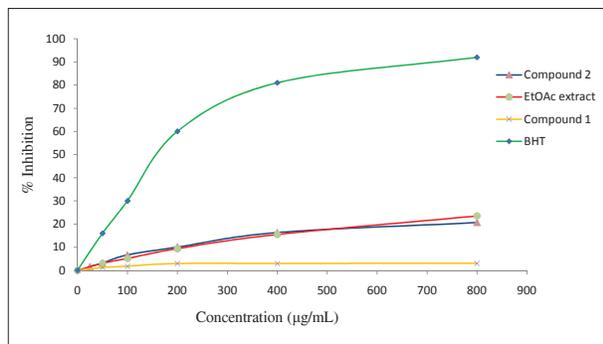
**Table 1:**  $^1\text{H}$  NMR Data (400 MHz,  $\text{CDCl}_3$ ) for compound 1

Position	$\delta$ $^{13}\text{C}$ (mult, nH, J/Hz)	$\delta$ $^1\text{H}$	HMBC
1	75.6	3.96 (m, 1H)	2, 3, 10, 14a
2	152.8	7.33 (dd, 1H, 4, 16)	1, 3, 4, 14a
3	117.0	5.80 (dd, 1H, 2, 16)	1, 2, 4, 14a
4	167.0		
6	72.0	4.76 (m, 1H)	4, 7, 9, 15
7	34.0	1.66 (m, 1H)	6
	1.46 (m, 1H)	10, 15	
8	31.8	1.76 (m, 1H)	7
	1.94 (m, 1H)	9, 7	
9	26.7	0.84 (m, 1H)	7
	1.78 (m, 1H)		
10	41.0	1.70 (m, 1H)	1, 9
	1.96 (m, 1H)	11, 12	
10a	44.3	2.26 (m, 1H)	1, 11, 12, 14, 14a, 13
11	136.8	5.22 (dd, 1H, 8, 16)	9, 10a, 14a
12	130.2	5.63 (m, 1H)	14a
13	42.9	1.41 (m, 1H)	10a, 14a, 14, 12
	2.11 (m, 1H)	14, 11, 14a, 10a	
14	72.1	4.20 (m, 1H)	13, 14a
14a	51.8	1.83 (m, 1H)	1, 10, 10a, 2
15	21.0	1.20 (d, 3H, 16)	7, 8, 6

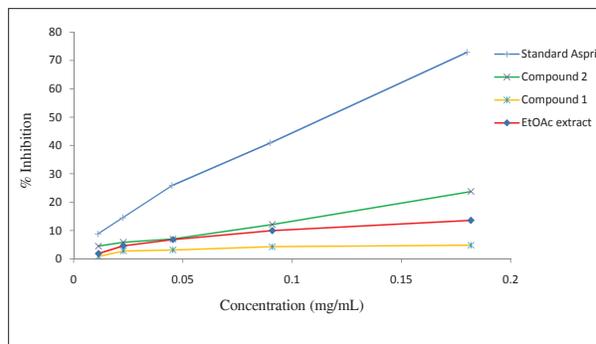
Compound 2 obtained as a fluorescent active semi - solid when analysed for  $\text{C}_{23}\text{H}_{30}\text{O}_6$  by a combination of FABMS and NMR data indicated 9 degrees of unsaturation with an isocoumarin ring. The UV  $\lambda_{\text{max}}$  at 238 nm indicated the presence of an  $\alpha,\beta$ -unsaturated lactone carbonyl chromophore (Schubert & Sweeney, 1955). Its IR spectrum had absorption bands at 3438, 1706 and 1641  $\text{cm}^{-1}$ , suggesting the presence of a OH group, carboxylic acid carbonyl, and  $\alpha,\beta$ -unsaturated lactone functionalities.

The  $^1\text{H}$  NMR spectrum of compound 2 (Table 2) indicated the occurrence of four 3H singlets, of which three were due to  $\text{CH}_3$  groups attached to carbons 4, 8 and 21 ( $\delta_{\text{H}}$  1.1, 1.4 and 2.1) and the fourth due to a  $\text{OCH}_3$  group ( $\delta_{\text{H}}$  3.8), and one 1H singlet at  $\delta_{\text{H}}$  6.5 due to an aromatic proton. DEPT spectrum of compound 2 indicated the occurrence of seven  $\text{CH}_2$  groups accounting for 27 out of 30 protons. The remaining three protons were suspected to be that of the two CH and  $\text{COOH}$  groups.

**Figure 4:** Selected HMBC correlations for compound 2



a. DPPH radical scavenging activity of the EtOAc extract of *C. trifolii* and the pure compounds (1 and 2) compared with the positive control, BHT after 15 minutes of reaction. Each value is expressed as mean  $\pm$  SD (n = 3)



b. Anti-inflammatory activity of the EtOAc extract of *C. trifolii* and the pure compounds (1 and 2) compared with the positive control, aspirin after 30 minutes of reaction. Each value is expressed as mean  $\pm$  SD (n = 3)

**Figure 5:** Results of DPPH radical scavenging activity (a) and the anti-inflammatory activity (b) of the EtOAc extract of *C. trifolii* and the pure compounds (1&2) compared with the positive control

**Table 2:**  $^1\text{H}$  NMR Data (400 MHz,  $\text{CDCl}_3$ ) for compound (2)

Position	$\delta$ $^{13}\text{C}$	$\delta$ $^1\text{H}$ (mult,nH,J/Hz)	HMBC
2	171.5		
3	112.8		
4	115.6		
5	163.0		
6	100.0	6.45 (s, 1H)	3, 4, 7
6a	159.3		
7	200.7		
8	44.2	3.31 (q, 1H)	7, 9, 10, 20
9	31.8	1.22 (m, 2H)	10
10	27.2	1.25 (m, 2H)	11
11	29.5	1.24 (m, 2H)	
12	14.1	0.84 (m, 2H)	11, 13
13	22.6	1.23 (m, 2H)	14
14	31.7	1.22 (m, 2H)	21
15	29.7		
15a	43.3	2.72 (dd, 1H, 8, 16)	6a, 16, 17
16	66.2(a)	4.72 (dd, 1H, 4, 12)	2, 6a, 15a
16	66.2(b)	3.96 (dd, 1H, 8, 12)	6a, 15a
17	90.8		
18	21.6	2.10 (s, 3H)	2, 3, 6
19	51.6	3.80 (s, 3H)	5
20	16.6	1.13 (d, 3H, 8)	7, 8, 9
21	21.7	1.38 (s, 3H)	15, 22
22	177.2		

The presence of two spin systems:  $\text{CH}_2(16)\text{-CH}(15\text{a})$  and  $\text{CH}_2(14)\text{-CH}_3(20)$  in compound 2 was evident from

its  $^1\text{H}\text{-}^1\text{H}$  COSY spectrum (Figure 4). The connectivity of the above two spin systems, tertiary methyls, and nonprotonated carbons was established by the analysis of the HMBC correlations (Figure 2) to constitute a structure with a twelve membered ring connected to a dihydroisocoumarin ring skeleton.

The  $^{13}\text{C}$  NMR spectrum (Table 2) assigned with the help of the HSQC data showed the presence of six conjugated aromatic/olefinic carbons of which one was oxygenated ( $\delta_{\text{C}} 163.3$ ) and one was protonated ( $\delta_{\text{C}} 100.0$ ), one methoxyl carbon ( $\delta_{\text{C}} 51.6$ ), three methyl carbons ( $\delta_{\text{C}} 16.6$ , 21.6 and 21.7), one lactone carbonyl ( $\delta_{\text{C}} 172$ ), one ketone carbonyl ( $\delta_{\text{C}} 201$ ) and a carboxylic acid carbonyl ( $\delta_{\text{C}} 177$ ). The proton at  $\delta_{\text{H}} 6.5$  of compound 2 showed HMBC correlations with methyl C-17 ( $\delta_{\text{C}} 21.6$ ), C-3a ( $\delta_{\text{C}} 112.8$ ) and C-15a ( $\delta_{\text{C}} 43.8$ ) suggesting that this proton is attached to the aromatic ring at C-14. The proton at H-15a  $\delta_{\text{H}} 2.7$  of compound 2 showed HMBC correlations with C-1 ( $\delta_{\text{C}} 66.2$ ), C-6a ( $\delta_{\text{C}} 159.3$ ), and C-16 ( $\delta_{\text{C}} 90.8$ ) conforming the presence of the proton ( $\delta_{\text{H}} 2.7$ ) attached to C-15a ( $\delta_{\text{C}} 43.8$ ). The proton at  $\delta_{\text{H}} 3.3$  showed HMBC correlations with C-7 ( $\delta_{\text{C}} 201$ ), C-9 ( $\delta_{\text{C}} 31.8$ ), C-10 ( $\delta_{\text{C}} 27.2$ ) and C-19 ( $\delta_{\text{C}} 16.6$ ) assigning it to H-8 and the carbonyl carbon to C-7 (Figure 2). The structure of the new compound 2 was thus established as 5-methoxy-4,8,15-trimethyl-3,7-dioxo-1,3,7,8,9,10,11,12,13,14,15,15 $\alpha$ -dodecahydrocyclo[dodeca[de]isochromene-15-carboxylic acid. The antioxidant assay results of compound 2 showed strong radical scavenging activity in the DPPH assay with an  $\text{IC}_{50}$  value of  $68.6 \pm 4.3$   $\mu\text{g/mL}$  and strong anti-inflammatory activity in the HRBC membrane stability assay with an  $\text{IC}_{50}$  value of  $310 \pm 48.2$   $\mu\text{g/mL}$  (Figure 5).

**Table 3:** Cytotoxicities of compound 1 against a panel of five tumor cell lines<sup>#</sup>

Compound	Tumor cell line <sup>a</sup>				
	PC3M	NCI-H460	SF-268	MCF-7	MDA-MB-231
1	96.8	92.1	98.5	95.5	99.5

<sup>#</sup> Results are expressed as percentage inhibition. Doxorubicin and DMSO were used as positive and negative controls.

<sup>a</sup> NCIH460, human nonsmall cell lung cancer; SF-268, human CNS cancer (glioma); MCF-7, human breast cancer; MDA-MB-231, human metastatic breast adenocarcinoma; PC-3, human prostate adenocarcinoma

## CONCLUSION

From the ethyl acetate extracts of *C. trifolii* grown in PDA, the macrocyclic lactone (compound 1) and macrocyclic ketone (compound 2) were obtained by column chromatography and preparative TLC. The present study identifies new anticancer, anti-inflammatory and antioxidant compounds from an endolichenic fungus, *Curvularia trifolii* isolated from the lichen, *Usnea* sp. in the Hakgala Botanical Garden, Sri Lanka. Two new polyketides were isolated from *C. trifolii* and their structures were determined as 1,14-dihydroxy-6-methyl-6,7,8,9,10,10 $\alpha$ ,14,14 $\alpha$ -octahydro-1H-benzo [f][1]oxacyclododecin-4(13H)-one and 5-methoxy-4,8,15-trimethyl-3,7-dioxo-1,3,7,8,9,10,11,12,13,14,15,15 $\alpha$ -dodecahydrocyclohexadeca[de]isochromene-15-carboxylic acid. This is the first report of the isolation of two new bioactive polyketides from an endolichenic fungus from *Usnea* sp. collected from Sri Lanka. The structures of the new compounds were elucidated using <sup>1</sup>H, <sup>13</sup>C NMR, DEPT, HMBC, HSQC and FABMS data. The results of this study suggest that endolichenic fungi available in Sri Lanka is a potential source of novel bioactive compounds.

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