ANTIOXIDANT ACTIVITIES OF BUTYROLACTONES FROM MARINE ENDOPHYTIC FUNGUS *ASPERGILLUS TERREUS*

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ABSTRACT

Marine organisms, particularly marine invertebrates, have proved to be a rich source of pharmaceutically active metabolites, some of which are under clinical trials. A fungal strain *Aspergillus terreus* isolated from red alga, *Laurencia ceylanica* was cultivated in bulk for 14 days and extracted with EtOAc. Above extract was purified using chromatography with Hexane/EtOAc mixtures as eluents to isolate butyrolactone-1 and butyrolactone-2. The positive CIMS of butyrolactone-1 showed its molecular ion peak at m/z 385.0 corresponding to $[M + H],^{+}$ while the positive FAB MS of butyrolactone-2 showed the molecular ion peaks at m/z 425.0 corresponding to $[M + H]^{+}$. Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage. In our studies, butyrolactone-1 and 2 were found to be significant radical scavenging potential against DPPH having the IC_{50} value of 88.2 ± 0.30 and 132.0 ± 3.77 μM respectively. Propyl gallate (IC_{50} = 30.0 ± 0.27μM), was used as a standard antioxidant in the assay.

Keywords: *Aspergillus terreus,* butyrolactone, *Laurencia ceylanica,* Antioxidants

Introduction:

Marine microorganisms, particularly marine fungi, have recently gained prominence as an important source of biologically active secondary metabolites. Among marine fungi, those living in association with marine algae are a particularly promising source of novel natural products due to the special ecological niche in which they exist. In our previous study, chemistry of endophytic microorganisms from *Sargassum wightii* Greville (Brown alga) was reported[1]. This paper describes chemistry and antioxidant activity of pure compounds isolated from an endophytic fungal strain obtained from marine red alga, *Laurencia ceylanica.* Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. Antioxidants protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite[2]

Methodology:

Extraction and isolation procedure

Laurencia ceylanica was collected from Arugambay shores, in the eastern province of Sri Lanka. The method developed to isolate endophytes from higher plants by Petrini[3] was adopted with slight modifications to isolate endophytic fungi from marine algae.

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Standard nutrient agar was used to isolate the fungal species from alga and the Czpex Dox medium was used for the large scale cultivation**.** EtOAc was added to the Erlenmeyer flasks in which the fungal strain grown and the EtOAc layer was separated and evaporated using rotary evaporator at low temperature (50 ^{o}C).

The EtOAc extract was subjected to column chromatography (CC) on Silica gel (230 -400 mess size) to yield 9 compounds using n-hexane, ethyl acetate, methanol and water as eluants.

DPPH radical scavenging Assay:

The assay was performed according to the method developed by Lee *et al*. 5 µL of each sample was dissolved in DMSO and mixed with 95 µL of DPPH in ethanol. The concentration of DPPH was at 300 mM with variable concentration of sample. The mixture was dispensed in a 96 microwell plate and incubated at 37 ^{0}C for 30min.The absorbance at 515 nm was measured by a microtitre plate reader (Spectra Max plus 384, molecular devices, CA, USA), and percent radical scavenging activity was determined in comparison with the DMSO treated control (Propyl gallate).

Results and discussions:

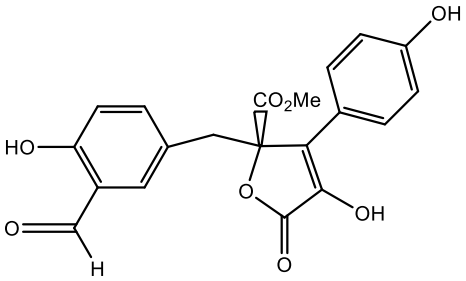
Nine compounds have been isolated and the structures of them were established using spectral data.

Butyrolactone -1

The compound butyrolactone -1 was isolated as a pale yellow colored powder with a melting point of 86-88 ^{o}C.Its specific rotation was found to be +128^{o} (c = 0.05, MeOH) which indicated the presence of chirality in the molecule. The positive CIMS of this compound showed the molecular ion peaks at m/z 385.0 corresponding to $[M + H]^{+}$ and the molecular formula was deduced to be $C_{20}H_{16}O_8$ with 13 double bond equivalents. The UV spectrum, which showed broad absorption at 203.0, 224.0, 257.8 and 306.0 nm, confirmed the presence of aromatic rings with an extended chromorphore in the molecule. The infrared spectrum of butyrolactone -1 showed typical absorption for ester carbonyls (1739.0 cm^{-1}), aldehyde group (2854.0 cm^{-1}), lactone carbonyl (1655.0 cm^{-1}) and hydroxyl groups (3411 cm^{-1}).

The ^{1}HNMR and ^{13}CNMR suggested that this compound also has the same butyrolactone skeleton as butyrolactone -2, differing only in the substitution pattern at the tri substituted aromatic ring.

The ^{1}HNMR spectrum showed signals due to seven aromatic protons, two methylene protons, one aldehyde proton and one methoxy group. Signals in the aromatic region indicated the presence of two benzene rings; one is *para* – disubstituted and the other one is tri -substituted.

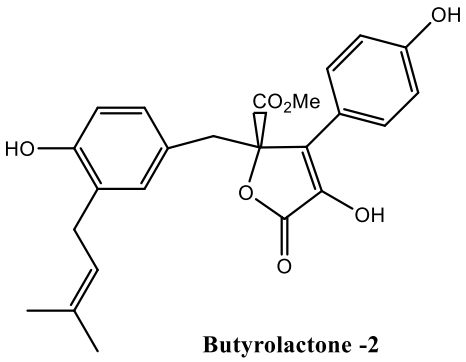


Butyrolactone -1

The *para* disubstituted aromatic ring protons, which resonated at δ 6.85 (2H, d, *j* = 8.5) and δ 7.60 (2H, d, 8.6) were assigned to H-6/H-2 and H-3/H-5 respectively. The tri -substituted aromatic ring protons which resonated at δ 6.69 (1H, d, *j* = 8.5), 7.14 (1H *s*) and 7.0 (1H, d, *j* = 8.5) were assigned to H-16, H-19 and H-15 respectively. In our previous studies, *β*-glucuronidase activity of this compound has been published[4].

Butyrolactone-2

The compound butyrolactone -2 was isolated as a white powder with a melting point of 94-96 ºC. Its specific rotation was found to be +70.0º (C = 0.08, MeOH) which indicate the presence of chirality in the molecule. The positive FABMS of compound LC-8 showed the molecular ion peaks at m/z 425.0 corresponding to $[M + H]^+$ and the molecular formula was deduced to be $C_{24}H_{24}O_7$ with 13 double bond equivalents. The structure of compound was finally identified as butyrolactone-2, through a comparison of spectroscopic data (UV, IR, MS, and NMR) with literature values[5].



Butyrolactone -2

Antioxidant activity of Pure compounds

The compound butyrolactone -1 and 2 were found to be significant radical scavenging activity against DPPH with the IC_{50} value of 88.2 ± 0.30 and 132.0 ± 3.77 µM (See table-1).

Table: 1 Antioxidant activity of pure compounds

Compounds	DPPH Scavenging Assay	
	% RSA	*$IC_{50}(\mu M)^a$
butyrolactone -1	91.6	132 ± 3.77
butyrolactone -2	93.4	88.2 ± 0.30
Propyl gallate (control)	90.3	30.0 ± 0.27

*IC_{50}: Stand for the concentration of compound that give 50% inhibition.

$^aIC_{50}$: Values are the mean ± standard mean (SEM) error of three concentrations

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