PERYLENEQUINONE DERIVATIVES FROM *ALTERNARIA* SP., AN ENDOPHYTIC FUNGUS ISOLATED FROM *GYNURA PROCUMBENS* (LOUR.) MERR. LEAVES*

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Abstract

The present study was conducted to investigate the bioactive metabolites from endophytic fungus isolated from *Gynura procumbens* (Lour.) Merr. leaves, which is locally known as Pyar-mee-ywet. A total of 17 strains were isolated from the leaf of selected medicinal plant. Among them, selection of the target fungus for further investigation was done based on chemical screening (spot pattern on TLC). From the culture broth of the selected fungus, perylenequinone derivatives, stemphyltoxin II (1), alterperylenol (2) and stemphyltoxin III (3), together with common fungal metabolites, 4-hydroxy-benzaldehyde (4), adenosine (5) and uridine (6) were isolated using various chromatographic methods. The structure elucidation of the isolated compounds was performed based on NMR and mass data.

Keywords: endophytic fungus, *Gynura procumbens*, perylenequinone, spectroscopically

Introduction

Endophytes play an important role in host plant. They can stimulate plant growth, increase disease resistance, improve the ability of plant to withstand environmental stresses and recycle nutrients (Sturz *et al*., 2000). Besides these, endophytes are also known as rich sources of important bioactive metabolites. Fungal endophytes living inside the plants could also produce metabolites which possess similar or more active compounds than that of their respective hosts (Strobel *et al*., 2003). Some of fungal metabolites include anticancer, anti-fungal, anti-diabetic and immunosuppressant compounds (Gunatilaka, 2006).

The objective of the present research work is to investigate bioactive metabolites from endophytic fungus. To achieve this aim, one important medicinal plant, *Gynura procumbens* was selected for isolation of endophytic fungus and structure elucidation of its fungal metabolites.

G. procumbens is a valuable medicinal plant. In Myanmar, the fresh leaves of this plant were used to treat diabetes. In Thailand, *G. procumbens* is used to treat topical inflammation, rheumatism and viral diseases of the skin. This plant can decrease blood sugar level and also induce insulin secretion. In Indonesia folk medicine, *G. procumbens* is used to treat fevers, skin rashes and as a remedy for ringworm infection.

Materials and Methods

General Experimental Procedures

^{1}H NMR spectra: Varian Unity 300 (300.542 MHz), Bruker AMX 300 (300.542 MHz), Varian Inova 500 (499.8 MHz). Coupling constants (*J*) in Hz. Abbreviations: s = singlet, d=doublet, dd= doublet of doublet, t = triplet, q = quartet, quint = quintet, m = multiplet, br = broad. – ^{13}C NMR spectra: Varian Unity 300 (75.5 MHz), Varian Inova 500 (125.7 MHz). Chemical shifts were measured relatively to tetramethylsilane as internal standard. - 2D NMR spectra: H, H COSY spectra (^{1}H,[1]H-Correlated Spectroscopy), HMBC spectra (Heteronuclear Multiple Bond Connectivity), HMQC spectra (Heteronuclear Multiple Quantum Coherence) and NOESY spectra

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(Nuclear Overhauser Effect Spectroscopy). - Mass spectra: EIMS at 70 eV with Varian MAT 731, Varian 311A, AMD-402, high resolution with perflurokerosene as standard. ESIMS with Quattro Triple Quadruple mass spectrometer Finigan MAT-Incos 50, ESIMS LCQ (Finnigan).

Materials

Thin layer chromatography (TLC): DC-Folien Polygram SIL G/UV$_{254}$ (Macherey-Nagel & Co.). - Column chromatography (CC): MN silica gel 60: 0.05-0.2 mm, 70-270 mesh (Macherey-Nagel & Co). Sephadex LH-20 (Pharmacia) was used for size exclusion chromatography.

Spray reagents

Anisaldehyde/sulphuric acid: 1 mL anisaldehyde was added to 100 mL of a stock solution containing 85 mL methanol, 14 mL acetic acid and 1 mL sulphuric acid. After spraying, the TLC cards were heated with hot air until colour development.

Microbiological Materials

Autoclave: Fedegari Autoclavi SPA, working temperature 121 °C, working pressure 1.2 kg/cm^2. - Antibiotic assay discs: 9 mm diameter, Schleicher & Schüll No. 321 261. - Culture media: glucose, yeast extract and malt extract were purchased from Merck, Darmstadt. – Petri dishes: 94 mm diameter, 16 mm height, Fa. Greiner Labortechnik, Nürtingen. – Celite: Celite France S. A., Rueil-Malmaison Cedex. - Sterile filters: Midisart 2000, 0.2 µm, PTFE-Filter, Sartorius, Göttingen. - Laminar-Flow-Box: Kojar KR-125, Reinraumtechnik GmbH, Rielasingen-Worblingen 1.

Work Up Procedure

Sample Collection and Isolation of Endophytic Fungi

The fresh leaves of *G.procumbens* were collected from Mandalay, Myanmar. To isolate endophytic fungi from *G. procumbens*, the fresh leaves were thoroughly washed with tap water and then the surface was sterilized by submerging them in 75% ethanol for 2 min, 5.3% NaOCl (v/v) for 1 min and thereafter dipped into 75% ethanol for 30 sec. After drying in sterile condition, small discs were cut and placed on isolation media (water agar; WA) (18 g/ L) supplemented with chloramphenicol (100 mg/ L) to suppress bacterial growth and incubated at 25°C until the outgrowth of endophytes was discerned. Individual fungal colonies were picked and transferred onto sterile water agar and periodically checked for purity.

A total of 17 fungi were isolated and each fungus was inoculated on M$_2$ medium (malt extract 10 g/L, yeast extract 4 g/L, glucose 4g/L at pH 7.8) for pre-screening. *Alternaria* sp. (fungus 1-92) was selected for further investigation due to its interesting zones on TLC both under UV and after spraying with anisaldehyde/sulphuric acid. The selected fungus was cultivated on M$_2$ medium (300 g malt extract, 120 g yeast extract and 120 g glucose in 30 L tap water pH 7.8) and transferred to 100 of 1 L Erlenmeyer flasks (300 mL each). After 14 days, the culture broth was harvested and extracted with ethyl acetate. The resulting culture extract was chromatographed to isolate the metabolites.

The culture extract (8.16 g) was chromatographed on silica gel using stepwise gradient of dichloromethane/methanol. The selected fraction I was subjected to Sephadex LH-20 using methanol only to obtain altertoxin II (1) which was isolated as red amorphous. It showed UV absorbing band at 254 nm and stained to reddish brown color with anisaldehyde/sulphuric acid on heating. After purification of fraction II on Sephadex LH-20 using methanol only, alterperylenol (2) and stemphyltoxin III (3) were isolated as red amporphous. Both of them showed UV absorbing band at 254 nm and stained to reddish brown color with anisaldehyde/sulphuric acid on heating.

Fraction III was further chromatographed on RP-18 using the gradient of methanol and water to isolate 4-hydroxy benzaldehyde (4), adenosine (5) and uridine (6). Compound (4) was islated as colorless solid and showed UV absorbing band at 254 nm. Compound (5) was isolated as colorless solid and showed UV absorbing band at 254 nm and tuned to blue with anisaldehyde/sulphurc acid on heating. Compound (6) was isolated as yellow compound and showed UV absorbing band at 254 nm and deep green color after spraying with anisaldehyde/sulphuric acid.

Figure 1 Selected fungus

Altertoxin II or Stemphyltoxin II (1)   Alterperylenol (2)   Stemphyltoxin III (3)

4-Hydroxy-benzaldehyde (4)   Adenosine (5)   Uridine (6)
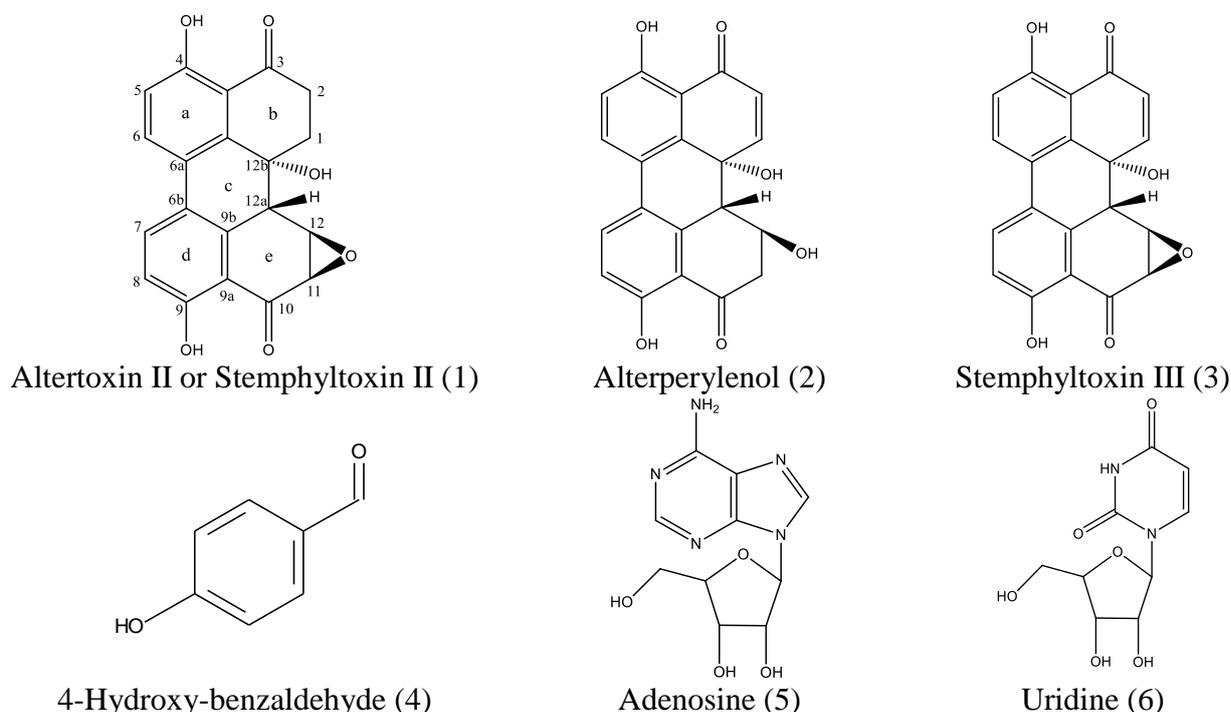
Figure 2 Compounds isolated from Alternaria sp., an endophytic fungus isolated from
G. procumbens leaves

Results and Discussion

Compound (1)

In the ^{1}H NMR spectrum, Figure 7(a), two chelating OH signals at δ 12.71 and 11.88 ppm were observed. Two doublets at δ 8.16 (J = 8.8 Hz) and 8.07 ppm (J = 8.7 Hz) in the downfield aromatic region, and another two doublets at δ 7.07 (J = 8.8 Hz) and 6.99 ppm (J = 8.7 Hz) in the upfield aromatic region, with the integration of one proton in each signals were detected. In addition, there was one OH signal at δ 5.56 ppm.

In the aliphatic region, two doublets at δ 4.37 ppm (1H) and 3.76 ppm (1H) with the same coupling constant of 3.8 Hz were observed. Furthermore, ^{1}H NMR spectrum displayed one singlet at δ 3.59 ppm (1H), two multiplets at δ 3.14 (1H) and 2.74 ppm (1H) and another multiplet at δ 2.50 ppm (2Hs). In the HMQC spectrum, Figure 7(e), the protons at δ 3.14 and 2.74 ppm

connected to the same carbon at δ 32.9 ppm and could be assigned as diastereotopic methylene protons.

According to ^{13}C NMR and HMQC spectra, Figure 7(b) & 7(e), total of 20 carbon signals were observed. Whereof, those at δ 205.5 and 197.5 ppm could be assigned as carbonyl of ketone. In addition, 12 aromatic carbon signals (four methine and eight quaternary) were visible. Among them, the two sp^2 quaternary carbons at δ 161.6 and 161.2 ppm were probably connected with oxygen. In the aliphatic region, one oxygenated quaternary carbon at δ 67.1 ppm, three methine signals at δ 56.2, 52.6, 44.1 ppm were observed. Moreover, there were two methylene signals at δ 32.9 and 31.9 ppm.

In COSY spectrum, Figure 7(c), doublet methine proton at δ 8.16 ppm showed ortho coupling with another doublet methine proton at δ 7.07 ppm with the coupling constant of 8.8 Hz. Similarly, one doublet methine proton at δ 8.07 ppm showed ortho coupling with another doublet methine proton at δ 6.99 ppm with coupling constant of 8.7 Hz. Therefore, two 1,2,3,4-tetrasubstituted benzene rings (a and b) could be drawn. In the HMBC spectrum, Figure 7(d), doublet methine proton at δ 8.16 ppm showed correlations to two sp^2 quaternary carbons at δ 161.2 and 140.5 ppm. Another doublet methine proton at δ 7.07 ppm showed correlations to two sp^2 quaternary carbons at δ 122.9 and 113.7 ppm. According to the chemical shift, the sp^2 quaternary carbon at δ 161.2 ppm could be connected to oxygen. In addition, one chelating OH signal at δ 12.71 ppm showed three HMBC cross peaks to carbons at δ 161.2, 118.0 and 113.7 ppm. It confirmed the attachment of OH group on sp^2 quaternary carbon at δ 161.2 ppm. According to HMBC data, the fragment (a) could be elucidated.

By analysis of the other HMBC correlations, the doublet methine proton at δ 8.07 ppm showed coupling with two sp^2 quaternary carbons at δ 161.6 and 136.1 ppm. Another doublet methine proton at δ 6.99 ppm showed the HMBC correlation to two quaternary carbons at δ 124.5 and 113.9 ppm. The sp^2 quaternary carbon at δ 161.6 ppm could also be connected to oxygen. As in previous fragment (a), one chelating OH signal at δ 11.88 ppm showed three HMBC cross peaks to carbons at δ 161.6, 116.1 and 113.9 ppm. According to HMBC data, the fragment (b) could be elucidated.
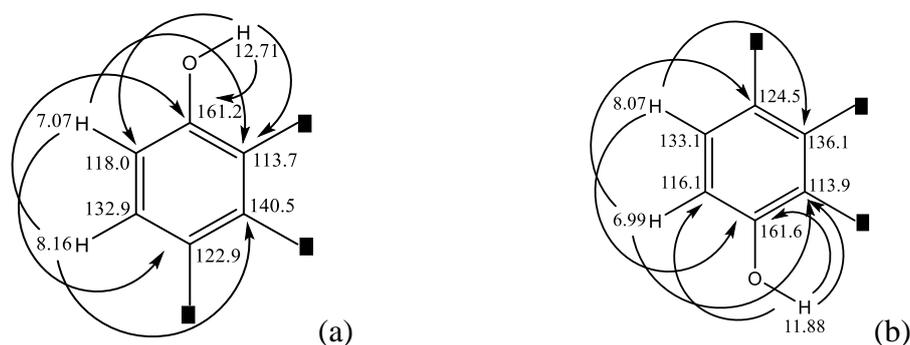
Figure 3 HMBC correlations ($\rightarrow$) in fragments (a & b)

Moreover, the methine proton at δ 8.16 ppm from fragment (a) showed HMBC correlation to one sp^2 quaternary carbon at δ 124.5 ppm from fragment (b) and similarly the methine proton at δ 8.07 ppm from fragment (a) showed correlation to one sp^2 quaternary carbon at δ 122.9 ppm from fragment (a). According to these correlations, the two benzene rings could be connected as shown in partial structure I.

Figure 4 Partial structures (I & II)

Because of downfield chemical shift of two OH groups, these two OH groups at δ 12.71 and 11.88 ppm could be assigned as chelating OH. Therefore, the carbon at δ 113.7 and 113.9 ppm could be connected to carbonyl of ketone at δ 205.5 and 197.5 ppm respectively. Therefore, the partial structure II could be assigned.

Moreover, the methane proton at δ 3.59 ppm which is attached to carbon at δ 44.1 ppm displayed HMBC correlation to sp^2 quaternary carbons at δ 140.5, 136.1, 124.5, 113.9 ppm and one sp^3 oxygenated quaternary carbon at δ 67.1 ppm. In addition, the OH signal at δ 5.56 ppm which is attached to sp^3 oxygented quaternary carbon at δ 67.1 ppm correlated to sp^2 quaternary at δ 140.5 ppm. It could confirm partial structure III.

Figure 5 Partial structures (III & IV)

Moreover, in the HMBC spectrum, Figure 7(d), the two diastereotopic methylene protons at δ 2.74 and 3.14 ppm showed correlation to sp^2 quaternary carbon at δ 140.5 ppm and one carbonyl of ketone at 205.5 ppm. In the COSY spectrum, Figure 7(c), there was a correlation between two diastereotopic methylene protons at δ 3.14 and 2.74 ppm and another methylene proton at δ 2.50 ppm. Thus, the partial structure IV could be assigned.

In addition, two methine protons at δ 4.37 and 3.76 ppm showed correlation to sp^3 methine carbon at δ 44.1 ppm. The former proton at δ 4.37 ppm showed correlation again with sp^2 quaternary carbon at δ 136.1 ppm. Thus, the partial structure V could be assigned with the partial molecular formula of $C_{20}H_{14}O_5$.
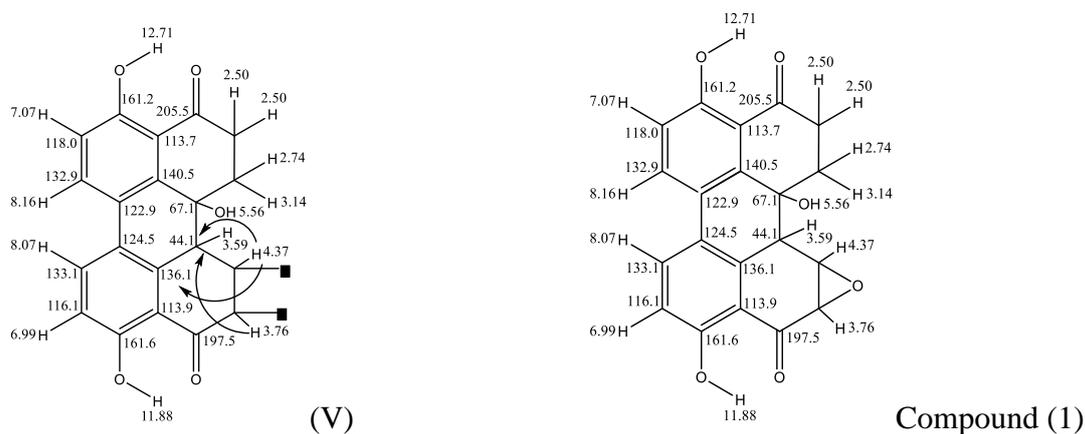
(V)   Compound (1)

Figure 6 Partial structure V and complete structure of compound (1)

The molecular mass of 350 Daltons was established by both ESI and EI mass spectra Figure 7(f), experiments. The HRESI mass spectrum gave the molecular formula $C_{20}H_{14}O_6$ with 14 double bond equivalents. Due to the high number of double bond equivalents, the isolated compound should be polycyclic. The remaining oxygen atom could be attached to carbons at δ 56.2 and 52.6 ppm as an epoxide ring. It was further confirmed by coupling constant of 3.8 Hz between two protons attached at δ 56.2 and 52.6 ppm.

(a)

(b)

(c)

(d)

(e)   (f)
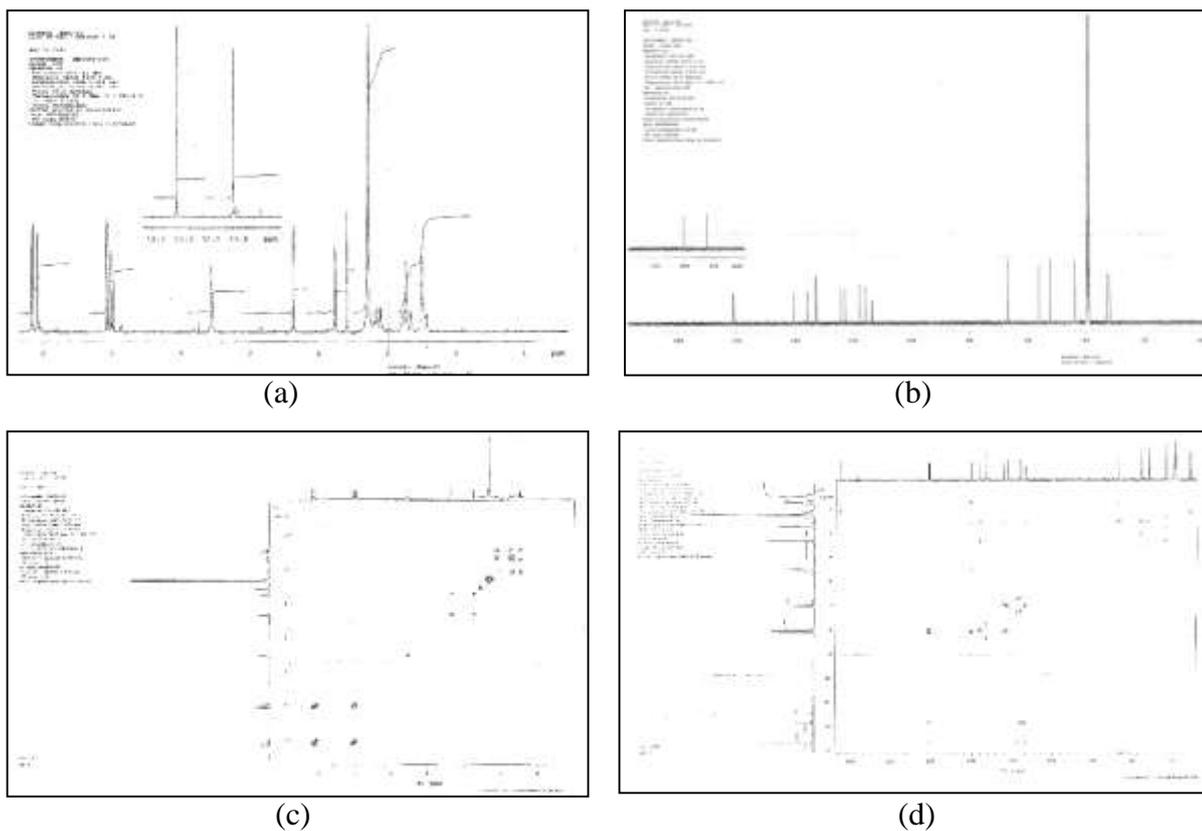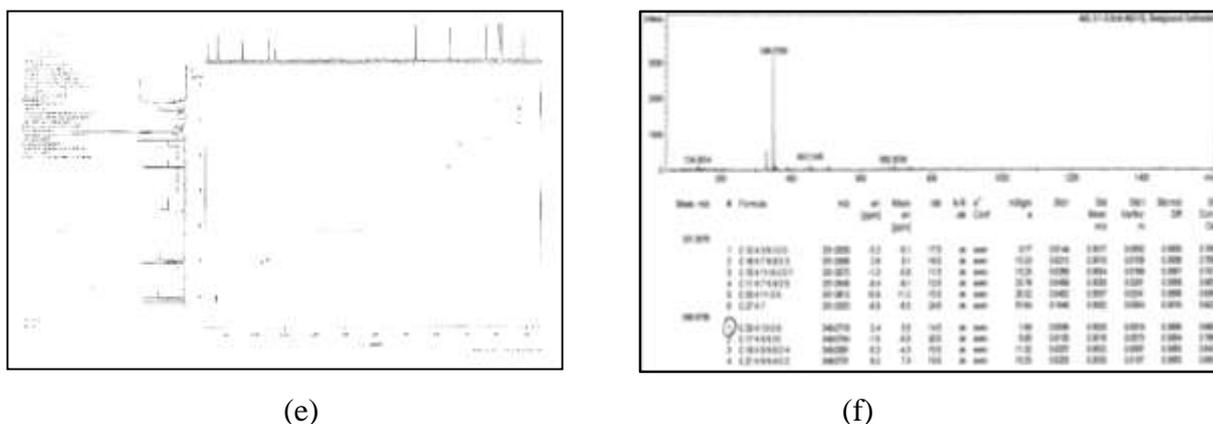
Figure 7 (a) ^{1}H NMR spectrum (DMSO-d$_6$, 300 MHz), (b) ^{13}C NMR spectrum (DMSO-d$_6$,
150 MHz) (c) COSY spectrum (DMSO-d$_6$, 600 MHz) (d) HMBC spectrum (DMSO-d$_6$,
600 MHz) (e) HMQC spectrum of (DMSO-d$_6$, 600 MHz) (f) (-) HRESI mass spectrum
of compound (1)

The relative configuration of compound **1** was determined by analysis of splitting pattern
and coupling constant as well as by comparison with literature data. H-11 and H-12 protons showed
the coupling constant of 3.8 *Hz*, which is matched with the cis coupling constant (~4 *Hz*) of the
two **vicinal** protons of the epoxide ring. So, H-11 and H-12 protons must be in the cis position.
Moreover, H-12a proton showed singlet and did not show doublet as expected. Therefore, the
dihedral angle between H-12 and H-12a must be approximately 90º with the coupling constant of
zero. H-12a and OH-12b are generally trans-axial arranged for combining two cyclohexanone
rings. All of the reported naturally occurring perylene derivatives have this stereochemistry for
H-12a and OH-12b. Thus, the structure of compound **1** could be assigned as altertoxin II or
stemphyltoxin II.

Compound (2)

Compound **2** was isolated as red amorphous powder. The molecular formula was
determined as C$_{20}$H$_{14}$O$_6$ on the basis of negative high resolution ESI mass spectrum at
m/z 349.0719 ([M-H]$^-$) with 14 degree of unsaturation. The ^{13}C NMR spectrum, Figure 8(b),
revealed the presence of 20 carbon signals which include two carbonyl of ketone, 14 *sp*^{2} carbons
(6 methine and 8 quaternary), four *sp*^{3} carbons (one CH$_2$ group, two CH and one C$_q$). Detailed
comparison of ^{1}H NMR and ^{13}C NMR spectral data of compound **2** with those of compound **1**,
indicated that the structures of these two compounds are very similar, except for the two doublet
methine protons of α, β-unsaturated carbonyl group at δ 6.31 (δ$_C$ 125.9, *J* = 10.46 Hz) and 7.87
(δ$_C$ 153.3, *J* = 10.46 Hz) in the aromatic region which were not observed in compound **1**. Moreover,
in the aliphatic region of ^{1}H NMR spectrum of compound **2**, the epoxide ring in compound **1** was
replaced by methylene protons (δ 2.84 and 3.01) and one oxygenated methine proton (δ 4.75).
Further analysis of 2D NMR data revealed that compound **2** was alterperylenol. H-12 and H-12a
must be trans-axial arrangement according to coupling constant of H-12 (*J* = 9.65 Hz).
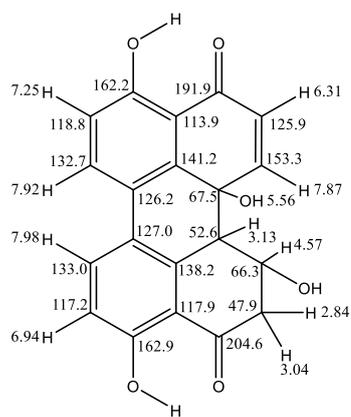
Alterperylenol (2)
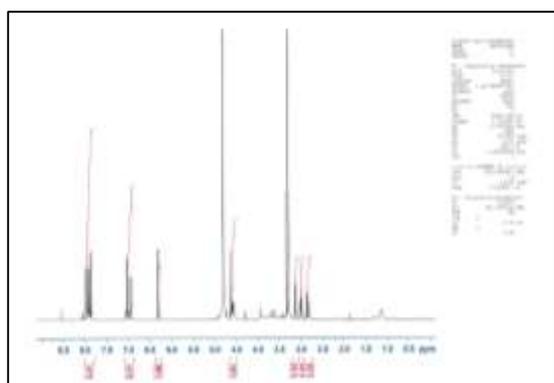
(a)
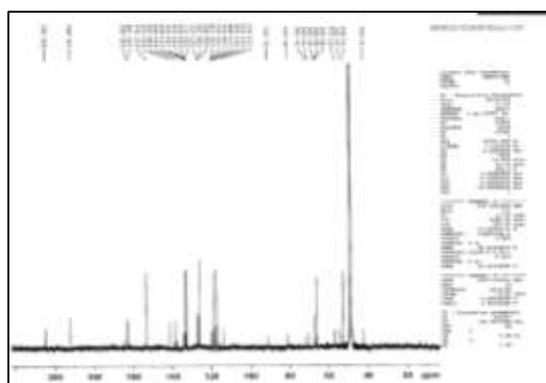
(b)
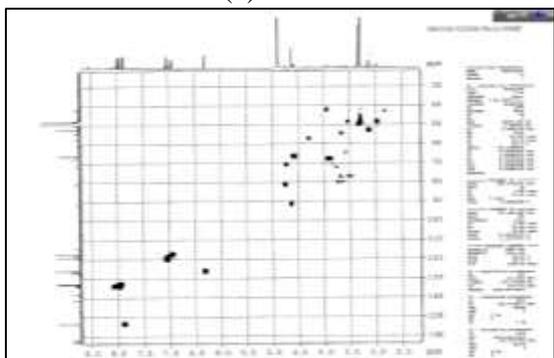
(c)
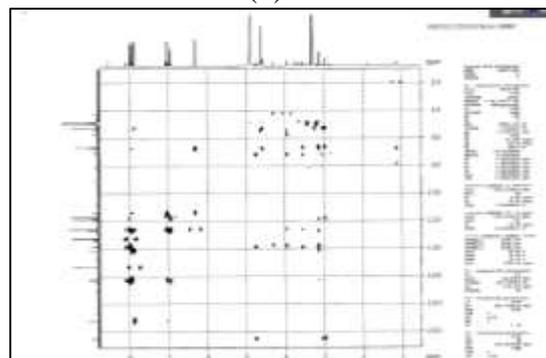
(d)
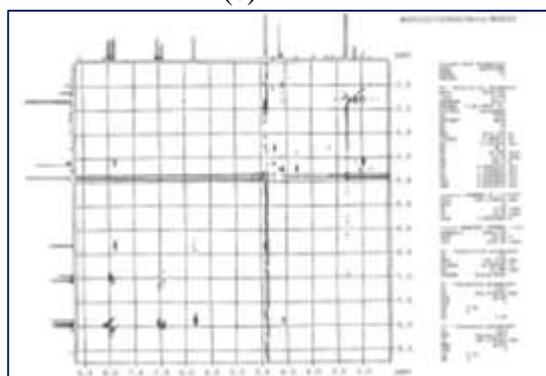
(e)
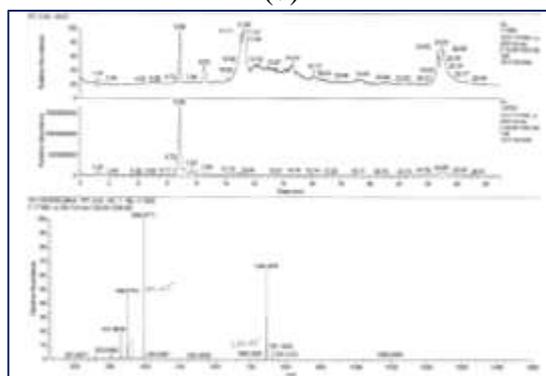
(f)

Figure 8 (a) ^{1}H NMR spectrum (CD$_3$OD, 300 MHz), (b) ^{13}C NMR spectrum (CD$_3$OD, 150 MHz)
(c) HSQC spectrum (CD$_3$OD, 600 MHz) (d) HMBC spectrum (CD$_3$OD, 600 MHz),
(e) NOSY spectrum (CD$_3$OD, 600 MHz) (f) (-) HR ESI mass spectrum of compound (2)

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Compound (3)

Another isolated compound is stemphyltoxin III and it was isolated as red amorphous. The sp^2 region of ^{1}H NMR spectrum of compound **3** is very similar to those of compound **2**. In the sp^2 region, two chelating OH, two sets of ortho-coupled protons and α,β-unsaturated carbonyl group were detected. In the aliphatic region, two doublet methine protons (H-11 and H-12) of epoxide ring at δ 3.78 and 4.60 ppm and one singlet methine proton (H-12a) at 3.80 ppm were observed. Those signals are very similar to the pattern of ring e in compound **1**. According to EI and ESI mass spectra, the molecular mass was deduced as 348 and compound **3** could be assigned as stemphyltoxin III.

The isolated perylenequinone derivatives are only soluble in DMSO and rather unstable, being quickly transformed into black insoluble products. They showed antibacterial activity *in vitro* against *Bacillus subtilis, Bacillus cereus,* and *Escherichia coli* (Arnone *et al.*, 1986). The presence of epoxy groups in compounds (**1, 3**) may well support the hypothesis that they are also phytotoxic; a respective test could not be performed, as the test organism was not available.

Some metabolites of fungi and plants containing perylenequinones have been used as folk medicine for the treatment of many diseases. Perylenequinones are a type of photosensitizers pigments widespread in nature, which have been isolated from fungi, as well as other organisms (Stack *et al.*, 1986, Davis *et al.*, 1998, Xu *et al.*, 2001 and Daub *et al.*, 2005). Due to their excellent photo-sensibilizing properties, they are expected to be developed as new phototherapeutic medicines.
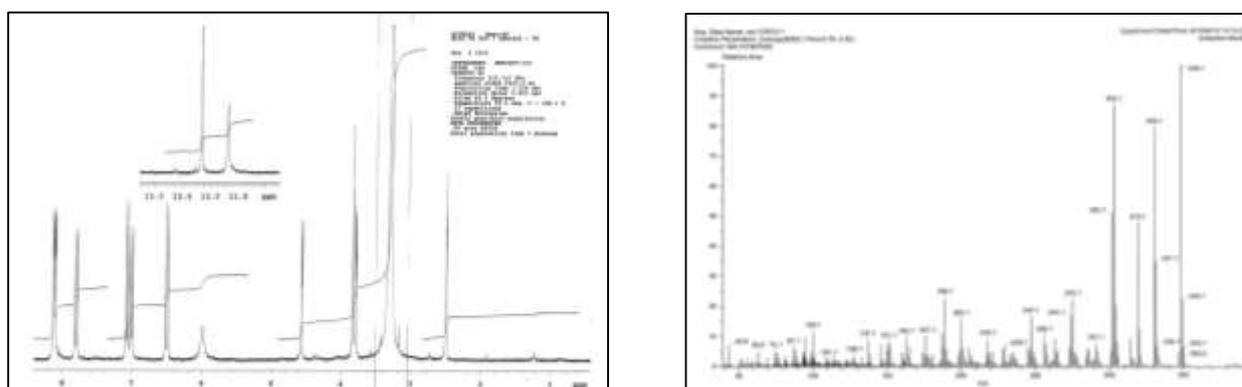
Figure 9 ^{1}H NMR spectrum (DMSO-d$_6$, 600 MHz) and ESI mass spectrum of compound (3)

Compound (4)

In the aromatic region of the ^{1}H NMR spectrum, Figure 10, two doublets at δ 7.68 and 6.85 with the integration of two protons in each signal indicated the presence of 1,4-disubstituted benzene ring. Moreover, the sharp singlet signal at δ 9.75 was observed for aldehydic proton. A search in Antibase as well as comparison with authentic spectrum led to *p*-hydroxy benzaldehyde. It was isolated frequently from aquatic organisms, e.g. by Fenical and McConnel (Fenical *et al.*, 1976) from the red seaweed *Dasya pedicellata* var. *stanfordiana* and has some antimicrobial activity against *Vibrio anguillarium*, *Candida albicans* and *Staphylococcus aureus*.
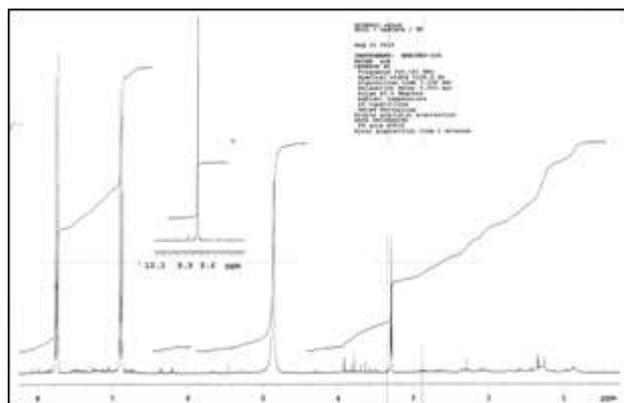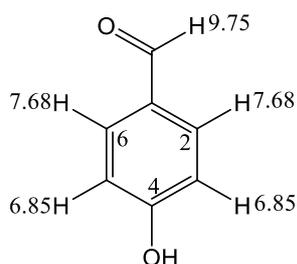
Figure 10 ^{1}H NMR spectrum of p-hydroxybenzaldehyde

Compound (5)

The ^{1}H NMR spectrum, Figure 11, showed two singlets at δ 8.38 and 8.18 for a heterocyclic aromatic ring, one anomeric proton at δ 5.90 (d) and three hydroxyl methine protons between δ 4.80 and 3.80, a diastereotopic methylene doublet of doublet at δ 3.50 and 3.70. The molecular mass 267 was deduced on the basis of negative ESI mass spectrum. According to spectroscopic data, the isolated compound could be assigned as adenosine (5). The structure was further confirmed by comparison with authentic spectra as well as literature data.
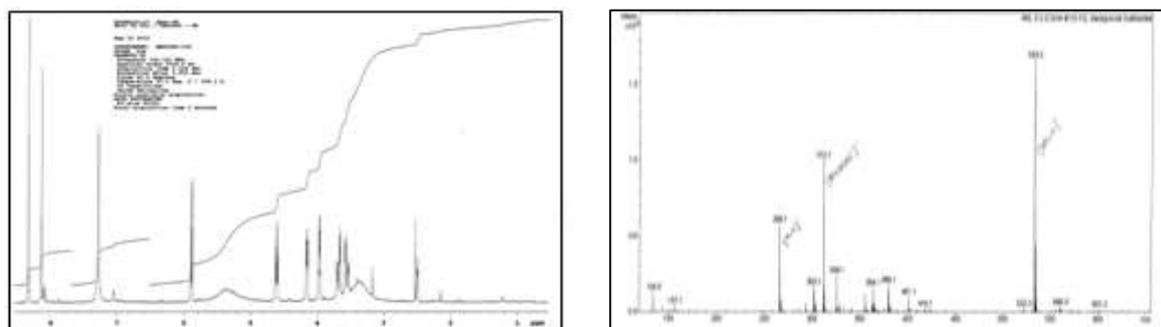
Figure 11 ^{1}H NMR spectrum and ESI mass spectrum of adenosine (5)

Compound (6)

The ^{1}H NMR spectrum of uridine exhibited two doublet peaks at δ 5.68 and 8.01 with the same coupling constant of 8.1 Hz. It indicated the presence of an α, β-unsaturated carbonyl group. The spectrum showed three oxymethine protons, two at δ 4.15 and third one at δ 4.01. Furthermore, one oxymethylene group was exhibited at δ 3.82 and indicated the presence of sugar moiety which was also confirmed by 1H doublet at δ 5.90 for the anomeric proton of the sugar.

The ESI mass spectrum showed a *pseudo*molecular ion peak at *m/z* 243 [M-H]⁻. A search in Antibase (Laatsch, 2012) as well as comparison with the authentic spectrum afforded uridine, which is widely distributed in nature in free state or in nucleic acid and can be produced by hydrolysis (Dictionary of Natural Products on CD-ROM, Chapman & Hall Chemical Database, 2010).
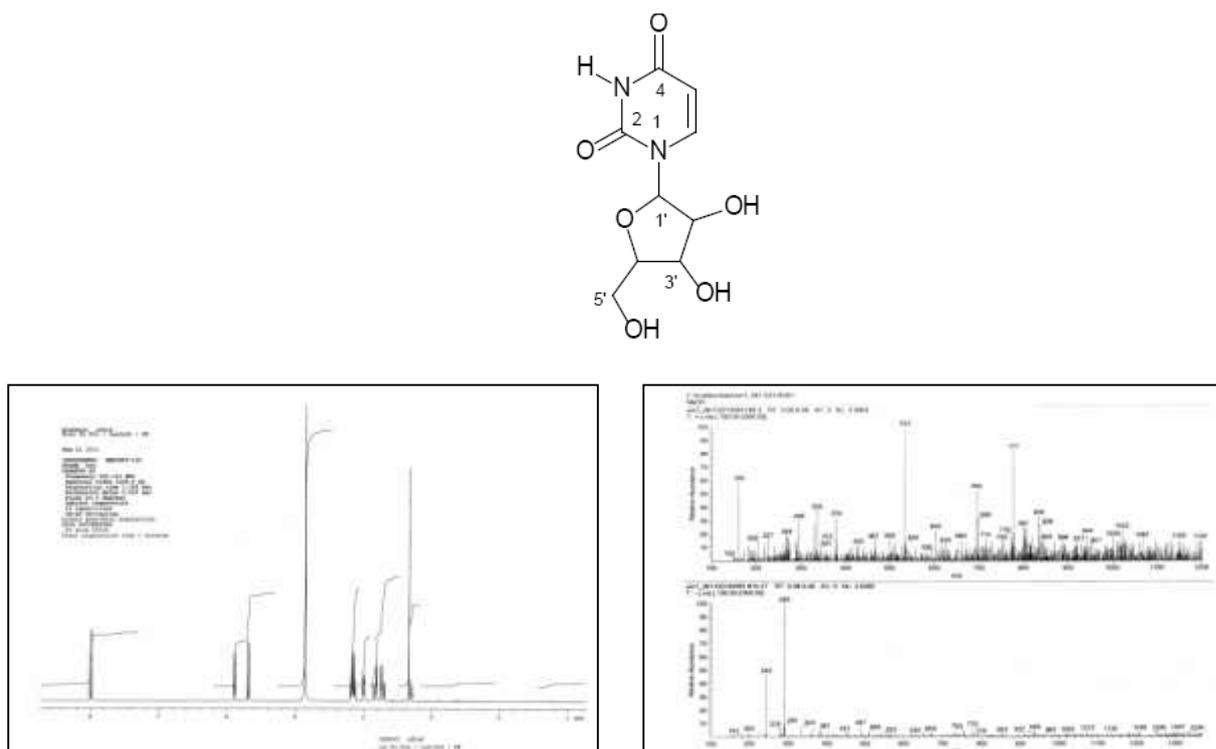
Figure 12 ^{1}H NMR and ESI MS spectra of uridine (6)

Altertoxin II or Stemphyltoxin II (1): red amorphous, strong UV absorbing band at 254 nm, deep green with anisaldehyde/sulphuric acid. – **^{1}H NMR** (DMSO-d$_6$, 300 MHz) δ 12.71 (s, 1H, OH-4), 11.88 (s, 1H, OH-9), 8.16 (d, ^3J = 8.8 Hz, 1H, H-6), 8.07 (d, ^3J = 8.7 Hz, 1H, H-7), 7.07 (d, ^3J = 8.8 Hz, 1H, H-5), 6.99 (d, ^3J = 8.7 Hz, 1H, H-8), 5.56 (s, 1H, OH-12b), 4.37 (d, ^3J = 3.76 Hz, 1H, H-12), 3.76 (d, ^3J = 3.76, 1H, H-11), 3.59 (s, 1H, H-12a), 3.14 (m, 1H, H-1), 2.74 (m, 1H, H-1), 2.50 (m, 2H, H-2). -**^{13}C NMR** (DMSO-d$_6$, 125 MHz) δ 205.5 (C$_q$-3), 197.5 (C$_q$-10), 161.6 (C$_q$-9), 161.2 (C$_q$-4), 140.5 (C$_q$-3b), 136.1 (C$_q$-9b), 133.1 (CH-7), 132.9 (CH-6), 124.5 (C$_q$-6b), 122.9 (C$_q$-6a), 118.0 (CH-5), 116.1 (CH-8), 113.9 (C$_q$-9a), 113.7 (C$_q$-3a), 67.1 (C$_q$-12b), 56.2 (CH-12), 52.6 (CH-11), 44.1 (CH-12a), 32.9 (CH$_2$-1), 31.9 (CH$_2$-2). - **(-) ESIMS** *m/z* 349 ([M-H]$^-$). – **EIMS** (70ev) *m/z* 350 ([M], 100), 332 (20), 321 (35), 305 (65), 263 (25). – **(-) HRESIMS** *m/z* 349.0709 [M-H]$^-$(calcd 349.0710 for C$_{20}$H$_{13}$O$_6$).

Alterperylenol (2): red amorphous, strong UV absorbing band at 254 nm, deep green with anisaldehyde/sulphuric acid. – **^{1}H NMR** (DMSO-d$_6$, 300 MHz) δ 7.98 (d, ^3J = 8.7 Hz, 1H, H-7), 7.92 (d, ^3J = 8.8 Hz, 1H, H-6), 7.87 (d, ^3J = 10.5 Hz, 1H, H-1), 7.25 (d, ^3J = 8.8 Hz, 1H, H-5), 6.94 (d, ^3J = 8.7 Hz, 1H, H-8), 6.31 (d, ^3J = 10.5 Hz, 1H, H-2), 4.57 (m, 1H, H-12), 3.13 (d, ^3J = 9.7 Hz, 1H, H-12a), 3.00 (dd, ^3J = 4.8, 15.9 Hz, 1H, H-11), 2.84 (dd, ^3J = 12.3, 15.9 Hz, 1H, H-11). -**^{13}C NMR** (DMSO-d$_6$, 125 MHz) δ 204.6 (C$_q$-10), 191.9 (C$_q$-3), 162.9 (C$_q$-9), 162.2 (C$_q$-4), 153.3 (CH-1), 141.2 (C$_q$-3b), 138.2 (C$_q$-9b), 133.0 (CH-7), 132.7 (CH-6), 127.0 (C$_q$-6b), 126.2 (C$_q$-6a), 125.9 (CH-2), 118.8 (CH-5), 117.9 (C$_q$-9a), 117.2 (CH-8), 113.9 (C$_q$-3a), 67.5 (C$_q$-12b), 66.3 (CH-12), 52.6 (CH-12a), 47.9 (CH$_2$-11). – **(-) HRESIMS** *m/z* 395.0771 [M-H]$^-$ (calcd 349.0719 for C$_{20}$H$_{14}$O$_6$).

p-Hydroxy benzaldehyde (4): colourless compound, R_f = 0.44 (CHCl$_3$/10%MeOH), UV absorbing band at 254 nm, – ^{1}H NMR (CD$_3$OD, 300 MHz) δ 9.75 (s), 7.68 (d, ^3J = 10.5 Hz, 2H, H-2,6), 6.85 (d, ^3J = 10.5 Hz, 2H, H-3,5).

Uridine (6): yellow compound, 7.9 mg, R_f = 0.11 (CHCl$_3$/10% MeOH), UV absorbing band at 254 nm, deep green after spraying with anisaldehyde/sulphuric acid. – ^{1}H NMR (CD$_3$OD,

300 MHz) δ 8.01 (d, ^3J = 8.1 Hz, 1H, H-6), 5.90 (d, ^3J = 6.1 Hz, 1H, H-1'), 5.68 (d, ^3J = 8.1 Hz, 1H, H-5), 4.15 (m, 2H, H-2',4'), 4.01 (d, ^3J = Hz, 1H, H-3'), 3.82 (m, 2H, CH$_2$-5'). – **(-)-**ESIMS *m/z* 243 ([M - H]$^-$, 100).

Conclusion

In this study, three perylenequinone derivatives and some common fungal metabolites were isolated and characterized. Perylenequinone derivatives are only soluble in DMSO and rather unstable, being quickly transformed into black insoluble products. They showed antibacterial activity against *Bacillus subtilis, Bacillus cereus* and *Escherichia coli*. Perylenequinone derivatives are photosensitizers with excellent photosensitizing properties. So, they are expected as new phototherapeutic medicines.

Acknowledgements

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