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Short communication

Cytotoxicity, antioxidant and antibacterial activity of four compounds produced by an endophytic fungus *Epicoccum nigrum* associated with *Entada abyssinica*

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ABSTRACT

Four compounds including beauvericin, parahydroxybenzaldehyde, indole-3-carboxylic acid and quinizarin were isolated from endophytic fungus *Epicoccum nigrum* and their cytotoxicity, antibacterial and antioxidant activity were evaluated. Beauvericin had remarkable activity against two Gram-negative strains (*Bacillus cereus* and *Salmonella typhimurium*) with respective MIC values of 3.12 and 6.25 μg/ml. All the compounds had weak cytotoxic effect on both normal and tumor cells. LC_{50} values ranged from 40.42 to 86.56 μg/ml, 31.87 to 86.57 μg/ml and 21.59 to 67.27 μg/ml on Vero cells, THP-1 and RAW 264.7 respectively. The present study showed that these compounds could be developed for the formulation of antioxidant-rich therapeutic diets and as a therapeutic agent against bacterial infections.

Introduction

Over the past few decades, multi-drug resistance in microorganisms has emerged as a serious problem in health care due mostly to the improper usage of antibiotics. In addition, accumulated evidence indicates that oxygen radicals such as superoxide are key molecules in the pathogenesis of various infectious diseases. Some pathologies arising during bacterial infections can be attributed to oxidative stress and generation of reactive species (Pohanka, 2013). Free radicals, especially reactive oxygen species (ROS), have been identified to play a crucial role in the pathogenic processes in many human disorders including cancer and microbial infectious diseases (Oyinloye et al., 2015). It is essential to investigate novel anticancer and antibiotic drugs with less chance of the development of resistance against them. Fungi are remarkably a diverse group, which can potentially provide a wide variety of metabolites such as alkaloids, flavonoids, phenols, steroids and terpenoids (Joel and Bhimba, 2013) Bioactive natural products from endophytic fungi, isolated from different plant species, have attracted considerable attention from natural product chemists and biologists in the last decades (Nisa et al., 2015). *Epicoccum nigrum* Link, Pleosporaceae is an endophytic fungus that has been associated with the biological control of phytopathogens, and the production of secondary metabolites (Fávaro et al., 2012). In search of new bioactive compounds of natural origin, this study was undertaken to evaluate the antibacterial and antioxidant activities and the cytotoxicity of four compounds isolated from the endophytic fungal species *E. nigrum* isolated from *Entada abyssinica* Steud. ex A. Rich., Fabaceae.

Materials and methods

Fresh leaves of *Entada abyssinica* Steud. ex A. Rich., Fabaceae, were collected in May 2012 at Balatchi (Mbouda), in the West region of Cameroon, and identified at the National Herbarium of Cameroon, by comparison to existing voucher specimen number HNC No. 10672/SFR/CAM. Endophyte fungi were isolated and identify according to Talontsi et al. (2013).

For the compounds isolation, the producing strain RAM10-1was cultured on PD agar at 25 °C for six days. Then, the culture was extracted with ethyl acetate (EtOAc) and concentrated to dryness *in vacuo* to afford crude extract (19.1 g). The extract was fractionated by silica gel column chromatography using CH_2Cl_2–MeOH gradient elution to provide three major fractions according to TLC.

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The second fraction was purified on silica gel CC and Sephadex LH-20 using MeOH to afford beauvericin (**1**; 15 mg; *Mw* 781; m.p. 147–148 °C) (Gupta et al., 1995). The third fraction was purified on Sephadex LH-20 using CH_2Cl_2–MeOH (1:1), and subfractions were further separated by preparative eluting with CH_2Cl_2–MeOH and purified with Sephadex LH-20 using MeOH to afford parahydroxybenzaldehyde (**2**, 6 mg; *Mw* 122; m.p. 112–114 °C), (Ayer and Trifonov, 1993), indole-3-carboxylic acid (**3**, 5 mg; *Mw* 161; m.p. 232–234 °C) (Bano et al., 1986) and quinizarin (**4**, 6 mg; *Mw* 240; m.p. 200–202 °C) (Farid and Ahmed, 2013). The structures of these compounds were identified by analysis of their spectroscopic data and by comparison with those reported in the literature as previously described (Melong et al., 2014). The antimicrobial and antioxidant activity as well as the cell growth inhibition were performed as previously reported (Supplementary data).

All experiments were conducted in triplicate and values expressed as mean ± standard deviation. Differences between values were assessed for significance using analysis of variance and results were compared using the Fisher's least significant difference (LSD) at 5% significance level.

Results and discussion

The antibacterial activity of compounds **1**–**4** against different test organisms is shown in Table 1. Results showed that their antibacterial potential against all the pathogens tested varied from weak to significant with MIC values ranging between 3.12 and 100 μg/ml (Kuete, 2010). Compounds **2**–**4** had moderate to weak activity. Interestingly, compound **1** (beauvericin) and compound **3** (indole-3-carboxylic acid) had remarkable activity against Gram-negative strains (*Staphylococcus aureus*) with respective MIC values of 3.12 μg/ml and 6.25 μg/ml. This finding is consistent with literature report on the antibacterial activity of indole-3-carboxylic acid and beauvericin. A novel series of indole-3-carboxylic acid derivatives were previously reported to possess potent antibacterial activity against *Enterococcus faecalis* (Himaja et al., 2010). Using the disk diffusion method, Meca et al. (2010) found that after 48 h of incubation time, the smallest amount of beauvericin that inhibited *Bacillus pumilus* was 0.1 mg per disk. Similarly, Xu et al. (2010) reported the median effective inhibitory concentration (IC_{50}) values of beauvericin against six test bacteria (*Bacillus subtilis*, *Staphylococcus haemolyticus*, *Pseudomonas*

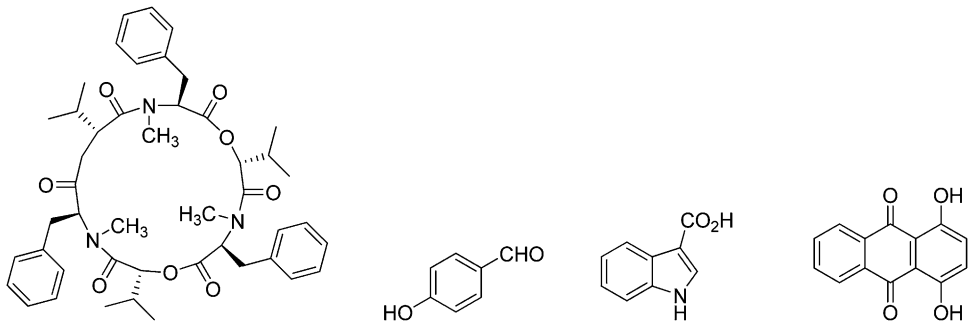

Table 1
Antibacterial and antioxidant activity of compounds isolated from *Epicoccum nigrum* (MIC in μg/ml; antioxidant values express as IC_{50}, μg/ml in DPPH and ABTS and μmol $FeSO_4$/g in FRAP).

Compounds	MIC (μg/ml)						IC_{50}, μg/ml		μmol $FeSO_4$/g
	Sa	*Bc*	*St*	*Pa*	*Ef*	*Ec*	DPPH	ABTS	FRAP
1	3.12	12.5	12.5	100	–	–	–	–	2.63 ± 0.14[a]
2	50	25	–	50	100	25	38.43 ± 4.85[a]	49.45 ± 6.52[a]	12.12 ± 1.25[b]
3	6.25	100	100	–	50	100	88.97 ± 8.91[b]	–	6.34 ± 1.14[c]
4	50	50	50	100	–	100	11.36 ± 2.83[c]	10.86 ± 2.73[b]	27.17 ± 1.66[d]
Gentamicin	0.5	0.5	2	0.25	0.25	1			
Trolox	nd	nd	nd	nd	nd	nd	8.71 ± 2.03[d]	10.38 ± 2.4[b]	nd
Ascorbic acid	nd	nd	nd	nd	nd	nd	3.44 ± 1.9[e]	4.15 ± 1.21[c]	nd

–, >100 μg/ml; *Sa*, *Staphylococcus aureus*; *Ef*, *Enterococcus faecalis*; *Bc*, *Bacillus cereus*; *Ec*, *Escherichia coli*; *Pa*, *Pseudomonas aeruginosa*; *St*, *Salmonella typhimurium*; nd, not determined. Data represent the mean ± SD of three independent experiments; values with different letters are significantly different at $p < 0.05$.

Table 2
Cytotoxicity (IC_{50} in μg/ml) of four compounds isolated from *Epicoccum nigrum* and their selectivity index (SI) against normal cell lines.

Compounds	Vero	THP-1		RAW 264.7	
		IC_{50}	SI	IC_{50}	SI
1	86.56 ± 3.94[a]	76.56 ± 5.76[a]	0.92	64.48 ± 6.17[a]	1.09
2	38.33 ± 0.60[b]	31.87 ± 4.07[b]	2.21	48.62 ± 4.70[b]	1.45
3	70.41 ± 5.69[c]	76.56 ± 4.44[a]	0.92	67.27 ± 4.12[a,c]	1.05
4	40.42 ± 2.18[b]	86.56 ± 7.76[a,c]	0.81	21.59 ± 4.22[d]	3.26
Doxorubicin	9.35 ± 0.66[c]	–	nd	0.5 ± 0.00[e]	nd
Puromycin	5.32 ± 0.90[c]	0.4 ± 0.02[d]	176.03	1.15 ± 0.17[f]	61.23

nd, not determined. Data represent the mean ± SD of three independent experiments; values with different letters are significantly different at $p < 0.05$.

lachrymans, *Agrobacterium tumefaciens*, *Escherichia coli* and *Xanthomonas vesicatoria*) to be between 18.4 and 70.7 μg/ml. Therefore, the antibacterial activity of beauvericin either determined by the diameter of inhibition zone or by IC_{50} is in agreement with the activity determined in terms of MIC in this study.

Free radical scavenging activities of the tested compounds were evaluated using the DPPH and ABTS assays while the FRAP assay was used to evaluate the reducing power. Results are presented in Table 1. The anthraquinone quinizarin had the greatest scavenging activity with IC_{50} values of 10.86 and 11.36 μg/ml in the ABTS and DPPH assays respectively. Similar to our findings, anthraquinone compounds have been reported to be very good electron and/or hydrogen donors (Zengin et al., 2015). The cytotoxicity results are shown in Table 2. Compared to doxorubicin and puromycin, compounds had no considerable cytotoxic effect on both normal and tumor cells. Compounds had LC_{50} values ranging from 40.42 to 86.56 μg/ml, 31.87 to 86.57 μg/ml and 21.59 to 67.27 μg/ml on Vero cells, THP-1 and RAW 264.7 cells respectively. Quinizarin (compound **4**) had moderate cytotoxicity against RAW 264.7 cells with LC50 value of 21.59 μg/ml and SI of 3.26. Rossi et al. (2010) previously reported the antiproliferative potential of quinizarin on B16-F10 melanoma murine cells, In addition, several data demonstrate the promising action of anthraquinones as anticancer agents (Kuete et al., 2015). The present study showed that the four compounds isolated from the endophytic fungus *E. nigrum* possess antibacterial and antioxidant activity with no toxic effect on normal Vero cells. Quinizarin had remarkable antioxidant activity while beauvericin had potent antibacterial activity. These compounds could be recommended for the formulation of antioxidant-rich therapeutic diets and as a therapeutic agent against bacterial infections respectively.

Authors contributions

JPD carried out the experiments and wrote the manuscript. RM, ATT and TM contributed to the compound isolation and identification. GDKWF and BTN supervised the chemical part of the study. JNE and LJM supervised the work and provided the facilities for biological activities study. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bjp.2016.08.011.

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