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Antitumor activity of extract and isolated compounds from *Drechslera rostrata* and *Eurotium tonophilum*

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ABSTRACT

Total extracts of *Drechslera rostrata* and *Eurotium tonophilum* in addition of two isolated compounds from their cultures [*di*-2-ethylhexyl phthalate (**H1**) and 1,8-Dihydroxy-3-methoxy-6-methyl-anthraquinone (**H2**)] were tested for their antitumor activity using four human carcinoma cell lines. Antitumor activity was assessed by performing MTT assay to check the % cell viability. The % viability of HCT-116 (colon carcinoma), HeLa (cervical carcinoma), HEp-2 (larynx carcinoma) and HepG-2 (hepatocellular carcinoma) cells decreased after treatment with *Drechslera rostrata* and *Eurotium tonophilum* extracts, these effects were ranged from 059.0 ± 0.1 to 217.0 ± 0.3 μg/ml on all types of cancer cells. The best activity was recorded for *Eurotium tonpholium* extract (054.5 ± 0.3, 059.0 ± 0.5 and 059.0 ± 0.1 for HEp-2, Hela, and HepG-2 respectively). The isolated compounds (**H1**&**H2**) were found to be responsible about the activities because they recorded the lowest IC_{50} on tested cell lines with range of 9.5–20.3 μg/ml. Vinblastine sulphate was used as a reference standard and showed *in vitro* anticancer activity. This study demonstrated that all extracts and isolated compounds have antitumor activity against HCT-116, HeLa, HEp-2 and HepG-2 cells.

1. Introduction

Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body and has become the most important health problem due effect on morbidity and mortality (Martin et al., 2013). Normal cancer chemotherapy has multidrug resistance (MDR) caused by overexpression of integral membrane transporters, which can decrease drug buildup inside the cell. MDR cells are resistant to cytotoxic effects of various chemotherapeutic agents (Ruefli et al., 2002; Shi et al., 2007). Developing new anticancer agents that are efficient to MDR cells is a reasonable approach to overcome MDR (Zhang et al., 2010).

Anticancer drugs can destroy tumors and arrest cancer progress but cancer treatment may damage healthy cells and tissues also (Liu et al., 2015). Thus, new anticancer agents from natural products are expected to play an important role in the development of more effective and safer drugs to inhibit the onset of cancer (Greenwell and Rahman, 2015).

Fungi contain some of the most unbelievable chemical factories known today. Accordingly, numerous bioactive agents such as mycotoxins, anticancer and antifungal agents have been reported in the literature (Frisvad, 2015; Frisvad et al., 2004). Despite there are many new compounds revealing meny biological activities but still being discovered, including well-known metabolites such as griseofulvin (Panda et al., 2005; Rebacz et al., 2007; Ho et al., 2001; Ronnest et al., 2009). The Large combinatorial libraries of fungal active compounds have not provided the estimated number of new chemical entities in addition it couldn't explains why the field of natural products is currently assuming new prominence (Barnes et al., 2016). It has been estimated that approximately 1.5 million or likely as many as 3 million fungal species exist on

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Earth, of which only around 100,000 species have been described so far (Sharma et al., 2016; Hawksworth, 2012). A multitude of new species are likely to be discovered from miscellaneous habitats, such as soils and tropical forest plants, associated to insects and in the aquatic environment (Bladt et al., 2013).

Therefore, our aim was to study the antitumor activity of the extracts and isolated compounds from *Drechslera rostrata* and *Eurotium tonophilum* in human cancer cells.

2. Materials and methods

Fungi under investigation [*Drechslera rostrata* (DSM 62596) *and Eurotium tonophilum* (ATCC 16440)] were pushed from DSMZ (German Collection of Microorganisms and Cell Cultures). For culturing the fungi malt extract agar (MEA) medium (Zain et al., 2012) was used for cultivation of the fungal.

2.1. Fungal extraction and isolation of compounds

The mycelia mat (800 g) of each fungus (*D. rostrata* and *E. tonophilum*) was separately harvested, washed with distilled water, and extracted by refluxing in boiled ethanol (2 liter) for 3 h and filtered off, this process was repeated for three times. The combined filtrates were concentrated under reduced pressure at temperature not exceeding 35 °C. The obtained residues of *D. rostrata* (88 g) and *E. tonophilum* (90 g) were symbolized as D1 & E1.

The total extracts (22 g each) were separately dissolved in ethanol and applied on the top of column (5 × 150 cm) packed with 100 g silica gel. Elution was carried out using chloroform - methanol (95:5 v/v), 150 hundred fractions (100 ml each) were collected, the similar fractions were collected together (according to color and number of spots) and concentrated using reduced pressure as previously described, they reduced into two sub fraction for each column. Both symbolized as DF and ET for *D. rostrata* and *E. tonophilum*. respectively.

DF fractions (12 g) were collected together were individually introduced to the top of a glass column (3 × 160 cm) packed with 360 g silica gel G. Elution were carried out using system Ethyl acetate: Methanol: Water (30: 5: 4 v/v/v), 110 fractions (100 ml each) were collected; each was concentrated under reduced pressure to a small volume. Similar fractions were collected together and re-applied on to other columns for final purification. **H1** was isolated (Awaad et al., 2014).

The sub-fractions ET (9) were dissolved in ethanol and applied on the top of column (5 × 150 cm) packed with 300 g silica gel G, Benzene: ethyl acetate (86:14 v/v) was used as eluent. Sixty fractions (100 ml each) were collected, similar fraction were collected together (according to color and number of spot) and concentrated as previously described, to produced two sub-groups with semi purified compounds for final purifications each fraction was reapplied on other columns packed with silica gel G and eluted with benzene: chloroform (90:10 v/v) from which compound **H2** was isolated (Awaad et al., 2014).

2.2. Cell culture

The tested human carcinoma cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD). The cells were grown on RPMI-1640 medium supplemented with 10% heat inactivated fetal calf serum, 1% L-glutamine, and 50 μg/ml gentamycin. The cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2 incubator (Shel lab 2406, USA) and were subcultured two to three times a week.

2.3. Antitumor activity assay

For antitumor assays, the tumor cell lines were suspended in buffer and added to the medium at concentration 5×10^4 cell/well in Corning® 96-well tissue culture plates, then incubated for 24 h. The tested compounds were then added into 96-well plates (six replicates) to achieve seven concentrations for each compound. Six vehicle controls with media or 0.5% DMSO were run for each 96 well plate as a control. After incubating for 24 h, the numbers of viable cells were determined by the MTT test. Briefly, the media was removed from the 96 well plate and replaced with 100 μl of fresh culture RPMI 1640 medium without phenol red then 10 μl of the 12 mM MTT (Sigma) stock solution (5 mg of MTT in 1 mL of PBS) to each well including the untreated controls. The 96 well plates were then incubated at 37 °C and 5% CO_2 for 4 h. An 85 μl aliquot of the media was removed from the wells, and 50 μl of DMSO was added to each well and mixed thoroughly with the pipette and incubated at 37 °C for 10 min. Then, the optical density was measured at 590 nm with the microplate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells.

The percentage of viability was calculated as:

$$[1 - (ODt/ODc)] \times 100\%$$

Where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells.

The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified compound. The 50% inhibitory concentration (IC_{50}), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each conc. using Graphpad Prism software (San Diego, CA, USA) (Mosmann, 1983; Elaasser et al., 2011).

2.4. Statistical analysis

Data were expressed as mean ± S. D. Statistical analysis was done by using GraphPad Prism 5 (San Diego, CA, USA).

3. Results and discussion

3.1. Isolated compounds

Two compounds were isolated from mycelial mat of *D. rostrate* (**H1**) and *E. tonophilum* (**H2**) using different spectroscopic analysis including ^{1}HNMR, ^{13}CNMR, HMBC, HMQC and EI-MS (Fig. 1).

H1: Colorless oil, (950 mg), R_f = 0.40 in system (n-hexane- benzene 30:70 v/v), b.p. = 385 °C; The ESI-MS at *m*/*z* 413 [M + Na]+ (100%), 390 g/mol, $[M + H]^+$ ion 391 (9.1), $[M]^+$ 390, $[M_2 + Na]^+$ 803 (5.4%).^{1}H-NMR in $CDCl_3$: δ; 0.94 (3H, t, *J* = 7.2 Hz,H8′, H8″), 0.93 (3H, t, *J* = 7.2 Hz, H10′,H10″), 1.25–1.40 (6H, m, (H-5′,5″), (H-6′, 6″ and (H-7′,7″), 1.50–1.42 (4H, m, H-9′,9″), 1.69 (2H, septet, *J* = 6 H_Z, H-4′, 4″), 4.10 (2H, dd, $J_{H3'b,\ H4'}$ = 6.1 H_Z and $J_{H3'b,\ H3'a}$ = 10.9 Hz,H-3′b,3″b), 4.26 (2H,dd, $J_{H3'a,\ H4'}$ = 5.7 H_Z and $J_{H3'a,\ H3'b}$ = 10.9 H_Z, H-3′a,3″a), 7.6 (2H, m, H4,H5) and 7.74 (2H, *m*, H3, H6).^{13}C-NMR and DEPT in $CDCl_3$: δ; 10.97 (C-10′,10″) and 14.07 (C-8′,8″)] δ; 22.97 (C-7′,7″), 23.24 (C-9′,9″), 28.90 (C-6′,6″) and 30.35 C-5′,5″), δ; 38.72 (C-4′,4″)], [δ 128.83 (C-3,6) and 130.86 (C-4,5), δ; 132.38 (C-1, C-2)] δ; 168.10 (C-1′, 1″). Spectroscopic data analysis (^{1}H-NMR.^{13}C-NMR and DEPT COSY, HSQC and HMBC) was compared with published (Rao et al., 2000; Amade et al., 1994), this compound is identified as; *di*-2-ethylhexyl phthalate.

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