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Letter to the Editor

Cytotoxicity of endophytic fungus *Chaetomium cupreum* from the plant *Mussaenda luteola* against breast cancer cell line MCF-7

Sir,

Natural products are the secondary metabolites derived from different sources as plants, animals and micro-organisms. Among these, the majority of the compounds were isolated from the plants and used as a therapeutic purpose and health supplement (Strobel and Daisy, 2003). Plant endophytes produce certain characteristic metabolites as produced by the host due to genetic recombination and thus it reduces the harvesting and usage of rare plants (Strobel et al., 2005). The endophytic fungi have been reported as a promising source of novel bioactive metabolites, particularly in therapeutic application especially anti-cancer and many others. For example, taxol is a plant-derived anti-cancer drug using commercially. It is also produced by the endophytic fungi *Taxomyces andreanae* from the host plant *Taxus brevifolia* (Chen et al., 2014).

The *Mussaenda luteola* (Family: Rubiaceae) is an ornamental plant possessing many medicinal properties like cytotoxicity, anti-inflammatory, anti-oxidant and many others with typical phytochemicals such as iridoids, triterpenes, saponins and phenols (Vidyalakshmi et al., 2008).

The plant leaves were surface sterilized according to the described method. The endophytic fungus was isolated from the leaf segments on potato dextrose agar plates and incubated at 30°C. The emerging endophyte was purified and maintained by subculturing (Arivudainambi et al., 2011). The isolated fungal strain was identified as *Chaetomium cupreum* by colony morphology and 18S rRNA sequencing (White et al., 1990). The 21 days grown fungal cultured broth was filtered and extracted with ethyl acetate. The crude extract was concentrated, dried and stored at 4°C for further analysis (Phongpaichit et al., 2007). The extract possesses phenolic-rich compounds and also showed the positive test for phenolic group (alc. FeCl₃ test).

Anti-proliferative assay of ethyl acetate extract was performed against breast cancer cell line MCF-7 and it was cultured in Dulbecco's Modified Eagle's Medium (DMEM) with added supplement of 10% fetal bovine

serum and 10 µg/mL streptomycin in a humidified CO₂ (5%) atmosphere at 37°C. The 100 µL of ethyl acetate extract with the various concentration of 10, 25, 50, 100, 150 µg/mL were seeded in 96-well plates and incubated for 24 hours. Then 10 µL of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution was added to the wells and incubated for 1-4 hours at 37°C. After the formation of formazan crystals, the MTT solution was discarded and 100 µL of solubilization solution was added to each well to dissolve the formazan crystals. The absorbance was read at 570 nm (Mosmann, 1983).

The results revealed that the cytotoxicity of ethyl acetate extract at various concentrations from 10 to 150 µg/mL showed significant inhibition and decline of cell viability in a dose-dependent manner (Figure 1). The cell viability decreased to 57.2% at the concentration of 150 µg/mL and the morphologic profiles of MCF-7 cell line treated with ethyl acetate extract of *C. cupreum* at various concentration is shown in Figure 2.

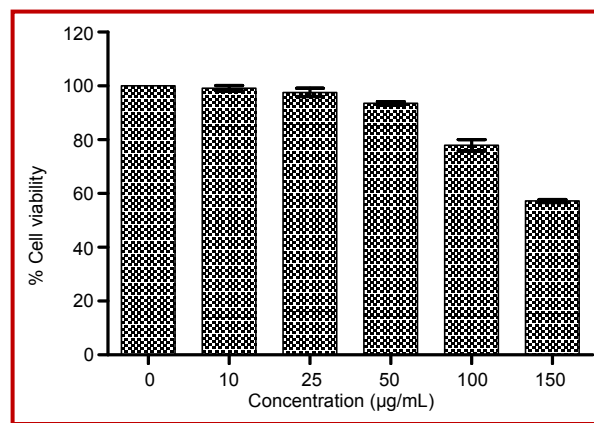


Figure 1: Percentage of cell viability at various concentrations of ethyl acetate extract of the endophytic fungus *C. cupreum*

The mode of action of various anti-cancer compounds had a strong correlation with the induction of apoptosis since many natural products were reported to have apoptosis and antiproliferative-inducing strategies in cancer cells (Giridharan et al., 2012). Paclitaxel, a novel diterpenoid from plant *Taxus* species, inhibits the depolymerization of microtubules during the cell division in cancer cells which leads to cell death (Chandra, 2012). Torreyanic acid from the endophyte *Pestalotiopsis microspora* of *T. taxifolia* which showed more potency against many cancer cell lines by inhibiting the protein



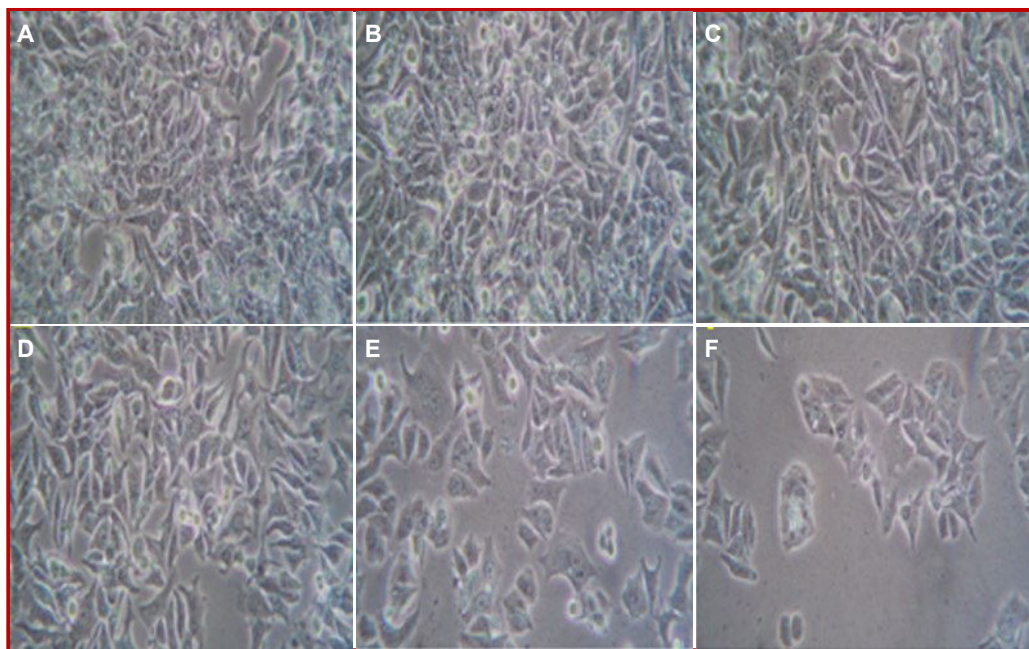


Figure 2: Morphologic profiles of MCF-7 cell line treated with ethyl acetate extract of *C. cupreum* at various concentration: A- Control; B- 10 µg/mL; C- 25 µg/mL; D- 50 µg/mL; E- 100 µg/mL; F- 150 µg/mL

kinase C and induced the cell programmed death of cancer cells (Lee et al., 1996).

Pestalactams A and B were isolated from the endophytic strain of *Pestalotiopsis* sp from the Australian plant *Melaleuca quinquenervia*. Four isoprenylated epoxy derivatives pestalofones F-H, and pestalodiols C were isolated from endophytic fungi *Pestalotiopsisfisci* from the plant *Camellia sinensis* were found to have cytotoxicity against mammalian breast cancer cell line MCF-7. 3b,5a-dihydroxy-(22E,24R)-ergosta-7,22-dien-6-one a phytoecdysteroids and 2,14-dihydrox-7-drimen-12,11-olide was isolated from the endophytic fungus *Aspergillus* sp of different plants *Bruguiera gymnohiza* and *Ipomoea batatas*. It showed strong cytotoxicity against MCF-7 cell line with the IC₅₀ value of 5.0 µg/mL and 41.7 µg/mL respectively (Chen et al., 2014).

Results from the study showed that the endophytic fungus isolated from the plant *M. luteola* have the potential anti-cancer activity against breast cancer cell line and it can be used as a source of the cytotoxic compound but further research needs to be carried out to identify the bioactive lead molecule for new anti-cancer drug development process.

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