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Research Article

Cytotoxic evaluation of endophytic fungal extract obtained through static fermentation against Human Larynx Cancer Cell Line (HEp2)

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ABSTRACT

Endophytic fungi apart from shedding light on the diversity on mitosporic fungi they are the reliable source of bio-active compounds. Several endophytic fungi isolated from different hosts produced valuable compounds which include alkaloids, flavonoids, terpenoids etc. Taxol is one of well anticancer compound produced by endophytic fungi of *Taxus brevifolia*. Hence, in the current study endophytic fungi isolated from *Ficus* plant was studied for their cytotoxic effect on human larynx cancer cell line (HEp2). Ethyl acetate extract of the fermentation broth of dominant endophytes such as *Phomopsis* sp*., Curvularia lunata*, *Botryodiplodia theobromae, Colletotrichum gleo-sporioid., Aspergillus* sp. and *Dreschlera* sp., were tested on human larynx cancer cell line (HEp2) using MTT assay. The IC_{50} value of the endophytic fungal extract effect were ranging between 50.87 to 91.01 and these results demonstrated that ethyl acetate extract of those endophytic fungi may act as a potent anticancer substance for drug development.

Keywords: Aquatic plants; antimicrobial; tannins; phenolic compounds; alkaloids

INTRODUCTION

Medicinal plants are reported to harbor endophytes which in turn provide protection to their host from infectious agents and they are unexpected producers of metabolites useful to pharmaceutical and agricultural industries (Petrini *et al.,* 1992). Endophytic fungi are probably one of the major potential sources for new, useful metabolites (Dreyfuss and Chapela, 1994). Many important anticancer, antifungal and antibacterial chemotherapautics are reported from endophytes (Rajagopal & Maheswari 1999). A single endophytic strain may produce multiple bioactive principles. Many of these compounds are alkaloids, steroids, terpenoids and peptides etc., (Tan and Zou, 2001). Some of the more interesting compounds produced by endophytic fungi are taxol, cryptocin, cryptocandin, jesterone, oocydin, isopestacin, the pseudomycins and ambuic acid (Strobel, 2000). Identification of endophytic fungi isolated from plant tissues have been the producers of the valuable drug taxol (Gary Strobel, 2003; Kusari *et al.*, 2009) is a great alternative method of natural product drug discovery which could be reliable, economical and environmentally safe. The present study was carried out to determine crude extract of endophytic fungus from *Ficus religiosa* on theHEp2 cell line

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Extraction of metabolites

Endophytic fungi was cultured in Petri dish, for 7 days. Liquid fermentation was carried out in a 500 ml Erlenmeyer flasks containing 200 ml PDB medium each and incubated for 21 days at 25° C in stationary condition. Before organic extraction the endophytic fungi mycelium was homogenised and separated by centrifugation at 7796 x g (SS- 34 rotor, RC5-B Plus Centrifuge, Sorval) for 10 minutes and then the supernatant was extracted thrice with equal volume of ethyl acetate. The organic extracts were concentrated in a rotary evaporator at 45°C. For further use (Maheswari, 2011).

Cytotoxicity and cell viability analyses

Viabilities of control and treated cells were evaluated using MTT assay, in triplicate. Cell ($1x10^4$/well) were seeded in 96- well microtiter plates containing 100 µl culture medium, and were permitted to adhere for 16-18 hrs, washed with PBS, and then treated with test compounds. After 4 hrs treatment, cells were allowed

Table 1: Cytotoxic assay on Human Larynx Cancer cell (HEp2) of ethyl acetate extracts of different endophytic fungi isolated from Ficus reliogiosa leaf

Concentration mg/ml	Ps	Bt	Dr	Cl	Cg	As
2.5	87.96	74.30	69.30	88.64	91.01	57.13
1.25	64.61	51.38	58.29	62.11	67.30	50.87
0.625	25.59	44.53	25.12	38.13	44.19	25.34
0.3125	20.00	28.67	26.53	27.48	43.63	23.45
0.156	32.52	23.02	34.62	40.47	38.96	21.78
0.078	43.15	32.52	42.24	53.51	43.69	31.89

Ps- *Phomopsis* sp., Bt- *Botryodiplodia theobromae,* Dr-*Drechslera* sp., Cl- *Curvularia lunata,* Cg- *Colletotrichum gleosporiodes and* As- *Aspergillus niger*

to grow for a further 48 hrs after medium was replaced by fresh, and then incubated at 37°C in 50 µl MTT solution (5 mg/ml) for 3 hrs. After removal of medium and MTT, 200 µl DMSO was added to each well, and the assay plate was read at 570 nm using a micro plate reader. Absorbance of untreated cells was considered as 100%. (Horton *et al*., 2007).

To calculate IC_{50} values of ethyl acetate extracts of *Botryodiplodia theobromae, Curvularia lunata, Dreschlera* sp., *Colletotrichum gloeosporioides*, *Aspergillus niger*, and *Phomopsis* sp., following formula is used:

$$\% \, Cell \, proliferation \, inhibition = 1 \frac{\sum treated \, cell}{\sum Control \, cell} \times 100\%$$

Cell cycle analysis

Human larynx cancer cells were treated with test compounds at indicated concentrations for 24 or 48 hrs. Cells were fixed in 70% cold ethanol, and then stained with a solution containing 45 µl/ml of PI and 50 µl/ml of RNase A. Flow cytometric analysis was performed using FAC Scan instrumentation (BD Biosciences, San Jose, CA, USA). Data were analysed using the Mod Fit LT program.

RESULTS

World is looking for new, novel and sustainable antimicrobial, anticancer, chemotherapeutic agents, agrochemicals etc which are very efficient, low toxicity with low environmental cause or impact. Natural bioactive compounds/metabolites/end or products from microorganisms, plants and animals are effective and promising candidates (Baker, et al., 2000). Endophytic fungi are microbes which are currently considered to be a wellspring of novel secondary metabolites offering the potential for medical, agricultural and industrial exploitation (Strobel and Daisy, 2003). Six endophytic fungal extract isolated from *Ficus religiosa* leaf were tested for cytotoxic effect by MTT assay. Out of six endophytic fungi *Collitotrichum glosporoides* and Curvularia lunata showed maximum activity at the highest concentration of the extract (Table 1). The morphological changes of the cancer cells treated with different concentration of crude extracts of the dominant endophytic fungi ranges from 0.078 to 2.5 mg/ml. All the endophytic fungal extracts showed varied cytotoxic activity at different concentrations (Table 1). The growth of all the cancer cells were markedly inhibited at 1.25, 2.5 mg/ml concentrations. At 1.25, 2.5 mg/ml concentrations most of the cells were arrested during cell division and the cell nuclei became condensed and segmented.

Colletotrichum gleosporiodes showed highest cytotoxicity in 1.25, 2.5 mg/ml concentrations with an IC_{50} value of 67.30% and 91.01% respectively (Table 1) followed by *Curvularia lunata* with an IC_{50} value of 88.64 and 62.11 and for *Phomopsis* sp. with the IC_{50} value of 64.61 and 87.96% at 1.25 and 2.5 mg/ml concentrations respectively. At these concentrations all other endophytes showed moderate activity (Table 1). Previous studies indicated the cytotoxic activity of crude extract as well the pure compound of some endophytic fungi supported the present work. Deoxypodophyllotoxin, a pro drug for anticancer have been extracted from the endophytic fungus *Aspergillus fumigates* (Kusari*et al*, 2009). Stierle *et al*., (1993) reported that a endophytic fungus, *Taxomyces andreanae* which was isolated from *Taxus brevifolia* could produce taxol, a product of as the host. This compound taxol is extensively used to treat human proliferating diseases (Rowinsky and Donehower 1991; Slichenmyer and Von, 1991). The endophyte *Rhinocladiella* sp. from *Tripterygium wilfordii* were found to produce three new chalasins that inhibited cell division of ovarian and colon tumour cell lines (Wagenaar*et al*., 2000). Crude extract from endophytic fungi from *Camptotheca acuminate* were found to be cytotoxic towards human tumour cell lines (Lin and Lu, 2007). Another compound that has antiproliferation mechanisms against human cancer cell lines is the enzyme asperaginase from the endophytic fungi *Colletotrichum* sp. (Theantana *et al.*, 2009).*Trichoderma longibrachiatum* showed a high anti-tumor ability against liver cancer cell-HEpG2, and reached its IC_{50} concentration after being diluted 20 times, (Li *et al*, 2009). Jamith Basha (2016) reported that polar and non-polar organic extracts showed anti-cancer activities against MCF7 and Hep2 cancer cell lines at the highest concentration 2.5 mg/µl. The *Chaetomium incomptum* and *Phomopsis* sp showed activity from dilutions concentration of 0.300

mg/ml to 2.5 mg/ml. In the same study the Hep2 cell line also inhibited by the crude extracts *Phomopsis* sp of endophytic fungi. The *Phomopsis* sp extract kills Hep2 cell line at all concentrations tested 0.775 mg/ml to 2.5 mg/ml in that study. Ganga devi and Muthumay (2007) showed that *Curvularia lunata* extract inhibited cancer cell lines at lowest concentration 0.078mg/ml similarly it was reported for *Botryodiplodia theobromae* against MCF7 (Pandi *et al*., 2010; Ruckdeschel *et al*., 1997). Suthep Wiyakrutta *et al*., (2004) showed 81 endophytic fungi from thai medicinal plant species of endophyte extract inhibits KB and BC-1 cancer cell lines.

The results indicate that endophytic fungi isolated from *Ficus religiosa* shown to be a good source of natural antitumor compounds. It was observed that the existing anticancer drugs have a limited selectivity and are highly toxic. Researchers in the molecular and cellular biology are regularly identifying novel potential targets, which are specific or selective for cancer cell (Kohn, 1996). In this study, extracts from six different isolates of endophytic fungi showed strong to moderate cytotoxicity against HEp2 cells indicating their potent anticancer activity and will be further purified to isolate the bioactive compounds for drug development. Further, different fermentation conditions, solvents and cell lines to be studied for their full potential.

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