Original Article

Bioassay-Guided Fractionation of Endophytic Fungal Extract of *Fusarium solani* (Saccardo) against Cancer Cell Lines and Zebrafish Embryo

Nael Mahmoud Abutaha[1,*], Muhammed Farooq Farooq[1], Fahd Ali Nasr[2], Fahd Abdu Ahmed Almekhlafi[1], Muhammad Wadaan[1]

[1]Bioproducts Research Chair, Department of Zoology, College of Science, King Saud University, Riyadh, SAUDI ARABIA.
[2]Medicinal Aromatic and Poisonous Plants Research Centre, College of Pharmacy, King Saud University, Riyadh, SAUDI ARABIA.

ABSTRACT

Background: This study aimed to explore the toxicity and apoptotic potential of *Fusarium solani* extracts on cancer cell lines and zebrafish embryo. **Materials and Methods:** *F. solani* was initially extracted with ethyl acetate and methanol. Further purification was performed using column chromatography. Each fraction was assayed for its cytotoxic potential against four cancer cell lines using MTT assay. The apoptotic potential of the HepG2 cells was investigated using Hoechst 33342 dye and caspase 3/7. The *in vivo* developmental toxicity of the active fractions was assessed using zebrafish embryos. **Results:** The ethyl acetate extract and active fractions affected the cell viability of tested cell lines on dose dependent manner. The IC_{50} values of F1 fraction for Jurkat, HEK 293, MDA-MB-231 and HepG2 cell lines were 48.07, 60.09, 97. 82 and 69.7 μg/ml respectively whereas, the IC_{50} values of F7 fraction were 58.17μg/ml and 34.29 for HepG2 and HEK 293 cell lines respectively. Morphological changes of cell such as chromatin condensation as well as the expression of caspase 3/7 were observed. In agreement with cell line data, the active fraction obtained from *F. solani* were toxic for zebrafish embryos at $\geq$100 μg/ml and lower concentration ($\leq$60μg/ml) induced various developmental defects. **Conclusion:** The *F. solani* ethyl acetate extract and its fractions showed anticancer activity in tested cancer cell lines and induced apoptosis via caspase 3/7 pathway and showed *in vivo* toxicity in zebrafish embryos.

Key words: Cell lines, Endophyte, *Fusarium solani*, Toxicity, Zebrafish.

Submission Date: 20-11-2019;
Revision Date: 06-02-2020;
Accepted Date: 12-03-2020

DOI: 10.5530/ijper.54.2s.87

Correspondence:
Dr. Nael Abutaha
Associate Professor of Microbiology and Biotechnology, Bioproducts Research Chair (BRC), Department of Zoology, College of Science, King Saud University, P.O. Box 2455, Riyadh-11451, SAUDI ARABIA.
Phone: +966 011 4699662
E-mail: nabutaha@ksu.edu.sa

INTRODUCTION

Cancer is considered among the top causes of mortality worldwide. It is accounted for 8.2 million deaths in 2012 and expected to cause 13.1 million of deaths by 2030.[1] The increasing demand for life saving medicine from new sources is an urgent necessity. The drug discovery via traditional synthetic chemical methods is not promising as compared to natural sources. The active compounds isolated from natural sources are a potential source for cancer chemotherapy due to their less toxic profile as compared to synthetic sources and will remain the main source of inspiration for chemists. Natural products drug discovery are never exhausted.[2-5] Of 22 thousand bioactive secondary metabolites isolated from micro-organisms about 38% (8,600) are of fungal origin.[5] Fungal endophytes area potential source for novel compounds.[6] Endophytic fungi survive within plant tissues causing no disease.[7] They benefit their hosts by improving drought tolerance of the plant,[8] enhancing plant growth[9] and protecting against phytopathogens.[10] Between 2000 and 2006 about 322 secondary metabolites were characterized[11,12] with a different range of structures[13,14] and biological activities.[15-19]

Saudi Arabia flora has about 2281 species in 853 genera.[20] Their endophytes as potential source of novel and bio-active compounds

www.ijper.org

are not well investigated. To date, very few reports have been published on the isolation and evaluation of endophytes and their bioactive extract from *Phoenix dactylifera* (Arecaceae family).[21]

Zebrafish is routinely used for the biological screening of small compounds and natural products and it has been well documented as the best vertebrate model for evaluating the toxicity of many compounds targeting molecular, morphological and safety pharmacological endpoints.[22-24] This value was accredited to zebrafish due to low cost, transparency and their small size that allows the screening in 96-well plates.[25] The zebrafish is perfect model to check the toxicity at the whole animal level that can be exploited to predict the toxicity on humans. We believe that this is the first report to evaluate the toxicity after the exposure of zebrafish embryos to *F. solani* extract and its fractions.

This study was performed with the objective of using zebrafish as an embryonic model system to study and evaluate the anticancer fraction from *F. solani* showing anticancer property to the HepG2 cell lines. We expect that our finding will provide insight into the probable toxicity and help in better understanding the natural products of therapeutic potential.

MATERIALS AND METHODS

Isolation, Purification and Identification of *F. solani* by Cultural and Morphological Methods

Endophytic *F. solani* was isolated from the roots of *Phoenix dactylifera* obtained from Riyadh. Isolation and purification of endophytic fungi was accomplished according to the method described by Abutaha *et al.*[26] Cultural characters such as color, shape, texture and type of the growth of were evaluated by visual observation. Morphological features of the fungus like conidia, conidiophores and mycelia were evaluated under microscope (Leica, Germany). Further confirmation was carried out by sending the fungi to Assiut University Mycological Centre (AUMC).

Extraction and Isolation of Secondary Metabolites

Ten plates with around 6 mm disks of *F. solani* (4 days old culture) were incubated at 30°C for 10 days. 10 plates of Potato dextrose agar (PDA) plate cultures were scrapped and blended in a blender containing ethyl acetate (500 ml) and transferred to flask and left to mix for 10 min at 140 x g at 30°C. The extraction process was repeated thrice and then pooled together and filtered through what man filter paper no 1. The extract was condensed and then centrifuged at 12000 x g (5 min). The extract was then evaporated under reduced pressure at 45°C. The sample was left at -80°C until required. The same extraction procedure was repeated for methanol solvent as mentioned above.

Approximately 500 mg of the ethyl acetate fraction was suspended in 2 ml of 95% methanol and loaded to a column filled with Sephardi LH-20 equilibrated with 95% methanol. The extract was eluted with methanol which yielded four fractions (F1, F2, F3 and F4). Each extract was further fractionated based on its bioactivity profile in cancer cell lines. Hence, F1 was dissolved in chloroform and further fractionated into two fractions i.e. chloroform soluble fraction (F 5, 51mg, yellow color) and chloroform insoluble fraction (F6, 9mg, yellow color). F5 Fraction was separated on silica gel 60 RP2 using $CHCl_3$, EtOAC and MeOH as an eluent to give three sub-fractions. Chloroform fraction (F7, 20mg, green color), ethyl acetate fraction (F8, 15mg, yellow color) and methanol fraction (F9, 18mg, yellow color) (Figure 1).

Studying the Morphological Changes by Apoptosis in Cancer Cells

Liver cancer cells (HepG2) (2×10^5 cells/ml) were left to attached overnight in 24 well cell culture plates and then treated at the concentration of 100 µg/ml of the extract and Methanol (0.01%) for 24 hr. Cells were assessed morphologically immediately under inverted light and fluorescence microscope (EVOS F1, USA) at 340-380 nm emissions using Hoechst 33258 (life technology, USA) staining method following the procedure of Abutaha.[27]

Caspase-3/7 Activity Assay

Cells were grown and treated as mentioned above. After 24hr of exposure, the caspase-3/7 activity was assessed using the Cell Event™ Caspase-3/7green

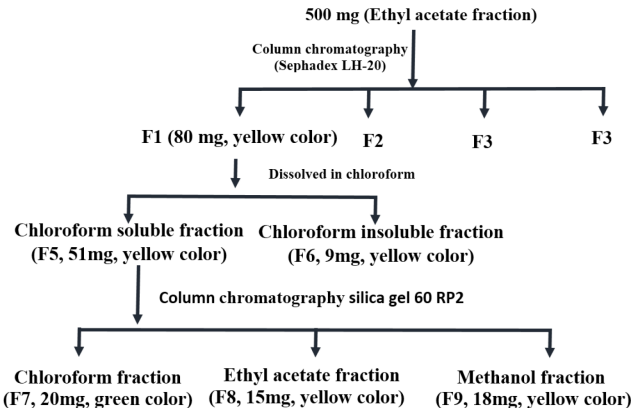


Figure 1: Bioassay guided fractionation of anticancer active fraction from *Fusarium solani* extract against different cancer cell lines. Each fraction was assessed using MTT assay to isolate the active fraction from the ethyl acetate extract.

detection reagent kit (Invitrogen, USA). Briefly, the cells were incubated for 30 min after adding the Caspase3/7 Detection reagent and the apoptotic cells were visualized as green cells using fluorescence microscope (EVOS F1, USA).

Animals

Wild type zebrafish were maintained in the laboratory under standard conditions on a 12-hr light/dark photoperiod in water (28°C). The later consists of reverse osmosis water augmented with a commercial salt (0.6% Instant Ocean) using the standard guidelines that are described in the literature. In this study the zebrafish embryos that were used were less than 120hpf, hence it does not need the consent of animal committee as stated by Strähle *et al.*[28] But all experiments were performed in accordance with the National and International Animal Use Guidelines.

Embryo Treatment

The zebrafish embryos (wild type) were obtained by natural pair-wise mating. All the extracts were dissolved methanol **(**HPLC, Sigma-Aldrich) at a stock concentration of 20 mg/ml. The embryos at one cell stage were exposed to serial dilutions of various extracts and the mock control (0.5% methanol (v/v). This treatment was repeated at least thrice using a different batch of embryos from different parents. The morphological defects and mortalities were evaluated after 24 hr of exposure.

FTIR Analysis of Bioactive Fractions

The bio-active fraction was evaluated using infrared (FTIR) spectroscopy, which was achieved by potassium bromide (KBr) plates (JASCO FTIR model 420, Japan).

RESULTS

The fungal strain grown on PDA medium showed the following feature: raised, circular, white, compact and, reverse light brown, hyphae profusely branched, hyaline, smooth, septate, phialide arising laterally on hyphae.[29] The fungal strain produced three types of spores chlamydospores, microconidia and macroconidia. Most of the morphological features of the strain (AUMC No. 10181) agree with the known features of *F. solani*. However, an expert microbiologist at Mycological center, faculty of science, Assuit, Egypt, further confirmed the identification of the fungal strain. The *F. solani* deposit number is (AUMC10181).

Cell Growth Inhibition

The crude ethyl acetate extract of *F. solani* exhibited percent survival (20±0.012%) whereas the crude methanol extract showed 91.69± 0.24 percent survival at the concentration of 200µg/ml for HepG2 cell lines. Therefore, we fractionated the crude ethyl acetate extract and monitored the activity by bioactivity-guided fractionation to isolate to the active principles. In the first fractionation process, we used sephadex as stationary phase. The ethyl acetate extract was divided in four distinct fractions of which F1 exhibited the anti-proliferative activity. Human breast cancer cell lines (MDA-MB-231), human hepatocarcinoma (HepG2) were treated with different concentration (25-200 µg/ml) of F1 extract. After 24hr of exposure percent survival was measured by MTT assay. The F1 fraction resulted in dose dependent decrease in the viability of the entire cell line tested but with different response. The LC_{50} value of F1 fraction was 99.62 and 72.03µg/ml (Figure 2) for MDA-MB-231 and HepG2 cell lines respectively. However, the other fractions obtained (F2-F4) were almost inactive and the percent survival ranged between 90-100%. F1 was further split yielding two fractions F5 and F6. Only one concentration of F5 was tested and exhibited anti-proliferative activity. The sub-fractionation of F5 on a reverse phase silica gel RP2 column yielded two fractions (F6-F7) among the fractions obtained fraction F7 exhibited anti-proliferative activity in HepG2 with the LC_{50} value 54.06µg/ml.

Apoptotic Potential of F7 Fraction on HepG2 Cell Lines

The apoptotic potential of the F7 fraction was checked by observing the alterations in morphological features

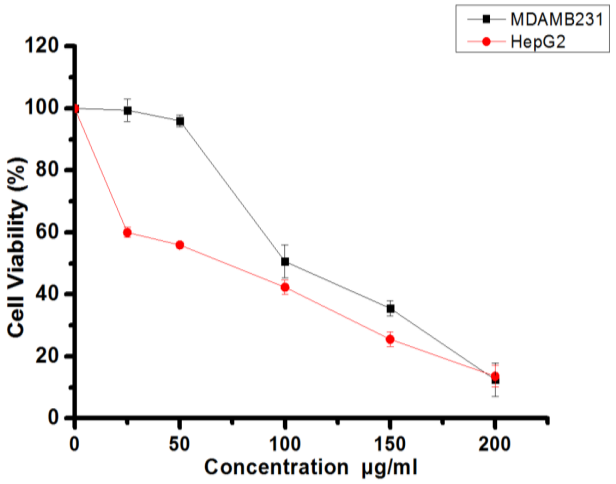


Figure 2: Dose response studies of the F1 fraction of *F. solani* extract on four cancer cell lines: MDA-MB-231 and HepG2 cell lines. The cells were treated with various concentrations (25-200 µg/ml) of F1 fraction for 24 h. The anti-proliferative effect was measured by MTT assay. Results were expressed as the means ±SD from three independent experiments.

of human liver cancer cells (HepG2). The treated HepG2 cells by F7 fraction at the concentration of 100µg/ml exhibited the typical features of apoptosis as mentioned by Moongkarndi *et al.*[30] These morphological changes are commonly used for the identification of apoptosis. The morphology of untreated cells (Figure 4A) revealed that control cells maintained their normal shape and were adhered to the cell culture plates. In contrast, HepG2 cells treated with F7 fraction (24 hr) showed that they were detached from the cell culture flask and lost contact with nearby cells. The cells changed to round shape and membrane blebbing and shrinkage was evident which the typical feature of apoptosis (Figure 3B). The Hoechst 33342 dye staining showed that, the control cells were normal shape and nuclei stained with a less bright blue fluorescence with no signs of fragmentation (Figure 3C). However, the nuclei of treated cells with F7 tested fraction clearly displayed highly fragmented chromatin (Figure 4D).

Activation of Caspase 3/7 on HepG2 Cell Lines

Program cell death is initiated via different pathways. A key part of this initiation is mediated by caspase-3, the enhancer of apoptosis. To assess the effect of F7 on the activities of caspases 3/7 in HepG2 cells, the cell line was treated the with F7 fraction for 24 hr. As it can be clearly visualized from florescent microscopy image (Figures 4E and F) that the cells, treated with F7 fraction, exhibited activation of caspase-3/7 activity (intense staining) whereas untreated cells without activated caspase 3/7 showed minimal fluorescence signal. These findings confirmed that F7 fraction induced apoptosis by activation of caspase 3/7 in HepG2 cells.

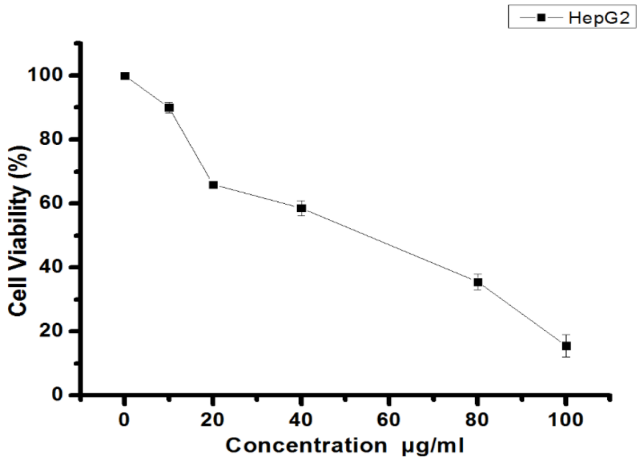


Figure 3: Dose response studies of the F7 fraction of *F. solani* on HepG$_2$ cell lines. The cells were treated with different concentrations (10-100 µg/ml) of F7 fraction for 24 h. The antiproliferative effect was measured by MTT assay. Results were expressed as the means ±SD from three independent experiments.

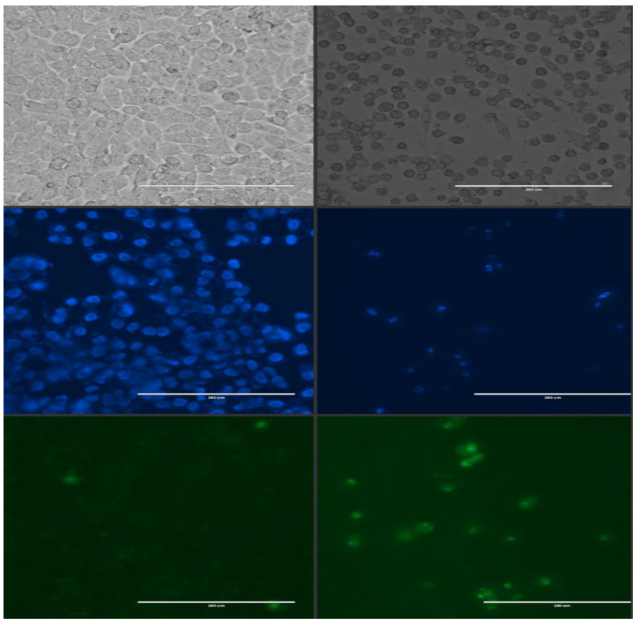


Figure 4: (A-F) Photomicrograph reveals the morphological changes of HepG2 cell line. Cells were treated with F7 fraction for 24 hr at the concentration of 100µg/ml and visualized by inverted light and florescent microscope (magnification 200x). A, C, D control and B, D, F treated cells for light microscopy, Hoechst staining and Caspase 3/7 activity respectively.

The *F. solani* Metabolites Induced Stunted Growth and Teratogenesis upon Exposure in Zebrafish Embryos

Presently, the developmental toxicity of *F. solani* was assessed in zebrafish embryos. The embryos were treated with different dilutions of the extracts and the numbers of affected embryos were counted in each case in order to assess the relative biological activity of each extracts. A consistent concentration of the tested extracts, which induced the prominent developmental defects, was chosen to evaluate the comparative biological activity between treated and untreated zebrafish embryos. The zebrafish embryos showed major developmental defects when they were treated at ≤60µg/ml with either of the extracts, so this concentration has been reported (Table 1) for the comparative biological activity of different fractions of *F. solani* in zebrafish embryonic development. We did not observe any developmental toxicity when embryos were treated below the concentration of 20 µg/ml. As shown in Figure 5. The developmental defects, which were seen in this study, are given in Table 1. The crude EtOAC extract showed mild level of activity by inducing bent spine and shorter yolk extension and larger yolk area as compared to mock (methanol) treated embryos.

The embryos exposed to crude *F. solani* extract did not hatch normally. The brain and notochord developed normally in crude extract treated embryos. The F1 fraction induced severe bending of the tail and notochord (Figure 5C). The yolk extension was also much smaller as compared to control. The fraction F7 (Figure 5D) induced most severe developmental delay in zebrafish embryos. The embryos were exposed to *F. solani* fractions at one cell stage, soon after spawning, The treated embryos did not develop properly and seem to be arrested at 5 somite stage (a stage after 9 hr of fertilization), whereas the mock treated embryos reached to 5 prim stage (almost 24 hr after fertilization) within same duration of time. The F7 treated embryos stayed in this arrested growth stage even up to 48 hr of post fertilization and died. As revealed in Figure 4, the F8 treated embryos did not show a significant level of developmental delay but all the embryos had enlarged yolk sac and also cardiac edema. The brain of F8 treated embryos did not formed normally (compare the images A and E, brain). The embryos, which were treated with *F. solani* F9, did not show any prominent abnormality at 60ug/ml concentration.

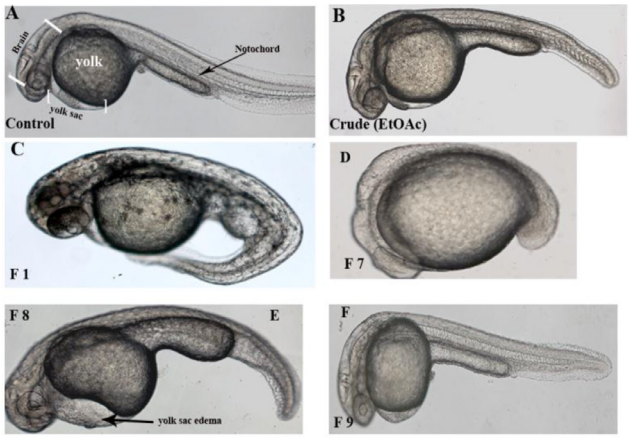


Figure 5: (A-F) induced severe developmental delay in treated Zebrafish embryos. Representative live images of zebrafish embryos after one day treatment; mock treated (A) and treated with various fraction of *F. solani* (B-F). It is evident from these images that solvent to solvent fractions of FS induced more embryonic abnormalities as compared to EtOAC crude extract (B). The overall development and organ formation in control embryos (A) was normal, whereas, EtOAC crude extract treated embryos also developed normally except they lack pigmentation. A severe bending of tail (due to undulated notochord is evident in fraction F1 treated zebrafish embryos (C). The zebrafish embryos treated with fraction F7 showed most severe developmental delay. The embryos were arrested at 5 somite stage (D). There were also severe developmental abnormalities observed in fraction F7 treated embryos which induced cardiac and yolk sac edema (arrow in E). The zebrafish embryos treated with fraction F9 (F) did not show any developmental abnormalities and were very much similar to control embryos.

IR Spectroscopic Analysis

The IR spectral data from *F. solani* F7 fraction showed broad band in the region 3396 cm^{-1} that was ascribed to hydroxyl (-OH) and amide (-NH) groups stretch. C-H stretch was seen in the region of 2926 cm^{-1}. The aromatic ring (C=C) group stretch was observed in the region of 1633 cm^{-1}. A peak observed in the region 1084 cm^{-1} is due to the presence of aromatic C, H bends. Bands observed at 2366 cm^{-1} and 2337.6 cm^{-1} region were due to the absorption of –COOH and enols. The band at 1741 cm^{-1} was due to the absorption of ketone (Figure 6).

Table 1: Detail of embryonic abnormalities induced by *F. solani* crude extract and fractions exposure in zebrafish embryos Hatching, growth, Brain deformation, Tail malformation, notochord defects and pericardial edema.

	Control	Crude (EtOAC)	F1	F7	F8	F9
	0.5% methanol	60µg/ml	60µg/ml	60µg/ml	60µg/ml	60µg/ml
Number of embryos	150	150	150	150	150	150
Stunted or delayed growth	0	0	0	150	0	0
Shortened bodies	0	50	0	NA	0	0
Pericardial edema	0	0	0	NA	150	0
Yolk sac malformation	0	120	100	NA	150	0
Bent spine (notochord defects)	0	150	150	NA	150	0
Brain deformation	0	0	0	NA	150	0
Eye development	0	0	0	0	150	0
Tail malformation	0	150	150	NA	150	0

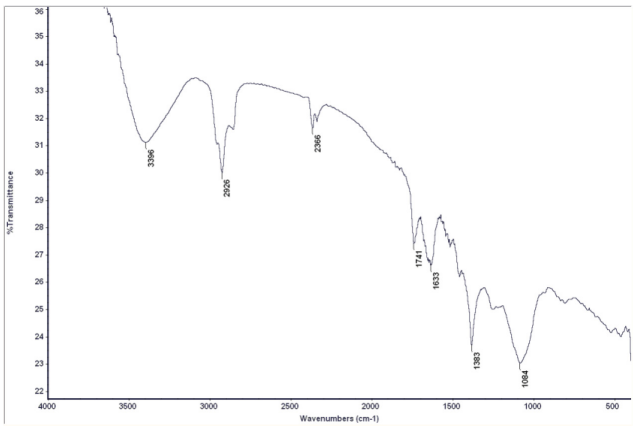


Figure 6: Infrared spectrum of F7 fraction of *F. solani.*

DISCUSSION

Natural products are considered the undoubted leaders among the different therapeutic tools humans have used to fight several diseases including cancer.[28,29] Endophytic fungi are a potential source for promising and novel compounds that are the focus of numerous research groups across the world.[30] Moreover, the increasing demand of promising drugs to face the increasing resistance to the anticancer drugs and sides effects brought these micro-organisms to spotlight. In the present study, an endophytic fungus *F. solani* isolated from the *P. dactylifera* was investigated for its anticancer potential and zebrafish toxicity.

In this study, ethyl acetate and methanol solvent were used to extract FS compounds because these two solvents possess different polarities. Ethyl acetate has medium polarity while methanol has a high polarity. Therefore, extracting with solvents of different polarity increases the chances of isolating different compounds. The results demonstrated that the extract possessed potent cytotoxicity against HepG2 and MCF-7 cell lines. Induction of apoptosis is an important characteristic feature of many anti-cancer drugs.[31] Our results also revealed that the extract of *F. solani* also induced apoptosis in HepG2 cells when analyzed using different techniques including inverted light microscope, Hoechst 33342 stain and Caspase 3/7 activity.

Several reports have shown that fungal extracts are toxic and cause organ and tissue damage.[32] Thus, assessment of fungal extract toxicity is imperative for human health. Unluckily, the assessment of fungal extract toxicity, their fractions and compounds isolated remains limited. The process of studying the toxicity on mammalian animal model such as monkeys, rabbits and rats is expensive and slow resulting in long process in drug discovery and development. Zebrafish is an ideal vertebrate model for drug screening and assessing the toxicity and the safety use of chemical compounds.[33]

In the present study, zebrafish embryos were used as a model for toxicity assessment of *F. solani* crude extract and its fractions. No developmental toxicity and toxic effects were observed when embryos were treated below the concentration of 20 µg/ml for all the extract tested However, this study showed that the *F. solani* extract has potential toxic effects as shown by the major developmental defects as well as the changes observed on the organs studied therefore caution should be exercise when using the extract. These results showed the diverse biological activity of different fractions of *F. solani* on zebrafish embryonic development. The diverse biological activity associated with each fraction clearly means that each fraction might contain different compounds/ active principles. The toxicity, which has been observed in zebrafish embryos, is in agreement with cell line data where the apoptosis was observed in HepG2 cell lines.

Natural products from different sources have been used for the treatment of many diseases. Therefore, quality control of natural products and their fractions are of major importance. For this reason, Infrared Spectroscopy (IR) is a rapid and non-destructive tool commonly used to validate unrefined crude extract and to determine natural extract biomarkers based on their functional groups and specific fingerprints.

CONCLUSION

Our results demonstrate that F7 caused concentration-dependent toxicity in cell lines as well as in zebrafish embryos. *F. solani* exerted cytotoxicity in tested cancer cells through activation of caspase-3. Although many reports are published using zebrafish model for drug screening and drug discovery, no studies have evaluated the toxicity of *F. solani* extract and its fractions using this model. Here, we report for the first time developmental toxicity of *F. solani* extract and its fractions. However, in future work, further investigation is required to find out the exact mechanism of action.

ACKNOWLEDGEMENT

Research Supporting Project number (RSP-2019/112), King Saud University, Riyadh, Saudi Arabia.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

IC_{50}: Half maximal inhibitory concentration; **MCF-7:** Human breast cancer cell line; **HEK 293:** Human embryonic kidney 293 cell; **MDA-MB-231:** Human mammary cancer cell line; **MTT:** 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; **EtOAC:** Ethyl acetate; **OD:** Optical density; **IR:** Infrared radiation.

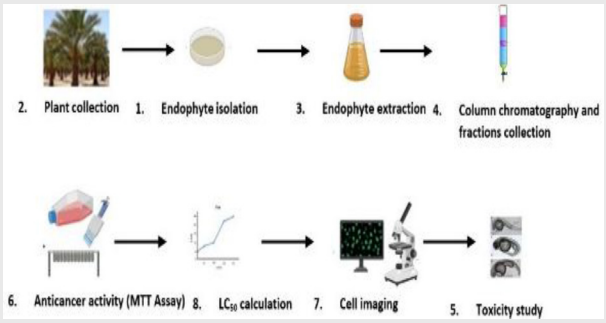
REFERENCES

1. World Health Organization. Cancer key statistics. World Health Organization. 2010. Available from: https://www.who.int/cancer/resources/keyfacts/en/.
2. Calderón AI, Smithy-WIlliams J, Gupta MP. Antimalarial natural products drug discovery in Panama. Pharm Biol. 2012;50(1):61-71.
3. Demain AL, Vaishnav P. Natural products for cancer chemotherapy. Microb Biotechnol. 2011;4(6):687-99.
4. Brdy J. Bioactive microbial metabolites. J Antibiot. 2005;58(1):1-26.
5. Venkateswarlu N, Reddy NV, Sureshbhargav D, Pushpalatha KCB, Anitha D. Endophytes: A natural source for novel compounds. World J Pharm Res. 2014;3(1):1352-70.
6. Petrini O, Sieber TN, Toti L, Viret O. Ecology, metabolite production and substrate utilization in endophytic fungi. Nat Toxin. 1992;1(3):185-96.
7. Hubbard M, Germida J, Vujanovic V. Fungal endophytes improve wheat seed germination under heat and drought stress. Botany. 2012;90(2):137-49.
8. Ren A, Li C, Gao Y. Endophytic fungus improves growth and metal uptake of *Lolium arundinaceum* Darbyshire ex. Schreb. Int J Phytoremediation. 2011;13(3):233-43.
9. Arnold AE, Mejía LC, Kyllo D, Rojas EI, Maynard Z, *et al*. Fungal endophytes limit pathogen damage in a tropical tree. Proc Natl Acad Sci. 2003;100(26):15649-54.
10. Tan RX, Zou WX. Endophytes: A rich source of functional metabolites. Nat Prod Rep. 2001;18(4):448-59.
11. Gunatilaka AA. Natural Products from Plant-Associated Microorganisms: Distribution, Structural Diversity, Bioactivity and Implications of their Occurrence. J Nat Prod. 2006;69(3):509-26.
12. Zheng CJ, Li L, Zou JP, Han T, Qin LP. Identification of a quinazoline alkaloid produced by *Penicillium vinaceum*, an endophytic fungus from *Crocus sativus*. Pharm Biol. 2012;50(2):129-33.
13. Molinar E, Rios N, Spadafora C, Arnold A E, Coley PD, *et al*. Coibanoles, a new class of meroterpenoids produced by *Pycnoporus sanguineus*. Tetrahedron Lett. 2012;53(8):919-22.
14. Xu J, Ebada SS, Proksch P. Pestalotiopsis a highly creative genus: Chemistry and bioactivity of secondary metabolites. Fungal Diversity. 2010;44(1):15-31.
15. Martínez-luis S, Della-togna G, Gerwick W, Cubilla-rios L. Antileishmanial constituents of the Panamanian endophytic fungus *Phoma herbarum* strain M16. Planta Medica 2008;74(09):PB67.
16. Varughese T, Riosa N, Higginbotham S, Arnold AE, Coley PD, *et al*. Antifungal depsidone metabolites from *Cordyceps dipterigena*, an endophytic fungus antagonistic to the phytopathogen *Gibberella fujikuroi*. Tetrahedron Lett. 2012;53(13):1624-6.
17. Cui JL, Guo SX, Dong H, Xiao P. Endophytic fungi from dragon's blood specimens: Isolation, identification, phylogenetic diversity and bioactivity. Phytother Res. 2011;25(8):1189-95.
18. Yan X, He L, Song G, Wang R. Antagonistic bioactivity of endophytic strains isolated from *Salvia miltiorrhiza*. Afr J Biotechnol. 2013;10(67):15117-22.
19. Alatar A, El-Sheikh MA, Thomas J. Vegetation analysis of Wadi Al-Jufair, a hyper-arid region in Najd, Saudi Arabia. Saudi J Biol Sci. 2012;19(3):357-68.
20. AlZubaide MA, AlZahrani HS, AlToukhy AA. Floristic Composition and Life Forms for Wild Plants of Shada Highest Mountain and Wadi Sagamah at Al Baha Area, Saudi Arabia. Middle East J Sci Res. 2017;25(6):1342-8. (cross reference)
21. Hill A J, Teraoka H, Heideman W, Peterson RE. Zebrafish as a model vertebrate for investigating chemical toxicity. Toxicol Sci. 2005;86(1):6-19.
22. Fan CY, Cowden J, Simmons SO, Padilla S, Ramabhadran R. Gene expression changes in developing zebrafish as potential markers for rapid developmental neurotoxicity screening. Neurotoxicol Teratol. 2010;32(1):91-8.
23. Peterson RT, Macrae CA. Systematic approaches to toxicology in the zebrafish. Annu Rev Pharmacol Toxicol. 2012;10(52):433-53.
24. Ali S, Vanmil HG, Richardson MK. Large-scale assessment of the zebrafish embryo as a possible predictive model in toxicity testing. PLoS One. 2011;6:e21076.
25. Abutaha N, Mashaly AM, AL-Mekhlafi FA, Farooq M, AL-Shami M. Larvicidal activity of endophytic fungal extract of *Cochliobolus spicifer* (*Pleosporales*: *Pleosporaceae*) on *Aedes caspius* and *Culex pipiens* (*Diptera*: *Culicidae*). Appl Entomol Zool. 2015;50(3):405-14.
26. Abutaha N. *In vitro* antiproliferative activity of partially purified *Withania somnifera* fruit extract on different cancer cell lines. J Balkan Union Oncol. 2014;1(20):625-30.
27. Butler MS, Newman DJ. Mother Nature's gifts to diseases of man: The impact of natural products on anti-infective, anticholestemics and anticancer drug discovery. Natural Compounds as Drugs. 2008;1:1-44.
28. Mondal S, *et al*. Natural products: promising resources for cancer drug discovery. Anti-Cancer Agents in Medicinal Chemistry. 2012;12(1):49-75.
29. Strobel G. Daisy B. Bioprospecting for microbial endophytes and their natural products. Mol Biol Rev. 2003;67(4):491-502.
30. Lowe SW, Lin AW. Apoptosis in cancer. Carcinogenesis. 2000;21(3):485-95.
31. Kumar A, Abnave P, Ahmad A. Cultural, Morphological And Molecular Characterization Of Vincaalkaloids Producing Endophytic Fungus *Fusarium solani* Isolated from *Catharanthus roseus*. Int J Bot Res. 2013;3(2):2277-4815.
32. Moongkarndi P, Kosem N, Kaslungka S, Luanratana O, Pongpan N, *et al*. Antiproliferation, antioxidation and induction of apoptosis by *Garcinia mangostana* (mangosteen) on SKBR3 human breast cancer cell line. J Ethnopharmacol. 2004;90(1):161-6.
33. Kanungo J, Cuevas E, Ali S, Paule MG. Zebrafish model in drug safety assessment. Curr Pharm Des. 2014;20(34):5416-29.

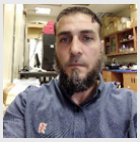
SUMMARY

- Endophytic *F. solani* was isolated from the roots of *P. dactylifera* from Riyadh region.
- The solvent extract of *F. solani* showed apoptotic potential against HepG2 and MDA-MB-231 cancer cell lines using Hoechst 33342 dye staining and caspase-3/7 activity
- The zebrafish embryos showed major developmental defects when they were treated at ≤60µg/ml by inducing bent spine and shorter yolk extension and larger yolk area as compared to mock treated embryos.
- The IR spectroscopy showed the presence of different functional group.

PICTORIAL ABSTRACT



About Authors



Nael Abutaha: Obtained his Ph.D. in 2010 from Dept. of Microbiology, Faculty of Science, Mysore University, India. He worked in the area of natural product as anticancer, antimicrobial and larvicidal agent.

Fahd Almekhlafi: Obtained his Ph.D. in 2010 from Dept. of Zoology, Faculty of Science, King Saud University, Saudi Arabia. He worked in the area of natural products and biological control.

Cite this article: Abutaha NM, Farooq MF, Nasr FA, Almekhlafi FAA, Wadaan M. Bioassay-Guided Fractionation of Endophytic Fungal Extract of *Fusarium solani* (Saccardo) against Cancer Cell Lines and Zebrafish Embryo. Indian J of Pharmaceutical Education and Research. 2020;54(2s):s301-s308.