Online ISSN: 2682-2628
Print ISSN: 2682-261X

IJCBR

INTERNATIONAL JOURNAL OF CANCER AND BIOMEDICAL RESEARCH

https://jcbr.journals.ekb.eg
Editor-in-chief
Prof. Mohamed Labib Salem, PhD

Antitumor and antimicrobial activities of endophytic fungi obtained from Egyptian *Urospermum picroides*

Nessma A. El-Zawawy, Metwally A. Metwally, Noha Abd El-Salam

PUBLISHED BY
EACR EGYPTIAN ASSOCIAN FOR CANCER RESEARCH
Since 2014

International Journal of Cancer & Biomedical Research
(IJCBR) https://jcbr.journals.ekb.eg

IJCBR is an Int. journal published by the Egyptian Society of Cancer Research (EACR, established in 2014, http://eacr.tanta.edu.eg) and sponsored by the Egyptian Knowledge Bank (EKB: www.ekb.eg).

IJCBR has been approved by the Supreme Council of Universities, Egypt with score 7 (http://egjournal.scu.eg). The journl is cited by google scholar and registered by Publons (https://publons.com). The journal has recently been evaluated in 2020 by Nature Springer with a good standing.

Scope of IJCBR

- Drug discovery from natural and synthetic resources
- BioMedical applications of nanotehnology
- Sem cell biology and its application
- Basic and applied biotechnology
- Inflammation and autoimmune diseases
- In slico models and bioinformatics
- In vitro and In vivo preclinical animal models
- Cellular and molecular cancer biology
- Cancer Immunology and Immunotherapy
- New methods for prediction, early detection, diagnosis prognosis and treatment of dieases.
- Immunology in health and dieases
- Anti-microbial defense mechanisms
- Cellular and molecular physhiology and pahthology of diseases

IJCBR Editor,
Prof. Mohamed Labib Salem, PhD
Professor of Immunology
Faculty of Science, Tanta Universiy, Egypt

INTERNATIONAL JOURNAL OF CANCER AND BIOMEDICAL RESEARCH (IJCBR)

RESEARCH ARTICLE

Antitumor and antimicrobial activities of endophytic fungi obtained from Egyptian *Urospermum picroides*

Nessma A. El-Zawawy[1], Metwally A. Metwally[1], Noha Abd El-Salam[2]

[1]*Botany Department, Faculty of Science, Tanta University, Tanta, Egypt*
[2]*Research Center and Measurements, Tanta University, Tanta, Egypt*

ABSTRACT

Urospermum picroides is a medicinal plant was founded in North coast, Egypt. Endophytic fungi of this medicinal plant in Egypt is poorly known and thus, this study aimed towards estimation of the biological activities of endophytic fungi from Egyptian *U.picroides*. *Fusarium oxysporum* and *Phoma herbarum* were isolated from *U.picroides* and identified based on phylogenetic analysis. Ethyl acetate extracts of *F.oxysporum* and *P.herbarum* gave high antioxidant activities (26.2 and 51.7%, respectively). Moreover, they showed highly antitumor activities with some significant morphological changes of characteristic apoptosis accompanied by up regulation of both p53 and Bax for *F.oxysporum* and *P.herbarum* extracts (1.55, 2.14 for p53 and 1.24, 2.2 for Bax, respectively) with down regulation of Bcl-2 (0.18 and 0.16, respectively). Ethyl acetate extract of *F.oxysporum* (EAFE) showed greatest antimicrobial activity against *Trichophyton mentagrophytes* (70 mm), while ethyl acetate extract of *P.herbarum* (EAPE) showed highest antimicrobial activity against *Bacillus cereus* (37 mm) and *Candida albicans* (35 mm). Scanning electron microscope micrographs showed major abnormalities for tested microorganisms after treatment with (EAFE and EAPE) resulting in complete alternation in their morphology. GC-MS results showed 30 biologically active compounds for both extracts. The most significant in EAFE was Diisooctyl phthalate with relative levels (74.9%), while Benzoic acid, 3, 5-bis (1, 1-dimethylethyl)-4-hydroxy- was the most active compound in EAPE (61.7%). These results proposed that ethyl acetate extracts of endophytic fungi from the Egyptian *U.picroides* showed to be promising novel as antioxidant, antitumor agents and antimicrobial with further phytochemical studies.

Keywords: Endophytic fungi, U. picroides, antioxidant, antitumor, antimicrobial

Editor-in-Chief: Prof. M.L. Salem, PhD - Article DOI: 10.21608/JCBR.2020.32713.1045

ARTICLE INFO

Article history

Received: June 14, 2020
Revised: August 28, 2020
Accepted: September 21, 2020

Correspondence to:
Nessma A. El-Zawawy, PhD,
Faculty of Science,
Tanta University, Egypt
Mobile: (+20) 1289102444
Email:
nesma.elzawawi@science.tanta.edu.eg

INTRODUCTION

Nowadays, many reports indicate that plants are considered as a repository of several microorganisms known as endophytes (Minirani *et al.,* 2017). Endophytes are microorganisms that colonize plant for all or part of their life cycle. They live in inside plant tissues below the epidermal cell layers without causing any disease to their host and It looks like they could penetrate the living cells (Strobel and Daisy, 2003). Most endophytic fungi can synthesize bioactive variable compounds with antimicrobial, cytotoxic and anticancer activities (Kharwar *et al.,* 2011) which become a hot spot of drug discovery.

Colletotrichum and *Nigrospora* spp endophytic fungal extracts isolated from *Uvaria grandiflora* have antibacterial, antioxidant and cytotoxic activity compared to mycelia extract (Israel *et al.,* 2019). The Nile delta region is flourished by many weeds which seem to be promising raw materials in drug discovery. One of these weeds is *U. picroides* which is a Mediterranean annual herb of flowering plant in the family Asteraceae was known by the common name prickly golden fleece. Previous studies suggested that this plant's root, flower and stem extracts are effective as an antimicrobial (El-Ghazooly *et al.,* 2003). Nevertheless, little is known about endophytic fungi isolated from *U. picroides*.

Owing to our knowledge, our study was considered the first study to isolate and identify these two endophytic fungi from *U. picroides* reporting their antimicrobial, antioxidant and antitumor activities and their effect on expressed tumor genes showing their apoptotic effect.

MATERIAL AND METHODS

Collection of host plants

U. picroides was collected from different areas in North coast, Alexandria, Egypt at October 2017 (Figure 1S). The identification of species was done according to Ahmed (2003). Plant flowers were cut, separated, dried and saved in plastic bags. Hold the samples at 4°C before fungal endophytes can be isolated.

Isolation of endophytic fungi

Based on the procedures mentioned by Xu *et al*. (2008), isolation of the endophytic fungi was carried out. The plant samples were cut into small cubes and then surface-disinfected for 1 min by washing in 75 percent ethanol, sterile distilled water twice, 0.05g/ml sodium hypochlorite solution for 3 min, followed by two rinses in sterile distilled water. The surface-sterilized samples were cut into small pieces using a sterile blade and positioned for incubation at 25°C on plates with potato dextrose agar (PDA). The endophytic fungi that were immersed from the tissues were transferred to new PDA dishes and sequential subculturing was performed until the identification of pure cultures was obtained.

Identification and phylogenetic analysis of endophytic fungi

Fungal identification was based on their internal transcribed spacer ribosomal DNA (ITSrDNA) sequences at Mubarak city for scientific research. A pair of primers ITS1 (sequence: 5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') was used for ITS-rDNA amplification (Phongpaichit *et al.,* 2006)]. The corresponding ITS-rDNA sequence of each endophytic fungus (Table 1S) was then used for similarity analysis using BlastN algorithm against the public database at the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov). Multiple sequence alignments were performed using the CLUSTALW program (Thompson *et al.,* 1994). Molecular evolutionary analyses were conducted using MEGA version 10.0.4. The phylogenetic trees were constructed using the neighbor-joining (NJ) algorithm (Naruya and Masatoshi, 1987).

Table 1. IC_{50} values (µg/ml) of antitumor activities of EAFE and EAFE.

Tested material	IC_{50} (µg/ml)	
	24 hours	48 hours
F. oxysporum extract	1269±0.002	645.7±0.004
P. herbarum extract	1590±0.003	627±0.004
Doxorubicin	0.5±0.03	0.3±0.05

Preparation of fungal fermentation broth

The two endophytic fungal isolates identified were cultivated at 25°C for 10 days in potato dextrose liquid medium. The crude fermentation broth was thoroughly blended for each isolate (107 CFU/ml) and centrifuged for 5 minutes at 4000r/min. Liquid supernatant has been extracted with an equal amount of thrice ethyl acetate. The organic solvent extract was then evaporated under reduced pressure to produce two fungal extracts of ethyl acetate (TEAFEs) for screening antioxidant, antitumor and antimicrobial activities (Lv *et al.,* 2010).

Antioxidant activity assay

The antioxidant activities of TEAFEs were determined by using the free radical scavenging method (DPPH) characterized by El-Amier *et al*. (2016). 2 ml of 0.15 mM DPPH has been added to 2 ml of different TEAFE concentrations (5, 10, 20, 25, 50, 75 µg / ml). A control was prepared in place of the sample by adding 2 ml solvent. At room temperature, the mixture was incubated in dark for 30 min. The absorbance was estimated at 517 nm, and graphically determined IC50. The estimation of antioxidant activity took the following equation:

$$\% \text{ Radical scavenging activity} = (A_o - A_s/A_o) \times 100$$

where A_o: is blank absorbance, A_s: is sample absorbance at 517nm.

Antitumor activity assay

Mosman and Alley tested antitumor activity *in vitro* using MTT assay (Mosmam, 1983; Alley *et al.*,1988) with minor modification on Human liver tumor cell line (HepG2) obtained from

Medical Research Institute (MRI), Alexandria University, Alexandria, Egypt. Briefly, HepG2 cells were seeded in 96 well plates of microculture (90 µl / well), and before sample addition, it was permitted to adhere overnight. The samples were applied to tumor cells (10 µl/well) with different concentrations of TEAFEs (200, 400, 600, 1000, 1200, 1500, 1700 µg/ml) in comparison to doxorubicin (chemotherapy) at different concentrations (0.21, 0.42, 0.85, 1.7, 3.4 µg/ml). Then incubated in a humidified 5% CO_2 incubator at 37°C for 24 and 48 hours. Analysis of each concentration in triplicate wells. At the end of the exposure, 20 µl of 5 mg/ml MTT has been applied to each well and the plates have been incubated at 37 ° C for 4 hours, then apply acidified isopropanol to crystal dissolve, shaking for 10 min. The optical density (OD) was read at a 630 nm wave-length on a plate screen. The growth inhibition rate was calculated by the following equation:

Growth inhibition rate % = $(OD_{control}/OD_{trated})/OD_{control} \times 100\%$

Gene expression & apoptotic effect of TEAFEs using quantitative PCR

Three groups were used as the following: Control group (negative control) contained HepG2 with density $0.5x10^5$/ml and (DEMEM) complete media, the other two groups contained HepG2 with the same density $0.5x10^5$/ml and 4 ml of complete DEMEM media containing the concentration of TEAFEs with IC_{50} at 48 hours, After incubation for 48 hours, RNA of control and treated cells were extracted using the commercial kits (Thermo scientific Gene JET RNA Co, #k0731, America) protocol. First cDNA strand was synthesized using RT-for-PCR advantage kit (SensiFAST™ cDNA Synthesis Kit Thermo Co, BIO-6505, America). The first-strand cDNA was used as a template in the PCR experiment to detect genes (p53, Bax and Bcl-2) using three primers designed by PRIMERPLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Table 2S) according to (Yu *et al.,* 2003; Yang *et al.,* 2010), respectively. Quantitative PCR is performed in a qRT-PCR (Rotor 5 plex QIAGEN gene). The GAPDH gene was utilized in the study as a housekeeping gene (reference gene).

Table 2. Inhibitory effect of different concentrations of EAFE and EAPE against different tested microorganisms

Different concentrations (µg/ml)	Diameter of inhibition zone (mm)																	
	Staphylococcus aureus			*Bacillus cereus*			*Salmonella sp*			*Candida Tropicalis*			*Candida albicans*			*Trichophyton mentagrophytes*		
	EAFE	EAPE	Tetracycline	EAFE	EAPE	Tetracycline	EAFE	EAPE	Tetracycline	EAFE	EAPE	Fluconazole	EAFE	EAPE	Fluconazole	EAFE	EAPE	Fluconazole
150	23±0.05	15±0.036	13±0.05	23±0.02	29±0.035	20.5±0.04	21±0.03	6±0.033	10±0.003	16±0.03	18±0.05	0.05±1.05	21±0.05	28±0.05	0.04±1	42±0.05	32±0.045	0.003±1
200	24±0.006	16±0.02	14.5±0.03	24±0.05	29±0.024	21.4±0.02	22±0.008	7±0.05	13±0.027	19±0.02	19±0.003	0.03±1.28	23±0.002	29±0.007	0.02±1.05	43±0.01	33±0.007	0.027±1.1
250	27±0.023	17±0.027	15±0.02	25±0.01	30±0.05	22±0.04	23±0.025	9±0.006	14.6±0.01	21±0.028	19±0.025	0.03±1.6	25±0.041	31±0.037	0.04±1.3	44±0.005	35±0.04	0.01±1.5
300	28±0.042	18±0.008	16.2±0.02	27±0.01	37±0.006	23.7±0.03	25±0.02	10±0.047	15±0.02	21±0.01	21±0.037	0.05±1.92	27±0.036	33±0.002	0.03±1.7	47±0.02	37±0.034	0.02±1.7
350	30±0.019	22±0.043	17.6±0.02	29±0.034	37±0.042	25±0.03	26±0.043	19±0.034	17.8±0.03	23±0.05	24±0.004	0.02±2.24	27±0.05	34±0.041	0.03±2.1	69±0.047	38±0.05	0.03±2
400	30±0.05	22±0.006	19±0.03	29±0.027	37±0.05	25.5±0.02	26±0.05	19±0.05	19±0.04	23±0.05	24±0.006	0.03±2.56	27±0.05	34±0.05	0.02±2.3	69±0.05	38±0.05	0.04±2.2

P value is significantly highly significant at the 0.001 level.

Antimicrobial activity assay

Antimicrobial activities of TEAFEs were measured using the modified agar well diffusion method. Different concentrations of TEAFEs (150, 200, 250, 300, 350, 400 µg/ml) were prepared compared to antifungal Fluconazole (Diflucan, Pfizer) and antibacterial Tetracycline (Thiophenicol, Sonafi aventis) and tested as a positive control in the same concentrations. One hundred micro liters of *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella* sp, the pathogenic fungus *Candida albicans, Candida tropicalis* and the pathogenic dermatophytes *Trichophyton mentagrophytes* (10^5CFU/ml) were inoculated on nutrient agar medium for bacterial isolates and SDA medium for candidal isolates and Sabouraud dextrose agar supplemented with cycloheximide (0.1%) for dermatophytes.

After inoculation of each isolate on plates of its media, regular wells were made aseptically filled up with 0.1 ml of different concentrations. The plates were incubated for 24h at 37°C (Gokhale *et al.,* 2017). The experiments were performed in triplicate. According to Radhika *et al*. (2008), MIC of the highest antimicrobial extract was calculated and identified as the lowest concentration at which growth was completely inhibited.

Scanning electron microscope

The electron microscope was used to investigate the mechanism of action of the most effective antimicrobial extracts of TEAFEs on *Bacillus cereus*, *Candida albicans* and *Trichophyton mentagrophytes*. The sample preparation for SEM was done according to Kaya *et al*. (2008). Small agar pieces from isolates were cut from the inhibition zone and they were fixed in 3% (v/v) glutaraldehyde buffered with 0.1 M sodium phosphate buffer (PH 7.2) for an hr. at room temperature, then washed four times in sodium phosphate buffer. The pieces were then post-fixed in 1% (W/V) osmium tetroxide (OsO_4) for an hr., then washed four times in the buffer. They were dehydrated in a graded alcohol series. The last stages of dehydration were performed with propylene oxide ($CH_3CH.CH_2.O$). The specimens were dried and were mounted onto stubs using double-sided carbon tape, and then were coated with a thin layer of gold by a sputter coater (DII-29030SCTR smart coater) then examined by scanning electron microscope (JEOL (JSM IT 100,30KV,300.000x) at Nano scientific technology institute, the University of Kafr El-Sheik, Kafr El-Sheik, Egypt.

Identification of bioactive metabolites by GC-MS analysis

Study of GC-MS for TEAFEs was performed at Center of Scientific Research and Measurements, Tanta University, Tanta, Egypt The Perkin Elmer model:(Clarus 580/560 S) was used in the column analysis (Rxi-5Sil MS column 30 m, 0.25 mm ID, 0.25 df) and the components were separated at a constant flow of 1ml / min using helium as a carrier gas. During chromatographic run the temperature of the injector was set at 280° C. 1 µl of extract sample was injected into the scanning instrument for 30 min, initial oven temperature 60° C for 10 min, ramp 10°C / min to 280° C for 6 min, split 20:1, solvent delay=3 min. The conditions for the mass detector were transferred line temperature 280°C, ion source temperature 200°C, and the effect of ionization mod electron at 70eV, a scan time of 0.2 sand scan-interval of 0.1s. Given fragments from 50 to 600 Da, the component spectrum was compared with the spectrum database of recognized components held in the GC-MS NIST library (Rukshana *et al.,* 2017).

Statistical analysis

Statistical analysis was conducted for the studied data of TEAFE extracts on microorganisms and tumor cells through the SPSS V17 one-way test of variance analysis (ANOVA), to evaluate the variation between the concentrations of both extracts.

RESULTS

Isolation and identification of endophytic fungi

Two endophytic fungi were isolated from flowers of *U. picroides*. Based on the results of molecular identification (Figure 1) these two fungi were identified as *F.oxysporum* and *P.herbarum*. Reports on the biological activities of *F.oxysporum* and *P.herbarum* as endophytic fungi from Egyptian *U.picroides* have been

documented to the best of author's knowledge. Therefore, this study was aimed at assessing the antioxidant, antitumor and antimicrobial activities of these two endophytic fungi.

Antioxidant activity

Ethyl acetate extracts of *F.oxysporum* (EAFE) and *P.herbarum* (EAPE) exhibited significantly higher antioxidant activities as shown in Figure 2. It was observed that the scavenging activity of the two extracts increased gradually by increasing concentration. EAPE showed the highest antioxidant activity (51.7%) followed by EAFE (26.2 %) opposed to ascorbic acid as a standard (24.5%) at a concentration of (75 µg/ml).

Antitumor activity

In vitro, the highly antitumor activity of TEAFEs against HepG2 cells at different concentrations (200, 400, 600, 800, 1000, 1200, 1500, 1700µg/ml) after 24 and 48 hours incubation compared to doxorubicin as chemotherapy was shown in (Figure 3). IC_{50} of EAFE and EAPE were 1269 µg/ml, 1590 µg/ml after 24 hours and 645.7µg/ml, 627µg/ml after 48 hours, respectively (Table 1).

Morphologic changes in cells treated with HepG2

The morphological examinations of the HepG2 cells treated with IC_{50} doses for 48 hours of EAFE and EAPE (Figure 4). Treated cells (Figure 4b, c) showed significant morphological changes, which were characteristic of apoptosis, such as cell swelling, shrinkage and reduced in growth with the destruction of monolayer which was not seen in untreated HepG2 cells (Figure 4a).

Gene expression & apoptotic effect of TEAFEs using quantitative PCR

The expression of p53, Bax, and Bcl-2 was expressed in treated HepG2 cells with 48-hour TEAFE IC_{50} and control untreated HepG2 cells in (Figure 5). The transcription rate induced in HEPG2 cells was analyzed compared with control cells by the change in relative quantity (RQ) or relative concentration in the treated HepG2 cells. The RQ values (fold change) obtained from the expression of p53 in treated HepG2 cells with EAFE and EAPE concentration IC50 were up to 1.55 and 2.14, compared to control untreated cells in HepG2 treated cells, respectively.

In addition, RQ values obtained from Bax expression in treated HepG2 cells with IC50 concentration of the two extracts were up to 1.24 and 2.2 for EAFE, EAPE, compared to control untreated cells in HepG2 treated cells, respectively. In HepG2 treated cells with IC50 concentration of EAFE a-nd EAPE, expression of Bcl-2 was reduced to 0.18 and 0.16 compared to control untreated cells in HepG2 treated cells.

Antimicrobial screening of ethyl acetate extracts produced by endophytic fungi

The inhibitory effect of different concentrations of TEAFEs against different microorganisms was shown in (Table 2). EAFE gave the greatest inhibition zone at a concentration (150 µg/ml) against *Trichophyton mentagrophytes* (42 mm) compared to Fluconazole followed up by *Staphylococcus aureus* and *Bacillus cereus* which measure (23 mm) for both compared to tetracycline. While EAPE gave the greatest inhibition zone at a concentration (150µg/ml) against *Trichophyton mentagrophytes* (32 mm) followed by *Bacillus cereus* which measure (29 mm) and *Candida albicans* (28 mm). MIC value of EAFE for *Staphylococcus aureus*, *Bacillus cereus*, *Candida tropicalis* and *Trichophyton mentagrophytes* is 350µg/ml which considered as MIC value with inhibition zones of (30, 29, 23 and 69 mm), respectively. However, 300 µg/ml of EAFE was recorded as MIC against *Salmonella* sp and *Candida albicans* with inhibition zones (26 and 27 mm), respectively (Table 1). While, MIC was detected in (EAPE) at a concentration of 350µg/ml against *Staphylococcus aureus*, *Salmonella* sp and *Trichophyton mentagrophytes* with inhibition zones of (22, 19 and 38 mm), respectively. While 300 µg/ml of EAPE was recorded as MIC against *Bacillus cereus*, *Candida tropicalis* and *Candida albicans* with inhibition zones (37, 21 and 33mm), respectively.

Scanning electron microscopy

Morphological alternations of *Candida albicans*, *Bacillus cereus* and *Trichophyton mentagrophytes* after treated with TEAFEs extracts were shown on SEM micrographs. In *C. albicans,* The EAPE cells had subjected distinct

morphological and cytological alternations. While control cells showed the normal structure of *Candida* cells. When the *Bacillus cereus* cells treated with EAPE were compared with untreated cells, the cells being treated seemed to be shrinking and there was a degradation of the cell wall and the cells were depressed from the middle as appeared in (Figure 6). Several changes in hyphae of treated *Trichophyton mentagrophytes* with EAFE, as it appeared shrinked with a rough surface, a flat ribbon-shaped structure with the demolition of the cell wall, cell wall thickening and disordered hyphal tip. However, the normal cells have smooth, thick-ring- shaped septum and stable surface decoration (Figure 7).

Identification of bioactive metabolites by GC-MS analysis

GC-MS is the best techniques for distinguishing the components of volatile matter, long chain, branched hydrocarbons, acids of alcohols, esters etc. The active principles were presented in percentage with their retention time (RT), molecular formula, molecular weight (MW), and peak area in (Table 3). GC-MS analysis of EAFE showed Diisooctyl phthalate was the most active compound. Whereas Benzoic acid, 3, 5-bis (1,1-dimethylethyl)-4-hydroxy- was the most active compound in EAPE as in (Table 4).

Table 3. Mass fractions of EAFE identified by GC-MS.

No.	RT (min)	Compounds	Relative proportion (%)
1.	**5.494**	p-Xylene	26.9
2.	**5.704**	Heptane, 3,4-dimethyl-	21.8
3.	**14.347**	Ans- pro	9.7
4.	**14.552**	Maprotiline	16.9
5.	**14.627**	Cinnamic acid, p-(trimethylsiloxy)-, methyl ester	22.8
6.	**14.767**	3,5-Di-t-butyl-4-methoxy-1,4-dihydrobenzaldehyde	15
7.	**14.937**	Perylene	11.1
8.	**14.967**	4-(1,1-Dimethylpropyl)phenol, trimethy-l silyl ether	26.3
9.	**15.218**	Tyr-Ala(Dstereo)	25.3
10.	**15.268**	Diclofenac	26.7
11.	**15.398**	Benzo[e]pyrene	27
12.	**15.598**	Maprotiline-M (desamino-di-HO-)	3.8
13.	**16.068**	6-Quinazolinecarboxamide,1,2,3,4-tetrahydro-1,3dimethyl-2,4-dioxo-N-(2-phenylethyl)	10.2
14.	**16.173**	Disperse Blue 26	34.7
15.	**16.423**	Benzo[b]naphtho[2,3-d]thiophene, 9,10-dihydro-7methyl	12.6
16.	**16.468**	Silane,9anthracenyltrimethyl-	13.3
17.	**17.078**	Methaqualone	9.6
18.	**18.789**	1,1'-Biphenyl, 2,4-dichloro-2',5'-dimethyl-	9.5
19.	**30.449**	Heptadecane, 9-hexyl-	5.1
20.	**31.229**	Di-n-decylsulfone	13.4
21.	**31.509**	Diisooctyl phthalate	54.6
22.	**31.794**	3-Benzo[g]quinoxalin-2-yl-propionic acid	32
23.	**31.824**	Asn-Pro	5.4
24.	**32.019**	2,2-Dimethyleicosane	5.3
25.	**32.815**	Oxalic acid, allyl pentadecyl este	6.6
26.	**33.765**	1-Hexadecanol, 2-methyl-	4.1
27.	**34.290**	Bismuthine, tripropyl-	22
28.	**34.851**	Di-n-decylsulfone	16.1
29.	**36.161**	1,4-Naphthoquinone, 6-ethyl-2,3,5,7-tetrahydroxy-	27.1
30.	**36.446**	Pendimethalin	19.9

Table 4. Mass fractions of EAPE identified by GC-MS.

No.	RT (min)	Compounds	Relative proportion (%)
1.	5.97	Tyr-Ala(Dstereo)	3.6
2.	6.03	4-Methoxy-N,N-bis-trimethylsilyl-aniline	6.6
3.	6.29	Glu-Ser	4.7
4.	6.53	1,2-Dimethyl-4,5-bis(trimethylsilyl)benzene	5.6
5.	6.7	-4'-Dimethylamino-2'-(trimethylsilyl)acetanilide	9.8
6.	6.79	Cinnamic acid, m-(trimethylsiloxy)-, methyl ester	2.3
7.	6.86	Zolpidem	13.6
8.	6.95	Phethalic acid, monoamide,N,N´-diphenyl	15.1
9.	7.18	1-Methyl-2,5-dichloro-1,6-diazaphenalene	10.6
10.	7.27	Benzo[e]pyrene	8
11.	7.34	Cinnamic acid,p-(trimethylsiloxy)-,methyl ester	14.9
12.	7.41	2-Myristynoyl-glycinamide	16.3
13.	7.68	Asn-Pro	2.8
14.	7.71	Disperse Blue 26	8.7
15.	7.86	Pyrazole-3-carboxylic acid, 4-iodo-1-methyl-	0.3
16.	8.05	Adenylosuccinic acid	2.6
17.	8.15	Metolachor	5.5
18.	8.3	Tyr-Ala(Dstereo)	7
19.	8.66	2-chloro-3-methoxydibenzo-p-dioxin	8.1
20.	8.97	Tyr-Ala(Dstereo)	3.2
21.	9.01	1,4-Naphthoquinone,6-ethyl-2,3,5,7-tetrahydroxy-	8.2
22.	9.18	Tyr-Ala(Dstereo)	3.1
23.	9.56	5-Methyl-4'-hydroxy-2-benzylidene-coumaran-3-one	11.9
24.	9.66	Tyr-Ala(Dstereo)	1.5
25.	9.83	Halosulfuron-methy	12.1
26.	10.47	Benzimidazole-5-carboxylic acid, 2-methyl-1-phenyl	2.4
27.	10.61	Maprotiline	11.8
28.	10.92	Tyr-Ala(Dstereo)	3.6
29.	11.89	Benzene,1-methoxy-3-[3-(trimethylsilyl)methyl-3-butenyl]-	7
30.	23.04	Benzoic acid, 3, 5-bis(1, 1-dimethylethyl)-4-hydroxy-methyl ester	26

DISCUSSION

Mycologists have used the name endophytes for fungi that occupy live, internal plant tissues without causing symptoms of the disease. Endophytic fungi that live inside plant tissues were present in virtually all species of plants (Glienke-Blanco *et al.,* 2002). Many economically important grasses carry fungal endophytes which may enhance host growth, may improve the ability of plants to tolerate abiotic stress, like drought, as well as improve their resistance to insect and mammalian herbivores (Huang *et al.,* 2008). Some endophytes protect their host from insect by producing bioactive metabolites(Jalgaonwala *et al.,* 2010). In addition, some of these compounds have proved useful as leads for the discovery of novel drugs (Yadav *et al.,* 2014). Endophytic fungi contain several bioactive metabolites that are used to treat wound burn infections and have a wide range of medicinal properties such as antimicrobial and antitumor activity (Aly *et al.,* 2008). Endophytes and their secondary metabolites therefore not only play an important ecological role but also have a positive impact on the medical sector. Also, their bioactive metabolites can be used as immunosuppressant, anticancer and biocontrol agent (Selvin *et al.,* 2004).

In our study, *F.oxysporum* and *P.herbarum* were isolated and identified in similar to Cui *et al*. (2011) who isolated *Fusarium* sp. from *Aquilaria sinensis* and Khan *et al*. (2014) who identified *Phoma sp.* as an endophytic fungus from *Moringa peregrine*. In this study, TEAFEs demonstrated high dose-dependent scavenging activity of DPPH radical compared with ascorbic acid as a standard, in accordance to Li *et al*. (2011) who reported that water extract of endophytic *F.oxysporum* from *Dioscoreazin giberensis* gave high antioxidant activity reached to (84.27%).

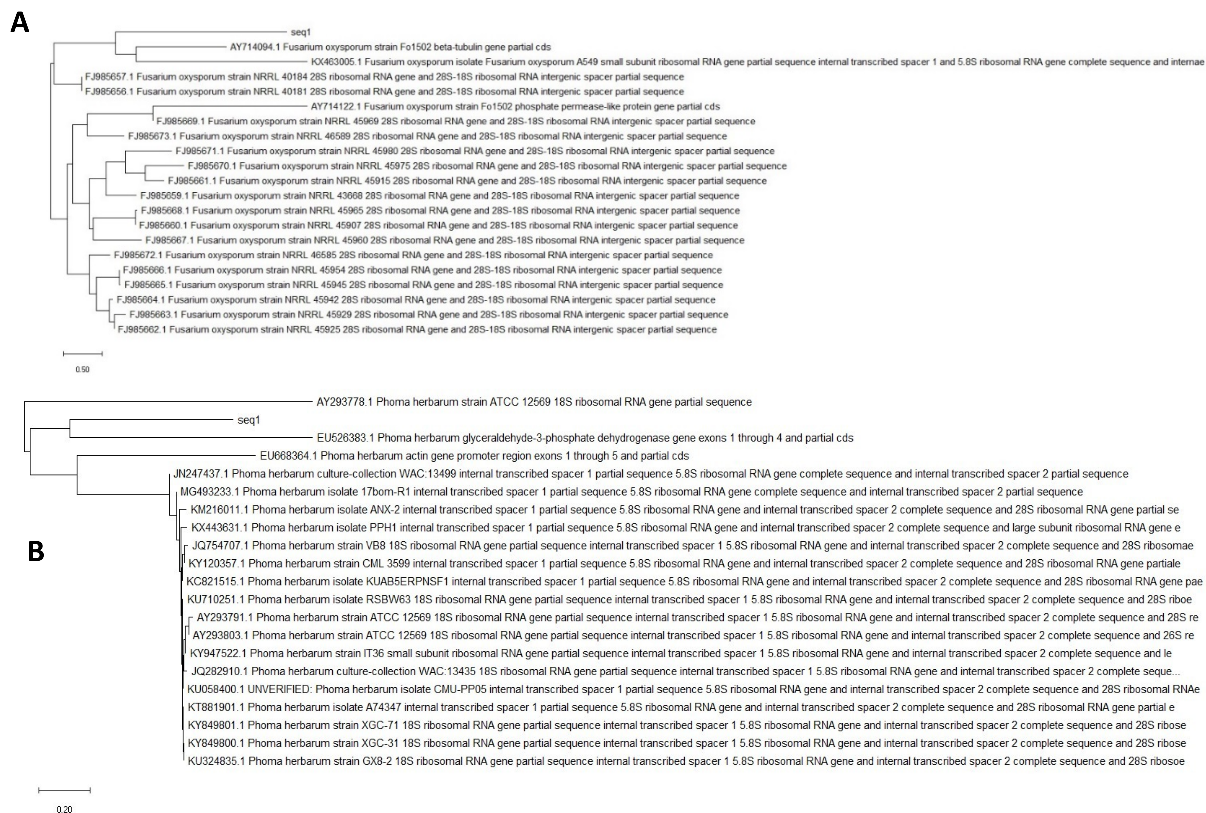

Figure 1. Phylogenetic analysis of isolated *Fusarium oxysporum* and *Phoma herbarum* from *urospermum picroides* (medicinal plant). A: Phylogenetic tree of isolated *F.oxysporum*. B: Phylogenetic tree of isolated *Phoma herbarum*. Both of Trees were constructed based on their internal transcribed spacer ribosomal DNA (ITSrDNA) sequences. The phylogenetic trees were constructed using the neighbor-joining (NJ) algorithm. Multiple sequence alignments were performed using the CLUSTALW program. Molecular evolutionary analyses were conducted using MEGA version 10.0.4.

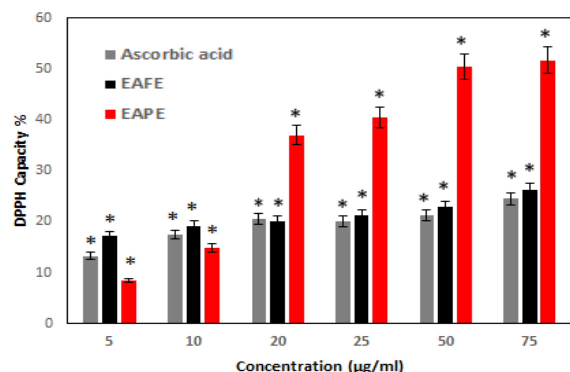

Figure 2. Quantitative analysis for antioxidant activity of EAFE and EAPE in comparison to ascorbic acid. EAFE: Ethyl acetate Fusarium Extract and EAPE: Ethyl acetate Phoma Extract. EAPE showed a potent antioxidant activity (51.7%) followed by EAFE (26.2%) compared to ascorbic acid (positive control) (24.5%) at a concentration of (75 µg/ml). Data are expressed as Mean ± SD. (n=3) of three independent experimental replicates. *Statistically significant antioxidant activities of EAFE and EAPE in relation to ascorbic acid (normal control). The data were statistically analyzed by Graph pad prism (version 5.01) indicating that the data were significant with P-value ≤ 0.05.

Moreover, polysaccharides from endophytic *P.herbarum* showed highly significant antioxidant activity (Yang *et al.,* 2005). Similarly, Huang *et al*. (2001) declared that *F.oxysporum* and *P.herbarum* as *Taxus mairei* and *Torreya grandis* endophytic fungi showed antitumor activity against BGC-823 cell line (Human gastric tumor cells). Further study by Minirani *et al*. (2017) showed anticancer activity of endophytic fungal extracts from *Annonam uricata* against MCF-7 cells with the same morphological changes in our study. Moreover, in accordance with our results, many morphological changes in treated HepG2 cells with *Terminalia arjuna* bark extract were reported by Sivalokanathan *et al*. (2006). These studies strongly supported our findings that endophytic medicinal plant fungi are a promising source of natural active antitumor compounds.

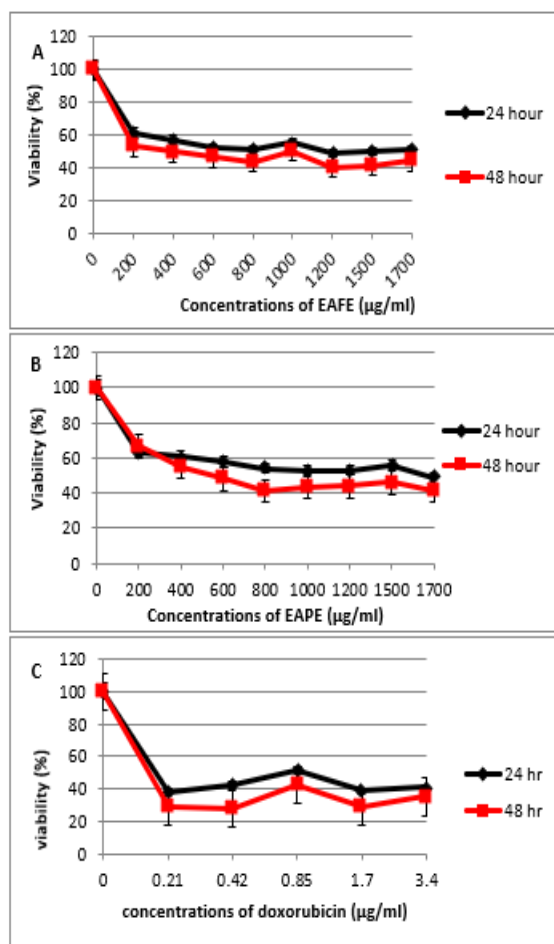

Figure 3. Quantitative analysis of anti-tumor activities of fungal extracts in comparison to doxorubicin against liver cancer cell line (HepG2). A: EAFE (Ethyl Acetate Fusarium Extract), B: EAPE (Ethyl Acetate Phoma Extract) and C: Doxorubicin. Both of extracts (EAFE and EAPE) showed highly antitumor activities with IC50% (1269, 1590 µg/ml) after 24 hours and (645.7, 627µg/ml) after 48 hours, respectively. Data are expressed as Mean ± SD. (n=3) of three independent experimental replicates. *Statistically significant antitumor activities of EAFE and EAPE in relation to doxorubicin (positive control). *P-value: versus non-treated control group; * all data is analyzed by Graph pad prism (version 5.01) significantly different as compared to non-treated control cell at P<0.05.

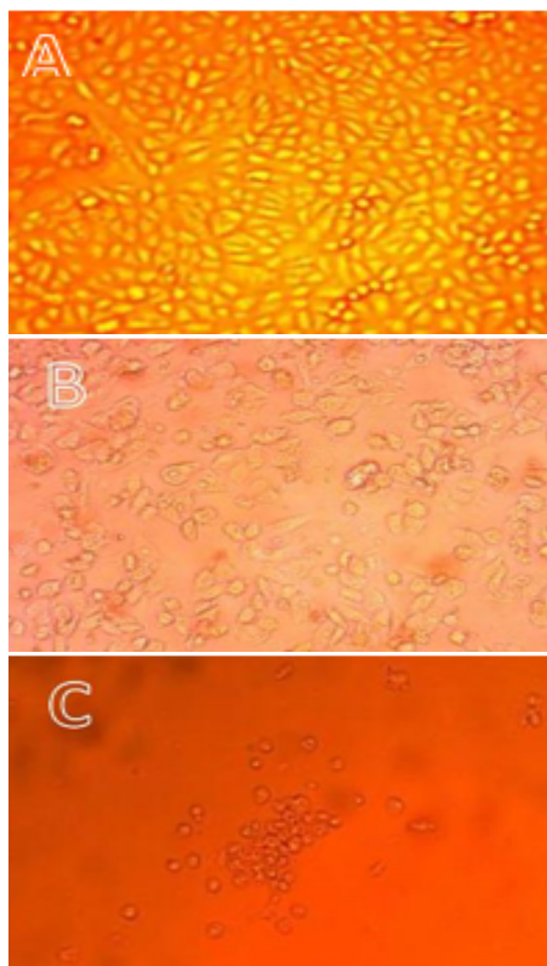

Figure 4. HepG2 liver-cancer cells treated with extract of two types of endophytic fungi. A= control, HepG2 liver-cancer cells without treatment, B = HepG2 liver –cancer cells treated with EAFE (Ethyl acetate Fusarium Extract) at a concentration of 645.7µg/ml, C = HEPG2 liver-cancer cells treated with EAPE (Ethyl Acetate Phoma Extract) at a concentration of 627µg/ml (20×magnification). Treated cells showed significant morphological changes, which were characteristic of apoptosis, such as cell swelling, shrinkage and reduced in growth with the destruction of monolayer which was not seen in untreated HepG2 cells.

Our study was the first study to study the gene expression and apoptotic markers of *Fusarium* sp and *Phoma* sp extracts which isolated from *U. picroides* using quantitative real-time PCR on Human liver cancer cell line (HepG2). which showed up of Bax regulation and p53 regulation and down of Bcl-2 regulation. Similarly, as in *Eurycomalo ngifolia* (Zakaria *et al.,* 2009) and Solanum nigrum (Yb *et al.,* 2008), several researchers reported induction of apoptosis in HepG2 cells through the up regulation of p53 and Bax, and down regulation of Bcl-2.

The TEAEs showed high antimicrobial activities which showed morphological alternations by SEM. Rantaweera *et al*. (2015), which examined the antimicrobial properties of endophytic fungi obtained from *Opuntia dillenii*, accordingly. Furthermore, some morphological changes appeared on *Cryptococcus neoformans, Candida albicans* and *Microsporum gypseum* treated with endophytic fungal extracts of *Penicillium* sp. *Fusarium* sp. *Trichoderma* sp and *Hypocreales* sp using scanning electron microscope as the cells seemed to shrink, and the cell wall degraded (Supaphon *et al.,* 2013).

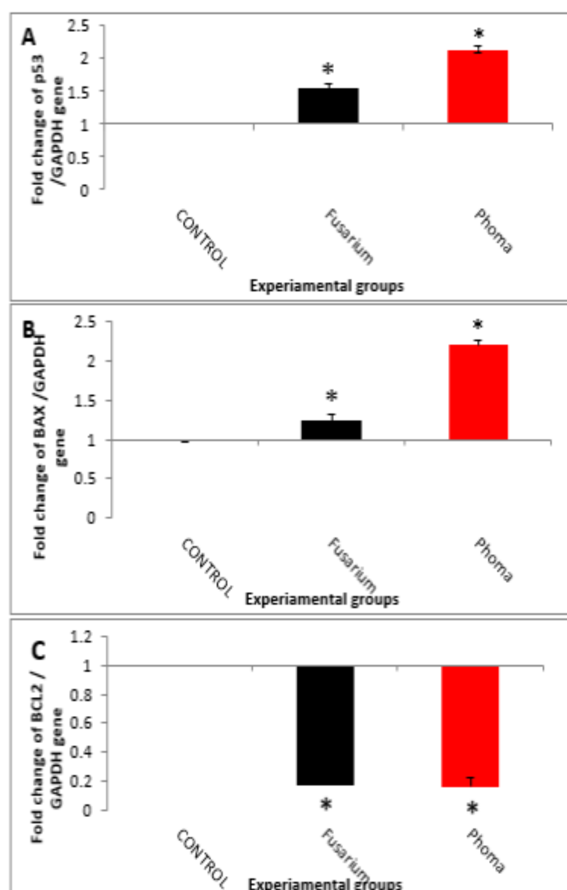

Figure 5. Quantitative analysis of gene expression of apoptotic markers on different treatments on HepG2 (liver cancer cells).p53 and Bax tumor marker genes indicated high expression in treatments with the two fungal extracts, While Bcl2 down regulated compared to control. a = control, HEPG2 liver-cancer cells without treatment, b = HEPG2 liver –cancer cells treated with IC50 concentration of EAFE, c = HEPG2 liver –cancer cells treated with IC50 of EAPE. P-value: versus the non-treated control group. Data represent the mean fold change ± SD of triplicate experiments. * All data is analyzed by Graph pad prism (version 5.01) significantly different as compared to non-treated control cell at P<0.05.

GC-MS is an effective method of identifying compounds that are present in the fungal extract. For EAFE and EAPE the GC-MS chromatography shows the presence of 30 compounds in each sample. In the same way as our findings, Gokhale *et al*. (2017) screened bioactive molecules of endophytic fungi by GC-MS spectroscopy. The highest three compounds were (Diisooctyl phthalate; Disperse Blue 26 and 3-Benzo[g]quinoxalin-2-yl-propionic acid) for EADF and (Benzoic acid, 3,5-bis (1,1-dimethylethyl)-4-hydroxy-methyl ester; 2-Myristynoyl-glycinamide and Cinnamic acid,p-(tri methylsiloxy)-,methyl ester) for EADP with different relative proportion.

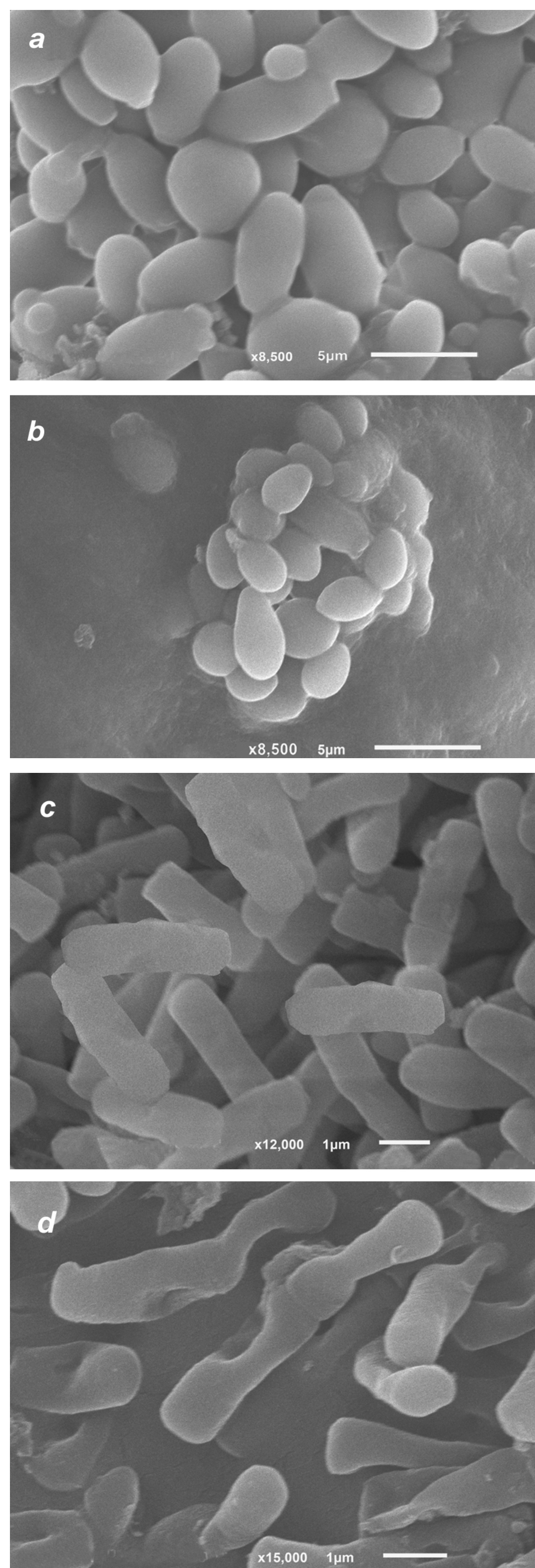

Figure 6. Scanning electron micrograph of treated *C.albicans* and *B.cereus* cells with EAPE. a: untreated *C.albicans* cells; b: treated *C.albicans* cells with EAPE; c: untreated *B.cereus* cells; d: treated *B.cereus* cells with EAPE.

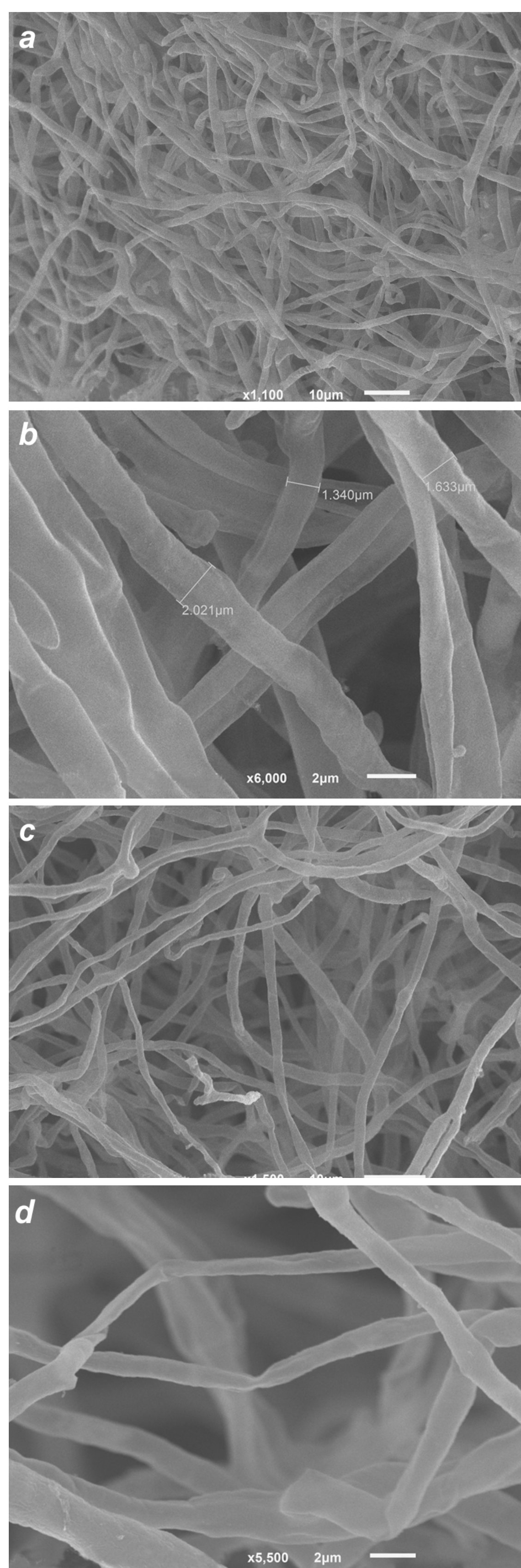

Figure 7. Scanning electron micrograph of treated *T. mentagrophytes* with EAFE. a,b: untreated *T.mentagrophytes* cells; c,d: treated *T.mentagrophytes* cells with EAFE.

Antioxidant, antimicrobial and antitumor activities of both extracts may be related to these active compounds. This was in accordance with the antioxidant and antimicrobial activities of di-isooctyl phthalate produced by *Cassia angustifolia* (medicinal plant) (Al-Marzoki *et al.,* 2016).

In addition, it has also described *Bauhinia variegate linn.* from secondary metabolites. Leaf extract has demonstrated colon cancer antitumor activity (Gunalan *et al.,* 2016). Similarly, Hazeldin *et al*. (2005) reported that 2-{4-[(7-Chloro-2-quinoxalinyl) oxyphenoxy] propionic acid and 2-[4-[(7-bromo-2-quinolinyl) oxy] phenoxy] propionic acid is the most highly analogues for antitumor agents. Park et al. (2001) have also reported a strong antimicrobial activity of benzoic acid for benzoic acid. As reported by Devereux *et al*. (2007), benzoic derivatives also have antitumor activity against human hepatic (HepG2), renal (A-498), and lung (A-549) cancer cell lines. Bashir *et al*. (2012) also demonstrated that cinnamic acid methyl ester (A. aspera), benzenepropanoic acid, and 3, 5-bis (1,1-dimethylethyl)-4-hydroxy-methyl ester have both antifungal and antioxidant activities.

CONCLUSION

This study showed that *F.oxysporium* and *P.herbarum* as novel endophytic fungi from *U.picroides* have significant antimicrobial, antioxidant and antitumor activities against HepG2 cancer cell line followed by gene expression analysis using (p53, Bax and Bcl-2) genes on real-time PCR which revealed the induction of apoptosis. Further studies were needed to confirm the efficacy of these endophytic fungal extracts by identifying the active components to be used as a promising drug thereafter.

Acknowledgements

This work was facilitated by of Scientific research Center and measurements (SRCM), Tanta University, Tanta, Egypt.

Conflict of interest

The authors declare that they have no competing interests.

References

Ahmed D (2003). Current situation of the flora and vegetation of Nile Delta region. Msc. Thesis, Faculty of science, Tanta University, Egypt.

Alley MC, Scudiero DA, Monks A, Hursey ML, Czerwinski MJ and Fine DL (1988). Feasibility of drug screening with panels of human tumor cell lines using a microculturetetrazolium assay. Cancer Res., 48: 589-601.

Al-Marzoqi AH, Hadi MY and Hameed IH (2016). Determination of metabolites products by *Cassia angustifolia* and evaluate antimicrobial activity JPP J., 8(2): 25-48.

Aly HA, Edrada-Ebel R, Wary V,Muller WEG, Kozytska S, Hentschel U, Proksch P and Ebel R (2008). Bioactive metabolites from the endophytic fungus Ampelomyces sp isolated from the medicinal plant *Urospermumpicroides*.Phyto.chemist., 69: 1716-1725.

Bashir A, Ibrar K, Shumaila B and Sadiq A (2012). Chemical composition and antifungal, phytotoxic, brine shrimp cytotoxicity, insecticidal, and antibacterial activities of the essential oils of *Acacia modesta*. J Med Plants Res., 6(31): 4653-4659.

Cui JL, Guo SX and Xiao PG (2011). Antitumor and antimicrobial activities of endophytic fungi from medicinal parts of *Aquilaiasinesis.* ZJUS-B., 12(5): 38-392.

Devereux M, O´shea D, O´connor M, Grehan H, Connor G, McCANN M, Rosair G, Lyng

F, Kellette A, Walsh M, Egan D and Thati B (2007). Synthesis, catalase, superoxide dismutase and antitumour activities of copper(II) carboxylate complexes incorporating benzimidazole, 1,10-phenanthroline and bipyridine ligands: X-ray crystal structures of [$Cu(BZA)_2(bipy)(H_2O)$], [$Cu(SalH)_2(BZDH)_2$] and [$Cu(CH_3COO)_2$(5,6-DMBZDH)$_2$] ($SalH_2$ = salicylic acid; BZAH = benzoic acid; BZDH = benzimidazole and 5,6-DMBZDH = 5,6-dimethylbenzimidazole). Polyhedron., 26(25): 4073-4084.

El-Amier YA, Al-hadithy ON and Abdullah TJ (2016). Antioxidant and antimicrobial activity of different extracts obtained from aerial parts of *Urospermumpicroides* (L.) F.W.fromEgypt. J ADV Chem.Sci., 2(3): 299-301.

El-Ghazooly, MagedG., El-Lakany, Abdalla M., Abou-Shoer, Mohamed I, Aly, Amal H (2003). Chemical constituents of *Helichrysumconglobatum* growing in Egypt. Nat. Prod. Sci., 9(4): 231-219.

Glienke-Blanco C, Aguilar-Vildoso C l, CarnerioVieria M L, Vianna Barroso P A and Azevedo J L (2002). Genetic variability in the endophytic fungus *Guignardiacitricarpa*isolated from citrus plants. Gene. Mol. Biol., 25(2):251-255.

Gokhale M, Verma M, Faraz R and Raj D (2017). Bioactive Molecules (GC-MS) of Endophytic fungi,Xylaria from Nyctanthes arbor-trists (Linn). H. K. B. J., 3(1):43-53.

Gunalan G, Vijayalakhshmi K, Tamilvannan T and Hopper W (2016). Anticancer activity of secondary metabolites from *Bauhinia variegate linn*. leaf- an in-silico approach. Indo American J Pharm. Res., 6(7): 6.

Hazeldine ST, Polin L, Kushner J, White K, Corbett TH, Biehl A and Horwitz JP(2005). Synthesis and biological evaluation of some analogs of the antitumor agents, 2-{4-[(7-chloro-2-quinoxalinyl)oxy]phenoxy}propionic acid, and 2-{4-[(7-bromo-2-quinolinyl)oxy]phenoxy}propionic acid. Bioorganic and Med.Chem. J., 13(4): 1069-1081.

Huang W Y, Cai, Y Z, Hyde, K D., Corke H and Sun M (2008). Biodiversity of endophytic fungi associated with 29 traditional chinese medicinal plants. Fungal div. J., 33:61-75.

Huang YJ, Wang JF, Li GL, Zheng ZH and SuWJ (2001). Antitumor and antifungal activities in endophytic fungi isolated from pharmaceutical plants *Taxusmairei, Cephalataxus fortune* and *Torreyagrandis.* FEMS Immunol Med. MIC., 31: 163-167.

Israel RK, Devanadera PKM, Mayor RBA, Cada ACM, Pecundo HM and Macabeo GPA (2019). Toxicity, Antibacterial, and Antioxidant Activities of Fungal Endophytes *Colletotrichum* and *Nigrospora* spp. Isolated from *Uvaria grandiflora.* Philippine J of Sci., 148(3):503-510.

Jalgaonwala R E, Mohite B V and Mahajan RT (2010). Evaluation of endophytes for their antimicrobial activity from indigenous medicinal plants belonging to North Maharashtra region India. Int. J. Pharma Biomedical Res., 1: 136-41.

kaya I, Yigit N and Benli (2008). Antimicrobial Activity of various extracts of *OcimumBasilicumL*. And observation of the inhibition effect on bacterial cells by use of scanning electron microscopy. Afr.J.Trad.CAM., 5(4):363-369.

Khan AL, Waqas M, Hussain J, Al-Harrasi A, Al-Rawahi A, Al-Hosni K, Kim MJ, Adnan M and Lee IN (2014). Endophytes *Aspergillus caespitosus* LK12 and *Phoma* sp. LK13 of *Moringa peregrine* produce gibberellins and improve rice plant growth. J Plant Interact., 9(1): 731-737.

Kharwar RN, Mishra A, Gong SK, Stierle A and Stierle D (2011). Anticancer compounds derived from

fungal endophytes: their importance and future challenges. Nat. Prod. Rep, 28: 1208-1228.

Li p, Luo C, Sun W, Lu S, Mou Y, Peng Yand ZhouL (2011). *In vitro* antioxidant activities of polysaccharides from endophytic fungus *Fusarium oxysporum* Dzf17, J Microbiol. Res., 5(32): 5990-5993.

Lv YL, Zhang FS, Chen J, Cui JL, Xing Y, Li XD and Guo SX (2010). Diversity and antimicrobial activity of endophytic fungi associated with the alpine plant *Saus-surea* involucrate. Biol. Pharm. Bull., 33(8): 1300-1306.

Minirani Artica IA, Julistiono H, Bermawie N, Riyanti EI, Hashim and Hasan AEZ (2017). Anticancer activity test of ethyl acetate extract of endophytic fungi isolated from soursop leaf (*Annonamuricata L*). Asian Pac. J Trop. Med, 10(6): 566-571.

Mosmman T (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol. Methods, 65: 55-63.

Naruya S and Masatoshi N (1987). The Neighbor-joining method: a new method for reconstructing phylogenetic tree. Mol. Biol.Evol., 4(4): 406-425.

Park ES, Moon WS, Song MJ, Kim NM, Chung KH and Yoon JS (2001). Antimicrobial activity of phenol and benzoic acid derivatives. Int.biodeterior. biodegradation, 47(4): 209-214.

Phongpaichit S, Rungjindamai N, Rukachaisirikul N and Sakayaroj J (2006). Antimicrobial activity in cultures of endophytic fungi isolated from *Garcinia species.* FEMSImmunol. Med. Microbiol., 48(3): 367-372.

Radhika P, Sastry B, Harica B and Madhu B (2008). Antimicrobial screening of *Andrographis Paniculata* (Acanthaceae) rot extracts. Res. Biotech. J., 3:62-63.

Rantaweera BP, Silva ED, Willias DE and Andersen RJ (2015). Antimicrobial activities of endophytic fungi obtained from the arid zone invasive plant *Opuntia dillenii* and the isolation of equisetin, from endophytic *Fusarium sp*. CAM J., 15(1): 220.

Rukshana MS, Doss A and Kumari P R T P (2017). Phytochemical screening and GC-MS analysis of Leaf extract of *Pergulariadaemia* (Forssk) Choiv. Asian J of Plant Sci. and Res., 7(1):9-15.

Selvin J, Joseph S, Asha KRT, manjusha WA, Sangeetha VS, Jayaseema DM, Antony MC and Vinitha AJD (2004). Antibacterial potential of antagonistic *Streptomyces sp*. Isolated from marine sponge *Dendrillanigra.* FEMS microbiology ecology, 20(2): 117-122.

Sivalokanathan S, Vijayababu MR and Balasubramanian MP (2006). Effects of *Terminaliaarjuna* bark extract on apoptosis of human hepatoma cell line HepG2. World J Gastroentro., 21(7):1018-1024.

Strobel G and Daisy B (2003). Bio prospecting for microbial endophytes and their natural products. Microbiol Mol. Biol. R, 67: 491-502.

Supaphon P, Phongpaichit S, Rukachaisirikul V and Sakayaroj J (2013). Antimicrobial potential of Endophytic fungi Derived from Three Seagrass Species: *Cymodoceaserrulata*, *Halophila ovalis* and *Thalassiahemprichii*. Plos one., 8(8).

Thompson JD, Higgins DG and Gibson TJ (1994). CLUSTALW, improving the sensitivity of progressive multiple alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Res., 22(22): 4673-4680.

Xu L, Zhou L, Zhao J, Li J, Li X and Wang J (2008). Fungal endophytes from *Dioscoreazingiberensis*rhizomes and their antibacterial activity. Lett. Appl. Microbiol., 46 (1): 68-72.

Yadav M, Yadav, Kumar S, Sharma D and Yadav J (2014). Evaluation of in vitro antimicrobial potential of endophytic fungi isolated from Eugenia Jambolana Lam. Intd. Pharm. Pharm. Sci., 6(5):208-11.

Yang J, Sun S and Yan Y (2010). a novel lipase gene cloned from exhibits enzymatic characteristics distinct from its previously identified family member. Biotechnoi. Lett., 32: 951-956.

Yang X, Gao X, Han F and Tan R (2005). Sulfation of a polysaccharide produced by a marine filamentous fungus *Phomaherbarum* YS4108 alters its antioxidant propereties*in vitro*, Biophys. Acta., 1725: 120-127.

Yb Ji, Gao SY, Ji CF and Zou X (2008). Induction of apoptosis in HepG2 cells by Solanine and Bcl-2 protein. J Ethnopharmacol., 115(2): 194-202.

Yu J, Mohawed s, Bhatnagar D and Cleveland T (2003). Substrate-induced lipase gene expression and aflatoxin production in *Aspergillus parasiticus* and *Aspergillus flavus.* Appl. Microbiol., 95: 1334-1342.

Zakaria Y, Rahmat A, Pihie AHL, Abdullah NR and Houghton PJ (2009). Eurycomanone induce apoptosis in HepG2 cells via up-regulation of p53. Cancer Cell Int., 9(1): 16.

Egyptian Association for Cancer Research (EACR)
http://eacr.tanta.edu.eg/

EACR is an NGO society that was declared by the Ministry of Social Solidarity (Egypt) No. 1938 in 19/11/2014 based on the initiative of Prof. Mohamed Labib Salem, the current Chairman of EACR. EACR aims primarily to assist researchers, in particular young researchers in the field of cancer research through workshops, seminars and conferences. Its first international annual conference entitled "Anti-Cancer Drug Discovery" was successfully organized in April 2019 (http://acdd.tanta.edu.eg). Additionally, EACR aims to raise the awareness of the society about the importance of scientific research in the field of cancer research in prediction, early diagnosis and treatment of cancer. EACR is also keen to outreach the scientific community with periodicals and news on cancer research including peer-reviewed scientific journals for the publication of cutting-edge research. The official scientific journal of EACR is "International Journal of Cancer and biomedical Research (IJCBR: https://jcbr.journals.ekb.eg) was successfully issued in 2017 and has been sponsored by the Egyptian Knowledge Bank (EKB: www.ekb.eg).

EACR **Chairman,**
Prof. Mohamed Labib Salem, PhD
Professor of Immunology
Faculty of Science, Tanta Universiy, Egypt

International Journal of Cancer & Biomedical Research (IJCBR) Online ISSN 2682-2628

Editor-in-Chief

Mohamed Labib Salem, PhD
Tanta University, Egypt

Managing Editor

Nehal Elmashad, MD
Tanta University, Egypt
Nabil Mohy Eldin, PhD
Kafrelsheikh University, Egypt
Doaa Al-Ghareeb, PhD
Alexandria University, Egypt
Abdel-Aziz Zidan, PhD
Damanhour University, Egypt
Wesam Meshrif, PhD
Tanta University, Egypt
Rasha Eraky, MD
Tanta University, Egypt

Associate Editor

Hesham Tawfik
Tanta University, Egypt
Mostafa El-Sheekh
Tanta University, Egypt
Yousry Albolkiny, PhD
Tanta University, Egypt
Gamal Badr
Assuit University, Egypt
Elsayed Salim
Tanta University, Egypt
Essam Elshiekh
Tanta Cancer Center, Egypt

Editorial Board

Alberto Montero
Taussig Cancer Center, Cleveland, USA
Marcela Diaz
Cleveland Clinic Foundation, USA
Yi Zhang
Zhengzhou University, China
Shengdian Wang
Chinese Academy of Sciences, China
Faris Alenzi
Prince Sattam bin Abdulaziz University, KSA
Mark Robunstein
Medical University of South Carolina, USA
Mamdooh Ghoneum, DSc
Charles Drew University of Medicine & Science, USA
Natarajan Muthusamy, DVM
The Ohio State University, USA
Hideki Kasuya MD, PhD, FACS
Nagoya University, Japan
Sherif El-Khamisy, MD
Sheffield University, UK
Mohamed Abou-El-Enein, MD
Charité Universitätsmedizin Berlin, Germany
Alaa Eldin Almostafa, MD
McGill University, Canada
Amr Amin
United Arab Emirates University, UAE
AbdelRahman Zekri
National Cancer Institute, Egypt
Mohamed Attia, MD
Tanta University, Egypt
Mohamed Elshanshory, MD
Tanta University, Egypt
Hussein Khamis
Alexandria University, Egypt
Magdy Mahfouz
Kafr Elsheikh University, Egypt
Ehab Elbedewey
Tanta University, Egypt
Abeer Badr
Cairo University, Egypt
Nadia Hamdy, PharmD
Ain Shams University, Egypt
Ibrahim El-Sayed
Menoufia University, Egypt
Tarek Aboul-Fadl, PharmD
Assiut University, Egypt
Mohamed Noureldin
Banaha University, Egypt
Haiam Abou Elela
National Institute of Oceanography and Fisherie, Egypt
Sameh Ali, MD
Nationa Liver Institute, Egypt
Maha EL-Demellawi
City for Scientific Research & Technology Applications, Egypt
Desouky A Abd-El-Haleem
City for Scientific Research & Technology Applications, Egypt
Ashraf Tabll
National Research Center, Egypt
Wael Lotfy, MD
Alexandria University, Egypt
Olfat Gadallah, MD
Tanta University, Egypt
Nahla Shoukry
Suez University, Egypt
Medhat Eldenary
Tanta University, Egypt
Nagla Sarhan, MD
Tanta University, Egypt
Naglaa Fathy, MD
Zagazik University, Egypt
Azza Hasan Mohamed
Menufia University, Egypt
Nanees Gamal Eldin
Tanta University, Egypt
Mohamed Mansour, UK
Sabbah Hammoury
Alexandria Ayadi Almostaqbal Oncology Hospital, Egypt
Nehal Aboulfotoh
Zewail City for Science and Technology, Cairo, Egypt
Amir Elkhami
Galaxo, San Francisco, USA
Rabab Khairat
National Research Center, Giza, Egypt
Ahmed Alzohairy
Zagazi University, Egypt
Wgady Khalil
National Research Center, Egypt
Sayed Bakry
Alazhar University, Egypt
Mohamed Ghanem, MD
Kafr Elshikh University, Egypt
Mohamed Salama, MD
Mansoura University, Egypt
Mona Marie, MD
Alexandria University, Egypt

For more information, contact

Hamdi Kandil
Tanta University, Egypt
Email: Ijcbr100@gmail.com