Original Article

Effects Of Some Fungal Secondary Metabolite Against Some Cancer Cell Line

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Abstract:

The promising feature of the fungi from the marine environment as a source for anticancer agents belongs to the fungal ability to produce several compounds and enzymes which contribute effectively against the cancer cells growth. Moreover, the compounds produced during the secondary metabolic process acts by changing the cell morphology and DNA fragmentation leading to apoptosis of the cancer cells. When compared to the other chemicals in the investigation, gliotoxin demonstrated the most cytotoxic effect. These findings suggest that gliotoxin could be a potential virulence factor of Aspergillus fumigatus during infection. Anti Cancer, the dreadful disease that spreads throughout the body, bringing its cells closer to death than life expectancy, will be defeated or at least slowed by natural plants. Many researchers and clinicians are familiar with the treatment of numerous plants, such as Rheum ribes. Cancer studies have shown that the usage of some medicinal herbs can help to minimize the severity of this dreadful disease, and plant care is also well understood.

Keywords: Marine; Fungi, Actinobacteria, Colorectal Cancer, Cytotoxicity, Secondary Metabolite

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Introduction:

Anethum graveolens L. (Apiaceae) is an annual plant with significant lipid-lowering activities and potential cardioprotective characteristics. It has long been linked to anemia, which can come from RBC confiscation for gastrointestinal issues such spleen disease, decreased red blood cell production or indigestion, stomachache colic, and primary bone marrow failure. The most prevalent therapies include oral hypoglycemics, monoterpenes, and insulin therapy. The seeds, leaves, and stems of mellitus, a chronic disorder of glucose, lipid, and protein metabolism, can yield a variety of compounds. The seeds of Anethum graveolens (AGS) have a strong, spicy scent and a bitter taste. Stomachaches, indigestion, dyspepsia, and other gastrointestinal issues are treated with this herb. There are anti-inflammatory and gastroprotective effects as well. Fungi have several medical applications due to their capacity to create numerous enzymes and antibacterial chemicals. According to studies, More antibacterial and anticancer chemicals are produced by fungi than by bacteria. The efficiency and functionalities of the active chemicals produced, on the other hand, are reliant on the genes found in a genetic cluster bundle called biosynthetic gene clusters (BGCs) [1] . Anethum graveolensis, for example, is ethanolic. Marine fungi, on the other hand, are abundant in bioactive molecules and have developed a wide range of anticancer compounds [2]. Kumar and his associates [3] Among other enzymes, the asparaginase enzyme was discovered to have the highest anticancer characteristics in Aspergillus niger [4], Aspergillus tubingensis [5]Trichosporon asahii [9-17] discovered a Microsporum sp. physcion that causes cell death by

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suppressing Bcl-2 expression. They also discovered that the Neosartorya siamensis chemicals Nortryptoquivaline, 2,4-dihydroxy-3-methylacetophenone, and chevalone C caused cytotoxicity and cell death in lung cancer cells, as well as DNA and intracellular accumulation. Purified from P. concentricum's secondary metabolism, the Epoxydon, 3-hydroxy, benzenemethanol, 2-bromogentisyl alcohol, 2-chlorogentisyl alcohol, and 2-bromogentisyl alcohol are some of the compounds that can be found in Compounds containing 6-dehydroxy-6-bromogabosine C showed anti-cancer action in a human colon adenocarcinoma cell line [18]. The anti-cancer activity of Pyrenochaetopsis sppentacyclic .'s decalinoylspirotetramic acid and pyrenosetin D against the noncancerous keratinocyte cell line and the melanoma cell line [19]. The goal of this study was to show how marine fungi can be used to make anticancer chemicals. To minimize disinformation and fill a vacuum in the literature, the current study used a SLR approach stands for systematic literature review . Furthermore, this article's The addition of bibliometric analysis of anticancer literature is significant to the creation of a roadmap that directs readers to the appropriate resources for learning more about the study. The current study's objective Some fungal secondary metabolites' effects on a cancer cell line

Materials and Methods:

REF cells were subcultured. After gently pressing the flask to release the cells, 20 ml of growth media supplemented with ten % fetal bovine serum was added, and the cells' viability was assessed using trypan blue dye, which stains dead cells. Using an automated micropipette containing (1x105 cells/well), 200 l/well was transferred to the 96 well flat bottom micro titer plate after properly mixing the cells. Plates were cultivated The REF cell line was grown at 37°C in an incubator with 5% CO2 until the internal surface area of the well was 60-70 percent confluent [20-22]. Analyze for cytotoxicity The cells were treated and incubated with purified extracts of gliotoxin, hemolysin, protease, and Melanin, three concentrations at triplicate form of each extract, to evaluate the cytotoxic effect of these extracts, respectively, to detect growth inhibition in the REF cell line , The following concentrations were used: gliotoxin 25,50,100 ng/ml, hemolysin 2.5, 5, 10 g/ml, protease 7.5, 15, 30 g/ml, and melanin 62, 125, 250 g/ml in triplicate form. Incubating the REF cell line with only maintenance media provided a negative control. The Cytotoxic Effect's Detection The cytotoxic effect was detected using a neutral red test. After the incubation period had passed, 100 l/well of freshly prepared neutral red dye was added to each well, and the plates were incubated for 2 hours; alive cells absorb the dye, but dead cells do not. After washing the plates in PBS to remove the excess dye, each well was filled with 100 l/well with eluent solution to remove the dye from the live cells. The optical density of each well was determined using an ELISA reader set to 492nm [22,21]. According to Wang (2003) [20], the inhibitory rate was calculated as follows: Percentage I.R = 100 [20]

In Vitro Anticancer Activity:

The anticancer activity was tested against the L20B cell line. The colorimetric MTT test for cell viability was utilized, as described by [21,22]. In a 96-well tissue culture plate, 100 L/well of L20B cells (106 cells/mL) were first cultivated. in water yielded various amounts test solution. After that, 100 L of varied concentrations . The anti-cancer activity of Pyrenochaetopsis sppentacyclic .'s decalinoylspirotetramic acid and pyrenosetin D against the noncancerous keratinocyte cell line and the melanoma cell line .

$$GI\% = \frac{(OD\ of\ control\ wells - OD\ of\ test\ wells)}{OD\ of\ control\ wells} \times 100.$$

Statistical Analysis:

The examined parameters' values were presented as By means of the mean standard error, differences between values are evaluated by analysis of variance (ANOVA) and by Duncan's test, all using version 7.5 of the SAS computer program. [21]. Differential outcomes were considered significant when the probability value was 0.05 or less.

Results And Discussion:

Effect of Leaf Ethanol Extract on HepG2 Parameters of Cells at Different Concentration.

Effects of a fungal secondary metabolite against a cancer cell line ethanolic extract was isolated from

(Anethum graveolensis ethanolic Ex.) leaves and its cytotoxic activity was assessed using HepG2 using several cell loss rate, membrane potential intensity, and cytochrome C realizing intensity parameters as shown in the table. (1) Effects of various fungal secondary metabolites on the viability of cancer cell lines, table (2) Hep g2 cell line result, and table (3) L20B cell line result. (4) L20B cell line result (Anethum graveolensis ethanolic Ex.)

Table (1) This report aimed to research the cytotoxic action against apoptosis behavior of Ethanolic Result against hep g2 cell line

Leaf extract isolated from a local plant of *Anethum graveolensis* ethanolic Ex. affected living Hep G2 cell line. The research used an in vitro assessment of the cytotoxic activity of the Ethanolic Result against hep g2 cell line

Leaf extract at various concentrations (1000, 500, 250, 125, 62.5 µg / ml) in serial dilutions on the HepG2 cell line and exposure period (28 h) to accomplish this objective. 62.5, 125 and 250 µg / ml is the most important drop in viable counting cells. Strong cell, tested but not impaired by all Ethanolic Result against hep g2 cell line

extract concentrations (no important significance P<0.001).

Table(1): Result against hep g2 cell line

Extract Conce.	Mean	S.D.	GI%
1(50)	0.404	0.049	0
2(25)	0.205	0.080	42.4
3(12.5)	0.141	0.053	60.3
4(6.25)	0.138	0.021	61.2
5	0.119	0.029	66.5
6	0.211	0.016	40.7
7	0.268	0.011	24.7
8	0.378	0.034	0
Control	0.356	0.080	

Table (2) This report aimed to research the cytotoxic action against apoptosis behavior of Ethanolic Result against hep g2 cell line

Leaf extract isolated from a local plant of *Anethum graveolensis* ethanolic Ex. affected living Hep G2 cell line. The research used an in vitro assessment of the cytotoxic activity of the Ethanolic Result against hep g2 cell line

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extract concentrations (no important significance P<0.001).

Table(2): Result against hep g2 cell line

Extract Conce.	Mean	S.D.	GI%
1(50)	0.206	0.033	41.9
2(25)	0.180	0.033	49.2
3(12.5)	0.112	0.004	68.5
4(6.25)	0.089	0.006	74.8
5	0.082	0.004	76.9
6	0.083	0.005	76.6
7	0.099	0.031	72.1
8	0.159	0.006	55.1
Control	0.356	0.080	

Table (3) This report aimed to research the cytotoxic action against apoptosis behavior of Ethanolic Result against L20B cell line

Leaf extract isolated from a local plant of *Anethum graveolensis* ethanolic Ex. affected living Hep G2 cell line. The research used an in vitro assessment of the cytotoxic activity of the Ethanolic Result against L20B cell line

Leaf extract at various concentrations (1000, 500, 250, 125, 62.5 µg / ml) in serial dilutions on the HepG2 cell line and exposure period (28 h) to accomplish this objective. 62.5, 125 and 250 µg / ml is the most important drop in viable counting cells. Strong cell, tested but not impaired by all Ethanolic Result against L20B cell line extract concentrations (no important significance P<0.001).

Table(3): Result against L20B cell line

Extract Conce.	Mean	S.D.	GI%
1(50)	0.060	0.001	83.1
2(25)	0.083	0.036	76.6
3(12.5)	0.079	0.026	77.8
4(6.25)	0.082	0.004	76.9
5	0.078	0.013	77.9
6	0.090	0.002	74.7
7	0.096	0.012	73
8	0.110	0.008	69.1
Control	0.356	0.080	

Table (4) This report aimed to research the cytotoxic action against apoptosis behavior of Ethanolic Result against L20B cell line (*Anethum graveolensis* ethanolic Ex.)

Leaf extract isolated from a local plant of *Anethum graveolensis* ethanolic Ex. affected living Hep G2 cell line. The research used an in vitro assessment of the cytotoxic activity of the Ethanolic Result against L20B cell line

Leaf extract at various concentrations (1000, 500, 250, 125, 62.5 µg / ml) in serial dilutions on the HepG2 cell line and exposure period (28 h) to accomplish this objective. 62.5, 125 and 250 µg / ml is the most important drop in viable counting cells. Strong cell, tested but not impaired by all Ethanolic Result against L20B cell line extract concentrations (no important significance P<0.001).

Table(4): Result against L20B cell line (*Anethum graveolensis* ethanolic Ex.)

Extract Conce.	Mean	S.D.	GI%
1(50)	0.218	0.009	38.6
2(25)	0.097	0.0007	72.6
3(12.5)	0.085	0.012	75.9
4(6.25)	0.081	0.011	77.2
5	0.078	0.018	78
6	0.099	0.011	72.1
7	0.085	0.009	76
8	0.160	0.000	55
Control	0.356	0.080	

The intrinsic pathway and the exogenous pathway associated with death receptors such as Fas (CD 95) leading to activation of caspase-8 are two main pathways leading to apoptosis[23,24,25]. The activation of these proteins contributes to permeability of the mitochondrial membrane , resulting in the reapplication of cytochrome C. The oleuropein isolated from Ethanolic Effects of some fungal secondary metabolite against some cancer cell line

Leaf was to investigate the toxicity of tamoxifen and the role of oleuropein in protecting against TAM-induced oxidative liver damage in Balb / c mice, and the ability to directly scavenge free radicals is at least partially linked to preventive action. Ethanolic Effects of some fungal secondary metabolite against some cancer cell line

Leaf has been shown to cause large levels of apoptosis in different cancer cells and to decrease cell viability, suppress cell proliferation , and apoptosis in MCF-7 human breast cancer cells. Ethanolic Effects of some fungal secondary metabolite against some cancer cell line against L20B cell line (*Anethum graveolensis* ethanolic Ex.)

In human breast cancer (MCF-7), human urinary bladder cancer (T24), and bovine brain capillary (BBCE), leaf rudimentary extracts have been shown to inhibit cell proliferation[28,29]. The effect of the Ethanolic Effects of some fungal secondary metabolite against some cancer cell line

Leaf extract and oleuropein on chronic UV-induced skin injury, carcinogenicity, and tumor growth was seen in several studies by inhibiting the expression of VEGF, MMP-2, MMP-9, and MMP-13 by a two-level COX reduction . Anethum graveolens seeds (AGS) have a pungent, spicy aroma and a bitter flavor. This plant is used to treat stomachaches, indigestion, dyspepsia, and other gastrointestinal conditions. Anti-inflammatory and gastroprotective properties are also present.

Conclusion:

When compared to the other substances in the investigation, gliotoxin demonstrated the most cytotoxic effect. These findings suggest that gliotoxin could be regarded a putative virulence component of Aspergillus fumigatus during infection. Ex. Anti Cancer Anethum graveolensis ethanolic , the deadly disease that travels across the body , taking its cells closer to death than life expectancy, will defeat or at least limit the magnitude of its spread by natural plants

throughout the body. Many researchers and physicians understand the method of treating several plants, like Rheum ribes, cancer studies have demonstrated that the use of some medicinal herbs helps to reduce the seriousness of this deadly disease, and plant care costs are very cheap.With all the findings of this research , it is possible to recognize Effects of some fungal secondary metabolite against some cancer cell line as a food antioxidant and anticancer agent. Our research through the findings obtained does not lead, through our use of this approach, to its use as a full cancer treatment.

References:

1. 1.Tran, P.N.; Yen, M.-R.; Chiang, C.-Y.; Lin, H.-C.; Chen, P.-Y. Detecting and prioritizing biosynthetic gene clusters for bioactive compounds in bacteria and fungi. Appl. Microbiol. Biotechnol. 2019, 103, 3277–3287. [CrossRef] [PubMed]
2. Deshmukh, S.K.; Prakash, V.; Ranjan, N. Marine Fungi: A Source of Potential Anticancer Compounds. Front. Microbiol. 2018, 8, 2536. [CrossRef] [PubMed]
3. Kumar, A.; Sørensen, J.L.; Hansen, F.T.; Arvas, M.; Syed, M.F.; Hassan, L.; Benz, J.P.; Record, E.; Henrissat, B.; Pöggeler, S.; et al. Genome Sequencing and analyses of Two Marine Fungi from the North Sea Unraveled a Plethora of Novel Biosynthetic Gene Clusters. Sci. Rep. 2018, 8, 10187. [CrossRef] [PubMed]
4. Vala, A.K.; Sachaniya, B.; Dudhagara, D.; Panseriya, H.Z.; Gosai, H.; Rawal, R.; Dave, B.P. Characterization of L-asparaginase from marine-derived Aspergillus niger AKV-MKBU, its antiproliferative activity and bench scale production using industrial waste. Int. J. Biol. Macromol. 2018, 108, 41–46. [CrossRef] [PubMed]
5. Doriya, K.; Kumar, D.S. Solid state fermentation of mixed substrate for l-asparaginase production using tray and in-house designed rotary bioreactor. Biochem. Eng. J. 2018, 138, 188–196. [CrossRef]
6. Paul, V.; Tiwary, B.N. An investigation on the acrylamide mitigation potential of l-asparaginase from Aspergillus terreus BV-C strain. Biocatal. Agric. Biotechnol. 2020, 27, 101677. [CrossRef]
7. El-Gendy, M.M.A.A.; Awad, M.F.; El-Shenawy, F.S.; El-Bondkly, A.M.A. Production, purification, characterization, antioxidant and antiproliferative activities of extracellular L-asparaginase produced by Fusarium equiseti AHMF4. Saudi J. Biol. Sci. 2021, 28, 2540–2548. [CrossRef]
8. Krishnapura, P.R.; Belur, P.D. Partial purification and characterization of L-asparaginase from an endophytic Talaromyces pinophilus isolated from the rhizomes of Curcuma amada. J. Mol. Catal. B Enzym. 2016, 124, 83–91. [CrossRef]
9. Baskar, G.; Sree, N.S. Synthesis, characterization and anticancer activity of β-cyclodextrin-Asparaginase nanobi-ocomposite on prostate and lymphoma cancer cells. J. Drug Deliv. Sci. Technol. 2020, 55, 101417. [CrossRef]
10. Jenila, A.V.; Gnanadoss, J.J. Formulation of A Suitable Medium and its Optimization for Maximizing L-Asparaginase Production from Endophytic Fungi Fusarium sp. LCJ273. Biosci. Biotechnol. Res. Asia 2018, 15, 887–898. [CrossRef]
11. Fan, B.; Dewapriya, P.; Li, F.; Grauso, L.; Blümel, M.; Mangoni, A.; Tasdemir, D. Pyrenosetin D, a New Pentacyclic Decalinoyltetramic Acid Derivative from the Algicolous Fungus Pyrenochaetopsis sp. FVE-087. Mar. Drugs 2020, 18, 281. [CrossRef]
12. Ashok, A.; Doriya, K.; Rao, J.V.; Qureshi, A.; Tiwari, A.K.; Kumar, D.S. Microbes Producing L-Asparaginase free of Glutaminase and Urease isolated from Extreme Locations of Antarctic Soil and Moss. Sci. Rep. 2019, 9, 1423.
13. 13.Elshafei, A.M.; El-Ghonemy, D.H. Screening and media optimization for enhancing L-asparaginase production, an anticancer agent, from different filamentous fungi in solid state fermentation. Biotechnol. J. Int. 2015, 1–15. [CrossRef]
14. Golbabaie, A.; Nouri, H.; Moghimi, H.; Khaleghian, A. L-asparaginase production and enhancement by Sarocladium strictum: In vitro evaluation of anti-cancerous properties. J. Appl. Microbiol. 2020, 129, 356–366. [CrossRef]
15. Nguyen, V.-T.; Lee, J.S.; Qian, Z.-J.; Li, Y.-X.; Kim, K.-N.; Heo, S.-J.; Jeon, Y.-J.; Park, W.S.; Choi, I.-W.; Je, J.-Y.; et al. Gliotoxin Isolated from Marine Fungus Aspergillus Sp. Induces Apoptosis of Human Cervical Cancer and Chondrosarcoma Cells. Mar. Drugs 2013, 12, 69–87. [CrossRef] [PubMed]
16. Qi, C.; Gao, W.; Guan, D.; Wang, J.; Liu, M.; Chen, C.; Zhu, H.; Zhou, Y.; Lai, Y.; Hu, Z.; et al. Butenolides from a marine-derived fungus Aspergillus terreus with antitumor activities against pancreatic ductal adenocarcinoma cells. Bioorganic Med. Chem. 2018, 26, 5903–5910. [CrossRef]
17. Malhão, F.; Ramos, A.A.; Buttachon, S.; Dethoup, T.; Kijjoa, A.; Rocha, E. Cytotoxic and Antiproliferative Effects of Preussin, a Hydroxypyrrolidine Derivative from the Marine Sponge-Associated Fungus Aspergillus candidus KUFA 0062, in a Panel of Breast Cancer Cell Lines and Using 2D and 3D Cultures. Mar. Drugs 2019, 17, 448. [CrossRef]
18. Chen, J.-J.; Wang, S.-W.; Chiang, Y.-R.; Pang, K.-L.; Kuo, Y.-H.; Shih, T.-Y.; Lee, T.-H. Highly Oxygenated Constituents from a Marine Alga-Derived Fungus Aspergillus giganteus NTU967. Mar. Drugs 2020, 18, 303. [CrossRef] [PubMed]
19. El-Hady, F.K.A.; Shaker, K.H.; Souleman, A.M.A.; Fayad, W.; Abdel-Aziz, M.S.; Hamed, A.A.; Iodice, C.; Tommonaro, G. Comparative Correlation Between Chemical Composition and Cytotoxic Potential of the Coral-Associated Fungus Aspergillus sp. 2C1-EGY Against Human Colon Cancer Cells. Curr. Microbiol. 2017, 74, 1294–1300. [CrossRef]
20. Wijesekara, I.; Zhang, C.; Van Ta, Q.; Vo, T.-S.; Li, Y.-X.; Kim, S.-K. Physcion from marine-derived fungus Microsporum sp. induces apoptosis in human cervical carcinoma HeLa cells. Microbiol. Res. 2014, 169, 255–261. [CrossRef] [PubMed]
21. Chih, P.L.,Wei, J.T., Yuang, L.L., & Yuh, C.K.: The extracts from Nelumbonucifera suppress cell cycle progression, cytokine genes expression, and cell proliferation in human peripheral blood mononuclear cells. Life Sci. 75, 699-716 (2004)
22. Freshney, R.I.: Culture of Animal Cell. 6th Edition.Wily-Liss, New York. (2012)