ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

Cytotoxicity of Fumaria Officinalis on Leishmania Tropica, L20B Cell Lines and Bacteria *In Vitro*

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Abstract

Fumaria officinal is (Shahatraja in Arabic), one of the popular herbal medicines used in Iraq. There are more than 60 species of Fumaria around the world, and many useful properties recognized for this herb. The aim of this study is to investigate the effects of the extracted alkaloids, and crude alcoholic extract on Leishmania tropica (L tropica), and L20B cancer cell lines, in additional to study the toxic effect of these two extracts on many types of bacteria and comparing the results in both cases. Viability rate of treating L tropica cells by the alkaloids and crude extracts were increased significantly in all concentrations. All concentrations of alkaloids extract showed toxic effect on L20B cell lines, except (0.015 mg/ml). While for crude extracts, there was no effect on Viability rates of L20B cell lines at all concentration, except (0.125 mg/ml), shown some decrease in Viability rate. The antibacterial activities of the alkaloids increased comparing with crude extracts in four strains of bacteria, *Staphylococcus aureus, Bacillus spp.*, *Klebsiella spp., and Proteus spp*. While for *Salmonella spp.,* the inhibition zone was increased in crude extracts more than alkaloids. The results for three remaining bacteria (*Streptococcus pyogenes, E. coli, and Enterobacter spp.*) were equal in their inhibition activity. From results of present study, the alkaloids that extract from Fumaria officinalis, made the best effect as anticancer and antibacterial agent.

Keywords: *Cytotoxicity, Anticancer activity, alkaloids, Fumaria officinalis, L20B cell line, antibacterial agent.*

Introduction

The genus Fumaria (Fumariaceae) comprise of 60 species spread around the world and especially in Mediterranean region and growing in the winter season [1, 3]. Fumaria officinalis (Shahatraja in Arabic), is one of the medicinal plant usually used in many traditional medical systems around the world [4], for treatment of several diseases [5]. Herb has many useful properties that recognized [6].

The plant is known for its activity as Antioxidant, well diuretic, expectorant, depurative, purgative, diaphoretic, anti-inflammatory, anti-arrhythmic; urinary, cardiac, digestive disorders, aphrodisiac, rheumatism, stomach ache, abdominal cramps, fever, diarrhea, syphilis, leprosy [7-10], treatment of some skin diseases, strengthens the teeth and lungs, gives luster to the eyes, stops vomiting, and in diseases of the spleen [11, 12].

The Fumariaceae family is highly rich in isoquinoline alkaloids, like aporphine, protoberberine, benzophenanthridine, and protopine types [13].

From Previous phytochemical analysis studies, Fumariae herb contains isoquinoline alkaloids expressed in protopine [14], polyphenols and Fumaric acid esters [15, 16]. Leishmaniasis, a health problem in more than 90 countries around the world, including Iraq, there are more than 350 million people at risk of infection around the world [17, 18]. Leishmania tropica and (L tropica) L20B, cell lines, were used at present study.

Materials and Methods

Plant Material and Preparation of Extracts

The Fumariae officinal is herbal were

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collected from local market. The leaves and stems of the plant were Dried, then grinded using an electric blender and placed in sealed bags until the work of extracting them. For extraction, 20 g of the grinded herb was placed in conical flask and treated with methanol, homogenized by vortex mixer for 3 min. The conical flask placed in an ultrasonic water bath (at 25 °C) and sonicated for two periods, 15 and 15 (min) continuously. Then, the suspension was filtered of using filter paper to remove stiff wreckage [19]. The extract was obtained divided into two parts; the first is dried using rotary evaporator under low pressure.

The second part is used to extract alkaloids by adding sulfuric acid 0.1 N, the solution was filtered, and similar volume of chloroform was added to the filtrate, then aspirate the solution and separate the layers with a separating seal and discard the chloroform layer Add sodium hydroxide to make the pH equal and then add a similar amount of chloroform and solution the solution in the separation funnel then separate the solution and neglect the water layer Add a similar amount of distilled water, release the solution with separation, then discard the water layer and evaporate chloroform to obtain alkaloids

Detection of Alkaloids

To detect the alkaloids, Dragendroff reagent was used, the stock solution was prepared by dissolving Bismuth carbonate (2.6 g) and sodium iodide (2 g) in 25 ml glacial acetic acid and boiled for a few min., after 12 hrs, the precipitated sodium acetate crystals were filtered off, ethyl acetate (80 ml) and distilled water (0.5 ml) was added to the clear red-brown filtrate (20 ml), and stored in colored bottle. The working solution prepared by adding 10 ml of acetic acid to the 4.5 ml of stock solution, and made up to 50 ml with distilled water. To about 3 ml of extract, a few drops of Dragendorff's reagent were added; reddish-brown precipitate was formed.

Cytotoxicity Assay

At present study, two types of cell lines; Leishmania tropica (L tropica) and murine L20B cells, were used. Cell lines were grown in RPMI-1640 medium with 10% inactivated fetal calf serum (FCS), streptomycin (100 mg/ml), and penicillin (100 U/ml) antibiotic. The cytotoxicity of alkaloids and crude extracts were tested by method of (20).

The alkaloids and crude extracts were dissolved in methanol (5 %) (The concentration of Stock solution was 0.5 mg/ml) and diluted with RPMI-1640 medium to give concentrations ranging from 0.25 – 0.007 mg/ml. The cells were grown in tissue culture flasks, containing growth medium, at an atmosphere of 5% CO_2 and 95% relative humidity in a CO_2 incubator, and temperature 37 ºC. The cells were gathered from the flasks at sub confluent stage, by handling with trypsin-versine solution (20 ml trypsin in 370 ml PBS containing 10 ml versine) and suspended in the medium. Cells with more than 97% viability (trypan blue exclusion) were used for determination of cytotoxicity. Cells were coated in multi well plate for 24 hrs in a CO_2 incubator at 37ºC.

Different concentration of the alkaloids and crude extracts (0.25, 0.125, 0.062, 0.031, 0.015, and 0.007 mg/ml) were added to the cells (three replicate wells were prepared for each individual concentration) and incubated again for 48 hours. Control cultures containing RPMI1640 alone was tested for back ground cytotoxicity. After that, 50 µl of crystal violate stain were added to the wells, and the plates were incubated in a CO_2 incubator for 30 minutes at 37 ºC. After washing spots with tap water (three times), it has been read at 492 nm. The inhibitory rate of cell growth was calculated as following formula [21]:

$$\textbf{Inhibition (\%)} = \frac{\textbf{Optical density of control wells} - \textbf{Optical density of test wells}}{\textbf{Optical density of control wells}} \times 100$$

Death cell rate (%) = 100 - Inhibition (%)

Antibacterial Activity

Antibacterial activity was examined for alkaloids and crude extracts *in vitro*, against eight pathogenic bacteria, (*Enterobacter spp., Streptococcus pyogenes, E. coli, Salmonella spp., Klebsiella spp., Proteus spp., Bacillus spp*, and *Staphylococcus aureus*), using the agar disk diffusion method (22-24). In

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dimethyl sulfoxide, the alkaloids and crude extracts were dissolved, filtered by using sintered glass filter, and then stored at 4 °C. For determination of inhibition zone, controls of Gram-positive and Gram-negative were taken as a standard for comparison of the results.

Alkaloids and crude extracts were prepared (250 µg/ml) using nutrient agar tubes. Sterilized Mueller-Hinton agar plates were cultivate with indicator bacterial strains (108 cfu) and incubated at 37 °C for 3 hrs. The growth inhibition zones around the disks were measured after 24 hrs of incubation at 37 °C. The sensitivities of the bacteria species to the alkaloids and crude extracts were determined by measuring the sizes of growth inhibition zones on the agar surface around the disks.

Results

Cytotoxicity of Extracts

The effect of treating L tropica cells by the alkaloids and crude extracts were shown in table 1. Viability rate in all concentrations (0.25, 0.125, 0.062, 0.031, 0.015, and 0.007 mg/ml) were increased significantly in L tropica cell line for alkaloids and crude extracts. All concentrations of alkaloids extract were affected on L20B cell lines Viability rates, except (0.015 mg/ml). While for crude extracts, there was no effect on Viability rates of L20B cell lines at all concentration, except (0.125 mg/ml), shown some decrease in Viability rate. The death cell rates effect were calculated according to the results of optical density.

For alkaloids extract, the best death cell rate in L20B cell lines was for concentration 0.062 and 0.031 mg/ml. in compares between effect of alkaloids and crude extracts, the alkaloids were more effect on L20B cell lines.

Antibacterial Activity

The Antibacterial activity of the alkaloids and crude extracts were studied in one concentrations (250 µg/ml) against eight pathogenic bacterial strains, three Gram-positive (*Staphylococcus aureus, Streptococcus pyogenes, and Bacillus spp.*), and five Gram-negative (*Salmonella spp., Klebsiella spp., Proteus spp., E. coli, and Enterobacter spp.*). Antibacterial of alkaloids and crude extracts were valued in terms of inhibition zones of bacterial growth.

The results of the antibacterial activities were shown in Table 2. The antibacterial activities of the alkaloids increased comparing with crude extracts in four strain of bacteria, tow Gram-positive (*Staphylococcus aureus, and Bacillus spp.*), and tow Gram-negative (*Klebsiella spp., and Proteus spp.*).

While for Gram-negative bacteria Salmonella spp., the inhibition zone was increased in crude extracts more than alkaloids. The other results for three remaining bacteria (*Streptococcus pyogenes, E. coli, and Enterobacter spp.*) were equal in their inhibition activity. The range of growth inhibition zones measured was from 2 to 17 mm for all the strains of bacteria as shown in Fig. 1.

Table 1: The Cytotoxicity effect of alkaloids and crude extracts on L tropica and L20B cell lines

Type of cell line	Concentrations (mg/ml)	Control (viability)	Alkaloids (viability)	ID	Crude extracts (viability)	ID
L tropica	0.25	0.113	0.310	0.0	0.559	0.0
	0.125		0.33	0.0	0.425	0.0
	0.062		0.298	0.0	0.210	0.0
	0.031		0.28	0.0	0.221	0.0
	0.015		0.29	0.0	0.275	0.0
	0.007		0.318	0.0	0.275	0.0
L20B	0.25	0,21	0.175	16.7	0.209	0.5
	0.125		0.162	22.86	0.199	5.3
	0.062		0.190	89.6	0.217	0.0
	0.031		0.190	89.6	0.209	0.5
	0.015		0.210	0.0	0.254	0.0
	0.007		0.200	4.8	0.223	0.0

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Table 2: inhibition zone (mm) for all the strains of bacteria

Bacteria	inhibition zones (mm)	
	alkaloids	Methanol (Crude extracts)
Staphylococcus aureus	5	3
Streptococcus pyogenes	2	2
Bacillus spp	17	10
Salmonella spp.	2	17
Klebsiella spp.	10	7
Proteus spp.	11	5
E. coli	2	2
Enterobacter spp.	2	2

A B C

Fig. 1: inhibition zone by alkaloids (1) and crude extracts (2) of (a) *Bacillus spp*, (b) *Klebsiella spp.*, and (c) *Proteus spp*.

Discussion

In this study, alkaloids and methanol crude extract, demonstrated different cytotocicity in vitro toward L tropica and L20B cell lines. Table 1 shows effects of concentration on death cell rates of L tropica and L20B cell lines activity. L tropica showed highly resistance to toxic effect of alkaloids extract and methanol crude extract. Comparison of alkaloids extract with methanol crude extract, allows the conclusion that the alkaloids was more active than crude extract in L20B cell lines studied, and that may refer to the reason of biological effect of Fumaria officinalis against L20B cell line was due to alkaloids in the herb. The results from present study, alkaloids made the best effect as antibacterial agent, and this result agree with many previous studies [25, 26].

Acknowledgments

The author wish to thank department of chemistry, department of biology - college of science for women, university of Baghdad and the Biotechnology Research Center, Al-Nahrin University for their support to finish this work.

References

1. Suau R, Cabezudo B, Rico R, Najera F, Lopez-Romero JM (2002) Direct determination of alkaloid contents in Fumaria species by GC-MS. Phytochem Anal., 13 (6): 363-367.
2. Jaberian H, Piri K, Nazari J (2013) Phytochemical composition and in vitro antimicrobial and antioxidant activities of some medicinal plants. Food Chem., 136 (1): 237-244.
3. Suresh K, Anil K, Anjoo K (2017) Fumaria parviflora Lam. (Fumitory): A traditional herbal medicine with modern evidence. Asian J. Pharm. Pharmacol., 3(6): 200-207
4. Anshul Sh, Shyam SCh, Vikas K (2012) Holistic Psychopharmacology of Fumaria indica (Fumitory). Chinese Medicine, 3: 182-199
5. Amin GH (1991) Popular medical plants of Iran. Tehran: Farhang Press, 51-52.
6. Khoshvaghti A (2013) Effect of fumaria officinalis hydroalcoholic extract on blood urea nitrogen and creatinine in New Zealand rabbits. Acad. J. Med. Plants 2013; 1(8): 137-140.

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7. Ahmad SS (2007) Medicinal wild plants from Lahore-Islambad motorway (M-2). Pak. J. Bot. 39(2): 355-375.
8. Sener B (2002) Molecular diversity in the alkaloids of Turkish Fumaria L species. Acta Pharm. Turc., 44: 205-212.
9. Maiza F, Khentache S, Bougoffa K, Chibane M, Adach S, Chapeleur Y, et al (2007) Antioxidant activities of alkaloid extracts of two Algerian species of Fumaria: Fumaria capreolata and Fumaria bastardi. Rec. Nat. Prod., 1(2-3): 28-35.
10. Ivan GI, Radka ZV, Andrey S, Nadezhda T P, Ina YA, Panteley PD, et al (2014) Antioxidant activities and phenolic compounds in Bulgarian Fumaria species. Int. J. Curr. Microbiol. App Sci., 3(2): 296-306.
11. Kirtikar KR, Basu BD (2005) Indian Medicinal Plants. Dehradun (India), International Book Distributors text I: 138-140
12. Jameel M, Islamuddin M, Ali A, Afrin F, Ali M (2014) Isolation, characterization and antimicrobial evaluation of a novel compound N-octacosan 7ß ol, from Fumaria parviflora Lam. BMC Complementary Altern. Med., 14(98): 1-9.
13. Ramona P, Anca T, Jean NW, Michel F, Monique T, Luc A, et al (2016) Phytochemical analysis of Fumaria officnalis L (Fumariaceae). Farmacia, 64: 3
14. European Pharmacopoeia, 8th ed. Council of Europe, Strasbourg; 2014.
15. Ravirajsinh NJ, Folami LP, Pamela MM (2016) Fumaric Acid Esters as Potential Therapies for Treating Degenerative Retinal Disease. J. Biomol. Res Ther., 5(4): 2167-7956
16. Patel A, Shenouda N, Tallis G (2007) PDR for Herbal Medicines. 4th ed. Thomson Healthcare, Montvale.
17. WHO (2010) Control of the leishmaniases. Report of a WHO Expert Committee. Technical Report Series, (949) 1-158.
18. Desjeux P (2004) Leishmaniasis: current situation and new perspectives. Comp. Immunol. Microbiol. Infect Dis., 27: 305-318.
19. Hanen F, Riadh K, Marrie-Elizabeth L, Chedly A, Christian M (2012) Ultrasound-Assisted Extraction: Effect of Extraction Time and Solvent Power on the Levels of Polyphenols and Antioxidant Activity of Mesembryanthemum edule L. Aizoaceae Shoots. Trop. J. Pharm. Res, 11(2): 243-249
20. Freshney RI (2005) Culture of animal cells: A manual for basic technique, 5th ed. Willey-Liss Inc, New Jersey. USA.
21. Gao S, Yu B, Li Y, Dong W, Luo H (2003) Antiproliferative effect of octreotide on gastric cancer cells mediated by inhibition of Akt/PKB and telomerase. World J. Gastroenterol., 9(10):2362-2365
22. Alzoreky NS, Nakahara K (2003) Antibacterial activity of extracts from some edible plants commonly consumed in Asia. Int. J. Food Microbiol., 80:223-230.
23. Bauer AW, Kirby WM, Sherris JC, Turck M (1966) Antibiotic susceptibility testing by standardized single disc method. Am J. Clin Pathol., 36:493-506.
24. Rios JL, Recio MC, Villar A (1988) Screening methods for natural products with antimicrobial activity: A review of the literature. J. Ethnopharmacol., 23:127-149
25. Tim Cushnie TP, Benjamart C, Andrew L (2014) Alkaloids: An overview of their antibacterial, antibiotic-enhancing and anti virulence activities. Int. J. Antimicrob. Agents 44(5): 377-386
26. Geoffrey AC (2013) Fifty years of alkaloid biosynthesis in Phytochemistry, 91: 29-51.

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