International Journal of Molecular and Clinical Microbiology 11(2) (2021) 1530-1539

International Journal of
Molecular and Clinical Microbiology

In vitro anticancer and antibacterial activities of *Avena ludoviciana* L.

Monavar-ul-Sadat Mousavizadeh[1], Hossein Aghajani[1], Mana Kheyri[1], Fatereh Rezaei [1*]

1. Department of Biology, Babol Branch, Islamic Azad University, Babol, Iran

ARTICLE INFO

Article history:
Received 04 September 2021
Accepted 30 October 2021
Available online 1 December 2021

keywords:
antibacterial,
anticancer,
skov3,
Avena ludoviciana L.

ABSTRACT

Cancer is one of the leading causes of death worldwide, with approximately 10 million people dying by 2020. Therapeutic advances in the spectrum of cancers continue at a rapid pace. Antibiotics are becoming increasingly ineffective as drug resistance spreads around the world, making infections and death more difficult to treat. Adverse drug reactions, as well as drug resistance, remain key challenges to treatment success. Natural resources play an important role in the development of anticancer and antimicrobial agents. The aim of this study was to evaluate the anticancer and antibacterial effect of *Avena ludoviciana* L. leaves. Chemical compounds were screened and identified using GC mass spectrometry. The anti-cancer effect of hydroalcoholic extract and fractions (hexane, chloroform, and ethyl acetate) of *Avena ludoviciana* L. leaves were evaluated by the MTT method on Skov3 and MRC5 cell lines. Antibacterial activity of hydroalcoholic extract of *Avena ludoviciana* L. Leaves on four bacterial strains was investigated by agar well diffusion method and the minimum inhibitory concentration was determined by dilution methods. The results showed that different concentrations of hydroalcoholic extracts and fractions significantly reduced the growth of the Skov3 cell line compared to the control group after 48 hours, dose-dependently ($P < 0.05$). Hydroalcoholic extracts except E. coli were tested on all gram-positive and gram-negative bacteria ($P < 0.05$). The largest growth-inhibitory diameter was observed in S. aureus. that was the most sensitive bacteria (lowest MIC) and B. cereus was the most resistant bacteria (lowest MIC) to the extract. Our results show that medicinal plants can be promising sources of natural products with potential anticancer and antimicrobial activity. Further research is suggested for clinical trials, identification, and extraction of effective compounds.

1. Introduction

The American Cancer Society annually estimates the number of new cancer cases and mortalities in the US and collects the latest data on population-based cancers. In 2020, the number of new cancer cases and mortalities in the US was estimated to be 1,806,590 and 606,520, respectively (Siegel et al., 2020). Hormone therapy, chemotherapy, radiotherapy, and surgery are typical ways of treatments, which are cytotoxic for most of the cells, i.e. besides damaging and killing cancer cells, they may also damage healthy and normal cells (Arora et al., 2021). In addition, resistance to antibiotics has globally been a major public health concern. in developing countries, untreatable infections *the second in* the *ranking* among the deadly diseases (Giacomini et al., 2021). If successful efforts are not made to find new drugs, these diseases will globally cost $ 100 trillion and the number of deaths will reach

*Corresponding authors: Fatereh Rezaei
Email address: faterehrezaei62@gmail.com

10 million by 2050. Therefore, the search for novel antibiotics of natural origin is a vital part of modern medicine to overcome the health and socioeconomic impacts of multidrug-resistant bacteria (Gomes et al., 2021). Natural remedies have long been utilized to improve health, and the success of modern medical science mostly depends on medicines derived from natural sources (Anand et al., 2019). Because >80% of the people worldwide rely on traditional medicines for their basic healthcare needs. The WHO encourages countries to develop strategic plans and evidence-based policies for herbal use (WHO, 2019). Various investigations have proven that herbal medicines contain secondary metabolites e.g. polyacetylenes, polypeptides, lectin, essential oils, tannins, terpenoids, alkaloids, phenolics, flavonoids, and coumarins (Upadhyay & Dixit, 2015). Various secondary metabolites, extracts, and essential oils of plants have antioxidant and antimicrobial effects with little or no toxicity. So, they are useful in the treatment of different diseases (Cavazos et al., 2021). In fact, phytochemicals, i.e. secondary metabolites with remarkable biological potential, ubiquitously exist in plants and are now utilized as the primary basis for the development of drugs (Ahad et al., 2021). Numerous studies have shown different pharmacological uses of plant extracts and compounds isolated from them. Utilizing the biological potential of medicinal plants gives an excellent opportunity to develop novel therapeutics. The bioactive plant extracts are considered a promising source of various medicines (Anand et al., 2019). *Avena ludoviciana* L. genus (Family Poaceae) is an annual tufted plant (Britannica, 2021). The possible antimicrobial and anticancer effects of the hydroethanolic extract and fractions of this plant have not been assessed yet. However, scientific evidence shows that it also contains other essential bioactive compounds, e.g. polyphenols that have a protective effect against degenerative and chronic diseases like cancer (Turrini et al., 2019). In addition to flavonoids and phenolic acids, *A. ludoviciana* L. is an excellent source of avenanthramides (AVAs), which are a group of phenolic alkaloids. Research indicates that AVAs also have antiproliferative and antioxidant activities that can prevent or treat cancer. AVAs can modulate various events, including metastasis, cell proliferation, and apoptosis, and may specifically be involved in all stages of cancer (Turrini et al., 2019).

However, there are limited data on the phytochemicals, as well as antibacterial and anticancer activities of *A. ludoviciana* L. Hence, this study aimed to analyze phytochemicals, as well as to evaluate the antibacterial (*Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Pseudomonas aeruginosa*) and anticancer (SKOV3) effects of the hydroalcoholic extract and fractions of *A. ludoviciana* L. leaf.

2. Materials and Methods

2.1 Plant materials

A.ludoviciana L. plant were collected from Marzoon-abad, Babol, Mazandaran, Iran in September. The plant was identified by a botanist in the Biology Department, College of Science, Mazandaran University, and prepared for extraction.

The fresh leaf were dried at room temperature under shade for 10 days which helps to prevent the loss of medicinal compounds and mixer dried leaf to powder for complete extraction of active compounds from the plant.

2.2 Preparation of extract

The powder (40g) extracted using soxhlet extraction with hydroethanolic solution in the ratio of 20:80 for 24hrs and the extract was then concentrated under reduced pressure at 40^{o}C using vacuum rotary evaporator. The yield of hydroalcoholic extract was 12.48g. Three solvent fractions (Hexane, Chloroform and Ethylacetate) were collected and concentrated with vacuum rotary evaporator. The yields of these fractions constituted 23.1%, 21% and 11.2% of the hydroethanolic extract respectively (Alfaifi et al., 2020).

2.3. GC-MS (Gas Chromatography-Mass Spectrometry) analysis

The phytochemical investigation of hydroethanolic extract was performed on a GC-MS equipment Agilent Technologies model 5975C- USA. Experimental conditions of GC-MS system were as follows: HP 5-MS capillary standard non-polar column, Film thickness: 0.25μm, ID: 0.25 mm, dimension: 30Mts. Flow rate of mobile phase (carrier gas: He) was set at

1.0 ml/min. In the gas chromatography part, temperature programme (oven temperature) was 30°C raised to 280°C at 5°C/min and injection volume was 1 µl.

2.4. In vitro anticancer activity evaluation by MTT assay

The cell line Skov3 (ovarian cancer cell line) and MRC5(fibroblast normal cell line) were acquired from Pasture Institute, Tehran, Iran. Cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM) complemented with 10% heat-inactivated Fetal Bovine Serum (FBS), 5 mM L^- Glutamine, 100 µg ml^- streptomycin and 100 U/mL penicillin. The cell lines were grown at 37°C in a humidified atmosphere containing 5% CO_2. The cytotoxic activity were measured using MTT assay(Malhao et al., 2021). The cells were grown in 96-well plates at a density of 1×10^5 cells per well. After incubation for 24 h, the cells were treated with different concentrations of hydroalcoholic extract and fractions incubated for 48 h. Later, the MTT solution (20 µl of 5 mg/ml, Roche) was added to each well, and the plate was re-incubated for an additional 4 h. Finally, the medium was removed and 180 µl of DMSO was added to solubilize the formed formazan crystals. The amount of formazan crystal was determined by measuring the absorbance at 490 nm using a microplate spectrophotometer (stat fax 2100)(Malhao et al., 2021; Nasiri et al., 2019). All assays were done in triplicate.

2.5 Antibacterial activity of the hidroalcoholic extract of A. ludoviciana L.

2.5.1. Disk Diffusion Test

Escherichia coli PTCC 1399, *Staphylococcus aureus* PTCC 1764, *Bacillus cereus* PTCC 1247 and *Pseudomonas aeruginosa* PTCC 1310, were purchased from the Persian Type Culture Collection (PTCC), IROST, Iran. Antibacterial activity of hydroalcoholic extract was assessed against bacterial strains by agar disk diffusion assay as previously described (Eloff, 1998). Initially, the required nutrient media for all bacterial strains were prepared from Mueller Hinton agar, and 100 μl inoculums of each tested bacterial culture were swabbed onto agar plates and incubated at 37°C for 30 minutes. Thereafter, different concentrations of hydroalcoholic extract (20, 40, 60, 80 and 100 µg/ml) were pipetted onto 5.5 mm sterile Whatman No. 1 filter paper disks and then placed on the surface of the bacteria-treated plates. After a 24 hours' incubation period at 37°C, the diameter of the zone of inhibition was measured (Ramdath et al., 2021).

2.5.2. Minimum Inhibitory Concentration (MIC)

Lowest dose of an antibiotic that dose the inhibition of the growth of a particular no (s) organism (s) upon which it acts is called the 'Minimal Inhibitory Concentration' (MIC). For MIC test the 'Serial tube dilution technique (Andrews, 2001) is used.

2.6 Statistical Data Analysis

The obtained data were analyzed by one-way analysis of variance statistical tests followed by Tukey posthoc method and EXCEL software was used. In all cases, $p < 0.05$ was considered as a significant level. The experiments were repeated three times and the results were recorded as Mean ±SEM.

3. Results

3.1 *GC-MS (Gas Chromatography-Mass Spectrometry) analysis*

The results of hydroalcoholic extract characterization by GC-MS are presented in Table 1. The results shows that highest combination with Peak area 44.64% belongs to 9,12-Octadecadienoic acid and also the lowest Peak area is Phytol with 0.63%.

3.2 Cytotoxic activity

3.2.1. Cytotoxic effect of A. ludoviciana L. on skov3

Cytotoxic activity of *A. ludoviciana* L. leaf on skov3 in different concentrations (0, 10, 50, 100, 200 µg/ml) of hydroalcoholic extract and fractions are illustrated in figures 1. *A. ludoviciana* L. significantly decreased cell viability of skov3 after 48 hours incubation intervals dose-dependently in hydroalcoholic extract and fractions compared with untreated control cells ($P<0.05$, figures 1). As shown, cisplatin significantly diminished the cell

viability of skov3 cells after 48 hours incubation compared with untreated control cells (P<0.05, figures 2). Extract and fractions cytotoxicity at all concentrations significantly increased in this order: chloroform fraction > ethylacetate fraction> total extract> hexan fraction in skov3 cells (P<0.05, figure 1).

3.2.2. Cytotoxic effect of A. ludoviciana L. on MRC5

Cell viability assessed in the MTT assay after, *A. ludoviciana* L. hydroalcoholic extract and fractions treatment on MRC5 is shown in Figure 3. *A. ludoviciana* L. reduced cell viability of MRC5 in hydroalcoholic extract and fractions in a concentration-dependent manner after 48 hours' incubation in comparison with untreated control cells (P<0.05). As shown, cisplatin diminished the cell viability of MRC5 cells after 48 hours incubation compared with untreated control cells (P<0.05, figures 2).

3.3 Assessment of antimicrobial effects of A. ludoviciana L.

The hydroalcoholic extract of *A. ludoviciana* L. showed inhibitory effects on the growth and proliferation of all four bacteria. The largest and the smallest diameter of growth inhibition zone belonged to S. *aureus* and *E. coli*, respectively. The mean (and SD) diameter of the growth inhibition zone due to the effect of hydroalcoholic extract of *A. ludoviciana* L. leaf on different microorganisms is shown in fig 3.

3.4. Minimum Inhibitory Concentration

The MIC of hydroalcoholic extract of *A. ludoviciana* L. were determined using serial dilution method. The highest antibacterial effect of *A. ludoviciana* L. extract was on S. *aureus* with MIC values of 62.5 µg/mL. The MIC values of this extract for different microorganisms are presented in Tables 2.

Table 1. Characterization by GC-MS of the chemical composition of the hydroalcoholic extract of *Avena ludoviciana* L. RT: retention time; %Air: percentage of compounds present.

	Chemical constituents	**RT**	**Peak Aria%5**	**MV**	**MF**
1	Tetradecanoic acid	33.676	5.99	228.37	$C_{14}H_{28}O_2$
2	Neophytadiene	34.490	11.96	278.52	$C_{20}H_{38}$
3	Pentadecanoic acid	36.091	0.85	242.40	$C_{15}H_{30}O_2$
4	Hexadecanoic acid, methyl ester	37.646	0.89	270.4507	$C_{17}H_{34}O_2$
5	Hexadecanoic acid	38.857	12.26	256.43	$C_{16}H_{32}O_2$
6	Oleic acid, methyl ester	42.092	1.07	296.495	$C_{19}H_{36}O_2$
7	Phytol	42.470	0.63	296.54	$C_{20}H_{40}O$
8	9,12-Octadecadienoic acid	43.184	44.64	294.47	$C_{19}H_{34}O_2$
9	cis-Vaccenic acid	43.576	10.95	282.46	$C_{18}H_{34}O_2$
10	Linoleic acid	45.845	0.66	280.445	$C_{18}H_{32}O_2$
11	Eicosanoic acid	47.589	0.85	312.53	$C_{20}H_{40}O_2$
12	cis-9-Hexadecenal	50.081	1.06	238.415	$C_{16}H_{30}O$
13	Stigmastane-3,6-dione	52.700	0.65	428.701	$C_{29}H_{48}O_2$
14	beta.-Tocopherol	53.098	5.99	416.69	$C_{28}H_{48}O_2$
15	Vitamin E	54.179	27.92	430.717	$C_{29}H_{50}O_2$
16	ZZbeta.-Sitosterol	57.550	12.56	414.718	$C_{29}H_{50}O$
17	Fucosterol	59.121	8.12	386.65	$C_{27}H_{46}O$

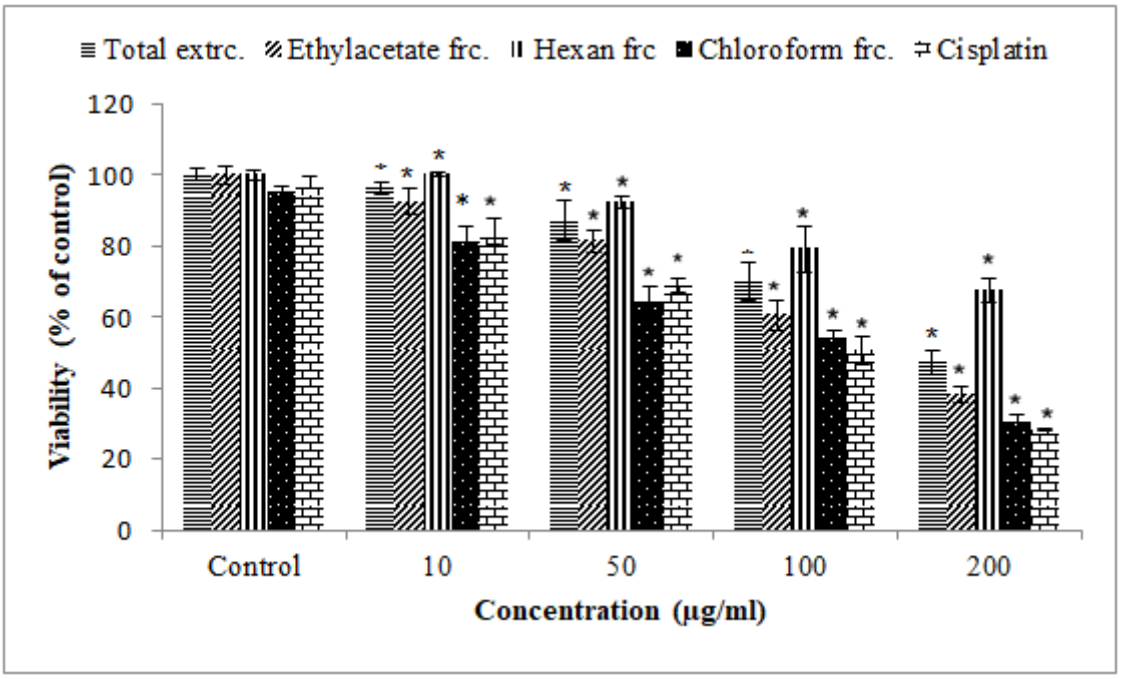

Figure 1. Rate (percentage) of skov3 cell viability in the presence of *A. ludoviciana* L. extract and fractions and cisplatin during 48 hours of incubation based on the MTT assay. Experiments were performed in triplicate. Significant difference between each concentration and control group (*p< 0.05).

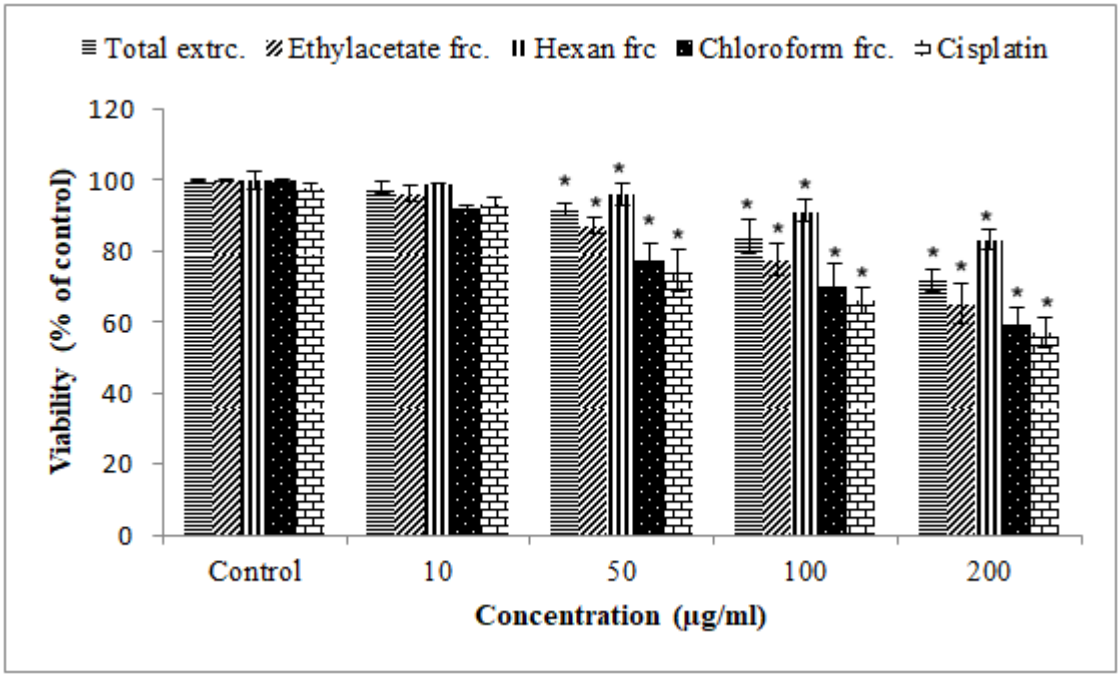

Figure 2. Rate (percentage) of MRC5 cell viability in the presence of *A. ludoviciana* L. extract and fractions and cisplatin during 48 hours of incubation based on the MTT assay. Experiments were performed in triplicate. Significant difference between each concentration and control group (*p< 0.05)

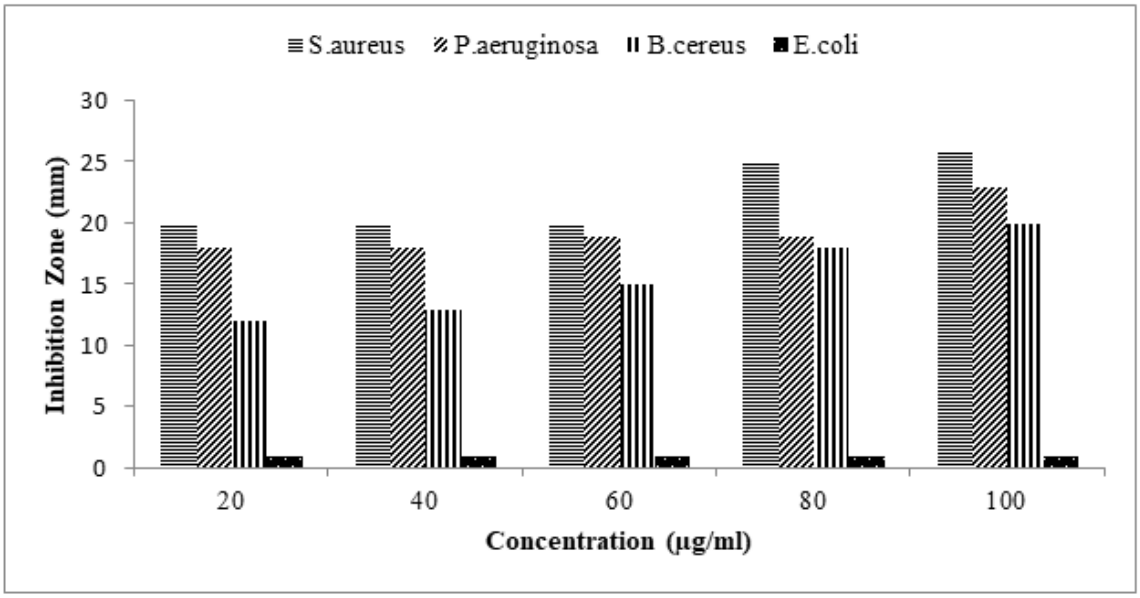

Figure 3. The mean and SD of the diameter of growth inhibition zone (mm) due to the effect of the hydroalcoholic extract of A. ludoviciana L. on different microorganisms.

Table 2. The Minimum inhibitory concentration of hydroalcoholic extract of *A. ludoviciana* L. against different microorganisms.

Marked test tubes	Medium added(Ml)	Sample solution(µg/Ml)	Inoculums added(µL)	Bacteria			
				Staphylococcus aureus	*Pseudomonas aeruginosa*	*Bacillus Cereus*	*Escherichia coli*
1	1	500	10	-	-	-	-
2	1	250	10	-	-	-	-
3	1	125	10	-	-	+	+
4	1	62.5	10	-	+	+	+
5	1	31.25	10	+	+	+	+
6	1	15.625	10	+	+	+	+
7	1	7.8125	10	+	+	+	+
T_{MI}	1	0	10	+	+	+	+
T_{MS}	1	0	10	-	-	-	-
T_{M}	1	0	0	-	-	-	-

MIC = Minimal inhibitory concentration, TMI = Test tube containing medium & inoculum, TMS = Test tube containing medium & solvent, TM = Test tube containing medium, (+) = Growth and (-) = No growth

4. Discussion

Cancer is a major cause of death in humans, and modern treatments are often ineffective and associated with adverse side effects. Therefore, considering the lack of optimal response to treatment and rapid disease growth, it is necessary to try to find more effective drugs with less toxicity (Falzone et al., 2018).

In recent decades, plants, as one of the main sources of biologically active substances, have become of global importance for the preparation of natural medicines for the treatment of cancer (Choudhari et al., 2020i). It is believed that the anti-cancer effects of plants are created by inhibiting cancer-causing enzymes, helping to repair DNA, stimulating the production of anti-tumor enzymes in cells, boosting the body's immune system and inducing antioxidant effects (Kopustinskiene et al., 2020). Although current treatments have been able to improve the prognosis of cancer patients, many of these tumors do not respond to current treatments. Thus, in the case of ovarian cancer, as in other cancers, efforts are underway to find more effective drugs with fewer side effects (Kurnit et al., 2021).

Infectious diseases are also one of the most common diseases in the world that impose a heavy financial burden on human societies. Fake antibiotics in the past decades, although they have been able to play an important role in the treatment of infectious diseases, but the problems associated with the emergence of microbial resistance to antibiotics have led to the tendency to use more and more herbal medicines (Ayukekbong et al., 2017).

In this study, the phytochemical characteristics of hydroalcoholic extract by GC-mass, anti-cancer activity of total extract and plant fractions in skov3 and MRC5 cancer cell lines by MTT method have not been reported so far. And antimicrobial activity was evaluated by disk diffusion method and determination of minimum concentration of growth inhibitor using dilution microbroth method. The present study was useful in identifying the compounds of the hydroalcoholic extract of *Avenaludoviciana L* by GC-Mass. The results of our study on methanolic extract of algae by GC-Mass showed that this extract has 17 compounds, the highest composition of which peaks area 44.64% belongs to 9,12-Octadecadienoic acid and and also, the lowest peak area for a compound called Phytol is 0.63.

Presence of bioactive antioxidant and antibacterial compounds such as Neophytadiene (Venkata Raman B et al., 2012), Vitamin E (Venkata Raman B et al., 2012), Stigmastane-3,6-dione (Lim et al., 2005), Phytol (Kim et al., 2018), Hexadecanoic acid (Chandrasekaran et al, 2011), Hexadecanoic acid, methyl ester (Chandrasekaran, M et al., 2011), Pentadecanoic acid (Farina Mujeeb et al, 2014), Tetradecanoic acid (Huang et al., 2019), Fucosterol (Khan et al., 2021), ZZbeta.-Sitosterol (Kasirzadeh et al., 2021), etc. in this plant suggests that more studies be done on the biological properties of the identified compounds to be of medical importance. This plant should be further identified and offered to drug centers as a promising anti-cancer and anti-bacterial compound.

In the study of cell culture, the effect of cytotoxicity of whole extract and *Avenaludoviciana L.* fractions on SKOV3 cancer cell line was investigated.With a brief look at the tables, diagrams and contents presented in the results, we can see that the plant leaf extract shows the effect of growth inhibition and cytotoxicity on SKOV3 and with increasing concentrations, the percentage of cell survival decreases. In other words, the effect of extracts is dose dependent.

Although the IC50 of extracts and fractions on the SKOV3 line are higher than that of the anticancer drug cisplatin, the growth inhibitory effects of the extracts and fractions studied on ovarian cancer cells have been adequate. In fact, plant extracts by affecting various metabolic processes in the cell, such as energy metabolism and protein synthesis, may affect cell activity by interfering with genetic mechanisms. Decreased cell viability in treatment with different doses of plant hydroalcoholic extracts at different time periods has been shown in other studies in this field (Abdalanet al., 2020).

Total extracts and fractions have the least toxic effect on normal control cells. This finding is important in terms of application because the specific effect of drugs on cancer cells is an advantage for them (Lin et al., 2020). This difference in effectiveness can be attributed to the ability of these cells to remove drugs and

extracts from the cell. Also, the methods or rate of response of normal cells to cancer cells in the face of various compounds is such that normal cells set up pathways to counteract the toxic properties of the compounds that lead to growth inhibition (Rezaei et al., 2014). Another reason for the morphological differences between cancer cell and normal cell membranes is the difference in their pore size. On the other hand, defense mechanisms in normal cells are less effective on these cells compared to cancer cells (Rezaei et al., 2014).

In the present study, the antimicrobial effect of chloroform extract in all studied methods showed that in general, with increasing the concentration of the extract, its inhibitory and bactericidal properties on the studied bacteria increase and the concentration of 100 is the most appropriate concentration to inhibit the growth of all bacteria.

In all methods, it can be said that aureuss is the most sensitive and E. coli the most resistant bacteria to glaucescens algae extract, while the other two species, aeruginosa P. and B. cereus, are in the middle position in terms of the degree of sensitivity to the extract). The difference in susceptibility of different microorganisms to antimicrobials is probably due to the different structure of microorganisms. Different concentrations of the extract are also effective in antimicrobial effect and in several studies (Idris et al., 2021; Boy et al., 2021; Oklaet al., 2021). By changing the concentration of the extract, the antimicrobial effects of the plant have changed. The culture medium used in antimicrobial tests also has a significant effect on the antimicrobial properties of the extracts (Bayot et al., 2020).

Due to the composition of *Avenaludoviciana L.* and the presence of various types of antioxidant compounds including flavonoids, terpenoids, vitamin E, etc., which in this article has proven the toxicity of cancer cells and its antimicrobial and its nativeness and according to this until now, no similar research has been done on this plant inside and outside of Iran. It is suggested that research on the clinical application of this medicinal plant in the concentration obtained for prevention and treatment, in the form of Invitro and Invivo can be the subject of further research.

Refereces

Abdalan S., Baghbani-Arani F., Sadat Shandiz S A. (2018). Evaluation of Anticancer Effect of Aqueous and Hydroalcoholic Extracts of Quercusin Fectoria Leaf against Colon Cancer HT29 Cell Line. J Arak Uni Med Sci. 21 (4) :48-57.

Ahad B., Shahri W., Rasool H., Reshi Z.A., Rasool S., Hussain T. (2021). Medicinal Plants and Herbal Drugs: An Overview. In: Aftab T., Hakeem K.R. (eds) Medicinal and Aromatic Plants. Springer, Cham.

Anand U., Jacobo-Herrera N., Altemimi A., Lakhssassi N.(2019). A Comprehensive Review on Medicinal Plants as Antimicrobial Therapeutics: Potential Avenues of Biocompatible Drug Discovery. Metabolites. 9(11):258.

Arora T., Mullangi S., Lekkala MR. Ovarian Cancer. [Updated 2021 Feb 22].(2021) In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; Available from: https://www.ncbi.nlm. nih.gov/ books/NBK567760/

Ayukekbong, J.A., Ntemgwa, M., Atabe, A.N.(2017). The threat of antimicrobial resistance in developing countries: causes and control strategies. Antimicrob Resist Infect Control , 6(47): 1-8.

Bayot ML., Bragg BN.(2021). Antimicrobial Susceptibility Testing. [Updated 2020 Aug 5]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK539714/

Boy FR., Casquete R., Martínez A., Córdoba MG., Ruíz-Moyano S., Benito MJ.(2021). Antioxidant, Antihypertensive and Antimicrobial Properties of Phenolic Compounds Obtained from Native Plants by Different Extraction Methods. Int J Environ Res Public Health. 18(5):2475.

Britannica, (2021). The Editors of Encyclopaedia. "Wild oat". Encyclopedia Britannica, Invalid Date, https://www.britannica.com/plant/wild-oat. Accessed.

Cavazos P., Gonzalez D., Lanorio J.(2021). Secondary metabolites, antibacterial and antioxidant properties of the leaf extracts of Acacia rigidula benth. and Acacia berlandieri benth. SN Appl. Sci. 3(522): 1-14.

Chandrasekaran M., Senthilkumar A., Venkatesalu V.(2011). Antibacterial and antifungal efficacy of fatty acid methyl esters from the leaves of Sesuvium portulacastrum L. Eur Rev Med Pharmacol Sci. 15(7):775-80.

Choudhari AS., Mandave PC., Deshpande M., Ranjekar P and Prakash O.(2020). Phytochemicals in Cancer Treatment: From Preclinical Studies to Clinical Practice. Front. Pharmacol. 10(1614):1-17.

Falzone L., Salomone S., Libra M.(2018). Evolution of Cancer Pharmacological Treatments at the Turn of the Third Millennium. Front Pharmacol. 9:1300.

Giacomini E., Perrone V., Alessandrini D., Paoli D., Nappi C., Degli Esposti L.(2021). Evidence of Antibiotic Resistance from Population-Based Studies: A Narrative Review. Infect Drug Resist. 14:849-858.

Gomes J., Barbosa J., Teixeira P.(2021). The Inhibitory Concentration of Natural Food Preservatives May Be Biased by the Determination Methods. Foods. 10(5):1009.

Huang L., Lin JS., Aris IM., Yang G., Chen WQ., Li LJ. (2019). Circulating Saturated Fatty Acids and Incident Type 2 Diabetes: A Systematic Review and Meta-Analysis. Nutrients. 11(5):998.

Idris FN., Mohd Nadzir M. (2021). Comparative Studies on Different Extraction Methods of Centella asiatica and Extracts Bioactive Compounds Effects on Antimicrobial Activities. Antibiotics (Basel). 10(4):457.

Kasirzadeh S., Ghahremani MH., Setayesh N. (2021). β-Sitosterol Alters the Inflammatory Response in CLP Rat Model of Sepsis by Modulation of NFκB Signaling. Biomed Res Int.2021:5535562.

Khan SA., Shahid S., Ayaz A., Alkahtani J., Elshikh MS., Riaz T.(2021). Phytomolecules-Coated NiO Nanoparticles Synthesis Using Abutilon indicum Leaf Extract: Antioxidant, Antibacterial, and Anticancer Activities. Int J Nanomedicine. 16:1757-1773.

Kim MH., Park DH., Bae MS.(2018). Analysis of the Active Constituents and Evaluation of the Biological Effects of Quercus acuta Thunb. (Fagaceae) Extracts. Molecules. 23(7):1772.

Kopustinskiene DM., Jakstas V., Savickas A., Bernatoniene J.(2020). Flavonoids as Anticancer Agents. Nutrients. 12(2):457.

Kurnit KC., Fleming GF., Lengyel E.(2021). Updates and New Options in Advanced Epithelial Ovarian Cancer Treatment. Obstet Gynecol. 137(1):108-121.

Lim JC., Park HJ., Budesinsky M.(2005). Antimutagenic Constituents from the Thorns of Gleditsia sinensis. chemical & pharmaceutical bulletin. 53(5):561-4 ·

Lin SR., Chang CH., Hsu CF.(2020). Natural compounds as potential adjuvants to cancer therapy: Preclinical evidence. Br J Pharmacol.177(6):1409-1423.

Malhão F., Ramos A.A., Macedo A.C., Rocha, E.(2021). Cytotoxicity of Seaweed Compounds, Alone or Combined to Reference Drugs, against Breast Cell Lines Cultured in 2D and 3D. Toxics. 9(24):1-32.

Mujeeb F., Bajpai P., Pathak N.(2014). Phytochemical evaluation, antimicrobial activity, and determination of bioactive components from leaves of Aegle marmelos. Biomed Res Int. 2014:497606.

Nasiri Tarzejani E., Nasri S.(2020). Cytotoxicity of methanol extracts of Ziziphora tenuior.L on Hela cell line by MTT assay. NCMBJ10 (38) :69-82.

Okla MK., Alatar AA., Al-Amri SS., Soufan WH., Ahmad A., Abdel-Maksoud MA. (2021). Antibacterial and Antifungal Activity of the Extracts of Different Parts of Avicennia marina (Forssk.) Vierh. Plants (Basel). 10(2):252.

Rezaei F., shokrzadeh M., Majd A., Nezhadsattari T.(2014). Cytotoxic activity of Ripe and Unripe cornellian cherry (cornus MasL.) Fruits. Emergencias. 3: 98 -104

Siegel RL., Miller KD., Fuchs HE., Jemal A.(2021). Cancer Statistics, 2021. CA Cancer J Clin. 71(1):7-33.

Turrini E., Maffei F., Milelli A., Calcabrini C., Fimognari C.(2019). Overview of the Anticancer Profile of Avenanthramides from Oat. Int J Mol Sci. 20(18):4536.

Upadhyay S., Dixit M.(2015). Role of Polyphenols and Other Phytochemicals on Molecular Signaling. Oxid Med Cell Longev. 2015:504253.

Venkata Raman B., Samuel LA., Pardha Saradhi M., Narashimha Rao B., Naga Vamsi krishna A., Sudhakar M., Radhakrishnan TM. (2012). Eupatorium odoratum. Asian Journal of Pharmaceutical and Clinical Research. 5(2):99-106.

WHO global report on traditional and complementary medicine 2019.(2019). Geneva: World Health Organization.