Original Article:

Antibacterial and anticancer activity of a bioflavonoid fractionated from *Allium Ascalonicum*

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ABSTRACT

Allium ascalonicum is a part of the diet of many populations of the world due to their long-held beliefs. *A. ascalonicum* extracts have been reported have antibacterial properties and prevent cancer cell proliferation. This study was conducted for the purpse of evaluating the anticancer and antibacterial activity of a flavonoid fraction isolated from *A. ascalonicum* bulbs. The HeLa and HUVEC cells were used as target cell line and some gram negative and positive bacteria were also targeted for antimicrobial activity. The *A. ascalonicum* plant was collected from the Zagros Mountains in the north of Dezful city- Iran, in September 2016 and confirmed by School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. The water extract of bulbs of this plant was extracted and the flavonoid fraction was isolated from aquous extract by ethyl acetate. The antibacterial and anticancer effects of isolated falavonoid were determined using MIC and MTT respectively. The best antibacterial effect of falvonoid extracted from *A.ascalonicum* was found against *C. diphtheria*. Furthermore, gentamicin resistant *P.aeruginosa* was the most resistant pathogenic bacterium. The MTT method showed that this fraction had a concentration-dependent anti-proliferative activity on HeLa cell lines and there was no cytotoxic effect against HUVEC cells. The inhibitory concentration 50% (IC50) values of the *A. ascalonicum* extract for Hela cell was 3 mg/mL but the treatment of HUVECs with the *A. ascalonicum* showed no considerable effect. The flavonoid fraction of *A.ascalonicum* bulbs had remarkable antibacterial and anticancer properties. Therefore, it could be used as an antibacterial and anticancer agent for control of cancers and infectious diseases.

Keywords: Anticancer; *Allium ascalonicum*; *Allium sativum*; flavonoid

INTRODUCTION

Allium plants are members of the *Liliaceae* family and consist of more than seven hundred species [1]. However, only three species, including *Allium ascalonicum* (Shallot), *Allium sativum* (garlic) and *Allium cepa* (onion) are well-known remedies in the prevention and treatment of diseases [2,3]. Traditional medicine has a very honorable past in Iran and medicinal plants play a key role in this country. Nowadays, traditional herbal medicine is still widely used in all cities and villages of Iran and there are "Attari stores", which sell herbals drugs [4-6]. Infectious diseases and cancers are the most common causes of death in many populations of the world and mankind have been grappling with this problem from the beginning. The adverse side effects and

resistance of synthetic antimicrobial and anticancer drugs have increased the necessity for the discovery of novel natural compounds. Recently, the use of herbal medicines has been considered for treatment of infectious diseases and cancers due to their fewer side effects, compression to chemical medicines [7-9]. *A.ascalonicum* is abundant in the Razavi-Khorasan and Lorestan provinces of Iran [1-7]. This herbal medicine has many potential benefits including antifungal and antibacterial properties, antioxidant activity, beneficial hematological effects and has been used for the treatment of rheumatism, kidney stones and decrease blood pressure [10,11]. *A. ascalonicum* plant contains saponin, sapogenine, ajoene and flavonoid extracts. Several studies have been conducted on the antibacterial and anticancer properties of total extract of *A. ascalonicum* and proved these activities against a variety of pathogenic bacteria and human cancer cell lines [12,13]. Despite the vast knowledge of herbal medicines in Iran, a few attempts have been performed to evaluate the antibacterial and anticancer effects of different fractions of shallot in this country. The aims of this study were the investigation of anticancer and antibacterial activity of flavonoid fraction of this plant against human cancer cell lines and some pathogenic bacteria using MTT method and MIC.

MATERIALS AND METHODS

Bacterial strains and Human cell lines

The current survey was a descriptive cross-sectional study. Bacterial strains including vancomycin resistant *Staphylococcus aureus* (PTCC: 29213), methicillin resistant *Staphylococcus aureus* (PTCC 33591), vancomycin resistant *Enterococcus faecalis* (PTCC 51299), gentamicin resistant *Pseudomonas aeruginosa* (clinical), tobramycin resistant *Escherichia coli* (clinical), *Bacillus cereus* (PTCC 11778), *Corynebacterium diphtheriae*, *Escherichia coli* (PTCC 25922) *Streptococcus pyogenes* (PTCC 12386), and *Klebsiella pneumoniae* (PTCC 700603), as well as human cell lines including human cervical carcinoma cell line (HeLa) and Human Umbilical Vein Endothelial Cell line (HUVEC) were purchased from the National Cell Bank, Pasteur Institute of Iran.

Preparation of target plant fraction

The *A. ascalonicum* plant was collected from the Zagros Mountains in the north of Dezful city-Iran, in September 2016 and confirmed by School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. For the preparation of plant extract, 500g bulbs of *A. ascalonicum* were washed completely with water and then mashed properly and after mixing with 500mL of distilled water, the mixture was soaked and stirred using a magnetic stirrer. The suspension was filtered through filter paper (Whatman No. 1) and the extract was mixed with ethyl acetate in 50/50 proportions and stirred by a magnetic stirrer for 10 minutes. Then the upper layer was separated accurately using a separating funnel and centrifuged at 7000 rpm for 10 min. The ethyl acetate layer was removed and the remaining residue was re-extracted twice and the fraction was dried in a rotary evaporator (Heidolph-Germany) at 50 ^{0}C. The separated fraction by column chromatography, which showed antibacterial activity, was used as an active fraction in this study. According to our previous study this fraction could be identified by chemical methods such as nuclear magnetic resonance (NMR) spectroscopy, infrared spectroscopy (IR) and gas chromatography-mass spectrometry (GC/MS) [14], and it was a flavonoid with C14H18O6 formula [3].

Antibacterial susceptibility test

Antibacterial activity of flavonoid fraction of *A. ascalonicum* bulbs against both Gram-positive and negative bacteria was determined by minimum inhibitory concentration (MIC), which were determined using an improved E test method (AB Biodisk, Solna, Sweden) in order to show the antimicrobial and anticancer activity. In the improved E test, several AB Bio discs impregnated with different dilutions of the extracts were used instead of strips. In fact, it was a simulated version of the standard E test (15). First, the extract was dried by Rotary Evaporator, then was double dilutioned in methanol and impregnated 10µl from each dilution in each blank disc and then, the dry amount in each disc was calculated. The ciprofloxacin and gentamicin powders were used as positive and DMSO10% as

negative controls, simultaneously.Six concentrations of the extract including 0.18, 0.37, 0.75, 1.5, 3 and 6 mg/mL were prepared. The bacterial suspension vancomycin resistant *Staphylococcus aureus* (PTCC: 29213) was adjustedto equal the turbidity of 0.5 McFarland standards giving a final inoculum of 1.5 X 10^8 CFU/mL. They were then subcultured on Mueller Hinton agar (MHA) medium and 8 blank discs were placed gently on the surface of MHA plates. Ten microliters of each concentration of the flavonoid fraction of *A. ascalonicum* plant was impregnated on the separate sterile blank disc using sterile micropipette and incubated for 24 hours at 37 ^{0}C. The test was carried out with quadruplicates for each bacterium and the disc which did not show inhibition zone was considered as MIC (3) and the amount of dried extract in this disc and it is MIC was calculated.

Anticancer susceptibility test

The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, Gaithersburg, MD) supplemented with 10% fetal bovine serum (FBS) (Gibco), penicillin (100 IU/mL), streptomycin (100 µg/mL) (Bioidea, Iran) and incubated in a humidified 5% CO_2 atmosphere at 37 ^{0}C. When the subcultures reached 50% to 80% confluence, they were trypsinized to a single cell suspension and subcultured in new culture flasks. After the subcultures reached more than 80% confluence (29 X 10^6), they were re-trypsinized to a single cell suspension and passaged in lower numbers in new culture flasks. For investigation of anticancer properties of the flavonoid fraction of *A. ascalonicum*, several concentrations of the extract (0.18, 0.37, 0.75, 1.5, 3 and 6 mg/mL) were prepared. The anticancer effect of flavonoid fraction of this plant was evaluated against HeLa and HUVEC human cancer cell lines using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) method [16]. This study was performed in 96-well plates. Cells were cultured at a concentration of (5×10^5) cells/well and incubated for 48 hours at 37°C in 5% CO_2. Microscopic observation showed that cell layers were suitable in each well. The passaged cells were treated with the dilutions of the fraction and their effects were examined at different times (24-72h). The cells in each well were incubated with 10 µL of the MTT solution (5 mg/mL) (Sigma-Aldrich Chemical, Germany). Then, 200µL Dimethylsulfoxide (DMSO) (Sigma) was added to dissolve the formazan crystals. The amount of purple formazan was determined by measuring the optical density (OD) using the ELISA reader (BioRad, USA) at 582 nm and the cell viability was calculated as the percentage of surviving cells after extract treatment. The effects of *A.ascalonicum* were expressed by IC50 values (the concentration required for a 50% viability inhibition).

Calculation of Cell Viability

The rate of viable cells = Number of Viable Cells / Total Cells x 100 . Total cells mean viable plus dead cells.

STATISTICAL ANALYSIS

Statistical analysis of experimental results and determination of inhibitory concentration 50% (IC50) was performed through SPSS software version 16. All data were expressed as means ± standard deviation. The statistical significance of the difference was determined and was considered significant at $p < 0.05$

RESULTS

Antibacterial activity of flavonoid fraction of *A. Ascalonicum* bulbs were evaluated against some selected bacteria using MIC method and the cytotoxic effect of the extract was determined on HeLa and HUVEC cells by the MTT assay. The antibacterial inhibitory activity of this flavonoid was found against all tested bacteria. The best antibacterial effect of the fraction was demonstrated in concentration of 1.5 µg/mL against the *C. diphtheriae*. However, in the current study, gentamicin resistant *P. aeruginosa* was the most resistant pathogenic bacterium (MIC=25 µg/mL) (Figure1). The inhibitory effects of flavonoid fraction of this herbal medicine on other tested bacteria are mentioned in Table 1. According to our data, MTT assay showed that this active extract of *A. ascalonicum* had a concentration-dependent anti-proliferative activity on HeLa cell lines and there was no cytotoxic effect against HUVEC cells (Table 2 and Table 3).


A

B

Figure1. Antimicrobial activity of flavonoid fraction of *A. ascalonicum* against *C. diphtheria* (A) and Gentamicin resistant *P. aeruginosa* (B)

Table 1. The antibacterial effects of flavonoid fraction of *A. ascalonicum*, against selected bacteria

Bacteria	*B.cereus*	*C.diphtheria*	*S. pyogenes*	*K.pneumoniae*	*E. coli*	TREC*[1]	GRPA*[2]	VREF*[3]	VRSA*[5]	MRSA*[4]
MIC(µg/mL)	3.1	1.5	12.5	6.25	3.1	6.25	25	6.25	6.25	6.25

*[1]: Tobramycin resistant *E. coli*, *[2]: Gentamicin resistant *P. aeruginosa*
*[3]: Vancomycin-resistant *Enterococcus faecalis*, *[4]: Methicillin resistant *Staphylococcus aureus*
*[5]: Vancomycin resistant *Staphylococcus aureus*

Table 2 . Anticancer activity and amount of OD after different times flavonoid fraction of *A. ascalonicum* against human cancer cell lines

Amount of OD after 72h		Amount of OD after 48h		Amount of OD after 24h		Extract concentration (mg/ml)
HeLa cell line	HUVEC cell line	HeLa cell line	HUVEC cell line	HeLa cell line	HUVEC cell line	
0.05	0.32	0.118	0.33	0.14	0.33	6
0.06	0.331	0.124	0.337	0.161	0.337	3
0.07	0.331	0.144	0.337	0.194	0.339	1.5
0.1	0.338	0.148	0.34	0.205	0.341	0.75
0.204	0.339	0.226	0.34	0.280	0.346	0.37
0.274	0.346	0.315	0.349	0.333	0.349	0.18
0.31	_	0.349	_	0.349	_	Control*

*It contains cancer cells, DMEM culture medium and DMSO solution without extract *A. ascalonicum*
Dulbecco's Modified Eagle's medium (DMEM) Dimethyl sulfoxide (DMSO)

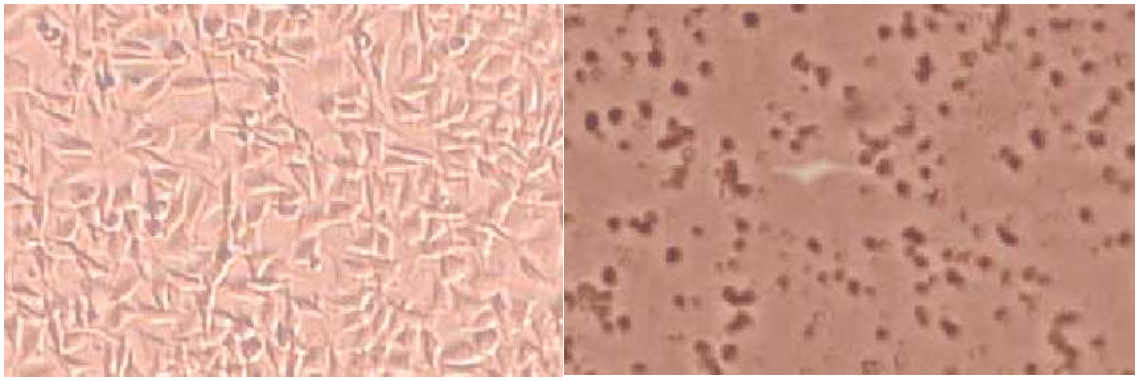
Table 3. Association between concentrations of flavonoid fraction of *A. ascalonicum* and Mean± SD biological survival rates (OD) of cells in different times

72h		48h		24h		COE mg/ml
HUVEC cell line	HeLa cell line	HUVEC cell line	HeLa cell line	HUVEC cell line	HeLa cell line	
94.0±4.8	16.15±4.08	94.0±3.9	34.0±4.05	94.0±4.1	40.25±4.11	6
94.0±4.1	20.1±5.01	96.0±4.0	36.5±4.25	96±.03.9	48.2±4.0	3
94.0±3.2	22.0±3.65	97.0±3.1	40.14±5.45	97.0±4.2	56.3±5.0	1.5
97.0±4.2	32.25±3.18	97.0±3.0	42.56±2.18	98.0±3.0	60.25±4.12	0.75
98.0±3.0	60.02±2.04	99.0±2.1	63.3±3.07	99.0±2.0	88.18±2.11	0.37
99.0±4.0	86.1±3.89	100±3.5	88.1±3.06	100±2.1	95.18±2.11	0.18
_	< 0.001	_	< 0.001	_	0.015	P value

COE: Concentration of extracts

The IC50 values of the *A. ascalonicum* extract for Hela cell was 3 mg/mL but the treatment of HUVECs with the *A. ascalonicum* showed no considerable effect. The potential of the

anticancer activity of the fraction to discriminate between normal and cancer cells is an important paradigm in the design and discovery of chemotherapeutic agents. The results of the current study demonstrated that HeLa cells were more sensitive to the cytotoxic activity of the plant while HUVEC showed no sensitivity to the extract. The highest sensitivity of cancer cell lines to flavonoid fraction of *A. ascalonicum* was presented after 72 hours. Detachment, shrinkage, and granulation of cytoplasm were seen in treated cells with flavonoid fraction of *A. ascalonicum* (Figure 2). Statistical analysis showed that at 24 hours incubation time of cell viability from 40% (at a concentration of 6 mg/mL) to 16% (after 72 hours) decreased and the difference was statistically significant. (P<0.05), (Figure 3).



A B

Figure 2. Microscopic images of Hela cell after 72 h incubation with flavonoid fraction of *A. ascalonicum.* Detachment and Shrinkage effects were visible in treated cells (A, untreated; B, treated cells). HUVEC, showed no sensitivity to the extract (not show images)

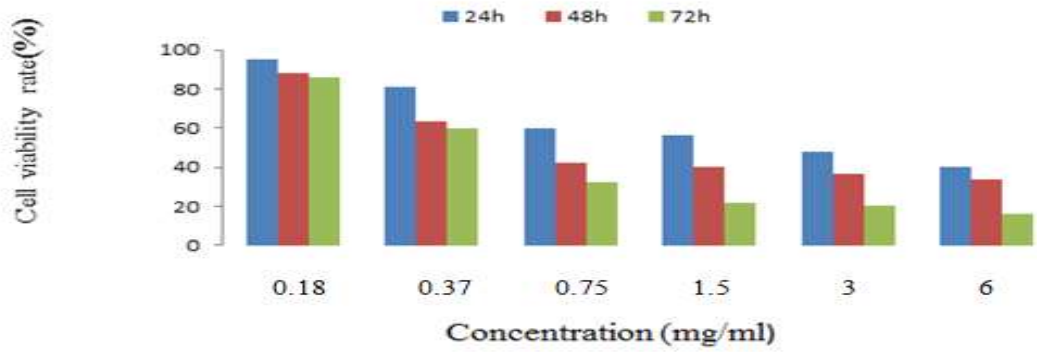


Figure 3. Association between concentrations of flavonoid fraction of *A. ascalonicum and* cell viability rate in different times

DISCUSSION

Infectious diseases and cancers are the main causes of morbidity-mortality worldwide and there is a constant demand for new therapies to treat and prevent these life-threatening diseases. Use of synthetic antibacterial and anticancer drugs have not succeeded within the broad range of clinically relevant bacterial pathogens and cell lines, probably because of their adverse side effects and antimicrobial resistance [17]. Nowadays, the use of herbal medicines has been considered for treatment of infectious diseases and cancers due to their fewer side effects in compression to chemical medicines. The previous studies have shown that the various extracts of *A. ascalonicum* have antibacterial activity against pathogenic bacteria [3-16]. In the current study, the best antibacterial inhibitory effect of flavonoid fraction of *A. ascalonicum* was found

against *C. diphtheria*. Furthermore, gentamicin resistant *P.aeruginosa* was the most resistant pathogenic bacterium. Amin *et al* in their study have evaluated the effect of the flavonoid fraction of *A ascalonicum* against a number of bacteria and showed the best effect of this fraction against *Bacillus cereus* [3]. To the best of the authors' knowledge, there are a few reports detailing the antibacterial effects of flavonoid fraction of *A. ascalonicum* bulbs against bacteria. Wang and Ng have isolated an antifungal peptide from bulbs of *A. ascalonicum* and showed that this peptide inhibited mycelial growth in the fungus *Botrytis cinerea*. However, this isolated antifungal peptide did not inhibit *Mycosphaerella arachidicola* and *Fusarium oxysporum* [18]. In another study, the MICs of the extract of shallot against *Trichophyton mentagrophytes*, *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigatus* were 0.62, 10, 20 and 20 mg/mL respectively [3]. The other studies which have been done in this field revealed the antibacterial effect of the extracts of *A. ascalonicum* against *Mycobacterium tuberculosis* and *Listeria monocytogenes*. As fraction of Shallot extract showed, antimycobacterial activity with a MIC value of 500 µg/mL and its essential oils had the highest antimicrobial effects against *L. monocytogenes* [19,20,21]. Many studies also demonstrated that the family of *Allium* possesses anticancer activities as shown through their capacity to prevent tumor proliferation in vitro and in vivo [22]. In spite of extensive consumption of *A. ascalonicum*, reports regarding its organic effects are rarely compared to other *Allium* species such as onion and garlic. *A. ascalonicum* is usually known for antidermatophytic, anti-angiogenic and Hypocho lesterolemic properties [23,24]. In the current study, flavonoid fraction of *A. ascalonicum* plant had a concentration-dependent inhibition activity on HeLa cells, but the treatment of HUVECs with the *A. ascalonicum* showed no considerable effect. In a study performed by Azadi *et al*. the in vitro effect of chloroformic and aqueous extracts of Iranian shallot (*Allium hirtifolium*) on the proliferation of HeLa, L929 (mouse fibroblast cell line) and MCF7 (human breast adenocarcinoma cell line) cells were investigated. Although Hela and MCF-7 cell lines were sensitive to Iranian shallot, the cell survival rate was almost unchanged in L929 cells. It means that *A. hirtifolium* did not affect the normal L929 mouse cells and it only decreased cancer cell populations [13]. *A.hirtifolium* showed growth inhibitory effect against MCF-7 and HeLa cells with IC50 values of 24 and 20 mg/mL, respectively, for 72 h. These results approved the data of the present study on the sensitivity of this cell line to flavonoid fraction of *A.ascalonicum*. In this study, there is an association between concentrations of flavonoid fraction of *A. ascalonicum* and cell viability rate at different times. According to data, increase in the concentration of *A. ascalonicum* extract decreases the cell viability count. This result confirms the reports of previous studies about the dose-dependent effect of *A. ascalonicum* extract on the inhibition of proliferation of cancer cells [12, 25]. In the study conducted by Pandurangan *et al*., anticancer effect of fresh and dry *A. ascalonicum* was assessed against liver cancer cell line HepG2 by MTT. The result shows that both extracts of shallot have anticancer potential (IC50 of 50 µg/mL) [11]. Finally, isolation of total extract components and identification of their combinations are recommended for future studies. Correspondingly, doing further studies to determine the cytotoxicity of other effective fraction is recommended. Moreover, it is hoped that through proven different extracts of *A. ascalonicum*, the product could be useful as a medicinal plant in one of the pharmaceutical forms in future.

CONCLUSION

This study highlights promoting increased consumption of this plant as a necessary means to inhibit or even to cancer therapy. However, supplementary studies are essential for assessment of the molecular mechanisms of anticancer effects of active composites from shallot.

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REFERENCES

1. Hanelt P, Schultze Motel J, Fritsch R, Kruse J, Maass H, Ohle H, et al. Infrageneric grouping of Allium-the Gatersleben approach. The genus Allium-taxonomic problems and genetic resources Inst Pflanzengenetik & Kulturpflanzenforschung. Gatersleben 1992:107-123.

2.Takahama U, Hirota S. Deglucosidation of quercetin glucosides to the Anglican and the formation of antifungal agents by peroxidase-dependent oxidation of quercetin on browning of onion scales. Plant Cell Physiol 2000;41(9):1021-9.

3.Amin M , Kapandis B . Heat stable antimicrobial activity of Allium ascalonicum against bacteria and fungi. Indian J Exp Biol 2005;43:751-754.

4.Amiri MS, Joharchi MR. Ethnobotanical investigation of traditional medicinal plants commercialized in the markets of Mashhad, Iran. Avicenna J Phytomed 2013;3(3): 254-271.

5.Hakemi- Vala M, Mehrara M, Pourramezan M, Asgarpanah J, Rahimifard N, Khoshnood S et al. Comparison the antimicrobial effect of methanolic total extracts and petroleum ether fractions of flowering aerial parts of Glaucium vitellinum Boiss. & Buhse and Gaillonia aucheri Jaub. & Spach. Novelty Biomed 2017;5(1):249.

6. Hakemi-Vala M, Eslamzadeh A, Bejestani FB, Asgarpanah J, Heidary M, Khoshnood S. Preliminary Evaluation of the Antimicrobial Activity of Total Extract and Fractions of Chloroform, Methanol, and Aqueous from the Aerial Parts of Salvia aegyptiaca. Avicenna Journal of Clinical Microbiology and Infection. 2017;4(3).

7.Razyfard H, Zarre S, Fritsch RM, Maroofi H. Four new species of Allium (Alliaceae) from Iran. Ann Bot Fenn 2011;48(4):352-360.

8.Heidary M, Hashemi A, Goudarzi H, Khoshnood S, Roshani M, Azimi H, et al. The antibacterial activity of Iranian plants extracts against metallo beta-lactamase producing Pseudomonas aeruginosa strains. J Paramed Sci 2016;7(1):13-19.

9.Hakemi-Vala M, Makhmor M, Kobarfar F, Kamalinejad M, Heidary M, Khoshnood S. Investigation of antimicrobial effect of Tribulus terrestris L. Against some gram positive and negative bacteria and Candida spp. Novelty Biomed 2014;2(3):85-90.

10.Block E, Birringer M, Jiang W, Nakahodo T, Thompson HJ, Toscano PJ, Uzar H, Zhang X, Zhu Z. Allium chemistry: synthesis, natural occurrence, biological activity, and chemistry of Se-alk (en) ylselenocysteines and their γ-glutamyl derivatives and oxidation products. J Agric Food Chem 2001;49(1):458-70.

11.Pandurangan V, Amanulla SSD, Ramanathan K. Anticancer efficacy of dry and fresh Allium ascalonicum (shallot) against HepG2 cell line. Natl J Physiol Pharm Pharmacol. 2016;6(3):196-199.

12.Azadi HG, Riazi GH, Ghaffari SM, Ahmadian S, Khalife TJ. Effects of Allium hirtifolium (Iranian shallot) and its allicin on microtubule and cancer cell lines. Afr J Biotechnol 2009; 8(19): 5030-5037.

13. Hamta A, Shariatzadeh S, Soleimani M, Tajali AM. Cytotoxicity effect of aquous and alcoholic total extract of shallot (Allium Ascalunicum) on cancer cell Derived from mammary Tumors in rat and cell line (4T1) in mouse, and comparison with taxol and Carboplatin Chemotherapy drugs. J cell tissue 2014;5(3): 253-61

14.Amin M, Montazeri EA, Mashhadizadeh MA, Sheikh AF. Characterization of shallot, an antimicrobial extract of Allium ascalonicum. Pak J Med Sci 2009;25(6):948-52.

15.Amin M, Choghakabodi PM, Hamidi MA, Najafian M, Sheikh AF. In vitro antimicrobial activities of metabolites from vaginal

Lactobacillus strains against Clostridium perfringens isolated from a woman's vagina. J Chin Med Assoc 2017;80(1):29-33.
16. composition, antioxidative activity and cell viability effects of a Siberian pine (Pinus sibirica Du Tour) extract. Food Chem 2009;112(4):936-43.
17.Solowey E, Lichtenstein M, Sallon S, Paavilainen H, Solowey E, Lorberboum-Galski H. Evaluating medicinal plants for anticancer activity. Sci World J 2014.
18.Wang H, NG, T. Ascalin, a new anti-fungal peptide with human immunodeficiency virus type 1 reverse transcriptase-inhibiting activity from shallot bulbs. Peptides 2002;23(6):1025-29.
19.Ehsani A, Mahmoudi R, Zare P, Hasany A. Biochemical Properties and Antimicrobial effect of Allium ascalonicum and Pimpinella anisum
Essential Oils against Listeria Monocytogenes in White Brined Cheese. J Food Ind 2011;21(3):318-328
20.Negi PS. Plant extracts for the control of bacterial growth: Efficacy, stability and safety issues for food application. Int J Food Microbiol 2012;156(1):7-17.
21.Amin M, Segatoleslami S, Hashemzadeh M. Antimycobacterial activity of partial purified extract of Allium ascalonicum. Jundishapur J Microbiol 2009;4(2):144-147.
22.Sengupeta *A*, Ghosh S, Bhattacharjee S. Allium vegetables in cancer prevention: an overview. Asian Pac J Cancer Prev 2004;5(3):237-245.
23.Leelarungrayub N, Rattanapanone V, Chanarat N, Gebicki JM. Quantitative evaluation of the antioxidant properties of garlic and shallot preparations. Nutrition 2006;22(3):266-74.
24.Trakranrungsie N, Chatchawanchonteera A, Khunkitti W. Ethnoveterinary study for anti dermatophytic activity of Piper betle, Alpinia galanga and Allium ascalonicum extracts in vitro. Res Vet Sci 2008;84(1):80-84.
25.Mohammadi Motlagh HR, Mostafaie A, Mansouri K. Anticancer and anti-inflammatory activities of shallot. Arch Med Sci 2011;7(1):38-44.