Herbal Medicines Journal **2021; 6(3):102-12**
DOI: 10.22087/hmj.v7i3.847 **ISSN:** 2538-2144

Original Article

The Effect of Foliar Application of Urea and Salicylic Acid on the Antibacterial Properties of *Physalis alkekengi* L. In vitro condition

Ali Pezeshki[1], Hassan Nourafcan[2*], Mehdi Oraei[3], Naser Mohebalipour[1], Assad Assadi[4]

[1] Department of Agriculture, Faculty of Agriculture, Miyaneh Branch, Islamic Azad University, Miyaneh, East Azarbaijan, Iran
[2] Department of Agriculture, Medicinal Plants and Organic Products Research Center, Miyaneh Branch, Islamic Azad University, Miyaneh, East Azarbaijan, Iran
[3] Department of Horticultural Sciences, Faculty of Agriculture, Miyaneh Branch, Islamic Azad University, Miyaneh, East Azarbaijan, Iran
[4] Department of Veterinary Medicine, Faculty of veterinary, Medicinal Plants and Organic Products Research Center, Miyaneh Branch, Islamic Azad University, Miyaneh, East Azarbaijan, Iran

Received: 31.10.2020; Accepted: 03.03.2022

Abstract

Background and Aim: The *Physalis alkekengi* is a medicinal plant that has long been used in pharmaceutical, food and health products. Increasing and indiscriminate use of synthetic antibiotics against bacterial infections has increased drug resistance. As a result, today there is an increasing tendency among people to use drugs of natural origin, particularly herbal ones. In this regard, this study was conducted to investigate the antibacterial effects of alcoholic extract of *Physalis alkekengi* on some common bacteria in vitro condition.

Materials and Methods: In this study, the alcoholic extract of *Physalis alkekengi* obtained from foliar application of urea fertilizer (0.25, 0.5, 1 and 2%) and salicylic acid (0.001. 0.0025, 0.005 and 0.01 mM) at four levels were used. For this purpose, antibacterial effects of the extracts were investigated by dilution methods in tube, disk and well diffusion method against *Staphylococcus aureus, Escherichia coli, Salmonella enterica, Proteus mirabilis, Pseudomonas aeruginosa, Bacillus cereus, Shigella dysenteriae* and *Klebsiella pneumonia*.

Results: The results of the present study indicated that the extracts of *Physalis alkekengi* have antibacterial effects under the influence of different treatments of urea and salicylic acid. Moreover, the results showed the effects of minimum inhibitory concentration and minimum bactericidal concentrations of different treatments of urea and salicylic acid. Among the tested concentrations, urea concentrations of 2% and 0.5% had the highest and lowest inhibitory effects on bacterial growth compared to the negative and positive control groups respectively. Salicylic acid with 0.01 and 0.001mM showed the highest and lowest bacterial growth inhibitory effects respectively.

Conclusion: The results of this study showed that the extract of *Physalis alkekengi* contain suitable antibacterial substances that can be used as an alternative in cases of drug resistance against pathogenic microorganisms.

Keywords: *Physalis alkekengi*, Dilution tube, Well diffusion, Disk diffusion, MIC, MBC

***Corresponding Author:** Hassan Nourafcan, Department of Agriculture, Medicinal Plants and Organic Products Research Center, Miyaneh Branch, Islamic Azad University, Miyaneh, East Azarbaijan, Iran. Email: hassannourafcan@gmail.com.
Please cite this article as: Pezeshki A, Nourafcan H, Oraei M, Mohebalipour N, Assadi. The Effect of Foliar Application of Urea and Salicylic Acid on the Antibacterial Properties of *Physalis alkekengi* L. In vitro condition. Herb. Med. J. 2021; 6(3):102-12.

Introduction

The *Physalis alkekengi L.* belongs to the Solanaceae family and is an annual or perennial herbaceous shrub

with a height of 40 to 60 cm tall, straight creeping rhizome and angular stems. This plant is also called Kakuneh, Jihudan cherry and Kachuman. Its juicy fruit is orange or red in color, and its size is the same as that of a hazelnut, like a cherry. There are small flat white seeds inside the fruit. The calyx parenchyma usually decomposes in autumn under the influence of cellulosic bacteria, so that only the vesicle network of the calyx remains in the form of a thin white net and the round fruit appears inside. This is why this plant is called "*Physalis alkekengi*" in Persian (1).

This plant is used in the treatment of urinary diseases, kidney and bladder stones, fever, inflammation, constipation, and arthritis rheumatism. In traditional medicine, it is used in abortion and as a contraceptive. One of the most important effects of the products of this plant is its antimicrobial effects. Today, a new approach has been adopted to the antimicrobial effects of this plant in medicine, pharmaceutical and food industries. Many studies and researches have been conducted on the antimicrobial effects of the extracts of this plant (2). Plant extracts contain important compounds such as alkaloids, flavonoids, vitamins and minerals in which various biological activities such as antibacterial, antiviral and antifungal have been observed (3).

Among the active ingredients of plants of the *Solanaceae* family are steroidal alkaloids and glucocorticoids. The fruit of *Physalis alkekengi* contains physalin, lycopene, alkaloids, alcohol and a large amount of vitamin C (4). Alkaloids and steroids are among the most important nitrogenous organic compounds from which many drugs and toxins are made. These substances are naturally biosynthesized in plants and extracted from them (5).

Studies have shown that the use of nitrogen has a positive effect on physiological parameters, yield and grain yield components that can improve plant yield. Urea fertilizer is widely and universally used to supply plants with nitrogen. Salicylic acid (SA) is a monohydroxy-benzoic acid, which is widely known as signaling molecule which is effective in local and systemic acquired resistance against pathogens and in acclimation to some abiotic stressors (6, 7, 8, 9 and 10)

It is possible to synthesize *Salicylic acid* (SA) via phenylalanine pathway. Phenylalanine is converted into cinnamic acid (CA) by phenylalanine ammonia lyase (PAL). PAL is considered as a significant regulator of the phenyl propanoid pathway and is induced under various kinds of biotic and abiotic stress conditions (11). SA can be synthesized from CA using two distinct procedures: it can be either hydroxylated to form ortho-hydroxycinnamic acid (oHCA) followed by oxidation of the side chain or, alternatively, the side chain of cinnamic acid can be initially oxidized to give benzoic acid as an immediate precursor, which is subsequently hydroxylated in the ortho position (12 and 13). Moreover, the phenylalanine pathway is used as a rich source of metabolites in plants, such as flavonoids, coumarins and lignans (14).

In an experiment, alkaloid steroids extracted from *Physalis alkekengi* with inhibitory concentration (MIC = 150 µg⁄ml) showed antibacterial effects by inhibiting the growth of four bacteria *E. coli, s. epidermidis, E. faecalis* and *B. cereus* (15). Alcoholic extracts of *physalis alkekengi* have also been shown to inhibit the growth and proliferation of *Trichomonas vaginalis* (16).

Moreover, the antibacterial effects of the alcoholic extract and *physalin D* on 5 Gram-positive bacteria (*Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis, Bacillus subtilis* and *Bacillus cereus*) and 5 Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Interobacter cloacae, Klebsiella pneumonia* and *Proteus vulgaris*) were investigated (17).

The positive effects of the alcoholic extract of *Physalis minima L.* on several bacterial species, including *Bacillus cereus, Bacillus subtilis, Citrobacter sp., Interobacter aerogenes, Klebsiella Pneumonia, Pseudomonas aeruginosa, Staphylococcus aureus* have been reported (18).

Hence, this study was conducted to evaluate the bactericidal effect of the alcoholic extract of *Physalis alkekengi* under the influence of foliar application of urea and salicylic acid on Gram-positive and Gram-negative bacteria.

Materials and Methods

Experimental groups

In order to evaluate the effect of foliar application of urea and salicylic acid on the antibacterial properties of Chrysalis alkekengi, a pot experiment in a completely

randomized design, including foliar application of urea (Merck, Germany) in four levels (0.25, 5/5). 0, 1 and 2%) salicylic acid (Merck, Germany) in four levels (0.001, 0.0025, 0.005 and 0.01 mM), foliar application with distilled water and no foliar application was performed in three replications, and each replication consisted of 10 experimental units (pots). Foliar application was done in three stages with an interval of 10 days from each other and after the complete establishment of the plant in the pot and when the plants had grown enough and reached about 15 cm. In order to better absorb urea and salicylic acid one ml of the tween liquid was added to each liter of the solutions of urea and salicylic acid. Finally, 15 days after the last foliar application, the plant was harvested.

Preparation of the Extract of *Physalis alkekengi*

To prepare the extract, first the shoots of each treatment were collected separately and dried in the shade at a temperature of approximately 30 ° C and pulverized separately in a blender. Then, 25 g of each of the powdered samples was weighed and poured into separate Erlenmeyer flakes. After that, 100 ml of ethanol was added to the contents of the Erlenmeyer flakes and stirred in a laboratory shaker for 48 hours. The alcoholic extract mixture was then passed through a filter paper and disinfected with a vacuum distillation model to obtain a crude extract (19).

The concentrated extract was placed in an electric oven for 72 hours at 45 ° C to produce a dry extract (19). Dimethyl sulfoxide (DMSO) solvent was used to dissolve the extracts. For sterilization, a 0.22-micron syringe filter was used and the filtered extracts were stored in a sterilized container at -4 ° C until the test (20).

Drugs

The drugs used included Urea (Merck, Germany) at four concentrations (0.25, 0.5, 1 and 2%), salicylic acid (Merck, Germany) at four concentrations (0.001, 0.0025, 0.005 and 0.01 mM), Gentamicin (Caspian, Iran), and Ampicillin (Daana, Iran).

Experimental Design

In this study, 8 samples of bacterial strains, including *Escherichia coli (ATCC 1399), Salmonella enterica (ATCC 13076), Proteus mirabilis (ATCC 43071), Pseudomonas aeruginosa (ATCC 9027), Shigella dysenteriae (RI 366), Klebsiella Pneumonia (ATCC 10031), Staphylococcus aureus (ATCC 1787)* and *Bacillus cereus (ATCC 11778)* were prepared as lyophilized ampoules according to the instructions of Iran Regional Center for Collection of Industrial Microorganisms.

In order to evaluate the effect of the foliar application of urea and salicylic acid on the antibacterial properties of *Physalis alkekengi*, a pot experiment in a completely randomized design including foliar application of Urea (Merck, Germany) at four levels (0.25, 0.5, 1 and 2%), salicylic acid (Merck, Germany) at four levels (0.001, 0.0025, 0.005 and 0.01 mM), foliar application with distilled water and without foliar application with three replicates were performed, and each replication consisted of 10 experimental units (pots). Foliar spraying was done in three stages with an interval of 10 days from each other, and after the complete establishment of the plant in the pot and when the plants had grown enough and reached about 15 cm, 1 ml of the tween liquid was added to each litter of the solution to improve its absorption. Fifteen days after the last foliar application, the plant was harvested.

These extracts were used in well diffusion and disk diffusion methods to determine the diameter of the bacterial growth inhibition zone and in the tube dilution method to find the minimum concentration of bacterial growth inhibition (MIC) and the minimum bactericidal concentration of the antibacterial agent (MBC). Microbial samples were prepared according to the instructions of Iran Industrial Microorganisms Collection Center. In order to prepare the microbial suspension for bacterial test, fresh and young culture of several colonies of newly grown bacteria were transferred to Müller-Hinton Broth culture and turbidity equivalent to 0.5 McFarland. The number of bacteria suspension was 1.5×10^8 CFU/ml (21).

Antimicrobial parameters

The antibacterial effects of the alcoholic extract of *Physalis alkekengi* were investigated in three ways as follows:

A) Tube Dilution Method

Tube dilution method is an accurate and sensitive method to determine the antimicrobial properties of plant extracts (22). Minimum inhibitory concentration (MIC) is the minimum concentration of extracts that can inhibit bacterial growth at the rate of 90 percent and a minimum bacterial concentration (MBC) is the

minimum amount of extracts that prevents the growth of bacteria at 99.9% (10). MIC and MBC of the antimicrobial activity can be determined using tube dilution method. To determine the MIC of *Physalis alkekengi* extract a series of 12 glass tubes were used. Nine test tubes were used to test various extract dilutions, a tube was used as a negative control (extract diluted plus medium), and a tube was used as a positive control (containing bacterial suspension plus medium). Moreover, a tube containing solvents, microbial suspension and culture was used to ensure the growth of bacteria in media containing the solvent used for extraction. The initial concentration was 5 mg/ml. By the serial dilution of 50% of tube No.1, its concentration reached 2.5 mg/ml, and a concentration of 0.00976 mg/ml was obtained for tube 9. Subsequently, 50 µ l of bacterial suspension with 1.5×10^8 CFU/ml was added to all tubes except tube No. 10 (negative control). Extract dilution for all bacteria was tested. All the test tubes were incubated for 24 hours at the temperature 37°C. Then, the turbidity of inoculated bacteria was examined. The last growth inhibition tube was recorded as the inhibitory concentration of the extract. Then, the tube in which the bacteria had not grown was used to determine MBC by surface culture method. To this end, 100 ml of the tubes that had shown no bacterial growth was spread on Muller Hinton agar medium. After incubating for 24 hours, plates were cultured for the presence of microbial growth control. The tube containing the lowest extract concentration, in which no bacterial growth was observed, was considered as MBC extract (23, 24).

B) Disk Diffusion Method

In this method, the antibacterial properties of *Physalis alkekengi* extract were evaluated based on agar diffusion bioassay (25). For this purpose, a suspension equivalent to 0.5 of McFarland (1.5×10^8 CFU/ml) was prepared, and then 0.1 ml of the bacterial suspension was cultured on Müller Hinton agar medium (Muller Hinton Agar, Merck) and completely spread on the medium by L-shaped glass bar. To inoculate the extract, 30 microliters of each *Physalis alkekengi* extract with a concentration of 5 mg/ml was injected into each sterile Whatman filter paper discs. Subsequently, the discs were placed on a sterile mesh plate for one hour to completely absorb the extract. The antibiotic discs of gentamicin and ampicillin at a concentration of 4 mg/ml. were used as a positive control and sterile distilled water disk as well as dimethyl sulfoxide (DMSO) disk were used a negative control. All the plates were incubated for 24 hours at 37 ° C, and antibacterial activity was performed based on measuring the diameter of the non-growth range around the discs in millimeters and compared with the control groups. All disc placements were repeated three times (26, 27, 28).

C) Well Diffusion Method

In this method, first a turbidity equivalent to 0.5 McFarland was prepared from the tested bacteria, and then cultured in Müller Hinton agar medium. Then, on the Müller Hinton, wells with a diameter of 5mm and intervals of 2.5mm from each other and 2.4 mm from the edge of the plate created by sterile Pasteur pipette. In each of the wells, 30 micro litters of different dilutions of the studied extracts were filled. Ampicillin and Gentamicin antibiotics with a concentration of 4 mg/ml were used as positive controls and DMSO (Di Methyl Sulfa Oxide) and distilled water were used as negative controls. All the cultures were incubated at 37°C for 24 h. Bacterial cultures were then measured by a caliper for the formation or non-formation of a growth range in millimeters and their mean was recorded (29).

Data Analysis

Data were analyzed using SPSS software, version 16, and analysis of variance, as wells as Duncan test at the significance level less than 0.01.

Results and Discussion

The antibacterial effect of different concentrations of urea and salicylic acid extracts of *Physalis alkekengi* showed that in the well and disc method the effect of the extract on all bacteria was significant at the level of 1% probability (Table 1).

Table 1: Analysis of Variance of the Mean Data of the Inhibition Zone of *physalis alkekengi* by Well Diffusion Method.

sov	df	Average of X^2							
		Escherichia coli	*Staphylococcus aureus*	*Salmonella enterica*	*Klebsiella Pneumonia*	*Bacillus cereus*	*Proteus mirabilis*	*Pseudomonas aeruginosa*	*Shigella dysenteriae*
Treatment	12	97**	70.7**	92.4**	63.2**	64.4**	41.1**	50.1**	40.2**
Error	26	4.8	1.6	2.1	1.4	1.4	1.8	3.4	3.8
Cv%	–	15.02	9.83	10.79	9.61	9.06	11.03	17.08	18.16

** Significant at less than 1%. 1% probability level

With increase in concentrations of extracts of urea and salicylic acid, the antibacterial effect of *Physalis alkekengi* extracts on *Staphylococcus aureus, Escherichia coli, Salmonella enterica, Proteus mirabilis, Pseudomonas aeruginosa, Bacillus cereus, Shigella dysenteriae* and *Klebsiella pneumonia* increases in disk and well diffusion methods.

The results of Table 1 shows analysis of variance of the mean diameter of the inhibition zone of *physalis alkekengi* extracts under the influence of foliar application of urea and salicylic acid by well diffusion method. Moreover, it shows that the results of the effect of all groups on the studied bacteria are significant at a probability level of less than 1%.

The results of Table 2 show that by increasing the concentration of foliar application with urea and salicylic acid, the inhibitory rate of *Physalis alkekengi* extract on the studied bacteria in the well method increased. Compared to the control group, significant inhibitory effects were observed for DMSO distilled water and the solvent group at urea concentrations of above 0.5% and at salicylic acid concentrations of above 0.0025mM against *Escherichia coli*, at salicylic acid concentrations of above 0.01mM against

Table 2: The Effect of Urea and Salicylic Acid Foliar Application on the Antibacterial Properties of *Physalis alkekengi* by Well Method.

Treatment		*Escherichia coli*	*Staphylococcus aureus*	*Salmonella enterica*	*Klebsiella Pneumonia*	*Bacillus cereus*	*Proteus mirabilis*	*Pseudomonas aeruginosa*	*Shigella dysenteriae*
Urea	0.25 %	10de	8f	8e	9f	11de	9e	8de	7f
	0.5 %	12de	9f	10de	10ef	12cde	10de	9cde	8ef
	1 %	13d	10ef	12cd	13cd	13cd	12cd	10cde	10cdef
	2 %	14d	12de	14bc	15c	14c	13c	12c	12bcd
SA	0.001 mM	10de	12de	10de	9f	10e	9e	8de	8ef
	0.0025mM	14d	12de	12cd	10ef	11de	10de	9cde	9def
	0.005 mM	18c	14cd	13bc	12de	12cde	12cd	10cde	11cde
	0.01 mM	22b	16c	15b	13cd	12cde	14c	11cd	13bc
Distilled water spray		10de	12de	14bc	8f	11/33de	9e	8de	9def
No spray (control)		10de	10ef	9e	12de	11de	12cd	10cde	10cdef
Amp		22b	21b	24/67a	17b	19b	17b	16b	15b
Gen		26a	24a	25a	24/67a	26a	20a	22a	20a
DMSO		8e	8f	8e	8f	8f	6f	7e	7f

Salicylic acid = SA, Ampicillin = AMP, Gentamicin = Gen, Di Methyl Sulfa Oxide = DMSO, Salicylic acid = SA extract concentrations are reported in parts per million (PPM), average diameter of growth inhibition zone (mm)

Table 3: Analysis of Variance of the Mean Diameter of the Inhibition Zone of *Physalis alkekengi* under the Influence of Foliar Application of Urea and Salicylic Acid by Disk Method.

sov	df	Average of X^2							
		Escherichia coli	*Staphylococcus aureus*	*Salmonella enterica*	*Klebsiella Pneumonia*	*Bacillus cereus*	*Proteus mirabilis*	*Pseudomonas aeruginosa*	*Shigella dysenteriae*
Treatment	12	53.1**	114.8**	44**	20.1**	22.7**	22.9**	21.7**	16.1**
Error	26	2.6	2.5	2.7	5.9	2.2	2.9	2.7	1.7
Cv%	–	12.22	10.2	10.94	23.8	15.41	17.36	16.66	14.8

** Significant at 1% probability level

Table 4: The Effect of Urea and Salicylic Acid Foliar Application on Antibacterial Traits of *Physalis alkekengi* by Disk Method.

Treatment		*Escherichia coli*	*Staphylococcus aureus*	*Salmonella enterica*	*Klebsiella Pneumonia*	*Bacillus cereus*	*Proteus mirabilis*	*Pseudomonas aeruginosa*	*Shigella dysenteriae*
Urea	0.25 %	8e	9gh	10fg	8cd	8cde	7e	8de	6d
	0.5 %	10de	10fg	12ef	10bcd	9bcd	8de	9cde	7cd
	1 %	12cd	12ef	14de	11bcd	10bc	10cde	10cd	8bcd
	2 %	12cd	15d	16cd	12abc	11b	12bc	12bc	9bc
SA	0.001 mM	12cd	14de	14de	8cd	7de	8de	7de	6d
	0.0025mM	14bc	16cd	16cd	9cd	8cde	9cde	8de	8bcd
	0.005 mM	16b	18bc	17cd	10bcd	9bcd	10cde	9cde	9bc
	0.01 mM	16b	19b	18bc	11bcd	10bc	11bcd	10cd	10b
Distilled water spray		12cd	14de	14de	8cd	8cde	8de	9cde	8bcd
No spray (control)		12cd	12ef	14de	9cd	10bc	8de	10cd	10b
Amp		16b	26a	20ab	14ab	14a	14ab	14ab	10b
GEN		24a	28a	22a	16a	16a	16a	16a	15a
DMSO		8e	7h	8g	7d	6e	7e	6.7e	8bcd

Salicylic acid = SA, Ampicillin = AMP, Gentamicin = Gen, Di Methyl Sulfa Oxide = DMSO, Salicylic acid = SA extract concentrations are reported in parts per million (PPM), average diameter of growth inhibition zone (mm).

Staphylococcus aureus, at urea concentrations of above 1% and at salicylic acid concentrations of above 0.01 mM against *Klebsiella pneumonia*, at urea concentrations above 0.5% and salicylic acid concentrations of above 0.005 mM against *Bacillus cereus*, at urea concentrations of above 2% and Salicylic acid concentrations of above 0.01 mM against *Salmonella enterica*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Shigella dysenteriae*. Among the studied groups, significant results were observed for Ampicillin group at salicylic acid concentrations above 0.01 mM due to *Escherichia coli*. However, none of the groups showed significant effects compared to the Gentamicin group. Also, salicylic acid concentration of 0.01 mM had the greatest effect on *Staphylococcus aureus*, *Salmonella enterica* and *Proteus mirabilis*, which were significant compared to the negative control groups but not significant compared to the positive control groups. Among different concentrations of *Physalis alkekengi* extracts, the urea concentration of 2% had the greatest effect on *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Bacillus cereus*, and *Klebsiella pneumonia*, which was significant compared to the negative control groups, but not significant compared to positive controls.

The results of Table 4 show that increase in concentration of foliar application with urea and salicylic acid increased the inhibitory rate of *Physalis*

Table 5: Minimum Inhibitory Concentration (MIC) of Extracts of Ten Treatments of *Physalis alkekengi* by Dilution in Tubes.

Microorganism / Treatment		*Escherichia coli*	*Staphylococcus aureus*	*Salmonella enterica*	*Klebsiella Pneumonia*	*Bacillus cereus*	*Proteus mirabilis*	*Pseudomonas aeruginosa*	*Shigella dysenteriae*
Urea	0.25 %	1250	1250	1250	2500	2500	1250	1250	2500
	0.5 %	625	625	625	1250	1250	625	625	1250
	1 %	156.25	313.5	313.5	1250	625	313	625	625
	2 %	156.25	156.25	78.125	625	313	156.5	313	313
SA	0.001 mM	313.5	313.5	313.5	1250	1250	625	1250	1250
	0.0025mM	313.5	313.5	313.5	625	625	313	625	625
	0.005 mM	156.25	156.25	156.25	625	313	156.5	313	313
	0.01 mM	156.25	78.125	156.25	313	156.5	78.25	156.5	156.5
Distilled water spray		625	313.5	313.5	2500	1250	1250	1250	1250
No spray (control)		313.5	313.5	156.25	2500	1250	1250	1250	1250

Salicylic acid = SA, Ampicillin = AMP, Gentamicin = Gen, Di Methyl Sulfa Oxide = DMSO, Salicylic acid = SA extract concentrations are reported in parts per million (PPM), average diameter of growth inhibition zone (mm).

alkekengi extract on the bacteria in the disk method. Compared to the control group, significant inhibitory effects were found for DMSO, distilled water and solvent treatment in salicylic acid concentrations of above 0.0025 mM against *Escherichia coli*, *Staphylococcus aureus* and *Salmonella enterica*, and in urea concentrations of above 0.25% and salicylic acid concentrations of above 0.001 mM against *Klebsiella pneumonia* and *Bacillus cereus*, were observed. Significant results were observed for *Pseudomonas aeruginosa* at urea concentrations above 0.5% and at salicylic acid concentrations of above 0.005 mM compared to the control group, and for *Shigella dysenteriae* at urea concentrations above 1%, and at salicylic acid concentrations of 0.0025 mM.

A significant inhibitory effect was observed for urea at concentrations higher than 0.5% and salicylic acid at concentrations above 0.001 mM against *Proteus mirabilis* compared to the control and Ampicillin groups. Compared to the ampicillin groups, significant results were obtained in salicylic acid at concentrations above 0.01 mM of *Shigella dysenteriae*, *Pseudomonas aeruginosa, and Bacillus cereus* and at concentrations above 0.005 mM of *Escherichia coli* and *Klebsiella pneumonia* and at urea concentrations of higher than 0.5% in *Klebsiella pneumonia* and in concentrations higher than 1% in *Bacillus cereus* and *Pseudomonas aeruginosa*. In none of the groups the results were significant compared to Gentamicin.

The results of Table 5 show the inhibitory effect of the extracts of different treatments of *Physalis alkekengi* on the studied bacteria, which increases with the increase of foliar application with urea and salicylic acid. Compared to negative and positive control groups, the minimum inhibitory concentration for extracts from urea foliar application was found in *Klebsiella pneumonia*, *Proteus mirabilis* and *Pseudomonas aeruginosa* at urea concentrations above 0.5%, and in *Escherichia coli, Bacillus cereus Shigella dysenteriae* was observed in urea concentrations higher than 1% and in *Staphylococcus aureus* and *Salmonella enterica* in urea concentrations above 2%. Compared to negative and positive control groups, the significant minimum inhibitory concentration of salicylic acid extracts was observed in *Bacillus cereus, Pseudomonas aeruginosa* and *Shigella dysenteriae* at concentrations above 0.0025 mM, in *Klebsiella pneumonia* and *Proteus mirabilis* at concentrations above 0.001 mM, and in *Escherichia coli, Staphylococcus aureus and Salmonella enterica* at concentrations of 0.005 mM

Table 6: Minimum Bactericidal Concentration (MBC).

Microorganism / Treatment		*Escherichia coli*	*Staphylococcus aureus*	*Salmonella enterica*	*Klebsiella Pneumonia*	*Bacillus cereus*	*Proteus mirabilis*	*Pseudomonas aeruginosa*	*Shigella dysenteriae*
Urea	0.25 %	2500	2500	2500	2500	2500	2500	2500	2500
	0.5 %	1250	1250	1250	2500	2500	1250	1250	2500
	1 %	313	1250	625	2500	1250	625	625	1250
	2 %	156.5	313.5	156.25	1250	625	313	313	625
SA	0.001 mM	625	2500	1250	2500	2500	1250	2500	2500
	0.0025m	313.5	1250	625	2500	2500	625	2500	1250
	M 0.005	156.25	625	313.5	1250	1250	313	2500	625
	mM	78.25	313.5	156.25	625	1250	156.5	1250	313.5
	0.01 mM								
Distilled water spray		1250	2500	2500	2500	2500	2500	2500	2500
No spray (control)		625	1250	1250	2500	2500	2500	2500	2500

Salicylic acid = SA, Ampicillin = AMP, Gentamicin = Gen, Di Methyl Sulfa Oxide = DMSO, Salicylic acid = SA extract concentrations are reported in parts per million (PPM), average diameter of growth inhibition zone (mm).

significant results were obtained.

The results of Table 6 show that increase in foliar application with urea and salicylic acid results in increase of lethality. Compared to negative and positive control groups, the minimum bactericidal concentration of urea foliar extracts was observed in *Proteus mirabilis* and *Pseudomonas aeruginosa* at concentrations above 0.5%, in *Escherichia coli, Bacillus cereus, Shigella dysenteriae* and *Salmonella enterica* at concentrations above 1% and in *Staphylococcus aureus* and *Klebsiella pneumonia* at urea concentrations above 2%. Compared to negative and positive control groups, the significant minimum bactericidal concentration of salicylic acid extracts *in Escherichia coli, Proteus mirabilis, Salmonella enterica* and *Shigella dysenteriae* was at concentrations above 0.0025 mM, in the case of *Staphylococcus aureus*, *Bacillus cereus* and *Klebsiella pneumonia* at concentrations above 0.005 mM and in the case of *Pseudomonas aeruginosa* at concentrations above 0.01 mM.

The alcoholic extract of the shoots of Physalis *alkekengi* contains large amounts of alkaloids, flavonoids, lycopene, glucocorticoids, ascorbic acid, alcohol and a large amount of vitamin (C) (4). Among the most important pharmacological activities of flavonoids and alkaloids are their antioxidant and antimicrobial properties (30). The fruit of *Physalis alkekengi* contains a lot of physalin. Physalins are one of the basic constituents of the *Physalis alkekengi* plant, which belongs to the group of tri terpenoids, but, structurally they are not a normal type of steroid, nor a normal type of tri terpenoid (31).

In an alkaloid study, steroids extracted from *Physalis alkekengi* had an antibacterial effect, stopping the growth of four bacteria, *Escherichia coli, Bacillus cereus, Staphylococcus epidermidis* and *Enterococcus faecalis*. The *Physalis alkekengi* showed bacteriostatic and lethal effect on *Bacillus cereus*, in which the values were the same for both indices at 150/g /ml. These results were also observed by disk diffusion method. Although a moderate bacteriostatic effect of the steroid alkaloids of this plant was observed on some bacteria, *Physalis alkekengi* alkaloids had no bactericidal effect on most bacteria in tested amounts (15).

Physalis alkekengi extract has been shown to inhibit the growth of Gram-positive *S.aureus* and Gram-negative *P.aeroginosa* bacteria both *in vitro* and *in vivo* (32).

In another study, the inhibitory effect of *P.peruviana* on bacteria *E.coli, P.aeruginosa, B.megaterium, P.vulgaris, K.pneumoniae, E.aeregenes, C.albicans, C.globrata, C.tropicalis, Trichophyton sp.* and *Epidermophyton sp* was shown (33). Based on the findings of Helvachi *et al*., *P.alkengengi* methanolic extract showed strong antibacterial effects against Gram-positive bacteria. Physalin D (100 mg / ml) also inhibited the growth of *S.epidrmidis, E. faecalis, s.aureus* and *B. subtilis* as methanolic extract at the same concentration. Thus, physalin D may be responsible for the main antibacterial effects of the extract (18).

Silva *et al*., reported that physalin B and physalin derivatives containing physalins B, D, F, and G inhibited the growth of *S. aureus* and *N. gonorrhea* species (34). Also, several antibacterial effects for aqueous, ethanolic and methanolic extracts of *P. angulata* and *P. Philadelphia* for a number of Gram-positive and Gram-negative bacteria have been reported by several authors (35, 36).

In the study conducted by Wajid *et al*., due to the antibacterial effect of different parts of *P.ixocara* extracted with butanolic solvents, ethyl acetate, hexane, aqueous extract and pure extract, it was shown that the extracts obtained from the calyx of the plant had inhibitory effects against *B. Subtilis, E. coli, K. pneumonia*, and *S. aureus bacteria*. Samples of leaf and fruit extracts showed inhibitory effect against *S. Aureus* and *K. pneumonia* and samples extracted from the stem had inhibitory effect against *K. pneumonia* and *E. coli*. Moreover, pure methanolic extract of stem and an-butanolic extract of fruits showed strong inhibitory effects against *K. pneumonia* at the highest concentrations (37).

In several studies, the antitumor, antibacterial and antiviral effects of the compounds in *P.alkekengi* have been investigated. For instance, Frisbey and Fu *et al*., investigated the antibacterial and antitumor effects of the aqueous extract of the *Physalis alkekengi* (38, 39). Alkaloids Steroids extracted from *Physalis alkekengi* also had antibacterial effects in such a way that at a concentration of MIC = 150µg stopped the growth of four bacteria, *E. Coli, S. epidermidis, E. faecalis* and *B.sereus*, (15).

In this study, the inhibitory and lethality effects of compounds in the alcoholic extract of the medicinal plant *Physalis alkekengi* on a number of bacteria were investigated. The results of this experiment showed the inhibitory effect (MIC) and lethality (MBC) of the extract on the studied bacteria. The inhibitory effect of different treatments of urea and salicylic acid on bacteria by dilution in the tube at different concentrations at a probability level of 1% were statistically significant. The same effect was also obtained by disk and well diffusion methods. It was also observed that the inhibitory effect increased with increasing the concentration of alcoholic extract. In the well and disc injection methods, salicylic acid concentrations of 0.01 mM and 2% urea had the greatest effects on the studied bacteria compared to the control groups. The minimum inhibitory and lethal concentrations for the studied bacteria start from 0.5% concentration for urea and from 0.0025 mM for salicylic acid. In determining the MBC/MIC between the tested concentrations compared to negative and positive control treatments, urea concentration of 2% had the highest effect and urea concentration of 0.5% showed the lowest effect in inhibiting the bacterial growth. Salicylic acid showed the highest effect on the growth and inhibition of the studied bacteria at concentrations of 0.01 mM and the lowest effect at 0.001 mM.

The physalins present in extracts of *Physalis alkekengi* plant have several biological properties, the most important of which are the inhibitory effect of F and B physalins on human leukemia cells *in vitro*, the antitumor effect of physalin F on 5 types of human tumor cells (40, 41) and the weak cytotoxicity effect of physaline M extracted from *Physalis alkekengi* on Hela cancer cells (42, 43).

The study on *Physalis alkekengi* showed that physalins are the main active compound of physalis species, and the presence of physaline D in the extracts could be the reason for their antibacterial activity. The lipophilic structure of the poly oxy functional physaline D may be related to the antibacterial mechanism (17).

Since physalins have a steroid-like structure, they can easily cross cell membranes and attach to steroid receptors in the cytoplasm, enter their receptors inside the nucleus, attach to a part of DNA, and interfere with protein or enzyme synthesis and cause abnormalities in fetal growth and development as well as disruption of various embryonic inductions. Antibacterial effects of

Physalines may be due to the structure of physaline F and the presence of epoxy group in it (40, 44, and 45).

Conclusion

The findings of this study indicate that the alcoholic extract of *Physalis alkekengi* has significant antibacterial effects due to its alkaloid and physaline compounds and can be used as a herbal medicine to eliminate pathogenic microorganisms.

Acknowledgment

This study was extracted from a PhD thesis at Miyaneh Branch, Islamic Azad University, Miyaneh, Iran. The research code was 21850251951001.

Conflict of Interest

The authors declare that they have no conflict of interest.

Funding

None.

References

1. Tong H, Liang Z, Wang G. Structural characterization and hypoglycemic activity of a polysaccharide isolated from the fruit of physalis alkekengi L. carbohydrpolym. 2008;71(2):316-23.
2. Vessal M, Fathi, N. and khoshdel, Z. Effect of aqueous extract of physalis alkekengi fruits on the activity of ovarian 3 beta –and 20alpha-hydroxysteroid dehydrogenases in late pregnancy in rat Iranian journal of Medical sciences. 2004;29(4):175-179.
3. Pinstrup-Andersen, P. Food security: definition and measurement. Food Security. 2009;1(1): 5-7.
4. Sadeghihe H.E, Ghiasi I, Mazroughi N. Sabzail S. the hepatoprotctive effects of doremaauchri on carbon tetrachloride induced liver damage in acts. Shahrekord University of Medical Sciences Journal. 2007;9(1):38-43.
5. Brantner A, Chakraborty A. In vitro antibacterial activity of alkaloids isolated from Adhatodavasica Ness. Pharm. Pharmacol. 1998;8:137-139.
6. Pezeshki A, Nourafkan, H, Safari, N. The effect of urea foliar application on morphophysiological traits of Chrysalis alkekengi. World Conference on New Approaches in Agriculture and Environment for Sustainable Development and Safe Production, Shiraz; 2015.
7. Raskin I. Role of salicylic acid in plants. Annu Rev Plant Physiol Plant Mol Biol.1992; 43:439-463.
8. Horváth E, SzalaiG, Janda T. Induction of abiotic stress tolerance by salicylic acid signaling. J Plant Growth Regul. 2007;26:290-300.
9. Szalai G, Krantev A, Yordanova R, Popova LP, Janda T. Influence of salicylic acid on phytochelatin synthesis in Zea maysduring Cd stress. Turk J Bot. 2013;37:708-14.
10. Pál M, Kovács V, Vida G, Szalai G, Janda T. Changes induced by powdery mildew in the salicylic acid and polyamine contents and the antioxidant enzyme activities of wheat lines. Eur JPlant Pathol. 2013;135:35-47.
11. Chen Z, Zheng Z, Huang J, Lai Z, Fan B. Biosynthesis of salicylic acid in plants. Plant Sign Behav. 2009;4:493-6.
12. Alibert G, Boudet, A, Ranjeva, R. Studies on the enzymes catalysts of biosynthesis of phenolic acids in Quercus pedunculata. III. Sequential formation of cinnamic, p-coumaric and caffeic acids from phenylalanine by isolated cell organelles.Physiol Plant. 1972;27:240-3.
13. Hayat Q, Hayat S, Irfan M, Ahmad A. Effect of exogenous salicylic acid under changing environment: A review. EnvironExpBot. 2010;68:14-25.
14. Fraser CM, Chappel C.The phenylpropanoid pathway in Arabidopsis. The Arabidopsis Book. 2011;9:e0152.
15. Talei G, Meshkatosadat M H, Delfan B. Effect of antibacterial steroid alkaloids from three Lorestan medicinal plants on Gram-negative/positive bacteria. Yafte. 2004;6(2):3-9.
16. Jalil Azar A, Farhadi M, Torabzadeh P, Momeni Z. In vitro Effects of alchoholic and aquatic Extract of Chrysalis alkekengi on proliferation of Trichomonas vaginalis. The Iranian Journal of Obstetrics, Gynecology and Infertility. 2017;19(38):48-56.
17. S. Helvacı, G. Kökdil, M. Kawai, N. Duran, G. Duran & A. Güvenç. Antimicrobial activity of the extracts and physalin D from physalis alkekengi and evaluation of antioxidant potential of physalin D, Pharmaceutical Biology. 2010;48(2):142-50.
18. Nathiya M and Dorcus D, Preliminary phytochemical and antibacterial studies on Chrysalis minima Linn., Int J Curr Sci, 2012; pp. 24-30.
19. Donkor A M, Glover RLK, Boateng JK. Antibacterial activity of the fruit extract of Physalis angulata and its formulation, Journal of Medical and Biomedical Sciences. 2012;1(4):21-26.
20. Das K, Tiwari RKS, Shrivastava DK. Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. Journal of Medicinal Plants Research. 2010;4(2):104-11.
21. Murray, P., Baron, R., Fauer, E., Tenoyer, M. Manual of clinical Microbiology 7ed, American scciety for Microbiolgy; 1999. p. 1567-70.
22. Scorzoni L, Benaducci T, Almeida AMF, Silva DHS, Bolzani VS, Mendes-Giannini MJS. Comparative study of disk diffusion and microdilution methods for evaluation of antifungal activity of natural compounds against medical yeasts Candida spp and Cryptococcus sp. Journal of Basic and Applied Pharmaceutical Sciences. 2007;28(1):25-34.
23. Vanden DA, Vlietinck AJ, In Dey, Harborne JB. Methods in plant biochemistry: screening methods for antibacterial and antiviral agents from higher plants. London: Academic Press. 1991;47-69.
24. Sindambiwe B, Calomme M, Cos P, Totte J, Pieters L, vlietinck A. Screening of seven selected Rwandan medicinal Plants for antimicrobial and antiviral activates. Journal Ethnopharmacol. 1999;65(1):71-7.
25. Andrews JM. Determination of minimum inhibitory concentrations. Journal of Antimicrobial Chemotherapy. 2001;48:5-16.
26. Baner AW, Kirby WMM, Sherries JC, Truck M. Antibiotic susceptibility testing by a stand-ardized single disc method. Am J ClinPathol. 1991;45:493-6.
27. Mangena T, Muyima NY. Comparative evaluation of the antimicrobial activities of essential oils of Artemisia afra, Pteroniaincana and Rosmarinus officinalis on selected bacteria and yeast strains. Lett ApplMicrobiol. 1999;28(4):291-6.
28. Declercq P, Neef H, Laekeman G. Hypoglycaemic activity of selected European plants. Phytother Res. 1995;9:45-8.
29. Platikanov, S., Evstatieva, L., Nikolov, S. Dinamic of acumulation of the flavonoids in Astragalus hamosus L. Proceeding of the 7th Symposium on Flora of Southeastern Serbia and

Neighbouring Regions. Dimitrovgrad. 2002;214-6.
30. Harborne, J.B: Phytochemical methods. 3th ed. Chapman and Hall, London, 1978.
31. Zunpeng S, Na X, Qiuhong W, Xinli L, Bingqing X, Zhenyu L, Haixue K. Antibacterial and Anti-Inflammatory Activities of physalis Alkekengi var. franchetii and Its Main Constituents. Hindawi Publishing Corporation Evidence-Based Complementary and Alternative Medicine. 2016; Article ID 4359394.
32. GöztokFerda, ZenginFikriye .The antimicrobial activity of Physalis peruviana L. BitlisErenUniv J Sci& Technol. 2013;15-7.
33. Silva MTG, Simas SM, Batista TGFM, Cardarelli P, Tomassini TCB. Studies on antimicrobial activity, in vitro, of Physalis angulata L. (Solanaceae) fraction and physalin B bringing out the importance of assay determination. Mem Inst Oswaldo Cruz100. 2005;779–82.
34. Caceres A, Alvarez AV, Ovando AE, Samayoa BE. Plants used inGuatemala for the treatment of respiratory diseases. 1. Screening of 68 plants against Gram-positive bacteria. J Ethnopharmacol. 1991;193–208.
35. Shim JS, Kyung-Min P, Chung JY, Hwang JK. Antibacterial activity of oleanolic acid from Physalis angulata against oralpathogens. Nutr Food 7. 2002;215–8.
36. Wajid Khan, JehanBakht and Mohammad Shafi. Antimicrobial potentials of different solvent extracted samples from Physalis ixocarpa. Pak. J. Pharm. Sci., Vol.29 (2). 2016;467-75.
37. Frisbey A, Rober JM, Jennig, JC, Gollshall RY, Lucas EH. Mich. Agric Exp. Sta quart Bull. 1953;392.
38. Fu Y, Zhu F, Ma Z, Lv B, Wang X, Dai C, et al. physalis alkekengi var. franchetii Extracts Exert Antitumor Effects on Non-Small Cell Lung Cancer and Multiple Myeloma by Inhibiting STAT3 Signaling. Onco Targets Ther. 2021;12(14):301-14.
39. Chiang HC, Jaw SM, Chen CF. Antitumor agent, physaline F from physalis alkekengi L., Anticancer Res. 1992;12(3):837-43.
40. Chiang, H.C., Chen, P.M. (1992). Inhibitory effect cells in vitro. Anticancer research. 12;1155-62.
41. Kawai O, Nakaishi M, Matssura T. Structure of physaline M isolated from physalis alkekengi Varfrencheti Bull. Chem. Soc. Jpn. 1998;61:2696-8.
42. Hong JM, Kwon OK, Shin IS, Song HH, Shin NR, Jeon CM, et al. Anti-inflammatory activities of physalis alkekengi var. franchetii extract through the inhibition of MMP-9 and AP-1 activation. Immunobiology. 2015;220(1):1-9.
43. Wang A, Wang S, Zhou F, Li P, Wang Y, Gan L, et al. Biomed Pharmacother. Physalin B induces cell cycle arrest and triggers apoptosis in breast cancer cells through modulating p53-dependent apoptotic pathway. 2018;101:334-41.
44. Kawai M, Yamamoto T, Makino B, Yamamura H, Araki S, Butsugan Y, et al. The Structure of Physalin T from physalis alkekengi var. francheti. Journal of Asian natural products research. 2001;3:199-205.