



Evaluation of Antibacterial Activity of Aqueous Extracts of Onion and some Antibiotics on a Number of Important Bacteria in Terms of Food Hygiene

Younes Anzabi¹*

Abstract

Objective: The purpose of this study was to evaluate the antibacterial effect of edible onion plant and a number of common antibiotics in the case of some important bacteria regarding food hygiene.

Materials and Methods: The sensitivity or resistance of standard strains of 9 important species of transmissible pathogenic bacteria, through food in laboratory Mueller Hinton agar medium and using blank paper discs containing onion extract, 9 standard synthetic chemicals, and antibiotics by agar disk diffusion method (disk diffusion agar), were investigated.

Results: The findings of this study showed that, of the 9 species of bacteria tested, the aqueous extract of onion only has relatively small antibacterial activity on the 2 species of *Staphylococcus aureus* and *Clostridium perfringens*. Statistical analysis of the results also indicated that there was no significant relationship among the different antibiotics used and the edible onion aqueous extract, and the resistance or susceptibility of isolates. Moreover, there was a difference between different antibiotics tested in this study and aqueous extract of onion, regarding the number of resistant bacteria, and intermediate and moderate susceptibility, and susceptibility to the antibacterial compounds.

Conclusion: It seems that the aqueous extract of onions cannot be used as an alternative to commonly used antibiotics to fight important bacteria in terms food hygiene.

Keywords: Antibacterial Effect, Aqueous Extract of Onion, Antibiotics, Pathogenic Bacteria, Food Hygiene

Introduction

One of the methods used today for controlling pathogenic microorganisms in food is using manmade chemical preservatives. However, the use of these chemicals in food has always been a concern, because the general belief among people is that antimicrobial chemicals may threaten their health. For this reason, the use of natural ingredients instead of chemicals has particular importance. Therefore, it seems that the use of alternative herbal extracts and essential oils can be very suitable for this purpose. Plant extracts contain substances that can be used against many microorganisms. Their antimicrobial activity against bacteria, yeasts, and fungi has been demonstrated (1-3).

Given the important role of antibiotics in the treatment of many diseases, the development of antimicrobial drugs is one of the most important advances in treatment procedure. Herbal medicine, due to its natural origin, is more consistent with body organisms and its complications are rare compared with chemical drugs (4). Search for new effective antibacterial agents among plant extracts and to discover new chemical structures which overcome the disadvantages mentioned above is in progress (5). Much attention has been paid to antimicrobial compounds with natural origin due to the Generally Recognized as Safe (GRAS) criteria, and their nutritional effects and impact on health (6,7).

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¹ Department of Pathobiology, Tabriz Branch, Islamic Azad University, Tabriz, Iran

*Corresponding Author: Younes Anzabi, Department of Pathobiology, Tabriz Branch, Islamic Azad University, Tabriz, Iran
Tel: +98 9141170856, Email: anzabi@iaut.ac.ir

Accordingly, edible plants, medical plants, and their derivatives (oils, herbal extracts, and hydrocele), due to their diverse and potent antimicrobial compounds, are largely used to avoid the growth of pathogenic bacteria and fungi. Antimicrobial compounds of plants can be found in extracts taken from different parts of the plant including leaves, flowers or buds, roots, seeds, rhizomes, fruits, and other parts. Essential oils (volatile or ethereal oils) are aromatic liquids that are obtained from different parts of plants. Considering the above, more than 340 plant species are identified with antimicrobial effects and more than 30 thousand phenolic compounds with antimicrobial properties of essential oils have been isolated (8,9).

In this regard, several studies have shown that terpenoids, the flavonoids kaempferol and quercetin, and alkylcysteine sulfoxides in the edible onion extract are the most important chemical compositions of antioxidants, disinfectants, and anti-inflammatory compounds, and as active and natural anti-pathogens. Moreover, they show antibacterial activity against many microorganisms both in vitro and clinical cases of some infectious diseases (10,11). Furthermore, for this purpose, the antibacterial activity of essential oils and plant extracts against pathogenic bacteria with food source, in media and in food, or food models has been studied in some researches (12,13).

Iran, in terms of climate and geographical location, is one of the best places in the world for medicinal plant growth, and in the past it had been the source of production and consumption of medicinal plants. Therefore, in this study, edible onion plant (scientific name: *Allium cepa* L.), which is considered a native plant in Iran and the Ilkhchi region of East Azerbaijan province, North West of Iran, and the antibacterial effect of its aqueous extract were examined on some of the standard strains of important bacteria in terms of food hygiene including *Staphylococcus aureus* (PTCC1112), *Enterococcus faecalis* (NCTC8213), *Bacillus subtilis* (PTCC1254), *Clostridium perfringens* (ATCC13124), *Listeria monocytogenes* (ATCC19114), *Escherichia coli* (PTCC1270), *Salmonella enterica* (CIP104115), and *Yersinia enterocolitica* (PTCC1151). They were purchased from the Iranian Research Organization for Science and Technology (IROST) in Tehran, Iran, and were maintained in the laboratory refrigerator of the Department of Microbiology, School of Veterinary Medicine of Islamic Azad University of Tabriz, Iran. In addition, in the present study, the antibacterial activity of some standard and commonly used antibiotics against any one of the aforementioned bacteria were simultaneously studied and compared with the antibacterial activity of aqueous extract of onion.

Materials and Methods

1. Preparation of the extract of onion: The liquid extract (water extract) of onion was obtained through squeezing the onion. Note that this extract

was freshly made in any one of the performed experiments (14).

2. Microbial suspension preparation: In this study, the standard strains of bacteria mentioned above were used which were provided from the Iranian Research Organization for Science and Technology (IROST). Microbial suspension preparation requires 24-hour culture of the bacterium. Therefore, 24 hours before each experiment, the stock bacteria were inoculated on nutrient agar medium and were incubated for 24 hours at 37 °C. The pure colonies formed on the surface of the culture medium were washed with normal saline solution in order to form a suspension. Then, bacterial suspension was diluted with normal saline so that the turbidity was equivalent to the McFarland turbidity standard set in tube No. 0.5, such that the suspension used contained 1.5×10^8 CFU/ml of the considered bacteria (15,16).

3. Antibacterial effects of aqueous extract of onion: In order to study the antibacterial effects of the extract, disk diffusion agar method was used. For this purpose, the extract was prepared using dimethyl sulfoxide and methanol solvent (in a ratio of 60 to 40) in sterile tubes with 10 micro grams per milliliter dilution. Then, blank sterilized disks, manufactured by Padtan Teb Company (Tehran, Iran), were placed in tubes containing prepared dilution from the extract. After a period of about 30 minutes when the disks absorbed the entire contents of the tube and were saturated, the prepared disks were placed in 37 °C temperature so that they were completely dried, and then used in the agar diffusion test (17,18).

Therefore, 100 ml of the suspension of each tested bacteria with a turbidity equivalent to McFarland turbidity standard tubes 0.5 was separately spread on the plate of Mueller Hinton agar medium and uniformly cultured. In the next step, using sterile forceps, the disks impregnated with the studied extract and the standard discs of antibiotics used, regarding each of the bacteria obtained from Padtan Teb Company, were placed on the surface of the culture medium at a certain space from each other on the edge of the plate and were fixed with a little pressure on the medium. Plates were then incubated for 24 hours at 37 °C, and the results of antibacterial activity were recorded by measuring the diameter of the zone of inhibition around the discs using calipers. To be certain of the results, this experiment was repeated 3 times in each case. The mean diameter of the zone of inhibition during the 3 times was recorded as the final diameter (antibacterial power of the plant extract and tested antibiotics according to the standard table of the antibiogram test of the mentioned company) (19,20). It should be noted that in all the stages of this experiment, blank disks impregnated with 10 micro liters of dimethyl sulfoxide and methanol solvent (in a ratio of 60 to 40) were used

as the control of the extract.

Statistical analysis: To compare the mean diameter of the zone of inhibition of the tested bacteria against onion extract and standard antibiotics, independent t-test and chi-square test were used

Results

The results of the experiments conducted in this study are shown in table 1.

The antibiogram test was performed on each bacterium using 5 types of common antibiotics effective on that bacterium. Therefore, a total of 9 types of antibiotics were used in this study, but not every antibiotic was used on every bacterium. Because not all types of antibiotics are used against all types of bacteria. For example, a group of antibiotics are used against gram-positive bacteria and another group of antibiotics are used against gram-negative bacteria.

In agar disc diffusion test, the comparison of inhibition zone related to the aqueous extract of Onion with that related to standard antibiotics against the tested bacteria did not show a statistically significant difference ($P < 0.05$, Subset for $\alpha = 0.05$).

Discussion

Nasim et al. in 2012 showed that the disk diffusion method of aqueous extract of fresh spinach has little inhibitory effect on *Proteus vulgaris*, *Micrococcus luteus*, and *Klebsiella pneumoniae*. In addition, old aqueous extract of spinach has no effect on the growth of *Salmonella typhimurium*, *Staphylococcus aureus*, and *Micrococcus luteus*, and fresh and old extracts of spinach leaves have a significant deterrent effect on *Escherichia coli*. They also revealed that the ethanol extract of fresh spinach leaves by disk diffusion method significantly inhibits the growth of *Salmonella typhimurium* (21). In the present study, the agar disk diffusion test on aqueous extract of onion showed only little antibacterial activity on gram-positive bacteria of *Staphylococcus aureus* and *Clostridium perfringens*. According to table 1, the diameters of the zone of inhibition for the mentioned bacteria were recorded, respectively, as 15 and 10 mm. While other gram-positive bacteria tested in this study (namely *Bacillus cereus*, *Bacillus subtilis*, *Enterococcus faecalis*, *Listeria monocytogenes*), and 3 species of gram-negative bacteria (*Escherichia coli*, *Yersinia enterocolitica*, and *Salmonella enterica*) were quite resistant to the presence of aqueous extract of onion. These results were inconsistent with the results of the study by Nasim et al. (21) regarding the tested bacteria, but were consistent regarding the fact that aqueous extract of onion had no specific antibacterial activity on most of the tested bacteria. The main cause of this problem is the lack of appropriate performance of aqueous extract of onion in terms of the antibacterial effect of the plant compared to other types of plant extracts. Some similar researches suggest the same point regarding other edible plants. For example, Ahmad and Aqil, in a study in 1998,

concluded that neither the old nor new n-hexane extract of spinach leaves show a significant result compared with ethanol and aqueous extracts. They also showed that active components of the extract were widely dependent on the type of solvent used and the method used for extraction (22).

Parekh and Chanda performed a research in 2007 using 34 species of medicinal plants in India on 3 different strains of *Staphylococcus aureus*. They found that the antibacterial effect of the alcoholic extract of all the plants in the study was more significant than that of their aqueous extract (23). In another study by Babic et al., the inhibitory effect of the extract of dried spinach powder on species of *Listeria monocytogenes* was studied. It was found that the active components of the extract were likely to affect the growth of *Listeria monocytogenes* (24). Based on the study by Dubey et al. which was performed by disk diffusion method, the most sensitive bacteria to spinach extract was *Escherichia coli* and the minimum diameter of the inhibition was related to *Bacillus subtilis*. They reviewed the glycoproteins purified lipid percentage in aqueous and methanol extracts and concluded that the glycolipid and glycerolipids content of methanol extract of spinach is higher than its aqueous extract. Moreover, the crude tannin isolated from the methanol extract was more than that from the aqueous extract (25). Furthermore, according to a survey conducted by Evanjelene and Natarajan, it was found that compounds such as flavonoids and steroids exist in aqueous extract of spinach and phenols, saponins, tannins, amino acids, and aldehyde group (CHO) exist in its alcoholic extracts. They showed that the alcoholic extract of spinach solves more active ingredients. Therefore, it has higher antimicrobial effect than the aqueous extract (26). It was also shown that spinach has a significant amount of flavonoids and terpenes, and these compounds have an inhibitory effect on the activity of polymerase in prokaryotes (12,27). These materials can also affect the cell wall of gram-negative bacteria on the outer membrane of the lipopolysaccharide layer and in this way the cell walls are destroyed and the gram-negative bacteria are prevented from multiplying (28).

The alcoholic extract of spinach, due to the presence of larger amounts of glycolipid and glycerolipids compared to its aqueous extract, can show strong antibacterial effects. The presence of a high percentage of flavonoid and terpene compounds, polyunsaturated fatty acids, and minerals were probably one of the most important inhibiting factors of spinach on *Escherichia coli* gram-negative bacteria (26). In this study, due to the high level of and the type of antibacterial active components of plants in products and different extracts of plants, the aqueous extract of plants have been emphasized on. Therefore, this matter can be the cause of the lack of antibacterial effect of edible onion aqueous extract.

However, regarding the antibacterial effect of plants discussed in this study, several studies in the past have shown that edible onion extract has antimicrobial effect on *Escherichia coli* bacteria,

Table 1. The diameter of the growth of bacteria against antibiotics and onion extract is recorded in millimeters

Antibiotic used	Penicillin	Vancomycin	Tetracycline	Streptomycin	Gentamycin	SXT	Oxytetracycline	Nalidixic acid	Ciprofloxacin	Aqueous extract of onion	Growth inhibition diameter
Staphylococcus aureus	25	17	23	19	-	-	-	-	13	15	The diameter of the inhibition
	S	S	S	S	-	-	-	-	I	S	Sensitivity or resistance of bacteria compared to tested antibiotics
Escherichia coli	-	-	10	-	34	25	0	30.5	-	0	The diameter of the inhibition
	-	-	R	-	S	S	R	S	-	R	Sensitivity or resistance of bacteria compared to tested antibiotics
Yersinia enterocolitica	-	-	20	-	24	40	17	30	8	0	The diameter of the inhibition
	-	-	S	-	S	S	I	S	-	R	Sensitivity or resistance of bacteria compared to tested antibiotics
Bacillus subtilis	12	20	23	24	-	-	-	-	30	0	The diameter of the inhibition
	R	S	S	S	-	-	-	-	S	R	Sensitivity or resistance of bacteria compared to tested antibiotics
Enterococcus faecalis	0	15	0	0	-	-	-	-	0	0	The diameter of the inhibition
	R	R	R	R	-	-	-	-	R	R	Sensitivity or resistance of bacteria compared to tested antibiotics
Bacillus cereus	0	20	30	30	-	-	-	-	28	0	The diameter of the inhibition
	R	S	S	S	-	-	-	-	S	R	Sensitivity or resistance of bacteria compared to tested antibiotics
Listeria monocytogenes	0	20	0	0	-	-	-	-	0	0	The diameter of the inhibition
	R	S	R	R	-	-	-	-	R	R	Sensitivity or resistance of bacteria compared to tested antibiotics
Salmonella enterica	-	-	13	-	20	24	13	22	-	0	The diameter of the inhibition
	-	-	R	-	S	S	R	S	-	R	Sensitivity or resistance of bacteria compared to tested antibiotics
Clostridium perfringens	28	32	35	0	-	-	-	-	35	10	The diameter of the inhibition
	S	S	S	R	-	-	-	-	S	I	Sensitivity or resistance of bacteria compared to tested antibiotics

SXT: Co-Trimoxazole (Trimethoprim/Sulphamethoxazole); S: Susceptibility of bacteria to antibiotics and onion extract are shown with the letter; R: Resistance with the letter; I: Semi-sensitivity is shown with the letter

Bacillus subtilis, *Salmonella typhi*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*. These properties in the mentioned plant have been attributed to their abundant content of antimicrobial compounds, such as terpenoids and flavonoids, and some volatile sulfur compounds with a pungent odor (11,29-31). In the study by Razavi Rohani et al., it was shown that organic onion extract has anti-listerial effects (32). On the other hand, research has shown that raw extracts of onion were effective on *Pseudomonas aeruginosa*, had weak effect on *Candida albicans*, and had no effect on *Staphylococcus aureus* and *Escherichia coli*. While hot water extract of onion had no antimicrobial effect on any microorganisms (11,31). Note that only a portion of the findings of recent research were similar with the results of the present study, and mainly discordance between these research findings and the present research is evident.

The results of the studies by Dankert et al. (33) and Momeni and Zamanzad (11) which were very similar, show that onion extract had little effect on *Listeria monocytogenes*. The present study results were consistent with this finding. Some researches had declared that some plants' methanol extracts, aqueous extract, and also some of their ether extracts had a clear effect on gram-positive bacteria. Given the abovementioned findings, it can be stated that the main reasons for the lack of antibacterial effect of onion extract in the present study and some other studies (whether in the case of gram-positive bacteria or gram-negative bacteria) can be the type of effective materials in the extract or essence, solvent type, and extraction methods. In fact, according to research findings, it seems that the type of extract is very effective in the antibacterial activity of edible onion (34-38). Some researchers have also reported that the comparison of the observed results regarding the antibacterial properties of plant extracts is very difficult. The reason for this can be the various methods used in different laboratories for studying the antibacterial properties of essential oils, strains, and species of plants and their sources, stages of plant growth, and the bacterial strains used (8). On the other hand, this may be due to differences in the plants of each region. One type of plant in different regions may display different compositions and properties (38).

Even for this specific case, studies have been carried out and the outcomes often showed that the type and preparation method of herbal extract can have an important role in the results of experiments on antibacterial properties of plants. For example, in 2006 the study by Chitsaz showed that despite the antimicrobial effects of *Berberis* plant extracts, no antimicrobial activity was reported for the aqueous and boiled extract of this plant (39). Considering some differences in the findings of this study and other similar studies, it seems that, in addition to type, herbal extract preparation method can also have an important role in the results of experiments on the

antibacterial properties of plants.

In the present study, it was observed that antibacterial effects of onion extract, in comparison with the effect of 9 antibiotics, were much lower (Table 1). Recent findings differed from the findings of the study of Nair et al. on the antimicrobial effects of *Nigella sativa* plant on *Listeria monocytogenes* in culture medium. Because in the study by Nair et al, it was found that anti-listeria effects of *Nigella sativa* were very strong and even more effective than the gentamicin antibiotic (40). The inconsistency of the results in the studies mentioned can be explained by the reasons mentioned above and the results of various studies cited on the antimicrobial effects of various plants, especially the edible onion.

Conclusion

Based on the statistical analysis of the results and findings of the study using chi-square test, it was shown that no significant relationship existed between different antibiotics and edible onion extract used in this study, and resistance or susceptibility of isolates. Furthermore, using the reviews and Students' independent t-test the difference between the different antibiotics used in this study as one group and the edible onion extract tested as another group, regarding the 3 characteristics that this study was testing, meaning the resistance of the bacteria, moderate resistance (semi-susceptible or intermediate state) of bacteria, and the sensitivity of bacteria to substances or compounds with antibacterial effects was studied. This showed that there was a difference between the antibiotics groups and plant extracts regarding the number of resistant bacteria, and moderate resistance and susceptibility. Therefore, it is concluded that the edible onion extract cannot be used as an alternative to synthetic chemical antibiotics used in this study to deal with the bacteria tested in the present study that include a large number of important bacteria regarding food hygiene. In fact, based on the results of this study and similar research, it can be argued that edible onion extract cannot be used as a good antibacterial compound, although the organic extracts (alcohol, ether, and etc.) of this edible plant have shown significant antibacterial effect on some microorganisms. Therefore, it seems that more research is needed to examine this issue more closely and determine the antimicrobial effects of the edible onion plant. Thus, maybe by using other methods of extraction or serum concentrations other than the one used in this study, the antibacterial effects of onion extract can be explained more clearly. Moreover, with further studies on the organoleptic properties of this extract, it can be used as a coating material for different food types.

Ethical issues

We have no ethical issues to declare.

Conflict of interests

We declare that we have no conflict of interests.

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