Antibacterial effects of the aqueous extract of *Lycopersicon esculentum mill* native in South Khorasan of Iran against four species associated with gastrointestinal infections

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Abstract

Introduction: Raising antibiotic resistance has led the human community to more frequent application of herbal medicines. The tomato fruit, scientifically called *Lycopersicon esculentum mill*, is an important source of vitamins, minerals, and antioxidants, all with beneficial effects on human health. The present study aimed to evaluate the antibacterial effects of tomato aqueous extract on four important bacterial species associated with gastrointestinal infections.

Materials and methods: After preparing the aqueous extract of native tomato, its minimum inhibitory concentration (MIC) was determined against *Staphylococcus aureus* (*S. aureus*) (ATCC116538), *Klebsiella pneumonia* (*K. pneumonia*) (ATCC700603), *Shigella flexneri* (*S. flexneri*) (ATCC12022), and *Proteus vulgaris* (ATCC6380) species using the microdilution broth method based on the Clinical and Laboratory Standards Institute (CLSI) guidelines. Each of the experiments was repeated three times. One-way Analysis of Variance (ANOVA) was used for examining normal variables.

Results: The results of this study showed that the most significant effect of the tomato extract is on *S. aureus.* The MIC of tomato extract for *S. aureus* was 31.25 mg/ml, while for *Proteus vulgaris*, *K. pneumoniae*, and *S. flexneri* was 62.5 mg/ml. The mean MIC of the extract was significantly different between the studied bacteria ($P < 0.05$).

Conclusion: The current study revealed a relatively significant antibacterial effect of tomato extract against different bacterial species and more studies are needed in this area

Keywords: *Lycopersicon esculentum mill*, Antibacterial effect, Micro dilution broth

Introduction

Infectious diseases are among the most common infections worldwide, incurring huge costs on human societies. While the synthesis of antibiotics has been a vital contributor to the treatment of infectious diseases in recent decades, antibiotic resistance problems in bacteria have led the human community to a more frequent application of herbal medicines (1). The increasing consumption of medicinal herbs

for the treatment of diseases in Iran can be attributed to their fewer side-effects compared to chemical drugs and the suitable climatic and geographic conditions of the country for the cultivation of various medicinal plants. Medicinal herbs are the primary source of pharmaceutical products worldwide. With more than 7,500 plant species, Iran is one of the main areas of biodiversity and the natural habitat of over 900 medicinal herbs (2).

Among medicinal herbs, tomato (*Lycopersicon esculentum mill*), known primarily as a fruit in human food basket, has been suggested as a new field of research. Advances in medicine, botany, agriculture, and traditional medicine have demonstrated the medicinal properties of this herb (3). Scientists have proposed numerous therapeutic properties for the tomato upon discovering compounds, such as carotenoids (especially lycopene), ascorbic acid, vitamin D, folate, flavonoids, potassium, solanine, saponin, and other compounds in this fruit. Among medicinal benefits, it is supposed that tomato reduces risks of cancer, particularly lung and prostate cancers, and cardiovascular diseases, as well as analgesic properties, especially for joint pain, kidney stone, and mouth diseases (4-6). Despite numerous studies on therapeutic properties of tomatoes, an inquiry into international research data banks shows that investigations on antibacterial effects of this fruit is still a matter of debate and more researches need to be done. On the other hand, according to the Food and Agriculture Organization (FAO) of the United Nations, Iran is among the first ten country of tomato producers and consumers. Then, the plant is of a high economic significance in Iran (7).

Hence, discovering antibacterial effects of this native herb may encourage the pharmaceutical industry to use it in the production of antimicrobials. One of the methods to use medicinal plants, especially the tomato, is to extract the plant (8). Some studies have shown that the tomato extract contains a variety of active bio-substances, such as gamma-aminobutyric acid, lycopene, and other compounds. These components have revealed anticancer and antioxidant properties and are contributory to the reduction of cardiovascular disease, mental disorders, osteoporosis, and harmful cholesterol to the body. They can also prevent constipation, inhibit the formation of kidney and bile stones, and improve skin and hair health (9, 10). The present study aimed to investigate the antibacterial effects of native tomato extract of Iran on four important bacterial species associated with gastrointestinal infections.

Materials and methods

Preparation of the extract

The aqueous method was used to prepare the tomato extract. Three kilograms of fresh tomato (*Lycopersicon esculentum mill*) native to Birjand (South Khorasan province, Iran) were collected, washed and mixed with a blender. Then, it was filtered through a cloth filter. The filtered tomatoes were put in a freezer at -80 °C. In the next stage, the filtered tomatoes were split into four dishes of the Freeze Dryer apparatus (Dena Vacuum Industry, model FD-5005-BT, Iran) to initiate the extraction process at -45 °C.

Experimental design

This study was conducted in Birjand University of Medical Sciences, Birjand, Iran (Ethics code: Ir.BUMS.REC.1395.163). An experimental study was performed to evaluate the antibacterial effects of tomato extract on four bacterial species associated with common gastrointestinal infections, including the standard strains of *S. aureus* (ATCC 116538), *K. pneumonia* (ATCC 700603), *Shigella flexneri* (ATCC 12022), and *Proteus vulgaris* (ATCC 6380). These

strains were obtained from Microbial Collection Department of Pasteur Institute of Iran.

Determining the minimum inhibitory concentration of growth

In this study, the minimum inhibitory concentration (MIC) of the tomato extract for the four standard bacterial species was investigated by the microdilution broth method. MIC is defined as the lowest concentration of the extract that inhibits the bacterial growth in a bacterial suspension. The method was conducted based on the Clinical and Laboratory Standards Institute (CLSI) guidelines (11). In brief, in the microdilution broth method, an 18-hour culture of the bacterium was done on the Muller Hinton Agar medium (Merck Co., Germany) for isolation of bacterial colonies. Then, a bacterial suspension equivalent to 0.5 McFarland's standard ($1.5*10^8$ CFU/mL) was prepared in physiological serum. Suspensions with a final concentration of 5×10^5 (dilution in the Muller Hinton Broth medium) were added to the tomato extract dilution sera in a 96-well microplate. Afterward, the microplate was placed on a shaker for 30 seconds until a perfect uniform mixture was obtained. The optical absorption was then read using an ELISA reader device in 620 nm wavelength. The plates were incubated at 37 °C for 24 hours. After the incubation period, the presence of spots in wells was examined by naked eyes, and the ELISA reader device measured the optical absorption. It should be noted that a broth culture medium containing only the extract was used as a negative control, and the bacterial suspension in the culture environment without the extract was taken as a positive control.

The obtained data were analyzed using SPSS.v.18. One-way Analysis of Variance (ANOVA) was used for examining normal variables. The significance level was considered when P values were less than 0.05.

Results

The results of this study showed that the highest antibacterial effect of tomato extract was on *S. aureus* with MIC value of 31.25 mg/mL. There was a significant difference between the MIC of tomato extract among the studied bacteria ($P < 0.05$). The MIC values of tomato extract for four studied bacteria are shown in Table 1.

Table 1. Results of the antimicrobial effect of the aqueous extract of tomato.

Bacteria	MIC (mg/ml)	P-Value
S. aureus	31.25	$P < 0.05$
S. flexinari	62.5	$P < 0.05$
K. pneumoniae	62.5	$P < 0.05$
P. vulgaris	62.5	$P < 0.05$

The data were analyzed by ANOVA, and the different letters indicate statistically significant difference ($P < 0.05$).

Discussion

This study aimed to investigate antibacterial effects of tomato extract on four important bacterial species associated with gastrointestinal infections. The results indicated that the extract of the tomato native to the Southern Khorasan region has a relatively significant antibacterial effect.

According to these results, the highest susceptibility to the aqueous tomato extract was found for *S. aureus*, and the lowest antibacterial effect of this extract was observed identically in *P. vulgaris*, *S. flexneri*, and *K. pneumoniae*. These findings confirmed the results of previous studies concerning the antimicrobial effects of tomato extract. In a study conducted by Pavlovic *et al.* on antimicrobial effects of ethanolic extraction of tomato by the microdilution method, the results showed that the extract had the highest impact on *Aspergillus niger* (MIC, 19.53 µg / ml),

Bacillus subtilis and *S. aureus* (MIC, 39.1 µg / ml) from among the eight bacterial and fungal strains (12). In another study, phytochemical, antioxidant, antimicrobial, and anti-inflammatory effects of aqueous tomato extract were investigated against *Streptococcus pneumoniae*, *S aureus*, *Escherichia coli*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* by dilution method. The results showed that the MIC of the tomato extract for *S. pneumoniae*, S. aureus, *E. coli*, *P. mirabilis*, and *P. aeruginosa* was comparable to the antibiotic ciprofloxacin (13). The results of these studiesare not consistent with our findings. This difference can be related to the indignity of the tomato strain, the type of extract, and the type of standard bacterial strain examined in our study. AL-Oqaili *et al.* compared the antibacterial effects of tomato extract in the forms of aqueous and diluted honey extracts on a set of pathogenic bacteria, such as *P. aeruginosa*, *Acinetobacter spp.*, and *E coli*. The results showed that the anti-bacterial effect of tomato diluted with honey is higher than that of the aqueous extract for all the studied strains (14).

Despite the antibacterial effects of tomato extract native to the southern Khorasan region, the question is which of its compounds is associated with the antimicrobial effects? Studies have shown that the tomato is among the herbs whose antimicrobial and anti-inflammatory effects may be due to the presence of lycopene, diphenesin-like protein, gamma-thionine, and lipid transfer-like proteins (15-17). Aiming to find antimicrobial peptides in extracts of the rapeseed leave (canola strain), Tomato solanum, and Tetragonia tetragonioides (New Zealand spinach) via mass spectrometry, Neubauerova *et al.* showed two proteins (NP24 and TPM-1) with antimicrobial activity in water-loving part of tomatoes (18). In another study, lycopene was isolated from tomatoes to test its antimicrobial effect against *Bacillus subtilis*. The study showed that lycopene can be a significant contributor to anti-bacterial effects of this fruit (19). Considering inconsistent findings of previous studies regarding the role of different compounds of tomato in inducing its antimicrobial effects, it seems that conducting more detailed researches on this field is helpful. Furthermore, it is supposed that the cytotoxic effects of the tomato extract might be usable for clinical application. The purification and use of antimicrobial compounds of Iranian tomato can ultimately be used as supplements in native mouthwashes or as additives in traditional healthy foods.

Conclusion

Native tomato grown in Southeast of Iran has relatively significant antibacterial effects and more studies are needed in this area.

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Conflict of interests

There is no conflict of interests to declare.

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References

1. Ateş DA, Turgay Ö. Antimicrobial activities of various medicinal and commercial plant extracts. Turk J Biol. 2003 Sep 4;27(3):157-62.
2. Akhani H, Djamali M, Ghorbanalizadeh A, Ramezani E. Plant biodiversity of Hyrcanian relict forests, N Iran: an overview of the flora, vegetation, palaeoecology and conservation. Pak J Bot. 2010;42(Special Issue): 231-58.
3. AbrishamChi P, Ijtehadi H, AdibiBidi F. Investigation of Phytochemical and Antibacterial Effects of Ribes Khorasanicum Sagha and Assadi Endemic species in northern Khorasan. J Med Plants. 2007;6(4):64.
4. Story EN, Kopec RE, Schwartz SJ, Harris GK. An update on the health effects of tomato lycopene. Annu Rev Food Sci Technol. 2010; 1:189-210. doi: 10.1146/annurev.food.102308.124120.
5. Heber D, Lu QY. Overview of mechanisms of action of lycopene. Exp Biol Med. 2002;227(10):920-3.
6. Sahlin E, Savage GP, Lister CE. Investigation of the antioxidant properties of tomatoes after processing. J Food Compos Anal. 2004;17(5):635-47. doi: 10.1016/j.jfca.2003.10.003.
7. Tilahun S, Seo MH, Jeong CS. Review on factors affecting the quality and antioxidant properties of tomatoes. Afr J Biotechnol. 2017;16(32):1678-87. doi: 10.5897/AJB2017.16054.
8. Abo-Elyousr KA, Asran MR. Antibacterial activity of certain plant extracts against bacterial wilt of tomato. Arch Phytopathol Plant Prot. 2009;42(6):573-8. doi: 10.1080/03235400701284740.
9. Lee J, Nonaka S, Takayama M, Ezura H. Utilization of a genome-edited tomato (Solanum lycopersicum) with high gamma aminobutyric acid content in hybrid breeding. J Agric Food Chem. 2018;66(4):963-71. doi: 10.1021/acs.jafc.7b05171.
10. Hirose A, Terauchi M, Tamura M, Akiyoshi M, Owa Y, Kato K, et al. Tomato juice intake increases resting energy expenditure and improves hypertriglyceridemia in middle-aged women: an open-label, single-arm study. Nutr J. 2015;14(1):34. doi: 10.1186/s12937-015-0021-4.
11. Waites KB, Bade DJ, Bébéar C, Brown SD, Davidson MK, Duffy LB, et al. Methods for antimicrobial susceptibility testing for human mycoplasmas; Approved guideline. Wayne (PA): Clinical and Laboratory Standards Institute; 2011 Oct. Report No.: M43-A.
12. Kim DS, Kwack Y, Lee JH, Chun C. Antimicrobial Activity of Various Parts of Tomato Plants Varied with Different Solvent Extracts. Plant Pathol J. 2019;35(2):149-155. doi:10.5423/PPJ.OA.07.2018.0132.
13. Omodamiro OD, Amechi U. The phytochemical content, antioxidant, antimicrobial and anti-inflammatory activities of Lycopersicon esculentum (Tomato). Asian J Plant Sci Res. 2013;3(5):70-81.
14. Al-Oqaili RM, Istabreq BM, Salman MA, Al-Satar DA. In vitro antibacterial activity of Solanum lycopersicum extract against some pathogenic bacteria. Food Sci Quality Manage. 2014; 27:12-7.
15. García-Olmedo F, Molina A, Alamillo JM, Rodríguez-Palenzuéla P. Plant defense peptides. Biopolymers. 1998;47(6):479-491. doi:10.1002/(SICI)1097-0282(1998)47:6<479: AID-BIP6>3.0.CO;2-K.
16. Dahan K, Fennal M, Kumar NB. Lycopene in the prevention of prostate

cancer. J Soc Integr Oncol. 2008;6(1):29-36.
17. Marcos NT, Magalhães A, Ferreira B, Oliveira MJ, Carvalho AS, Mendes N, et al. Helicobacter pylori induces β3GnT5 in human gastric cell lines, modulating expression of the SabA ligand sialyl–Lewis x. J Clin Invest. 2008;118(6):2325-36.
18. Neubauerová T, Doležílková I ,Králová M, Schevchenko I. Antibacterial effect of compounds of peptide nature contained in aqueous extract of Brassica napus, Solanum lycopersicum and Tetragonia tetragonioides leaves. J Microb Biotechnol Food Sci. 2015; 4(5): 427-433. doi: 10.15414/jmbfs.2015.4.5.427-433.
19. Dhanawade SS, Sakhare AV. Isolation of lycopene from tomato and study of its antimicrobial activity. Int J Sci Res. 2014;3(12):673.