



Antibacterial effects of *Solanum tuberosum* peel ethanol extract *in vitro*

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ARTICLE INFO

Article Type:

Original Article

Article History:

Received: 2 December 2014

Accepted: 21 February 2015

Keywords:

Solanum tuberosum

Ethanol extract

Antibacterial activity

In vitro condition

ABSTRACT

Introduction: Today, medicinal plants are being widely used due to being natural, available, and cheaper than synthetic drugs and having minimum side effects. Since there were reports about the antibacterial properties of *Solanum tuberosum* (SE), the aim of this study was to investigate the antibacterial effects of SE ethanol extract *in vitro* condition on *Streptococcus pyogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*

Methods: Ethanol extract of SE peel was prepared by maceration method. Initially, antibacterial activity of ethanol extract of SE was qualitatively determined by disk diffusion test; then, the minimum inhibitory concentration and minimum bactericidal concentration were qualitatively determined by micro-dilution method.

Results: SE peel extract had antibacterial properties and its effect was more pronounced on gram-positive bacteria, especially *S. aureus* (0.62±0.00 mg/ml). The extract had antibacterial activity on gram-negative bacteria, *P. aeruginosa*, too (8.33±2.88 mg/ml).

Conclusion: SE peel extract has antibacterial activity and its effect on gram-positive bacteria was more pronounced than the investigated gram-negative bacteria. Therefore, it is suggested that SE peel constituent compounds be determined and to determine the exact mechanism of its antibacterial properties, and more comprehensive research be done to apply it, clinically.

Implication for health policy/practice/research/medical education:

Solanum tuberosum peel ethanol extract has good antibacterial properties against gram-positive bacteria. Nevertheless, more investigations need for identification of the active chemical constituents of SE peel ethanol extract responsible for its antibacterial properties as well as their specific mechanisms of action.

Please cite this paper as: Amanpour R, Abbasi-Maleki S, Neyriz-Naghadehi M, Asadi-Samani M. Antibacterial effects of *Solanum tuberosum* peel ethanol extract *in vitro*. J HerbMed Pharmacol. 2015; 4(2): 45-48.

Introduction

Today, infectious diseases are of the most important reasons for mortality (particularly in third-world countries). Synthetic (artificial) antibiotics, in addition to being expensive and having side effects, can cause drug resistance. Therefore, developing natural antibiotics is necessary (1). In other words, resistance to antimicrobial and antiparasitic drugs has become a global challenge, which is due to inappropriate and excessive consumption of these drugs. Because of the importance of this issue, the slogan "Resistance to antimicrobial drugs is a global threat" was determined by World Health Organization

in 2011 (2). Therefore, as a result of rapid growth of antibiotic resistance, side effects of chemical drugs, and production of environmental pollutions (by them), which threatens the earth, the researches on herbs and herbs-derived products have increased day to day because herbs, compared with synthetic drugs, have less side effects. Consequently, we are observing an increased attention to treatment using the extractions from and development of herbs per traditional methods and widespread use of essence and extract of herbs in various pharmacologic and food products (3,4).

Potato, scientifically called *Solanum tuberosum* (SE), is

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an herbaceous, perennial, and shrub plant whose height reaches to less than one meter. Underground stem of this plant, which is a tuber, contains large amounts of starch. In addition, the research has indicated that *Solanum tuberosum* peel mainly contains ferritin, vitamin C, riboflavin, alpha-tocopherol, flavone aglycones, quercetin, glutathione, etc (5,6). The research has demonstrated analgesic, antioxidant, and antibacterial properties on some of the bacteria, and blood pressure lowering, sputum collecting, muscles strengthening, and appetizing properties by SE (7). Given this background knowledge, the present study is aimed to investigate the antibacterial effects of SE ethanol extract on four gram-positive and gram-negative bacteria in *in vitro* condition.

Materials and methods

Preparation of ethanol extract

To prepare *Solanum tuberosum* ethanolic extract, maceration method was used. For this, 10 g of ground peels were extracted by mixing using a magnetic stirrer, with 200 ml of ethanol (80%) at room temperature over night. The extract was filtered through wathman No. 42 filter paper and the residue was re-extracted under the same conditions. The combined filtered was evaporated in a rotary evaporator below 40°C. Then centrifuged at 3000 × g for 10 minutes at 5°C and stored in a refrigerator.

Antibacterial activity

An *in vitro* experimental study was conducted in 2012-2013 in Urmia branch, Islamic Azad University. Tested bacterial strains including *Staphylococcus aureus* PTCC 1113, *Streptococcus pyogenes* PTCC 1447, *Klebsiella pneumonia* PTCC 1053 and *Pseudomonas aeruginosa* PTCC 1430 were prepared as lyophilized granules from microbial collection of Iranian research organization for science and technology (IROST).

The antibacterial activity of the extract was determined by disk diffusion and broth micro- dilution MIC (minimal inhibitory concentration) testing methods (8).

For disk diffusion test, initially 100 mg/ml concentration of SE extract was prepared in 10% dimethyl sulfoxide (DMSO) as solvent and sterilized by filtration with 0.45 µm Millipore filters. Overnight cultures of the bacteria were prepared in Mueller-Hinton broth (Merck KGaA, Darmstadt, Germany) and their turbidity was adjusted to 0.5 McFarland standards. Then, bacterial suspensions containing about 10⁸ CFU/ml was swabbed on Mueller-Hinton agar (Merck KGaA, Darmstadt, Germany). Sterile blank discs (6 mm in diameter) were impregnated with 30 µl of the SE extract (100 mg/ml) and placed on the inoculated agar. Negative control was prepared using 10% DMSO. Gentamicin (30 µg/disc) was used as positive reference standard to determine the sensitivity of each bacterial species tested. The inoculated plates were incubated at 37°C for 24 hours. Antibacterial activity was evaluated by measuring the zone of inhibition against the test organisms (9).

The minimal inhibitory concentration (MIC) and

minimal bactericidal concentration (MBC) values were studied for the bacterial strains, being sensitive to the extract in the disc diffusion assay. Bacterial suspensions prior adjusted to 0.5 McFarland standards, 0.01 diluted in sterile saline to achieve inoculum size about 10⁶ CFU/ml. The extract serially diluted using twofold dilutions in sterile test tube containing 10% DMSO to achieve a concentration range from 0.78 to 100 mg/ml. The 96-well sterile micro-dilution plates with U-bottom wells were prepared by dispensing into each well 160 µl of Mueller-Hinton broth and 20 µl of the bacterial inoculum. A 20 µl aliquot of various prepared concentration of the extract was added into the wells. The total volume in each well was 200 µl. For every experiment negative (growth) and positive (sterility) controls were used. The wells consisting of broth with inoculum and broth containing of 10% DMSO and inoculum were used as negative controls. The wells consisting of broth and broth containing of lowest concentration of the extract were used as positive controls. Contents of each well were mixed on a plate shaker at 250 rpm for 20 seconds and incubated at 37°C for 24 hours. The lowest concentration of the extract showing visually no growth by comparing the negative control was taken as its minimal inhibitory concentration (MIC) and confirmed by plating 10 µl samples from clear wells onto plate count agar (Merck KGaA, Darmstadt, Germany). In this study, 90% inhibition of inoculum was defined as MIC and 99.9% inhibition of inoculum was defined as MBC. The tests were run in triplicate and the mean of three repetitions were considered as results (10).

Results

SE peel ethanolic extract had antibacterial effect and this effect was greater on gram-positive bacteria, particularly *S. aureus*, so that the highest zone of inhibition was obtained for *S. aureus* out of the studied bacteria. However, out of gram-negative bacteria, this extract had antibacterial effect on *P. aeruginosa*, as well (Table 1). Out of the studied bacteria, SE peel ethanolic extract at minimal concentration was able to inhibit and kill *S. aureus* (Table 2).

Discussion

The findings of the present study indicated that SE peel ethanol extract *in vitro* was more effective on positive gram bacteria (particularly *S. aureus*). This extract was effective only on *P. aeruginosa* out of gram-negative bacteria, and exerted no effect on *K. pneumoniae*. Consistent with the

Table 1. Inhibitory zone of SE peel extract (mm) on the tested bacteria

Test bacteria	Means ± SD
<i>S. aureus</i>	14.66±0.47
<i>S. pyogenes</i>	12.00±0.00
<i>P. aeruginosa</i>	8.66±0.47
<i>K. pneumoniae</i>	00.00±0.00

Table 2. MIC and MBC of SE peel extract (mg/ml) on the tested bacteria

Test bacteria	Means \pm SD	
	MIC	MBC
<i>S. aureus</i>	0.62 \pm 0.00	1.25 \pm 0.00
<i>S. pyogenes</i>	1.25 \pm 0.00	2.5 \pm 0.00
<i>P. aeruginosa</i>	8.33 \pm 2.88	>10
<i>K. pneumoniae</i>	>10	>10

findings of the present study, quercetin (flavonoid) in SE peel was demonstrated to have bacteriostatic effect on all food pathogenic bacteria such as *Listeria monocytogenes*, *Escherichia coli*, *Pseudomonas fluorescens*, *Salmonella enterica*, *S. aureus*, *Bacillus stearothermophilus*, and *Vibrio cholerae* (11).

In view of the studies, there are several compounds in SE structure including alpha-tocopherol (12). SE also contains flavone aglycones that comprise a significant group of phenolic compounds in SE and have antioxidant property (5). In SE peel, there are also chlorogenic acid and glutathione that have antioxidant properties (6).

In SE structure, there are also phenolic compounds called flavones that were demonstrated to have antibacterial property. In fact, flavonoids exert antiviral, antibacterial, and antifungal properties (13). Consistently, flavones at 3.9-15.6 g/ml concentrations were demonstrated to cause inhibition of growth of 15 strains of toxic, methicillin-resistant *S. aureus* and five susceptible strains of *S. aureus* (14).

There are two steroidal alkaloids in SE namely solasodin and solanidin, as well. In a study by Kumar *et al.*, *Solanum dulcamara* was similarly demonstrated to have alkaloids such as solanin and solasodin and these alkaloids all have antibacterial properties and cause growth inhibition of *E. coli* and *S. aureus* (15), which is consistent with the findings of the present study. Also, the anthocyanins in SE were demonstrated to exert strong antimicrobial, antioxidant, and antioxidant property (16).

In agreement with the findings of the present study, the anthocyanins existing in SE were demonstrated to exert strong antibacterial effects against gram-positive bacteria (such as *S. aureus*) and gram-negative bacteria (such as *P. aeruginosa*), but their effect on gram-positive bacteria was greater than that on gram-negative bacteria (17). Of course, it should be mentioned that SE peel contains other phenolic compounds such as chlorogenic acid derivatives, which are derived from caffeic acid and quinic acid that could also be examined for antibacterial activity.

In addition, except for peel and cortex that contain large amounts of phenolic compounds compared with other parts, there is little difference in content of phenolic compounds among the parts of a SE tuber (18). Phenolic compounds are mainly distributed between peel and cortex of SE, so that approximately 50% of total phenolic compounds are located in peel and surrounding tissues and the remaining amount is reduced from outside towards the center of SE (19).

Of course, the type of SE contributes greatly to its

compounds. Consistently, anthocyanins, flavonoids, and phenolic acids present in SE peel are twice higher in peel red tubers than normal peel types, a less but significant difference has been noted in flavonoids concentration between peel red tuber and normal peel, and there are a lot of anthocyanins in colored bulbs. In fact, the concentrations of anthocyanins and phenolic acids are greatly correlated, which confirms that more colored bulbs contain more phenolic compounds, thus the variation in the amount of phenolic compounds relates to the plant species. Therefore, the time of planting and harvesting, soil fertility, SE genus, etc. all play an important role in the compounds and consequently the characterization of SE (20).

Conclusion

In light of the present study findings and consistent with other studies, SE peel extract has good antibacterial properties against gram-positive bacteria. Various compounds such as phenolic compounds, anthocyanins, and flavonoids present in SE structure contribute to exerting its antibacterial effects; determination of precise, antibacterial mechanism of this extract requires conduction of gas chromatography-mass spectrometry (GC-MS), and SE peel constituents should be separately investigated more comprehensively by further studies.

Acknowledgements

This paper is a part of a MSc. thesis (No. 10330513901006) that was conducted in Islamic Azad University, Urmia Branch. The author wishes to express his sincere thanks to Mr. Reza Delshad.

Authors' contributions

All the authors wrote the manuscript, contributed to the work, equally, and confirmed the final version of the article.

Conflict of interests

The authors declared no competing interests.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

Funding/Support

This project was financially supported by Islamic Azad University, Urmia Branch (Grant No: 10330513901006).

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