Evaluation of Antibacterial Effects of Aqueous and Alcoholic Extracts of *Nasturtium Officinale* on Some Pathogenic

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ABSTRACT

Background and Objective: Although antibiotics are commonly used for treatment of infectious diseases, these treatments are often associated with several problems such as unwanted side effects and resistance to antibiotics. The aim of this study was to evaluate the antibacterial effect of aqueous and alcoholic extracts of *Nasturtium officinale* on *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Listeria monocytogenes*.

Methods: All experiments were performed using the well diffusion method. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the aqueous and alcoholic extracts of *N. officinale* against the pathogenic bacteria studied were determined by microdilution method.

Results: In the well diffusion method, *S. aureus* and *L. monocytogenes* were the most sensitive bacteria with MIC of 8 μg/ml, while *E. coli* and *S. typhimurium* were the most resistant bacteria to the aqueous and alcoholic extracts. In addition, the inhibitory activity of the alcoholic and aqueous extracts of *N. officinale* was higher against gram-positive bacteria compared to gram-negatives. The lowest MIC (6.25 μg/ml) and MBC (12.5 μg/ml) of the plant extract were against *S. aureus*.

Conclusion: The aqueous and alcoholic extracts of *N. officinale* affect the growth of gram-positive bacteria (*S. aureus* and *L. monocytogenes*) but not the gram-negatives (*S. typhimurium* and *E. coli*). These extract could be used for treatment of infections.

Keywords: *Nasturtium Officinale*, Aqueous Extract, Alcoholic Extract, Antibacterial Effect.

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INTRODUCTION

Some compounds extracted from plants have natural antimicrobial activity against a large number of pathogenic and spoilage bacteria. Most of these compounds have phenolic active groups in their structure. In fact, medicinal plants have received a lot of attention due to possession of large amounts of volatile aromatic compounds that are also used for flavoring foods. The volatile compounds have natural oxidative and antimicrobial properties and play an important role in plant defense system against diseases caused by microorganisms. Thus, these compounds can be used as a functioning components, flavors and preservatives in foodstuff (1).

Secondary metabolites are produced as inactive precursors stored in plant tissues that are released in response to environmental stress. The precursors of the plant tissue include phenolic compounds, flavonols and flavonoids, glycosides, alkaloids and polyacetylene. These compounds have been recently considered for their inhibitory and bactericidal effects against pathogenic microorganisms (1). The extract of *Nasturtium officinale* has strong anti-scurvy effect so that its consumption shortly resolves the complications of vitamin C deficiency. It also has blood purifying, energizing, diuretic, appetizing, stomach tonic, antipyretic and neuropathic pain relieving effects. It is commonly used as antipyretic in traditional medicine. It has beneficial effects on diabetes and reduces the amount of glucose in urine of diabetic patients. In addition, it is effective for treatment of eczema and hair loss (2). Given the mentioned properties and activities of plant extracts for controlling the growth of pathogenic and spoilage bacteria, they can be used as food preservatives (1).

N. officinale is a member of the Brassicaceae family and a climbing herbaceous plant with square stem and alternate feather-like leaves (3). A cluster of white flowers grows at the end of the stem and its fruit has a saddlebag-like curve. Its main origin is Central and Western Europe, but today, it is spread all over the world. This plant grows along streams, springs and mainly around clear waters (3). Recent studies have proposed anti-cancer effects for this plant due to presence of isosulphocyanide ethylbenzene (beta-phenylethyl isothiocyanate), because this compound inhibits the enzymes that are activated by carcinogens in animals. It also inhibits histamine release, increases the concentration of intracellular free calcium and decreases oxidative metabolites of acetaminophen probably through inhibition of its oxidative metabolism (4).

Different parts of the plant such as root, leaf, stem and flower have been used for medical purposes (4). This in vitro study investigated the antibacterial effect of alcoholic and aqueous extracts of *N. officinale* on standard strains of four pathogenic bacteria including *Escherichia coli, Salmonella typhimurium, Staphylococcus aureus* and *Listeria monocytogenes*.

MATERIAL AND METHODS

The plant was dried at room temperature away from sunlight. The dried plant was then powdered by an electric mill and Soxhlet method was used for extraction. Briefly, 30g of *N. officinale* powder were poured into the soxhlet cartouche set, and then soaked with a little methanol. The mixture was placed into the soxhlet apparatus in such a way that the plant powder could not exit the cartouche. About 300 ml of pure methanol was added to flask attached to the soxhlet. After heating the flask, oven at temperature of 40 °C was used to obtain the pure extract without solvent (5). To prepare the aqueous extract, about 30g of powdered *N. officinale* was mixed with 300 ml of distilled water in a beaker. After boiling the mixture for 30 min followed by cooling, the mixture was passed through clean tampons and filter paper to separate the extract. The mixture was placed in an oven at 40 °C to obtain pure and solvent-free extract (5).

The important gram-positive and gram-negative pathogenic and spoilage bacteria including *S. typhimurium* ATCC 13311, *E. coli* ATCC 43894 O157 H7, *L. monocytogenes* ATCC 19118 and *S. aureus* ATCC 6538 were used in this study. The well-diffusion method was used to measure the antibacterial effect of the extract. The Wells with a diameter of 5 mm were created on Mueller-Hinton agar and filled with concentrations of 50%, 25% and 12.5% of the extract. The plate was incubated at 35 °C for 18 hours. Finally, 5mg of chloramphenicol and sterile dimethyl

sulfoxide (DMSO) solution was used as the positive and negative controls, respectively (6). The positive control was used for antibiotic sensitivity testing and the negative control for insensitivity to the desired substance (DMSO). After incubation, the growth inhibition zone was measured in mm using a caliper.

Evaluation of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of this extract against the bacteria was performed according to the method described by Gulluce et al. (7). The bacteria were cultured in brain heart infusion (BHI) broth for 12 hours. A bacterial suspension equivalent to 0.5 McFarland standard was prepared. The plant extract was dissolved in 10% DMSO solution at the highest concentration used in this study. Then, 10 two-fold serial dilutions in the 3.78-100 µg/ml range were prepared from the extract in sterile tubes containing 10 ml broth. The MIC of aqueous and alcoholic extracts of *N. officinale* against the pathogenic bacteria studied was determined using the well microdilution assay. Each well of the microplate contained 95 µl nutrient broth and 5 µl single bacterial cultures equal to 0.5 McFarland standard. Later, 100 µl of the stock solutions of extract prepared with desired concentrations was added to each well in a descending order. The positive and negative controls were used for each phase of the experiments. At end of the incubation period (24 hours), MIC and MBC values (µg/ml) were calculated. Microbial growth was assessed by measuring absorbance at 600 nm and culturing 5 µl of the transparent contents of the wells on nutrient agar medium.

RESULTS

Table 1 shows the effects of different concentrations of the aqueous and alcoholic extracts of *N. officinale* in the well diffusion method. Tables 2 and 3 show the results of the MIC and MBC of the alcoholic and aqueous extracts of *N. officinale*, respectively.

Table 1- Effect of different concentrations of the alcoholic and aqueous extracts of *N. officinale* in the well diffusion method

Negative control disk	Positive control disk	Alcoholic extract			Aqueous extract			Extract Bacterium
DMSO	Chloramphenicol	50%	25%	12.5%	50%	25%	12.5%	
---	18	8	8	8	8	8	8	*S. aureus*
---	20	8	8	8	8	8	8	*L. monocytogenes*
---	10	---	---	---	---	---	---	*S. typhimorium*
---	6	---	---	---	---	---	---	*E. coli*

***The unit of measurement is mm.**

Table 2- MIC and MBC (µg/ml) of the alcoholic extract of *N. officinale* against bacteria studied in the microdilution method

MBC (µg/ml)	MIC (µg/ml)	Bacteria
12.5	6.25	*S. aureus*
25	12.5	*L. monocytogenes*
100	50	*S. typhimorium*
100	50	*E. coli*

Table 3- MIC and MBC (µg/ml) of the aqueous extract of *N. officinale* against bacteria studied in the microdilution method

MBC (µg/ml)	MIC (µg/ml)	Bacteria
12.5	6.25	*S. aureus*
25	12.5	*L. monocytogenes*
100	50	*S. typhimorium*
>100	100	*E. coli*

DISCUSSION

Herbal extracts have been used for treatment of diseases since ancient times. Considering the compatibility of these compounds with body and their beneficial pharmaceutical effects, it is of great value to study the anti-bacterial effects of plants that have been used in traditional medicine (5). In this study, the antibacterial effect of alcoholic and aqueous extracts of *N. officinale* against gram-positive and gram-negative bacteria was investigated using the well diffusion and microdilution methods. Inhibition zones were detected for some concentrations of the ethanolic and aqueous extracts against gram-positive strains (*S. aureus* and *L. monocytogenes*) in the well diffusion method. However, no growth inhibition zone was detected at any concentration of the extracts for gram-negative strains. In the microtiter broth dilution method, the MIC-MBC values of the alcoholic extract of *N. officinale* against *S. aureus* and *L. monocytogenes* were 6.25-12.5 and 12.5-25 µg/ml, respectively. MIC-MBC value of the alcoholic extract against *S. typhimurium* and *E. coli* was 50-100 µg/ml. In the same method, the MIC-MBC values of the aqueous extract of *N. officinale* against *S. aureus* and *L. monocytogenes* were 6.25-12.5 and 12.5-25 µg/ml, respectively. The MIC-MBC values of the aqueous extract against *S. typhimurium* and *E. coli* were 50-100 and 100-100 µg/ml, respectively. It has to be noted that the inhibitory effect of the alcoholic extract was slightly higher than that of the aqueous extract, which may due to higher solubility and extraction of active antibacterial compounds by the alcoholic solvent. Moreover, the inhibitory effect of the alcoholic and aqueous extracts was higher against gram-positive bacteria compared to gram-negatives. This difference could be due to structural differences in the bacterial wall of these two groups of bacteria. Gram-negative bacteria have an outer membrane that acts as a barrier against the passage of large and hydrophobic molecules. Since most effective compounds found in the essential oils and extracts have hydrophobic nature, it can be concluded that these compounds might not enter or reach the active points within gram-negative bacteria. Therefore, gram-negative bacteria are expected to show more resistance against these compounds compared to gram-negative bacteria (8). Flavonols and flavonoids of plants have phenolic structure and antimicrobial effects. Their antimicrobial effect may be due to composition of extracellular proteins, formation of complex with the cell wall and disruption of the cell membrane of microorganisms. In addition to the antimicrobial effects, the phenolic compounds such as flavonoids and flavonols are useful for their strong antioxidant properties. The plant used in this study has the aforementioned compounds and can be used as food preservative and flavor. It should be noted that due to presence of low levels of these compounds in plants, they should be condensed or used with other preservatives for improved quality. Sharifa et al. investigated the effect of ethanolic, methanolic and aqueous extracts of *Plantago major* on gram-positive and gram-negative bacteria and yeast. The mentioned study showed that the methanolic extract has antibacterial activity against *E. coli* (MIC 120 mg/ml) and gram-positive bacteria such as *S. aureus* (MIC 100 mg/ml). The ethanolic extract also had antimicrobial effects at concentration of 140 mg/ml. None of the extracts had any effects on *Bacillus subtilis* (9). Jalali investigated the antimicrobial effects of hydroalcoholic extracts of five Iran's native plants including thyme, eucalyptus, chamomile, rosemary and clary on two pathogenic serotypes of *L. monocytogenes.* In the mentioned study, MIC and MBC were determined by broth macrodilution method. The results showed that the extract of eucalyptus could be considered as an anti-Listeria compound and food preservative (10). Study of Razzagi et al. evaluated the antimicrobial effects of saffron on *E. coli*, *S. aureus* and *P. aeruginosa* and reported that safranal content of saffron had inhibitory effects on the growth *of E. coli* and *S. aureus* (11). Study of Freitas et al. evaluated the impact of alcoholic and aqueous extracts of *N. officinale*, 2-phenylethyl isocyanate and some antibiotics (extended spectrum beta lactamase) on *E. coli*. They attributed the antimicrobial effects of the *N. officinale* extracts to the 2-phenylethyl isocyanate content. They also observed that combination of the extract with antibiotics significantly increased the antimicrobial effects and could help prevent

emergence of beta-lactamase-resistant bacteria. Since the *N. officinale* extract did not affect *E. coli* in the present study, the combination of the extract with antibiotics may create a synergetic effect, indicating that the extract is unable to affect the bacteria on its own (12).

In the study of Penecilla, the antibacterial activity of hexane, acetone, ethanol and aqueous extracts from 12 medicinal plants commonly used in the Philippines including *N. officinale* (13), was evaluated against *S. aureus, B. subtilis, E. coli* and *P. aeruginosa*. The growth inhibition zone of *S. aureus* for the ethanol and aqueous extracts was determined as 9 mm and 12 mm, respectively. In addition, the ethanol and aqueous extracts could not inhibit the growth of *E. coli* in the mentioned study. In the present study, both the methanolic and aqueous extracts of *N. officinale* created 10 mm inhibition zones against *S. aureus*, while the growth of *E. coli* was not inhibited. The results of the two studies are similar when neglecting the difference in the type of alcoholic solvent used.

Camacho et al. evaluated the anti-tuberculosis properties of nine plants used in traditional medicine in Mexico and reported that *N officinale* has the highest activity against. *Mycobacterium tuberculosis* (MIC=100 mg/ml). However, different effects have been reported for multidrug-resistant strains of *M. tuberculosis* (14).

Study of Sefidkon et al. compared the anticancer effects of nanocapsules of *N. officinale* extract with the methanolic extract and its fractions, and reported that the *N. officinale* extract had anti-cancer properties (2).

CONCLUSION

Alcoholic and aqueous extracts of *N. officinale* affect the growth of gram-positive bacteria (*S. aureus* and *L. monocytogenes*) but not gram-negative bacteria (*S. typhimurium* and *E. coli*). This could be due to the structural differences in the bacterial cell wall of these two groups of bacteria. Gram-negative bacteria have an outer membrane that acts as a barrier, preventing the passage of large molecules.

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CONFLICT OF INTEREST

We have no conflict of interest to declare.

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