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RESEARCH ARTICLE

Antibacterial and Antioxidant Activities of *Nasturtium officinale* Essential Oil on Food Borne Bacteria

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Abstract:

Introduction:

The use of synthetic preservatives has been increasing in the food industry, and this leads to an increased incidence of gastrointestinal diseases and cancers in humans in the long run.

Aims & Objectives:

The aim of this study was to investigate the antibacterial and antioxidant activities of *Nasturtium officinale* essential oil on some important food borne bacteria.

Materials & Methods:

In this study, the antibacterial activity of *N. officinale* essential oil was evaluated on *Staphylococcus aureus, Bacillus cereus*, *Escherichia coli* and *Salmonella enteric* by microdilution method. Also, the antioxidant activity of this essential oil was evaluated by inactivating free radicals produced by 2,2-diphenyl-1-picrylhydrazyl (DPPH). Finally, the chemical compounds of the *N. officinale* essential oil were evaluated by gas chromatography- mass spectrometry (GC/MS).

Results:

The results showed that *S. enteric* and *E. coli* isolates had the most resistance and *B. cereus* isolates had the most susceptibility to *N. officinale* essential oil. The evaluation of antioxidant properties showed that in the same concentrations, the antioxidant effect of *N. officinale* was less than BHT. The obtained results from GC/MS showed that Phytol (30.20%) was the highest proportion and Megastigmatrienone 2 (0.18%) was the lowest proportion of essential oil.

Conclusion:

In general, the results of this study showed that *N. officinale* essential oil has an appropriate antibacterial activity against gram positive bacteria and can be used as a new antibacterial and antioxidant compound in the food industry.

Keywords: Antibacterial, Antioxidant, *Nasturtium officinale*, Essential oil, Concentrations, Megastigmatrienone.

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1. INTRODUCTION

Today, although advances have been made on food industry hygiene, diseases caused by microbial contamination of foodstuff have become a major problem [1]. In some countries, even in developed countries, 30% of the population is affected by diseases caused by the consumption of contaminated foods, once a year [2]. Overuse of preservatives and antibiotics in the food industry and treatment of patients have greatly expanded drug resistance [3]. Therefore, natural resources, especially medicinal and edible plants have been considered as ecological reservoirs [4]. Due to the tendency of people to consume food with natural preservatives, plant

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sources are not only used as flavoring, but are also used as antimicrobial compounds [5]. Recent studies have shown that the extract and essential oil of a large number of traditional medicinal plants have inhibitory and sometimes lethal effects on various pathogenic microorganisms [7, 8]. Therefore, many plant species have been used in the food industry due to their antimicrobial and anti-oxidant properties [4, 6].

Nasturtium officinale belongs to the Cruciferae family. The main origin of this plant is the Central and Western Europe, but today it is spread throughout the world, including Asia, Europe and throughout North America [9]. For many years, *N. officinale* has been used to treat high blood glucose, high blood lipids, high blood pressure, and cardiovascular and pulmonary diseases [7]. It also contains beta-carotene, ascorbic acid, calcium, folic acid, iron, phosphorus, iodine and amino acids, and it is effective in inhibiting the growth of cancer cells [10]. *N. officinale* has a significant antioxidant capacity due to the presence of numerous chemical compounds such as flavonoids quercetin, carotenoids, beta-carotene, lutein, vitamin C and zeaxanthin [11]. Also, recent studies have shown that the extract and essential oil of this plant have antimicrobial activity against different types of human and food pathogens [4, 12].

Since food health is a fundamental issue, and due to the negative attitude of consumers to the use of food containing chemical preservatives, identification and use of herbal and natural ingredients with antimicrobial and antioxidant properties as preservatives are very important. Therefore, the aim of this study was to evaluate the antioxidant and antibacterial activities of *N. officinale* essential oil on food borne bacteria.

2. MATERIALS AND METHODS

2.1. Preparation of Ethanolic Essential Oil

After collecting *N. officinale* from Bonab city (Qara Ghoshun area, April 2016), it was identified and approved by the Herbarium of the Islamic Azad University, Maragheh Branch. For isolation of the essential oils, the dried aerial parts of the plants (50gr) were separately hydrodistilled in a Clevenger-type apparatus for 3 h. The oils were dried over anhydrous sodium sulfate and kept at 4°C in sealed brown vials until required.

2.2. Preparation of Isolates and Bacterial Strains

The isolated bacteria from foods were used to study the antibacterial activity of *N. officinale* essential oil. Gram positive bacteria included *Staphylococcus aureus* and *Bacillus cereus*, and gram negative bacteria included *Escherichia coli* and *S. enteric*. Also, *S. aureus* (PTCC 1112), *B. cereus* (ATCC 11778), *E. coli* (PTCC 1270) and *S. enteric* (PTCC 1709) were purchased from the Persian Type Culture Collection (PTCC) as standard strains.

2.3. Evaluation of Antibacterial Activity by Microdilution Method

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) methods were used to determine the antibacterial activity of the essential oil of *N. officinale*. 100µl of sterile Brain Heart Infusion (BHI) (Merck, Germany) was poured into each micropellet (from No. 2-10). Then, 100µl *N. officinale* essential oil was poured into the first and second micropellets and 100µl essential oil was poured from the second well to a third well; this continued to the 10th well. Therefore, dilutions of 100-0.39% of essential oil were prepared. 100µl of new bacterial culture (the equivalent of concentration of 0.5 Mc Farland test) with 1:100 diluted ratio was added to each well. Then, 30 µl of resazurin index (Sigma-Aldrich, USA) was added to all of the wells. The well that showed a color change was the essential oil MIC. The well had changed its color with two wells, before and after it had been cultured in BHI agar medium, and was incubated (Labtech, South Korea) at 37°C for 24 h. The first plate associated with the well that did not show bacterial colony was considered as the essential oil MBC. The MBC was defined as the concentration in which no microorganism growth was observed.

2.4. Evaluation of Antioxidant Activity by DPPH Method

Total antioxidant activity was measured by inactivating free radicals produced by 2,2-diphenyl-1-picril-hydrazil (DPPH) (Sigma-Aldrich, USA) and decolorization of dark purple solution. A 500µM methanolic solution of DPPH was prepared. Different concentrations (50ppm, 100ppm, 200ppm, 300ppm, 400ppm, 500ppm, 1000ppm) of synthesized antioxidants of Butylated Hydroxytoluene (BHT) (Sigma-Aldrich, USA) were prepared as reference antioxidants. Then, 4ml of each concentration was transferred to the test tubes and mixed with 1ml of DPPH solution. After 30 minutes, the absorbance of the solution was measured at 517nm using a spectrophotometer (UNICO-SQ2800, USA). This experiment was also repeated for *N. officinale*. The percentage of Radical scavenging activity (RSA%) was calculated using the following formula: RSA% = (Ac-As)/Ac × 100 (Ac = control absorption and As = sample absorption).

2.5. Evaluation of Chemical Compounds by GC/MS

The gas chromatograph (Shimadzu-QP2010, Japan) with ZB-WAX column (length 20m, inner diameter 0.18mm, thickness 18.1µm) were used to identify the compounds of the essential oil of *N. officinale*. The essential oil of *N. officinale* was diluted with normal hexane and 1µl was injected into gas chromatography/mass spectrometry (GC/MS). The initial temperature of the oven was 50°C, maintained at this temperature for 5 minutes (thermal gradient: 3°C per minute) and then the temperature was increased to 240°C. The final temperature of the oven was 300°C and maintained at this temperature for 3 minutes (thermal gradient: 3°C per minute). The temperature of the injector was 300°C and split/split less (1 to 50). Helium (99.9999%) was used as the carrier gas at a flow rate of 1ml/min. Then, mass spectrometry (Agilent 5973, USA) (length 20m, inner diameter 0.25µm, thickness 0.25mm) was used. The temperature of the ionization chamber was 150°C, the temperature of the detector was 230°C, the ionization energy was 70eV and the mass analyzer was Quadrupole. The scan mass range was 40m/z to 550m/z. The mass spectrometry was used to determine the compounds of the essential oil of *N. officinale*. The spectral values were compared with Kovatz index values in the standard tables and

the compounds of the essential oil of *N. officinale* were identified according to data and information available in the GC-MS library. The conditions of the compounds identified from the essential oil of *N. officinale* using GC/MS method are shown in Table **1**.

Table 1. The conditions of GC/MS to identification compounds of *N. officinale* ethanolic extract.

Conditions and Compounds		Instrument
Model QP2010, Shimadzu Co., Japan	Instrument model	Gas chromatograph
ZB-WAX model	Column model	
Length: 20 m Inner diameter: 0.18 mm Thickness: 0.18 µm	Column dimensions	
Initial temperature: 50°C for 5 min Final temperature: 300°C for 5 min Temperature gradient: 3°C per min	Oven temperature program	
1µl	Injection volume	
Split/split less (ratio 1:50)	Split ratio	
300°C	Injector temperature	
Helium (99.9999%)	Carrier gas	
1ml/min	Flow rate of the carrier gas	
Model 5973, Agilent, USA	Instrument model	Mass spectrometer
Length: 20m Inner diameter: 0.25µm Thickness: 0.25mm	Column dimensions	
70eV	Ionization energy	
150°C	Ionization chamber temperature	
Quadrupole	Mass analyzer	
230°C	Detector temperature	

3. RESULTS

3.1. Antibacterial Activity of *N. officinale* Essential Oil

S. enteric and *E. coli* (gram negative) showed the most resistance (growth of all isolates in ≥25% concentrations), and *B. cereus* (gram positive) isolates had the most sensitivity (growth of all isolates in ≤1.56% concentration) against *N. officinale* essential oil (Table **2**).

3.2. Antioxidant Activity of Essential oil of *N. officinale*

The obtained results showed that in the same concentrations, the antioxidant effect of *N. officinale* essential oil was less than BHT. The antioxidant effect of *N. officinale* essential oil, such as BHT, increased with increasing concentrations (Table **3**).

3.3. Compounds of Essential Oil of *N. officinale*

The chemical compounds extracted from the essential oil of *N. officinale* using GC/MS method are shown in Table **4**. According to the obtained results, phytol was the most frequent compound and Megastigmatrienone 2 was the least frequent compound. However, further studies on the extracts and essential oil of *N. officinale* and especially other bacterial pathogens may be necessary.

Table 2. Antimicrobial effect of *N. officinale* ethanolic extract on bacteria isolated from food.

Bacteria	Ethanolic extract (%)					
	1.56	**3.12**	**6.25**	**12.5**	**25**	**50**
E. coli PTCC 1270	+	+	+	+	+	-
E.coli	+3	+3	+3	+3	+3	-3
S. enteric PTCC 1709	+	+	+	+	+	-
S. enteric	+3	+3	+3	+3	+3	-3
S. aureus PTCC 1112	+	+	+	+	-	-
S. aureus	+3	+3	+3	+3	-3	-3
B. cereus ATCC 11778	+	-	-	-	-	-
B. cereus	+3	-1	-2	-3	-3	-3

(+) Bacterial growth; (-) Bacterial non-growth; (PTCC) Persian Type Culture Collection; (ATCC) American Type Culture Collection

Table 3. Comparison of antioxidant effect of *N. officinale* ethanolic extract with BHT.

Sample	Concentration (ppm)						
	50	**100**	**200**	**300**	**400**	**500**	**1000**
N. officinale extract (%)	4.18	9.34	16.39	24.37	28.6	32.83	37.99
BHT (%)	77.97	90.41	92.53	92.86	93.24	93.51	93.75

4. DISCUSSION

Recently, secondary metabolites of medicinal plants such as essential oils and extracts have been investigated for antimicrobial effects [13], and it has been shown that the most obtained essential oil from medicinal plants have anti-fungal, anti-parasitic, anti-bacterial and anti-viral properties [14]. Therefore, essential oil of medicinal plants has been used in pharmacological fields, herbal pharmacology, clinical microbiology, phytopathology, and food, fruits and vegetables preservatives [15]. Traditional medicinal plants have been recognized for many centuries in many parts of the world for the treatment of various diseases and use of these antibacterial agents has revolutionized the treatment of various bacterial infections [16]. The results of MIC and MBC analysis in the present study showed that *N. officinale* ethanolic essential oil has a bacteriostatic effect on *S. aureus*, *E. coli*, *B. cereus* and *S. enterica* which is in agreement with the results of Lanciotti *et al*,. 2003 research [17].

Hexanal is an organic alcohol and previous studies on this alcohol have reported its antimicrobial properties on *Salmonella* spp and *Listeria* spp. Also, 2-E hexanal has a protective effect against *Salmonella* spp. The presence of these two compounds in a higher degree in the phytochemicals of essential oil and extracts of *N. officinale* can be a reason for its inhibitory properties on Salmonella strains [17]. Patrignani *et al*. (2008) showed the antimicrobial effects of hexanal and 2-E hexanal on *S. aureus*, *S. enterica* and *E. coli* strains [18].

Table 4. The obtained compounds of *N. officinale* ethanolic extract using GC/MS.

No.	Compound	Frequency	No.	Compound	Frequency
1	Hexanal	1.06%	17	2-methoxy-4-vinyl phenol	3.12%
2	Normal hexanol	0.42%	18	3-carene-10-acethyl-methyl	9.41%
3	2-pentyl furan	1.32%	19	Neryl asetone	1.53%
4	Normal nontanal	0.91%	20	Megastigma Trianon	0.49%
5	Decanal normal	0.54%	21	Anthracene	0.49%
6	Trimethyl	0.55%	22	Eucusan	1.44%
7	Beta-Dumas Senon	7.42%	23	2-E hexanal	0.96%
8	3-carene-10-acethyl-methyl	9.41%	24	Benzaldehyde	0.25%
9	E-beta-lavonone	7.15%	25	Normal Octanol	0.55%
10	Megastigmatrienone 2	0.18%	26	Safranal	0.74%
11	Phytol	30.20%	27	1-cyclohexene-1-acetaldoyde	0.55%
12	2-E hexanal	0.96%	28	Cyclohexane	1.41%
13	2-heptane	0.28%	29	2-Butanone	4.10%
14	Bnz- E- acetaldehyde	0.74%	30	Alpha Humolin	0.48%
15	2-Nonnal	0.34%	31	Hexadekan	0.25%
16	Naphthalene-2,1-dihydro-6,1,1-trimethyl	1.25%	32	2-Pentadecanone-14,10,6-trimethyl-25/1% -15,12,9-octo-deca tri-acetic acid	1.20%

Previous studies indicate that the presence of normal hexanol in essential oils and extracts of medicinal plants refers to the antimicrobial effect of these plants on *S. aureus* and *E. coli* bacteria [19]. The presence of normal hexanol in medicinal plant phytochemicals is one of the inhibitory factors for the mentioned bacteria, which is compatible with the present study [19]. The presence of 1-cyclohexan acetaldehyde in the extract and essential oil of some medicinal plants indicates their antimicrobial ability, which has an inhibitory effect on *S. aureus*, *E. coli* and *K. pneumoniae*. This inhibitory property can be involved with this chemical compound. Our results in phytochemical section indicate the presence of this compound in the essential oil of *N. officinale*.

Butnariu and Bostan (2011) reported that the most antimicrobial activity of *N. officinale* was found in *S. aureus*, *E. coli* and *S. enterica*, respectively. Furthermore, it was reported that the antimicrobial effect of the essential oil of this plant was more than its extract [20]. Jang *et al*. (2010) reported that the inhibitory effect of *N. officinale* essential oil on gram positive bacteria (*S. aureus*, *Listeria monocytogenes*, *B. cereus*) was more than gram negative bacteria (*Aeromonas hydrophila* and *Shigella sonnei*) [21], which is compatible with the results of the current study.

The measurement of inhibition of DPPH free radicals is one of the valid, accurate, easy and inexpensive methods with high repeatability, which is used in the evaluation of antioxidant activity of medicinal plant essential oil *in vitro*. In the present study, it was shown that increasing the concentration of *N. officinale* essential oil leads to an increase in antioxidant activity and consequently, the percentage of inhibition of free radicals was increased. Previous studies have also shown that the inhibitory activity of DPPH-free radicals by medicinal plants essential oil depends on the concentration, and with increasing concentrations, inhibitory effects increased [22 - 24]. The compounds of *N. officinale* essential oil are capable of releasing electrons to free radicals and thus stop the free radical chain reaction, which matches the results of the current study.

CONCLUSION

According to the results obtained in this study, *N. officinale* essential oil showed appropriate antibacterial and antioxidant activity against tested gram-positive bacteria. Therefore, it can be used as a natural preservative and antibacterial compound in food.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

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