 pueblo, Colorado, for a preliminary screening
for antimicrobial activity. The results of the
screening indicated the presence of antibac-
terial activity of the essential oil of nigella
sativa against the Gram-positive bacteria
Staphylococcus aureus and Bacillus subtilis,
Gram-negative bacteria Escherichia coli
and Salmonella typhimurium. The antibac-
terial activity of the essential oil of nigella
sativa was determined by the agar disk diffu-
sion method.

The crude oil of nigella sativa was isolated
by steam distillation from the seeds of nigella
sativa. The oil was further fractionated into
three fractions viz. Fraction A, Fraction B,
and Fraction C. The oil and its fractions
were subjected to GC/MS analysis to
study the chemical composition. The main
components of the oil were thymol, p-
formylation, and cinamaldehyde. The major
components of fraction A were cymene,
β-pinene, and 1,8-cineole. The composition
of fraction B was similar to that of the
oil. However, the major components of
fraction C were trans-caryophyllene and
carvone. These results indicated that the
chemical composition of the oil of nigella
sativa was influenced by the fractionation
process.

M. Chaudhry et al. (2014) investigated the
antibacterial activity of the essential oil
of nigella sativa against various Gram-
positive and Gram-negative bacteria. They
reported that the essential oil of nigella
sativa was effective against all the tested
bacteria, including Staphylococcus aureus,
Escherichia coli, Salmonella typhimurium,
and Enterococcus faecalis. The minimum
inhibitory concentrations (MICs) of the
essential oil of nigella sativa were found
to be 2.5, 1.25, and 1.25 mg/mL, respectively.

The present study was conducted to
investigate the antibacterial activity of the
essential oil of nigella sativa against a
broader range of pathogenic bacteria. The
essential oil of nigella sativa was subjected
to GC/MS analysis to study its chemical
composition. The essential oil of nigella
sativa was found to be effective against
all the tested bacteria, including Staphylo-
coccus aureus, Escherichia coli, Salmonella
typhimurium, and Enterococcus faecalis.

The results of the present study suggest
that the essential oil of nigella sativa has
the potential to be used as an alternative
for the treatment of infections caused by
bacteria. Further studies are required to
investigate the mechanism of action of
the essential oil of nigella sativa and its
safety profile in humans.

Keywords: Essential oil, Nigella sativa,
Antibacterial activity, GC/MS

INTRODUCTION

Infectious diseases pose serious problems to health and
they are a main cause of morbidity and mortality
worldwide. Nowadays, multiple drug resistance has
developed due to indiscriminate use of commercial
antimicrobial drugs commonly used in treatment of
infectious diseases. Herbs and spices are invaluable
resources useful in daily life as food additives, flavors,
fragrances, pharmaceuticals, colors or directly in
medicine. These plants contain medicinal properties which
make them potent to cure or prevent diseases. According
to World Health Organization (WHO), more than 80% of
world’s population relies on traditional medicine for their
healthcare needs. The uses of herbs in treatment of animal
and human diseases have long been established. Most plant
extracts have been shown to possess antimicrobial agents
active against microorganisms in vitro. Some medicinal
plants used in traditional Iranian medicine are effective in
treating various ailments caused by bacterial and oxidative
stress. Studies have shown that the phenolic compounds
play an important role in the antimicrobial properties of
plants. These compounds spoil microorganisms through
destroying the cell walls and proteins, interfering in the
work of membrane enzymes and affecting DNA and RNA
replication. Aromatic oils are used in many industries
including food preservation, pharmacy and medicine. They
are expected to form new sources of antimicrobial
drugs especially against bacteria. The antibacterial
effectiveness of aromatic oils has been divided into a good,
medium or bad. These oils can also produce some
defense products against several natural enemies. In
addition, and in order to continue their natural growth and
development, aromatic oils may produce some secondary
metabolites in response to some external stress. N. sativa
also known as nigella or kalonji often called black cumin
is an annual flowering plant in the family Ranunculaceae.
In fact, N. sativa is an annual herb of the Ranunculaceae
family grows in countries bordering the Mediterranean
Sea, Pakistan, India and Iran. The historical tradition of
N. sativa use in medicine is substantial. N. sativa is known
to have beneficial effects on a wide range of diseases,
antimalarial, antiasthmatic, antitumor, antiviral,
antimicrobial, anti-inflammatory, gastroprotective,
antihypertensive, antidiabetic, anti-atherosclerotic,
protective and antioxidant, nutritional and anti-
cholesterol. Thymoquinone, the main constituent of the
essential oil of N. sativa seeds, was capable to also exert
beneficial effects on acute gastric ulcer. In addition,
thymohydroquinone and its reduced product thymohydroquinone have been reported to have an antimicrobial activity and beneficial interaction with some antibiotics\(^28\). The aim of the study was to screen the in vitro antibacterial activity of the plant oil on some bacteria including *E. coli*, *S. aureus*, and *B. subtilis*.

**MATERIALS AND METHODS**

**Plant sample collection**

In this empirical-experimental study, medicine plant collected from Kermanshah. The sample was cleaned from any strange, plants, dust, or any other contaminants.

**Oil extraction of *N. sativa* by Steam Distillation:**

The hot steam helps to release the aromatic molecules from the plant material since the steam forces open the pockets in which the oil is kept in the plant material. The molecules of the volatile oil then escape from the *N. sativa* material and evaporate into the steam. The temperature of the steam needs to be carefully controlled just enough to force the *N. sativa* material to let go of the oil, yet not too hot as to burn the *N. sativa* material or the oil. The steam which then contains the oil is passed through a cooling system to condense the steam, which forms a liquid from which the essential oil and water is then separated. The steam is produced at greater pressure than the atmosphere and therefore boils at above 100 degrees Celsius which facilitates the removal of the oil from the plant material at a faster rate and in so doing prevents damage to the oil.

**Gas chromatography mass spectrometry (GC/MS)**

To analyze oil of *N. sativa* by GC-MS, fused silica DB-5 column with 0.25 μm thickness film was used. The oven temperature was kept at 500°C for 5 minutes and then programmed from 50-2800°C for 40 minutes. Helium flow rate was maintained at 2 ml/min, with the split ratio of 1:3.

Sample injection of 1μl and ionization voltage of MS-analysis was run by EI technique at 70ev. The volatile oil constituents were identified by matching their MS and retention index data with those of the standards spectra and by matching their fragmentation pattern in Mass Spectra\(^29\).

**RESULTS**

**Chemical composition**

In examining the chemical composition of the oil of *N. sativa* using Gas chromatography mass spectrometry (GC/MS), the presence of α-hellandrene, α-pinene, β-pinene, p-cymene, cis-carveol, trans-anethole, Thymoquinone, Thymol, α-Longipinene and Longifolene in this herb were specified.

**Agar disk diffusion test**

All test bacteria were sensitive to undiluted oil of *N. sativa*. Growth inhibition zones due to different dilutions are listed in table 1. No inhibition zone was observed due to DMSO.

**MIB and MBC ascertaining**

The most and the least values for MIC were acquired in 0.031 g/ml for *E. coli*, *S. aureus* and 0.015 g/ml for *B. subtilis*. Toward oil of *N. sativa*, MBC was 0.031 g/ml for all bacteria (table 3). As the table shows, the oils of *N. sativa* have prevented the growth of *E. coli*, *S. aureus* and *B. subtilis*. Also, by increasing the concentrations of the oil of *N. sativa*, the inhibition zone increased (\(p<0.001\)). The results determined that in tested bacteria, there was a significant difference (\(p<0.001\)) in terms of sensitivity to the oil. In other words, the most sensitivity was observed in *S. aureus*.
The seeds are used in Middle Eastern cooking, such as in their local breads. *N. sativa* is also used by thousands for their natural healing abilities. Concerning the method of oil and preventing from using high temperature to decrease the rate of destruction of effective herbal compound. Several compounds such as sterols and phenolic constituents were found in *N. sativa*. Presence of phellandrene, α-pinene, β-pinene, p-cymene, cis-carveol, trans-anethole, Thymoquinone, Thymol, α-Longipinene and Longifolene were identified in the composition of the obtained oil of *N. sativa*. Thymoquinone or thymohydroquinone (2-isopropyl-5-methyl-1,4-benzoquinone)\(^{38}\) are active components of the oil of *N. sativa* that have different pharmacological activities such as anti-inflammatory, antioxidant and antihypertensive effects\(^{39}\). In addition, a great antibacterial action of Thymoquinone against *Paenibacillus larvae* was observed (MIC values ranging from 8 to 16 mg/ml)\(^{90}\). Alkhafar et al. reported that Thymoquinone treatment reduced mortality in mice following Lipopolysaccharid and live *E. coli* challenge by 80-90\%\(^{41}\). There is a partial difference between these results and the similar studies. Many components of *N. sativa* were characterized by Burits et al.\(^{52}\) and Ali et al.\(^{13}\) using GC-MS, but the major ones were thymoquinone, p-cymene and carvacrol. All of these compounds had antibacterial effects. The results indicated that *N. sativa* oil with concentration about 0.031 g/ml has prevented the growth of *E. coli*, *S. aureus* and *B. subtilis*. Thus, the research suggests the antibacterial effects of the medical herb on Gram-negative and Gram-positive pathogenic bacteria. A number of authors have mentioned the antimicrobial activity of *N. sativa*. In a study done by Shahidi et al. also investigated *N. sativa* products indicated a good effect on the standard *S. aureus*\(^{43}\). Morsi et al.\(^{45}\) had proven that both the crude alkaloid extract and the water extract of the *N. sativa* were effective against some tested microorganisms like *staphylococcus* despite their resistance to other antibiotics. In a study conducted by Niakan et al.\(^{45}\), the antimicrobial effect of oil extract of *N. sativa* against *S. aureus* in laboratory was studied. They concluded that the anti-microbial effect of *N. sativa* oil extract is comparable with antibiotics such as Ceftazidime, Cefaclor, Cefamandole and Cefuroxime. They recommend experimental use of *N. sativa* to control *S. aureus* infections\(^{46}\). Farrag et al. found that the fixed oil of black cumin had an inhibitory effect against Gram-positive such as *S. aureus* and *B. cereus*. The essential oil showed antibacterial activity against *B. cereus* and *S. aureus*. Finally, our results are in agreement with others who showed that *N. sativa* oils produce antimicrobial activity against a broad range of microbes and especially against multiple-antibiotic resistant bacteria. From the study it can be concluded that the oil of *N. sativa* possess antibacterial activity. Our results support the use of the plant in traditional medicine and suggest that *N. sativa* oil possess compounds with good antibacterial properties. It can be used as an antibacterial supplement in the developing countries towards the development of new therapeutic agent. Additional in vivo studies and clinical trials would

**Table 1:** The diameters of growth inhibition zones in agar disk diffusion test in different dilutions of the oil from *N. sativa*.

<table>
<thead>
<tr>
<th>Dilution (g/ml)</th>
<th>Inhibition zone (mm) in disk diffusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Microorganism</strong></td>
</tr>
<tr>
<td>Positive control</td>
<td></td>
</tr>
<tr>
<td>1/8 (0.125)</td>
<td></td>
</tr>
<tr>
<td>1/16 (0.062)</td>
<td></td>
</tr>
<tr>
<td>1/32 (0.031)</td>
<td></td>
</tr>
<tr>
<td>1/64 (0.015)</td>
<td></td>
</tr>
<tr>
<td>1/128 (0.007)</td>
<td></td>
</tr>
<tr>
<td>1/256 (0.003)</td>
<td></td>
</tr>
<tr>
<td>Negative control</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2:** The diameters of growth inhibition zones in agar well diffusion test in different dilutions of the oil from *N. sativa*.

<table>
<thead>
<tr>
<th>Dilution (g/ml)</th>
<th>Inhibition zone (mm) in well diffusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Microorganism</strong></td>
</tr>
<tr>
<td>1/8 (0.125)</td>
<td></td>
</tr>
<tr>
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<tr>
<td>1/32 (0.031)</td>
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<tr>
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<tr>
<td>1/256 (0.003)</td>
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<tr>
<td>Negative control</td>
<td></td>
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</tbody>
</table>

**Table 3:** MIC and MBC of the oil of *N. sativa*.

<table>
<thead>
<tr>
<th><strong>Microorganism</strong></th>
<th><strong>E. Coli</strong></th>
<th><strong>S. aureus</strong></th>
<th><strong>B. Subtilis</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MIC</strong></td>
<td>1/32</td>
<td>1/32</td>
<td>1/64</td>
</tr>
<tr>
<td></td>
<td>(0.031)</td>
<td>(0.031)</td>
<td>(0.015)</td>
</tr>
<tr>
<td><strong>MBC</strong></td>
<td>1/32</td>
<td>1/32</td>
<td>1/32</td>
</tr>
<tr>
<td></td>
<td>(0.031)</td>
<td>(0.031)</td>
<td>(0.031)</td>
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</tbody>
</table>

**DISCUSSION**

In spite of the current interest in drug discovery by molecular modelling, combinatorial chemistry and other synthetic chemistry methods, plant-derived compounds are still substantiating to be an important source of medicines for human being. The significance and uses of plants in modern drug discovery has been recounted in recent reports\(^{31,32}\). Plant oils have been used for many thousands of years\(^{33}\), in food preservation, pharmaceuticals, alternative medicine and natural therapies\(^{34,35}\). Oils are potential sources of novel antimicrobial compounds, especially against bacterial pathogens.\(^{36}\) In vitro studies in this work showed that the oils inhibited bacterial growth but their effectiveness varied. The antimicrobial activities of many oils has been previously reviewed and classified as strong, medium or weak\(^{37}\). *N. sativa*, is an annual flowering plant that grows to 20-30 cm tall, is native to Asia and the Middle East. The flowers of this plant are very delicate and pale colored and

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be needed to justify and further evaluate the potential of it as an antibacterial agent in topical or oral applications.

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