Study the Effect of Cold and Hot Water Extracts of Parsley Plant *Petroselinum crispum* on the Growth of some Enterobacteriaceae

May H. Mohammad  
Department of Ecology, College of Ecology, Al-Qasim Green University, Babil-Iraq.  
E-mail: mayhameed85@yahoo.com.

Abstract  
This study was conducted to determine the effect of hot and cold water extractsof parsley leaves on five genus of bacteria belong toenterobacteriaceae family nomely; *E.coli, Enterobacter, Klebsiella, Shigella* and *Salmonella* after 24 or 48 hrs of incubation where the number of growth colonies on macConkey agar appears. The efficiency of these extracts to prevent bacteria from growth was indicated by using the percentage of dilution value. The resultsshowed that the hot water extract was more efficient in most treated bacteria compared with cold water extract. Also results indicated that both *Salmonella* and *Enterobacter* were more sensitive toward both extracts in comparison with other studied bacteria, such as *Klebsiella* showed higher resistance toward both extracts. Additionally, significant differences between number of colonies grown on MacConkey medium that treated with hot or cold water extracts were recorded.

Keywords: Parsley, Water Extracts, Enterobacteriaceae, Resistance.

Introduction  
Food poisoning affects both the consumer and food industry specially that treated with different conservation or using machines to prepare foods. For that the researchers of food safety and organizational agencies are interested to pursue high level of diseases caused by certain pathogens, which include micro-organisms in foods [1, 2]. In addition to development the resistance to antibiotics that used clinically especially after increasing use. This state leads to produce resistant strains more pathogenesis than others strains. For that the attention shifted to find natural antimicrobes factors that suitable for treating these organisms and do not appear any resistance to it, or creating a new types of nontoxic antimicrobes compounds to the consumer.

Many studies have concentrated on natural compounds derived from type of herbs, as food additives, and created numerous studies on the possibility of finding, or preparing a single compound of anti-bacterial, Viral or fungal isolation or extraction from natural sources, which vary with the diversity of these microorganisms and safety to consumer, such as volatile oils, especially essential oils consisting of basic turbinates and hydrocarbons used as anti-microbes but unfortunately some studies did not appear full understand to their mechanism [3].

Many studies have addressed the effect of plant extracts on microorganisms, especially those isolated clinically. Some of these studies deal with water and alcohol extracts as study of Sule and Agbabiaka[4] that is used three type of plants to find active constituents on four genus of Enterobacteriaceae: *Escherichia coli, Klebsiella* sp., *Salmonella* sp. and *Shigella* sp., which is the results showed the water extract was not affected on bacteria while alcohol extract was more effective for some plants. Other study details with 55 plant extracts, which is the results showed greater Antimicrobial ativities for acetone and ethanol extracts against twelve microbial genus compared with water extracts, and this study show it could be the potential source to develop antimicrobial agents [5]. In another study, the antimicrobial activity extracts has been evaluated with antibiotic susceptible and microorganisms resistance by usingethanolic extracts of some kind of plant. The results also showed the highest antimicrobial potentials of clove and jambolan plants while sage and yarrow extracts didn’t present any antimicrobial activity [6].

Many of the plants are widespread. Many of these plants have therapeutic uses especially...
as antioxidants, anti-tumor and anti-microbe
carriers [7]. Large number of the world's
population still depends on the benefits of
these plants as food, which provides effective
compounds on many diseases [8,9]. Parsley
plant as food was important medically and has
an important role in treatment of many
diseases because of containing effectively
compounds responsible on therapeutic
efficacy, such as antioxidants, anti-microbes,
anti-clotting contents, liver anti-toxins and
anti-fat accumulation in the blood [10]. Leaves
are rich with minerals, natural vitamins,
ascorbic acid and oils, and others such as
coumarines (Bergapten and Imperatorin),
flavonoids (Glycosides and Appin), essential
oils (Apiole and Miriszticin) and other
[11,12]. It is an important food that contain
fats, fibers, proteins, sugars, many minerals
and vitamins, such as vitamin (C) and vitamin
(A) that useful for the eyes. And its warehouse
for vitamin (B), such as (B1, B2, B3 and B6)
with helpfully to absorb iron from other foods
[13].

Enterobacteriaceae is a family of intestinal
bacteria, small bacillary form, gram negative,
non forming spores, mobile and others
immobile, possess capsule and others do not
and containing more than 100 types of bacteria
that inhabit normally in human and animals
intestines. Some of it are normal flora and
others are known as pathogens [14]. Many of
these bacteria can fermentation different
types of carbohydrates and developing
resistant to many antibiotics. It is constituted
of 80% gram negative bacteria, 50% of
it are isolated diagnosing in the laboratories
of most hospitals [15], such as E. coli,
Klebsiella pneumonia, Proteus mirabilis,
Enterobacter spp. and Serratiamarcescens
[16]. Some species that important to human
can survive in soil, water and decomposition
material and can produce external toxins called
exotoxins and internal enterotoxins [17] that
effectedly on gastrointestinal tract.

This study aimed to investigate the effect
of hot and cold water extract of parsley
plant on the growth of five genus of
Enterobacteriaceae: E.coli, Enterobacter,
Klebsiella, Shigella and Salmonella that can
growth on macConkey media dependent on the
fact that these bacteria are the most common
contaminants in foods, which is effected
directly on the human healthy. In addition to
that these bacteria in some studies showed
more resistance to some plant extracts as
compared with gram positive bacteria, such as
study of Osman et al. [14] and Shan et al. [1].

Materials and Methods

Parsely plant Petroselinum crispum, is a
herb, bilateral strabismus from umbrella
family (Umbelliferae or Apiaceae [11], native
to the Mediterranean region and cultivated in
various parts of the world in present time [18],
the height was 6-200 cm and the stem
standing, rounded and branched with many
stalks grow from one root. The leaves are
compound, the flowers clustered in groups
with white color and the fruits take shape
umbrella compound. Parsley recognized by
pungent aromatic odor and bright green leaves
with high content from chlorophyll. Also the
parsley oil can be obtained from the seeds
[19].

Preparation of Extracts

Similar method has been used as stated by
Muraliet al. [20]and the way followed by
Mohammad and Al-Jibory [21] were collected
the leaf parsley and washed with fresh tap
water to remove dust and dried for 24 hrs at a
temperature of 70-80°C. After that the dry
leaves grinded by electrical mill and sieved
through clean passage gauze to remove the
solid parts that did not grind well. The weight
of 300 g of the preparing powder has been
taken to sift and mix well with 500 ml of cold
distilled water by an electric mixer until
blended contents well. These components
separated first by using normal nomination
papers and second by using the centrifuge on
speed of 3000 r / min for 15 minutes to get the
filtrate; which is left to dry for getting a
vegetable powder extract that 5 g of it has
been mixed with 100 ml of distilled water to
obtain the standard solution have 50%
concentration. Finally the aqueous extract
sterilized through special leaves passage have
0.45 µm diameter holes and transferred to
sterile bottles under refrigeration until a test on
it. The same method has been done to prepare
hot water extracts after boil the sample for one
hour [22].

Preparation of Bacteria
The bacteria activated before the test in a nutrient broth after taken from laboratory of hospital and isolated from other types of bacteria by using differentiation culture medium. A biochemical test has been used to confirm the diagnosis of the genus of bacteria: E.coli, Enterobacter, Klebsiella, Shigella and Salmonella from each other that under the study. The procedure of food and drug administration [23] for detection, isolation and identification was followed, and the pour plate method on facultative nutrient media has been used to know the numbers of these bacteria after counted the colonies of bacteria that developing in the test [24].

Examination of Water Extracts

A similar method as stated by food and drug administration [23] was used to evaluate the inhibition of bacterial growth and to identify the effectiveness of antimicrobes after mixing 10 ml of extract with a litre of bacterial nutrient medium. The media were poured in sterile plates in average of five replicates for each treatment. This media was left to harden then incubated upside down at a temperature of 37°C for 24 and 48 hrs. Finally the colonies of Enterobacteriaceae were counted.

Experimental design and statistical analysis

A completely randomized design (CRD) was used. Data were analyzed statistically by using less significant differences (LSD) at 0.05 after subjection to the analysis of variance [25].

Results and Discussion

Table 1 shows the most important genus of Enterobacteriaceae: E.coli, Enterobacter, Klebsiella, Shigella and Salmonella that isolated from Hospital laboratory; as a result of continuing pathogenesis to humans and contamination various foods and environments. Results showed that these gram negative bacteria grown on MacConkey agar were affected by hot and cold water extract. Results exhibited that Salmonella and Enterobacter colonies were more sensitive to the hot water extract than E.coli, Klebsiella and Shigella, as it didn't show any growth on the culture media treated with extracts. This could be due to the presence of the active substances in hot water extracts that can break the surface of the outer membrane of the bacteria-rich multiple liposaccharide molecules [26]. It was damaged the enzyme or associated with shattered enzyme particles [27], to prevent the enzyme from performing its duties. These substances could be phenolic compounds [28] that extracted by hot water extract and appears as compounds that have inhibition of bacterial growth, or may be some basic oil materials [29, 30] that could be melted in hot water extracts which have the ability to analyze the cell wall and can interfere with the components of cytoplasmic membrane.

Results also indicated that some bacteria did not inhibit by components of the hot water extract, such as E.coli and Klebsiella while other genus of bacteria were less resistant compared to cold water extract as shown in the Table (2). E.coli was the most resistant after Klebsiella as mentioned earlier, followed by Shigella that showed a kind of low resistance (Table (3)). Some studies indicate that the reason for the growth of these bacteria may due to the structure of membrane that prevents other molecules from penetration. Additionally the presence of enzymes in this structure [27]. The studied bacteria can be arranged according to their resistance to hot water extracts as follows: Klebsiella > E.coli > Shigella > Enterobacter > Salmonella.

The result pointed to the lowest number of colonies of bacteria developing in media indicating that colonies of Salmonella were more sensitive to cold water extract compared with Enterobacter and other kinds of bacteria that developed on MacConkey medium, as it did not show any growth, while Klebsiella showed over resistance for cold water extracts as shown in the Table (2) and Fig. (1). This sensitivity may return to the components in cold water extract affecting this type of bacteria. As study of Brantner and Grein [31] indicated that many parsley extracts can broke the bacterial cell completely. While the study of Peter et al. [32] pointed to the potential for these compounds to break down the cell wall of bacteria.

Results also indicated that E.coli had less growth in cold water extracts after 24 hrs of incubation, which means this bacterium in the first time has sensitivity to components of the
cold water extract than the components of hot water extract as shown in Table (2). Shigella appeared more resistant to cold water extract after 24 hrs than E.coli (Table (3)) but, after 48 hrs the E.coli showed higher resistance than of Shigella. In addition to that results indicated that Klebsiella was the most resistant bacterium to the cold water extract than any other bacteria under investigation with high average of developing colonies on macConkey medium. This may be caused by possessing external membrane and the obligatory unique peripheral plasmic not present in other types of bacteria, or the presence of specific enzymes [27] that break these materials before implemented into the bacterial cell. The bacteria can be arranged according to their resistance to their cold water extracts as follows: Klebsiella>E.coli>Shigella>Enterobacter>Salmonella.

Results showed that Shigella, Klebsiella and E.coli despite their growth on agar treated with hot water extracts, the number of the colonies were few compared to control. Number of colonies were 56-60, 35-43, 0.5-2 colony/ml. Number of colonies in control treatment were 110-121, 55-57, 60-68 colony/ml respectively. This suggesting perhaps that some components of the hot water extract had stopped or slowed the growth of bacteria instead of killing them. As the study of Moazedi et.al. [33] which is pointed that some plants have hampered compounds to bacterial growth and movement instead of breaking it. Other study indicated to some plant extracts that can not only effect bacteria from growing but also hampered growth by prolonging the life cycle of bacteria by stopping or limiting the movement of bacteria [5].

It can be said that most of the studied bacteria have shown growth on the medium treated with cold and hot water extracts, and there were some differences between the sensitivity of the bacteria to both extracts.

It can be concluded that the materials or compounds in hot and cold water extracts can be very important as ant materials, mostly antioxidants. There are many previous studies indicated that in spite of the outer membrane responsibility for the classification of bacterial strains, it is responsible for many of the genetic characteristics which make the response to extracts vary from one bacterium to another [28].

Conclusions
1- Hot water extract was more effective in inhibiting or reducing the growth of studied bacteria than shown in cold water extract.
2- Salmonella and Enterobacter were more sensitive to both extracts.
3- Klebsiella was higher resistant to both extracts.

Table (1)
Numbers of bacterial colonies that developed on MacConkey medium treated with hot and cold water extracts of Parsley leaves after 24 and 48 hrs of incubation.

<table>
<thead>
<tr>
<th>Time (hour)</th>
<th>Type of extract</th>
<th>Genus of bacteria that used</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>Hot</td>
<td>E.coli, Enterobacter, Klebsiella, Shigella, Salmonella</td>
</tr>
<tr>
<td></td>
<td>Cold</td>
<td>E.coli, Enterobacter, Klebsiella, Shigella, Salmonella</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>E.coli, Enterobacter, Klebsiella, Shigella, Salmonella</td>
</tr>
<tr>
<td>48</td>
<td>Hot</td>
<td>E.coli, Enterobacter, Klebsiella, Shigella, Salmonella</td>
</tr>
<tr>
<td></td>
<td>Cold</td>
<td>E.coli, Enterobacter, Klebsiella, Shigella, Salmonella</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>E.coli, Enterobacter, Klebsiella, Shigella, Salmonella</td>
</tr>
</tbody>
</table>

- No growth, + number of colonies less than 15 colonies, ++ number of colonies greater than or equal to 15 colony.

Table (2)
The Percentage of resistant bacteria that developed on MacConkey medium treated with hot and cold extracts of Parsley leaves after 24 and 48 hrs of incubation.

<table>
<thead>
<tr>
<th>Time (hour)</th>
<th>Type of extract</th>
<th>Genus of bacteria that used</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>Hot</td>
<td>E.coli, Enterobacter, Klebsiella, Shigella, Salmonella</td>
</tr>
<tr>
<td></td>
<td>Cold</td>
<td>E.coli, Enterobacter, Klebsiella, Shigella, Salmonella</td>
</tr>
<tr>
<td>48</td>
<td>Hot</td>
<td>E.coli, Enterobacter, Klebsiella, Shigella, Salmonella</td>
</tr>
<tr>
<td></td>
<td>Cold</td>
<td>E.coli, Enterobacter, Klebsiella, Shigella, Salmonella</td>
</tr>
</tbody>
</table>

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Table (3)
The Average of colonies numbers of bacteria developed on MacConkey medium treated with hot and cold water extracts of Parsley leaves after 24 and 48 hrs of incubation.

<table>
<thead>
<tr>
<th>Time (hour)</th>
<th>Type of extract</th>
<th>Genus of bacteria that used</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>E.coli</td>
</tr>
<tr>
<td>24</td>
<td>Hot</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>Cold</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>9.6</td>
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<tr>
<td>48</td>
<td>Hot</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>Cold</td>
<td>82.64</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>115.5</td>
</tr>
</tbody>
</table>

Fig.(1) The effect of hot and cold water extracts of parsley leaves on some genus of Enterobacteriaceae after 48 hrs of incubation.

References


الخلاصة

أجريت هذه الدراسة لتحديد تأثير المستخلصات المائية الحارة والباردة للأوراق المعدنية على خمسة أنواع بكتيرية تعود لعائلة البكتيريا المعوية: E.coli و Klebsiella و Shigella و Enterobacter و Salmonella. بعد 24 و 48 ساعة من الحضن اذ يمكن ان تظهر المستعمرات النامية على وسط ماكونكي. وتم الاطلاع على كفاءة تلك المستخلصات في منع البكتيريا من النمو عبر ايجاد النسبة المئوية للقيم. وقد أظهرت النتائج أن المستخلص المائي الحار كان أكثر كفاءة في الحد من نمو المثلية المعوية من المستخلص المائي البارد، كما اشارت النتائج إلى أن كل من E.coli و Shigella و Enterobacter و Salmonella كانت الأكثر حساسية تجاه المستخلصين مقارنة ببقية البكتيريا الأخرى المدروسة، وأيضا اظهرت اختلافات كبيرة جدا تجاه كلا المستخلصين. كما اظهرت النتائج وجود فروق معنوية بين اعداد المستعمرات النامية على وسط ماكونكي المعامل بالمستخلص المائي الحار والبارد.