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

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Abstract

This study was conducted to determine the effect of hot and cold water extractsof parsley leaves on five genus of bacteria belong toenterobacteriaceae familynomely; *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 extractsto prevent bacteria from growthwas indicated by using the percentage ofdilution value. The resultsshowed that the hot water extract was more efficientin most treated bacteria compared with cold water extract. Also resultsindicated that both *Salmonella* and*Enterobacter*were more sensitive toward both extracts incomparsion with other studied bacteria, such as*Klebsiella*showed higher resistance toward both extracts. Additionally, significant differencesbetween number of colonies grown on MacConkeymedium 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 pursuit 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 useit. This state leads to produce resistant strains more pathogenesis than others strains. For that the attention shifted to find natural anti-microbes factorsthat suitable for treating these organisms and do not appear any resistance to it, or creating a new types of nontoxic anti-microbescompounds 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 ani-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 turbines and hydrocarbons used as anti- microbes but unfortunately some studies did not appear full understand to their mechanism [3].

Many studies haveaddressed theeffect of plant extracts onmicroorganisms, 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 no effected 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 somekind of plant. The results also showed the highest antimicrobial potentials extracts of cloveand jambolanplants 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 materials [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 wasimportant 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].

Enterobacteriaceaeis 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*, *Klebsiellapneumonia*, *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 effectedonly on gastrointestinal tract.

This study aimed to investigate the effect of hot and cold water extractsof parsley plant on the growth of five genus of Enterobacteriaceae: *E.coli, Enterobacter, Klebsiella, Shigella*and *Salmonella* that can be 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 positivebacteria, such as study of Osman *etal.* [14] and Shan *etal.* [1].

Materials and Methods

Parsely plant *Petroselinumcrispum*, 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 Murali*etal.* [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 70-80ºC.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 culturemedium. 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 evalute 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^{o}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. Datawere analyzed statistically by using less significant differences (LSD) at 0.05 after subjection to the analysis of variance [25].

Results and Discussion

Table 1shows 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 shatteredenzyme 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 maydue 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 that 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 were56-60, 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*etal.* [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 higherresistant 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.

Time (hour)	*Type of extract*	*Genus of bacteria that used*				
		E.coli	*Enterobacter*	*Klebsiella*	*Shigella*	*Salmonella*
24	Hot	+	-	+	+	-
	Cold	+	-	+	+	-
	Control	+	++	++	+	++
48	Hot	++	-	++	+	-
	Cold	++	+	++	++	-
	Control	++_	++	++	++	++

- 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.

Time (hour)	*Type of extract*	*Genus of bacteria that used*				
		E.coli (%)	*Enterobacter (%)*	*Klebsiella (%)*	*Shigella (%)*	*Salmonella (%)*
24	Hot	25	0	1	2	0
	Cold	6	0	13.4	27	0
48	Hot	50	0	69.64	6.25	0
	Cold	71.55	10.39	94.64	51.56	0

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.

Time (hour)	*Type of extract*	*Genus of bacteria that used*				
		E.coli	*Enterobacter*	*Klebsiella*	*Shigella*	*Salmonella*
24	Hot	2.4	0	0.2	0.2	0
	Cold	0.6	0	2.68	2.7	0
	Control	9.6	56	20	10	23
48	Hot	58	0	39	3.5	0
	Cold	82.64	8	57.73	32.99	0
	Control	115.5	80	61	64	35

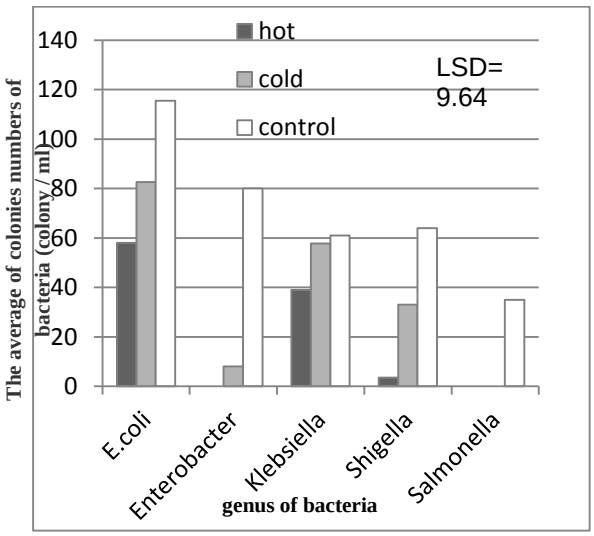

Fig.(1) The effect of hot and cold water extracts of parsley leaves on some genus of Enterobacteriaceae after 48 hrs of incubation.

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الخلاصة

اجريت هذه الدراسة لتحديد تأثير المستخلصات المائية الحارة والباردة لأوراق نبات المعدنوس على خمسة اجناس بكتيرية تعود لعائلة البكتريا المعوية :*E.coli* و*Enterobacter* و*Klebsiella* و*Shigella* و *Salmonella* بعد 24 و 48 ساعة من الحضن اذ يمكن ان تظهر المستعمرات النامية على وسط ماكونكي.وتم الاشارة الى كفاءة تلك المستخلصات في منع البكتريا من النمو عبر ايجاد النسبة المئوية للقيم. وقد اظهرت النتائج ان المستخلص المائي الحار كان اكثر كفاءة في الحد من نمو اغلب البكتريا المعاملة من المستخلص المائي البارد، كما اشارت النتائج الى ان كل من *Salmonella* و *Enterobacter* كانت الاكثر حساسية تجاه المستخلصين مقارنة ببقية البكتريا الاخرى المدروسة، وايضا اظهرت *Klebsiella* مقاومة كبيرة جدا تجاه كلا المستخلصين. كما اظهرت النتائج وجود فروق معنوية بين اعداد المستعمرات النامية على وسط ماكونكي المعامل بالمستخلص المائي الحار والبارد.