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Antimicrobial activity of the mint extract against campylobacter jejuni isolated from chicken sample

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Abstract--Foodborne infectious diseases are seen around the world. One of the most common contaminants found in chicken meat products is *Campylobacter jejuni*. This study tries to: To assess the utilization of Mint extract in various concentrations as natural antimicrobial against *C. jejuni*, 100 chicken samples were collected From the city center of AL- Diwaniyah, 50 samples of fresh poultry meat and 50 samples of frozen poultry meat, and the evaluation was done on them after preparation. The antimicrobial activity of methanolic, ethanolic, and aqueous extracts of mint was evaluated in multi-well micro-titer plates using the well diffusion method. Mint extract was evaluated for antimicrobial property against *C. jejuni* at different concentrations (80%, 40%, and 20%), and the highest inhibitory effect was observed in the ethanolic extract of mint, followed by the methanolic extract, while the aqueous extract was active only at a high concentration of 80% using the well diffusion method. Due to the biologically active properties of the compounds in mint, which are antimicrobial, antibacterial, anti-inflammatory, anticancer, antioxidant, and antifungal, mint extracts exhibit biological activity against *C. jejuni*.

Keywords--mint, extracts, antimicrobial activity, campylobacter jejuni, chicken.

Introduction

The chicken meat sector is the largest source of acceptable animal protein, with a high meat yield, low cooking shrinkage, and a rich source of amino acids, vitamins, and minerals (1). *Campylobacter jejuni* is the most common pathogen that causes food poisoning around the world (2). *C. jejuni* is also one of the most common causes of human bacterial enteritis. Nowadays, practically all consumers

are concerned about consuming food that is high in nutritional content, contains natural preservatives, and is devoid of chemical preservatives and microbial hazards. In addition to being safer, healthier, and less susceptible to hazards than foods containing artificial food additives, especially in meat that is highly susceptible to microbial growth, such as various meat kinds, which considered favorable media for food degradation and food-borne infections in humans, causing major health problems (3). Consumer desire for all-natural products has led industry and regulatory agencies to investigate at the possibility of using natural antimicrobials to prevent or minimize the growth of foodborne pathogens and spoilage bacteria.

Despite the fact that several research have suggested that Essential oils (Eos) could be employed as a natural antibacterial preservative in the food industry(4). Mint (*Mentha* spp.) is one of the plant species from which essential oils are extracted, and it is used as a flavoring agent in cosmetics, pharmaceutical items, food (including candy and gum) and liqueur all over the world (5). Mint species *Mentha spicata* is native to North Africa, Egypt, and Morocco. It is a member of the *Mentha* genus of the Labiateae family (Lamiaceae). Because of its fragrant and flavorful properties, it is commonly utilized in commercially manufactured products, food, and medication (6,7). Mint leaves are widely used in herbal teas and in the cooking to enhance flavor and aroma. *Mintha* spp. are known for their peculiar smell and flavor, which is attributed to the naturally occurring cyclic terpene menthol (8). *Mentha* has 25-30 species and is known for its antibacterial, antiviral, and antifungal properties. Mint essential oils are widely utilized in the cosmetics, food, and pharmaceutical industries due to their fragrant, stimulating, and carminative properties(9). There is a correlation between the chemical structure of the essential oil's most abundant components and its antibacterial activity(10).

Materials and Methods

Plant Collection

The mint plants were obtained from local AL- Diwaniyah's markets after being separated from their leaves, washed thoroughly, and dried for a month in a dark, dust-free environment at room temperature, following which they were ground and stored until needed.

Preparation of extracts

Aqueous extract

The powder was extracted with distal water according to the maceration method by taken 50 g of mint powder was prepared and placed in 500 ml of distilled water and placed at a temperature of 38°C and a shaker for two hours, then left for a period of 5- 7 days after that, filter it with filter paper(11).

Alcoholic Extract **Ethanolic extract**

The powder was extracted with Ethanol according to the maceration method used 70% ethyl alcohol (350 ml of alcohol and 150 ml of distilled water), it was taken 50 g of mint powder, added 500 ml of alcohol, so the ratio of solvent to solute became 1:10.. It was left in a place away from light for 7 days (11).

Methanolic extract

The powder was extracted with Methanol according to the maceration method used 80% methyl alcohol (320 ml of alcohol and 80 ml of distilled water), it was taken 40 g of mint powder, added 400 ml of alcohol, so the ratio of solvent to solute became 1:10.. It was left in a place away from light for 7 days.. (11). The extract was filtered by Whatman no.1 filter paper. The filtrate was concentrated in a rotary evaporator at 40°C. The concentrated extract was oven dried at 40°C for 3 days and freeze dried for 48 h. The freeze dried extracts were stored at -20°C until use.

Isolation of Campylobacter

A total of 100 samples of fresh and frozen chicken meat were collected from various markets in Al - Diwaniyah, with 50 samples of fresh chicken and 50 samples of frozen chicken chosen at random. 25 g of each sample was taken from the chest, wing, and thighs muscles and placed in 90 ml of peptone water, mixed well, and incubated at 37 °C under microanaerobic conditions for 48 hours. The broth was then streaked onto selective Campylobacter base Agar (Oxoid) containing antibiotic supplement (Oxoid) and Blood agar at 42°C for 48 hours under microaerophilic conditions (5 % O₂, 10 % CO₂, and 85 % N₂) using Campylobacter gas generating kits (Oxoid) under microaerophilic conditions (5 % O₂, 10 % CO₂, and 85 % N₂) (12). According to (12) the isolates were biochemically identified using catalase, oxidase, urea hydrolysis, hydrogen sulphide (H₂S) generation, Nitrate reduction test and quick hippurate hydrolysis test. PCR was used to identify pathogenicity genes in 20 pure positive isolates for *C. jejuni* using primers 16s rRNA

Antimicrobial activity Of Extraction

The organisms (*C. jejuni*) were enrichment under microaerobic conditions in Muller Hinton broth at 42°C/24 h to obtain 10⁵ cfu/mL. The tubes are turbid by growth on liquid media with 0.1-0.5 ml sterile saline solution containing 10⁵ colonies (McF Mac frland) then strains were cultured under microaerobic condition on Muller Hinton agar plates. Disc Diffusion Method used to determine antimicrobial activity according to the zone of inhibition measurement. Sterile filter paper discs (5.0 mm diameter) were soaked with 1 µL of mint extract and placed in the holes (wells) made on the inoculated agar plates (5.0 mm diameter). At the same time, a control pit is made by placing 1 µl of sterile distilled water or ethanol and methanol in the pit, and also placing an antibiotic tablet (tetracycline). All plates were left at room temperature for 30 min to allow diffusion of oil before inverting the plates for incubation under microaerobic

conditions at 42°C/48 h. Then read the result by measuring the diameter of the inhibition area which represents the area of no bacterial growth surrounding the hole by the ruler.

Statistical Analysis

The means and variance analysis (anova across the different data and treatments in this study were obtained using standard error and analysis of variance ($P < 0.05$) using the statistical technique GraphPad Instant version 3 for Windows.

Results

Cultural Characteristic

The results indicates that the rate of positive isolated of *Campylobacter jejuni* from fresh chicken meat was 6:50 (12 %) and from frozen chicken meat was 8:50 (16 %) by culture methods. The characterization of *Campylobacter* spp. isolates on primary selective media, such as *Campylobacter* agar base and blood agar, reveals that *Campylobacter* colonies are small, mucoid colonies that are usually grayish in coloration but some must be creamy grey in color, moist, slightly raised, and often produce discrete colonies, flat with irregular edges, and non-hemolytic at 24-48 hours (13).

Antimicrobial Activity of Mint Extract

The results of a sensitivity test for Mint plant extract demonstrated that the ethanolic extract was superior to the methanol and aqueous extracts, and that the effectiveness of the 80% concentration exceeded the 40% concentrations, where the effectiveness of ethanol, methanol, and aqueous extracts were (22 ± 1.02 , 20 ± 1 , 15 ± 0.6), respectively, at the 80% concentration, and at the 40% concentration was (16 ± 0.45 , 15 ± 0.4 , 0) respectively. While at a concentration of 20%, the effect of aqueous methanol was superior to ethanol it was (10 ± 0.36 , 13 ± 0.2 , 0) at 20%, respectively. It's interesting to note that the aqueous extract was ineffective at concentrations of 40% and 20%. Statistical analysis revealed substantial differences in the efficiency of the three Mint extracts, with significant differences between concentrations of 80 %, 40 %, and 20 %. Probability level of less than 5%.

Table 5-1
Diameter of zone of inhibition (mm) of Antimicrobial extracted from Mint plant against *C. jejuni*.

Strain of Bacteria	Concentration% Mg/Ml	Inhibition zone /MM		
		Mint extract		
		methanol	ethanol	aqueous
<i>Campylobacter jejuni</i>	80%	20 ± 1 Aa	22 ± 1.02 Ab	15 ± 0.6 Ac
	40%	15 ± 0.4 Ba	16 ± 0.45 Bb	0 ± 0 Bc
	20%	13 ± 0.2 Ca	10 ± 0.36 Cb	0 ± 0 Cc
LSD($P < 0.05$)		1.21		

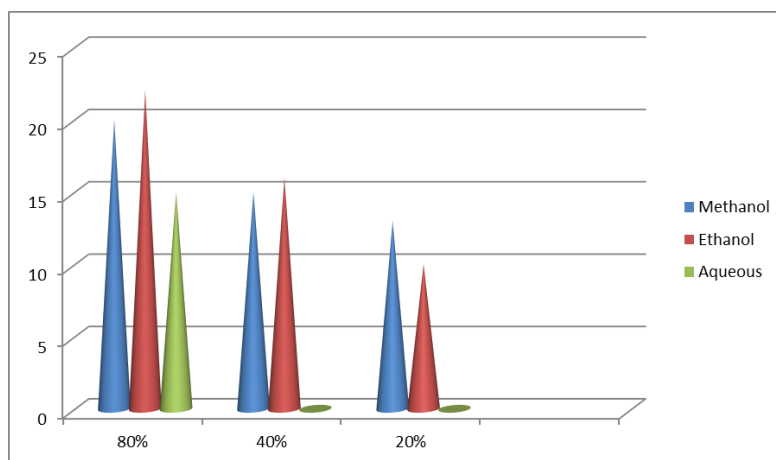


Figure 5-6. Antimicrobial activity of Mint extract agents *C.jejuni* in different concentration

Discussion

Campylobacter jejuni has become one of the most common bacterial causes of food poisoning worldwide. There are obviously on going food safety issues posed by *C. jejuni*, which are exacerbated by the rising spread of antibiotic resistance among isolates. As a result, finding alternative antimicrobials, both for prevention and treatment, is critical. Essential oils, with their antimicrobial properties, could be a potential solution to these issues. Different mechanisms have been identified and proposed to contribute to their antibacterial activities. Because certain components are hydrophobic, they can cause lipid partitioning in the bacterial cell membrane, resulting in increased membrane permeability, lipid depolymerization, and thus a disruption in coordinated ion flow, resulting in lower membrane potential and ATP generation. Other EO components can obstruct the transfer of vital chemicals into the cell by interfering with cell wall proteins. However, the precise manner of antibacterial activity of most EOs has remained a mystery until now, despite several studies emphasizing the role of pore formation and subsequent oxidative stress in this process(14). *Campylobacter* is a sensitive organism that requires sophisticated media containing blood in order to grow in vitro(15).

Without the application of selective procedures, isolating this organism is challenging(16). The results revealed that cultural characterisation on selective media produces small, mucoid colonies that are usually grayish in coloration but can be creamy grey in color, moist, slightly elevated, and often create discrete colonies that are flat with irregular borders and non-hemolytic. These findings are consistent with earlier research (17, 13, 18). The colonial morphology of *Campylobacter* has been used as a guideline for species identification, but it has not been used as a significant indication factor for a variety of reasons, including bacterial strain, basal medium, moisture level on the agar surface, incubation temperature, and incubation time (19). The percentage of *C.jejuni* isolated on *Campylobacter* base agar and Blood agar was 14%. These result agree with (20) was the percentage of isolated15% Isolated from random samples. While not

agree with(21),(22) and(23) all found much greater value (76.9 %, 75.6 % and 79 % respectively).(24) and(25) Isolated from questionable samples , on the other hand, found substantially lower isolation rates in Egypt (3 %and 6 %respectively).

The differences in *Campylobacter* isolation rates between studies could be due to a variety of factors including the type of samples tested, location, climate conditions, sanitary level, and isolation as well as identification methods. The study's major goal was to investigate natural antimicrobials against *Campylobacter jejuni* to see if they could be used as active ingredients to inhibit growth colonization in chickens. . The natural extract from mint where prepared with (ethanol ,methanol and aqueous) then tested against *Campylobacter* strains using disc diffusion method. The mint extract with ethanol showed higher inhibition zone against *Campylobacter jejuni* (22 - 10) than mint extract with methanol (20 - 13) due to their main active components for mint and alcohol .while the aqueous extract show activity only in higher concentration (80%,15) it very low value comparative with alcoholic extract. The extracts of the *Mentha* plant were shown to be highly effective against bacteria, with the intensity of the activity varying depending on the solvent employed and the concentration of the extract.

The ethanol extract inhibited bacteria more effectively, with an average diameter of (22-10) mm. when the level of inhibition reached methanolic (20-13) With the maximum concentration of the extract, the aqueous extract inhibited bacterial growth less (15, 80)% . This is in agreement with a study by (26)which found that the aqueous extract had a high inhibitory activity against various types of positive bacteria. *Staphylococcus aureus*, *Bacillus fastidiosus*, and the negative bacteria *Escherichia coli*, *Salmonella choleraesuis* The findings revealed that the effects of methanol and ethanol extracts of the plant were identical. The presence of second-hand compounds was attributed for the inhibition. These extracts contain saponins, flavonoids, fucomarines, and terpenes. This study corroborated. Maysa Yazigi (27) conducted an experiment to demonstrate the great efficacy of mint extract in inhibiting fungus growth. Mint extract have strong antifungal effectiveness is attributed to the presence of essential oils such as *Menthon* *Menthol*, *Menthofuranem*, and *Menthhe Acetate*, where it is known that essential oils, whether in mint or other plants, have antifungal activity as pathogenic fungi (28)This is attributed to the high efficiency of the extract method utilized in this study against bacteria of the mint plant's active compounds, as well as the use of crude extracts (a combination of compounds) rather than fractionally isolated compounds. The majority of research has indicated that the benefit of utilizing the natural extract is the combined action of a variety of components. It has a diverse microbial population and a minimal incidence of disease resistance (29).

Conclusion

Many herbal plants are used not only as a food flavoring agent, but also as a food preservative to keep meals from spoiling due to microorganisms. Because of their antibacterial properties, mint extract can be used as a natural preservative in perishable foods, particularly protein-rich foods. can halt the growth of *campylobacter jejuni* and some *C. jejuni*, virulence genes as Gram negative and positive bacteria, especially in high concentrations in vitro, and may be suitable

for preventing foodborne disease, particularly in highly perishable food. More research is needed to better understand and apply other herbs' essential oils.

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