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Antibacterial effects of Mentha piperita extract and their synergistic effects with Some Antibiotics Against *Escherichia coli* Isolated From minced Meat

[1] Mussa M Alkhatib, [2] Entesar Hussain madi, [3] Mohammed Khadim Wali

[1] Department of Veterinary Public Health, College of Veterinary Medicine, University of Al Muthanna, Samawah, Iraq
[2] Zoonoses Research Unit, College of Veterinary Medicine, University of Bagh
[3] Department of Veterinary Public Health, College of Veterinary Medicine, Al-Qasim Green University, Iraq

Corresponding Author: **Mussa M Alkhatib**

Abstract

Mentha piperita extract and an antibiotic were tested against Escherichia coli isolated from minced meat for their antibacterial and synergistic effects. Ninety-nine samples of minced meat were procured from local supermarkets in the province of Babylon. In order to determine the presence of E. coli in the minced beef samples, the samples were tested for microbiological quality (Aerobic plate count and total coliform count). A total of 18 (or 16.2%) of the 90 minced meat samples tested were positive.

The result indicated that high significance ($p \geq 0.05$) between Mentha piperita, sulfonamide, and tetracycline different concentrations (0.5 and 8 mg\ml) (11.5±0.748 to 21.2±1.2), (3.8±0.374 to 24.4±1.630) and (1.6±0.244 to 17.4±0.871) respectively.

A significant ($p \geq 0.05$) correlation was found between the synergistic effects of Mentha piperita and both sulfonamide and tetracycline when used together against E. coli in this study.

The obtained demonstrated that minimum inhibition of the synergistic effect of Mentha piperita extract with sulfonamide could inhibit *E. coli* in different concentrations (6.4 ± 0.678 to 26 ± 0.836) while antibiotic drugs of tetracycline in some concentrations could inhibit *E. coli* (1.6 ± 0.244 to 17.4 ± 0.871).

Keywords: Minced Meat, Plant Extract, Antibiotic

1. Introduction

Meat and meat products are a great source of important nutrients like protein, fat, and minerals [1]. Food spoilage outbreaks occur on a yearly basis at all points along the production chain, from slaughter to packaging to packaging to transportation. Quality degradation can result in significant economic and ecological harm due to factors like lipid oxidation and autolytic enzymatic spoilage as well as microbial deterioration [2]. Microorganisms like Clostridium spp., Salmonella spp., Campylobacter jejuni, Escherichia coli O157:H7, and Listeria monocytogenes can be transmitted through the improper storage and processing of meat and meat products [3, 4]. Meat and meat products deteriorate in quality as a result of the proliferation of these microorganisms in abundance [5, 6], bad smell and taste, discoloration, inability to maintain texture, gas production and pH change may be due to the deterioration of the products' proteins and fats [3].

There are numerous bioactive compounds found in essential oils (EOs) that can extend the shelf life of beef and other protein-rich foods. EOs are widely used in the food industry due to their ability to retard food spoilage, enhance organoleptic quality, and inhibit pathogen growth [7]. According to the FDA, they are "generally recognized as safe" food additives for human consumption with antimicrobial, anti-fungal and antioxidant properties.

The Lamiaceae family includes peppermint (Mentha piperita), whose aerial parts are traditionally used for their antiseptic properties during the flowering season. Mentha spp. essential oils and extracts are now being used in food production. In food, this essential oil may be used as an alternative to chemical-based antibacterial and flavoring agents because of its antioxidant and anti-microbial properties [8].

In light of these findings, the use of EOs in meat products could reduce the risk of foodborne outbreaks in consumers, according to these studies [9]. Some studies have been done in vitro on plants used in traditional medicine, particularly on pathogenic bacterial growth; and some of these studies were about the antimicrobial activity of Mentha piperita (mint) [10]. There is still a need for more research into the synergistic effects of plant extracts and antibiotics on the human body [11]. Mentha piperita extract was tested for antibacterial effects and synergistic effects with antibiotics against Escherichia coli

isolated from minced meat as part of this study.

2. Materials and methods

2.1 Samples collection

E. coli was isolated from ninety minced meat samples taken from a Babylon supermarket and transported in an ice box to the laboratory of the Department of Veterinary Public Health.

2.2 Mentha extracts

Herbal plants hopping market in Babylon province sold the Mentha piperita plant. Distillation flasks were used to distill the dried plant, which was ground in a mixer grinder and added to the mixture with ethyl alcohol. The ethanol method was used to extract the MEO, and the temperature was kept at 100°C for three hours. In order to get enough MEO for further experiments, this procedure was repeated several times. Filters of 0.45 microns were used to sterilize the essential oil before it was evaporated and stored at 4oC for further analysis [12].

2.3 Bacterial isolated

45 ml of peptone water was used to homogenize 5 grams of minced meat (0.1 percent). Eosin-methylene blue (LEMB) agar was incubated at 37O C for 24 hours after the dilutions were spread on it. The purification of suspected E. coli isolates on trypticase soy agar (and confirmation by Gram stain, growth in brilliant green bile) [13].

2.4 Antibiotic susceptibility

In this study, antimicrobials such as tetracycline and sulfonamide were tested as pure powders. Dissolving the powder in TSB at various concentrations (0.5, 2, 4, and 8 mg/ml) yielded the stock solution [14].

2.5 Antibacterial activity test

Bacterial culture was diluted to 0.1ml on Muller Hinton agar media and the discs were placed in the center of wells and kept at room temperature. There were three sets of wells made in each plate, and 100l volumes of each dilution were added aseptically into the wells in each set using the sterile corn borer with an 8.0mm diameter, and the discs were incubated at 37°C for an hour with various concentrations of plant extract (0.5, 2,4 and 8 mg/ml). The wells were then filled with the various concentrations of plant extract (0.5, 2,4 and 8 mg/ml) (18 hours). After 24 hours of incubation, the area of inhibition (measured in mm) [14].

2.6 Synergism test between mentha extract and antibiotics

The interference test between the antibiotics under investigation and the natural substances (mentha) was carried out for bacterial isolates, depending on the method, and then 10l of mentha extract and antibiotic were placed in the wells and then the dishes were incubated at a temperature of 37° C for 18 - 24 hours, and the diameter of the inhibition zone of the antibiotic with mentha extract was measured [15].

2.7 Statistical analysis

In accordance with the Statistical Analysis System [16], the data were analyzed, and the statistically significant differences were determined at ($p \geq 0.05$). This data set was subjected to statistical analysis using ANOVA, and then the least significant differences (LSD) test was used to determine whether or not there were significant differences between the different mean values.

3. Results and discussion

3.1 Total viable and coliform bacterial counts

This study demonstrates that the microbiological study (total viable and coliform bacterial counts) of minced meat is demonstrated in (Table 1). It has been discovered that minced meat samples collected during the study period have significant contamination between total viable bacterial counts and coliform bacterial counts, as demonstrated by these findings. The average mean value (log cfu\g) of total viable bacterial counts 6.273 ± 0.189 (log cfu\g) and the average mean value (log cfu\g) of coliform bacterial counts 5.933 ± 0.312 (log cfu\g). The total Plate Count of minced meat ranging between 1.3×10^4, 2.5×10^8 cfu/g of minced meat has been detected by different workers in geographical areas [17, 18]. In our study, the average mean value of total viable bacterial counts 6.273 ± 0.189 (log cfu\g) of minced meat compare [19] who detect that total bacterial counts values of 3.9×10^4 cfu/g and 7.6×10^4 cfu/g respectively, while [20] reported the mean values obtained (expressed in log CFU/g) were 4.94 of total bacterial counts of minced meat in butcher shops of Oujda city, Morocco. In present study, the average mean value (log cfu\g) of coliform bacterial counts 5.933 ± 0.312 (log cfu\g) lower than [21] who detect that the total coliform counts 9.30×10^9 cfu. g^1 from minced meat in Sokoto, Nigeria [22], minced meat in Baqubah city-Diyala Province-Iraq had the highest average coliform plate count (125 log cfu/g) of all samples tested.

Table 1: Total aerobic and coliform bacterial counts (log cfu/ml) of samples of minced meat samples

Bacterial counts	Mean ±SE	LSD
Total bacterial count (log cfu/ml)	6.273± 0.189	2.3
Coliform counts (log cfu/ml)	5.933±0.312	

3.2 Isolation of *Escherichia coli* from minced meat

The present study shows the prevalence of Escherichia coli isolated from minced meat in Babylon city. In this study, 90 samples 18(16.2%) were positive, 72(64.8%) negatives. These results show that the presence of microbial contamination of *E. coli* bacteria from minced beef meat lower than [22] reported high contamination of minced meat (88.88%) positive samples with E. coli isolated from minced meat in certain locations of Baqubah city-Diyala Province-Iraq. While [23] found that 27 (48.2%) of the ground beef was contaminated with *E. coli*. [24] found that E. coli was positive in 30% of ground beef samples. Contamination of minced meat coming from the carcasses as well as from various equipment used during personnel, processing, water, and air [25]. the mincing meat process spreads the distribution of bacteria to the mass inside. However, the mincing process releases meat fluids which are a good medium for bacterial growth [26]. The high level of *E. coli* generally correlates in meat products with higher levels of food-borne disease originating from fecal origin [27].

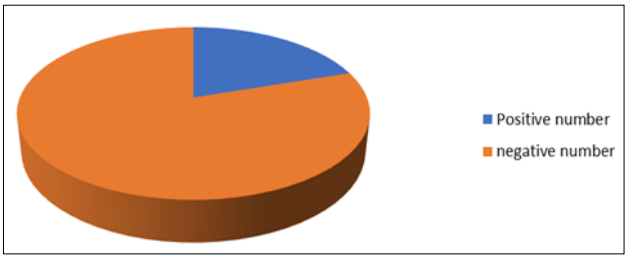

Fig 1: Prevalence of Escherichia coli isolated from minced meat

3.3 Antibacterial activities of the Mentha piperita and antibiotic alone:

The present study indicated that average mean value of the antibacterial activity of the alcoholic plant extract alone (Mentha piperita) and antibiotic alone (sulfonamide and tetracycline) in different concentrations (0.5 mg\ml, 1 mg\ml, 2 mg\ ml, 4mg\ml, and 8 mg\ml) against *Escherichia coli* isolated from minced meat were shown in Table 2. The average means value of antibacterial of Mentha piperita against *Escherichia coli* (11.5 ± 0.748, 14.2 ± 1.113, 15.8 ± 0.583, 19.2 ± 1.319, and 21.2 ± 1.2) respectively. The result shows high significance ($p > 0.05$) of average mean minimum inhibition concentration of alcoholic plant extraction of mentha (11.5 ± 0.748 to 21.2 ± 1.2) in concentration (0.5 mg\ml and 8mg\ml) respectively. In present study, concentration 0.5mg\ml, 1mg\ml and 2 mg\ml higher than [28] reported that antibacterial activity of mentha investigated by agar disc diffusion method range from 9.0 ± 0.2 to 10.0 ± 0.4 mm of inhibition zone in concentration from 0.625-2.5mg\ml against *E. coli.* The differences in antibacterial and anti-oxidant activities with the reported one may be attributed to different procedures followed or a different geographical environment, cultivar type, seasonality, physiological age of the plant, and the method of oil isolation [29]. Mentha piperita oil was a stronger bactericide against *E. coli* [30]. Peppermint oil could be used as a good conservation agent by inhibiting some food-borne pathogens [29].

The results demonstrated that different concentrations from sulfonamide 0.5, 1, 2, 4 and 8 µg/ml could inhibit *E. coli* 3.8 ± 0.374, 5.8 ± 0.663, 10.6 ± 0.871, 20 ± 0.707 and 24.4 ± 1.630 respectively. While antibiotic drugs of tetracycline in some concentrations could inhibit *E. coli* 1.6 ± 0.244, 2.4 ± 0.583, 4.6 ± 0.678, 9.4 ± 0.707, and 17.4 ± 0.871 respectively. These results showed mean ± SE of Mentha piperita, sulfonamide, and tetracycline high significant ($p \geq 0.05$) between different concentrations (0.5 and 8 mg\ml) respectively.

Table 2: Minimum inhibitory concentration of plant extracts alone and antibiotics alone against *Escherichia coli*

Concentration Mg\ml	Sulfonamide Mean ±SE	Tetracycline Mean ±SE	Mentha Mean ±SE	LSD
0.5	3.8±0.374	1.6±0.244	11.5±0.748	
1	5.8±0.663	2.4±0.583	14.2±1.113	
2	10.6±0.871	4.6±0.678	15.8±0.583	
4	20±0.707	9.4±0.707	19.2±1.319	1.86
8	24.4±1.630	17.4±0.871	21.2±1.2	

3.4 The Synergistic Effect between Mentha piperita and antibiotics.

In vitro synergistic effects between Mentha piperita and antibiotics (sulfonamide and tetracycline) against Escherichia coli isolated from minced meat were evaluated using well diffusion methods in different concentrations (o.5, 1, 2, 4 and 8 mg\ml respectively).

In the present study show that high significant ($p \geq 0.05$) between minimum inhibition concentration (Mean ±SE) the synergistic effect of Mentha piperita with sulfonamide and Mentha piperita with tetracycline against *Escherichia coli* in different concentration o.5, 1, 2, 4 and 8 mg\ml) respectively.

The results demonstrated that minimum inhibition of the synergistic effect of Mentha piperita extract with sulfonamide could inhibit *E. coli* in different concentrations 6.4±0.678, 10.8±0.748, 11.8±0.8, 19±.0.447, and 26±0.836 respectively. It had the same synergistic effect with both Mentha piperita extract with tetracycline could inhibit *E. coli* in different concentrations 5.4±0.748, 6±0.707, 8.2±1.019, 12.2±0.916, and 19.8±0.663 respectively. Depending on the method of extraction, the plant extracts displayed various synergistic powers to suppress the growth of bacteria. Plant antimicrobials are synergistic enhancers, meaning that even if they don't have any antimicrobial characteristics on their own, when combined with regular medications, they boost the effectiveness of the latter [31]. It has long been recognized that restoring antibiotic efficacy through the synergistic action of antibacterial compounds derived from natural and synthetic sources is one of the most effective ways to combat bacterial resistance [32].

Table 3: Synergism Between Mentha piperita and antibiotics against *E. coli*

Concentration Mg\ml	Sulfonamide and Mentha Mean ±SE	Tetracycline and Mentha Mean ±SE	LSD
0.5	6.4±0.678	5.4±0.748	
1	10.8±0.748	6±0.707	
2	11.8±0.8	8.2±1.019	
4	19±.0.447	12.2±0.916	1.81
8	26±0.836	19.8±0.663	

4. Conclusion

The fact that these studies were done shows that mentha has antibacterial properties against Escherichia coli, which is a type of bacteria that causes infections. When these plant extracts were combined with antibiotics (sulfonamide and tetracycline), the bacteria were kept from growing.

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