

Antibacterial Effect of Aqueous Extracts of Spices and Herbs against Bacteria Isolated from Frozen Meat

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ABSTRACT

This study investigated the antibacterial activity of nine aqueous plant extracts (bitter fennel, black tea, ginger, turmeric, nutmeg, coriander, cubeb, dry black lemon and senna), some sterilizers and some Hand Sanitizers against bacteria (Escherichia coli and Staphylococcus aureus isolated from meat), with the aim of sterilizing the contaminated hand with meat, where the results showed that the sterilizers used had variant effects against obtained bacteria from this experiment, and also showed the antibacterial activity of aqueous plant extracts against these bacteria, where Black dry lime and Senna extracts had a strong antibacterial activity against both bacteria in this study, while Nutmeg extract had a weak antibacterial activity, the other extracts did not have any effect against these two bacteria.

It appeared from the well diffusion technique that Black dry lime inhibited E. coli and S. aureus and the inhibition zones were (30, 29, 25, 15, and 9) mm and (33, 30, 30, 25 and 20) mm respectively at (100, 75, 50, 25 and 12.5) %, and the inhibition zone was 22 mm for Senna against E. coli at 100%, while Nutmeg inhibited S. aureus at the concentration 100% and the inhibition zone was 17 mm. The MIC was determined for all extracts against both bacteria, and S. aureus in general was more susceptible comparing with E. coli.

Key words: Food borne, Escherichia coli, Staphylococcus aureus, Sterilizers, Antibacterial, Aqueous plant extracts.

INTRODUCTION

Food borne infections have been one of the major public health concerns worldwide and account for considerably high cases of illnesses attacking human and animals. More than 250 different food borne diseases have been described. Most of these diseases are due to microbial infections. The illnesses as a result of eating food contaminated with bacteria and/or their toxins range from stomach upset to more serious symptoms include vomiting, diarrhea, and abdominal cramps (1-3).

Growth of microorganisms in food may cause spoilage or food borne disease. Synthetic additives have been widely used. The trend is to decrease their use because of the growing concern among consumers about such chemical additives. Consequently, search for natural additives, especially of plant origin, has notably increased in recent years. Therefore, the development and application of natural products with both antioxidants and antibacterial activities especially in meat products may be necessary and useful to prolong their storage shelf life and potential for preventing food diseases (4).

Herbs and spices are generally considered safe and proved to be effective against certain ailments. They are also extensively used, particularly, in many Asian, African and other countries. In recent years, in view of their beneficial effects, use of spices/herbs has been gradually increasing in developed countries also (5).

Herbal spices, being a promising source of phenolics, flavonoids, anthocyanins and carotenoids, are usually used to impart flavor and enhance the shelf-life of dishes and processed food products (2).

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Plant derived products have been used for medicinal purposes for centuries. At present, it is estimated that about 80% of the world population rely on botanical preparations as medicines to meet their health needs. Herbs and spices are generally considered safe and proved to be effective against certain ailments, Spices occupy a prominent place in the traditional culinary practices and are indispensable part of daily diets of millions of people all over the world (6), and various reports have been published on the antibacterial effect of spices on pathogenic bacteria including *E. coli* O157 (7).

MATERIALS AND METHODS

Experimental Design

The experiment included sterilizing the hand, then taking the swab from it and culturing it on nutrient agar, MacConkey agar and blood agar. The second step was contaminating the sterilized hand with a piece of raw meat (previously freezing), then taking the swab from it and culturing it on nutrient agar. The third step was sterilizing the contaminated hand with meat by some types of Dettols and some hand sterilizers, and then culturing it on nutrient agar by taking a swab (repeating the first and second step with using each sterilizer solution). The fourth step was as third step but instead of using the sterilizer solutions, the plant extracts were used (for each extract the previous steps were repeated).

Bacteria obtained

The bacteria obtained from step one and step two were identified depending on their smear, cultural and some biochemical properties.

Plant Extraction

Collection and preparation of plant samples

The plants (bitter fennel, black tea, ginger, turmeric, nutmeg, coriander, cubeb, dry black lemon and senna) were obtained from market in Erbil city, then were washed with tap water, then with distilled water, then left for air drying until become completely drying, after drying the plants converted into powder form and stored in polyethylene sacks in refrigerator at 4°C for further process.

Extracts preparation

150 ml of sterilized distilled water was added to 15 g of ground dried plant, heated below the boiling point and stirred for 2 - 3 h. The extract was filtered by muslin

cloth, then by filter paper (Whatman No 1). Half quantity of prepared extract was also evaporated to dryness and stored in the refrigerator at 5°C for using (8 and 9).

Preparation of inoculums

Two to three colonies from pure growth of each tested organism were transferred to (5) ml of nutrient broth. Broths were incubated overnight at 37°C. The suspension was diluted with sterile distilled water to obtain approximately 1×10^6 CFU/ ml (10).

Well diffusion technique

Screening of antibacterial activity was performed by well diffusion technique (11). The Nutrient agar (NA) plates were seeded with (0.1) ml of the inoculums of each tested organism. The inoculums were spread evenly over plate with loop. A standard cork borer of (8) mm diameter was used to cut uniform wells on the surface of the NA and (100) μ l of each concentration of plant extracts was introduced in the well, the plates were incubated for 24 hours at 37°C, and the zones of inhibition was measured to the nearest millimeter (mm).

All experiments were applied in triplicates.

MIC determination

The minimum inhibitory concentration (MIC) of medicinal plant extracts were determined by turbidity method (spectrophotometric method) at 600 nm, and serial dilutions were prepared for each extract (500-9000) g/ml (12). In addition to the control sample that consists of (10ml) of nutrient broth and (0.1ml (1×10^6 CFU/ ml)) of overnight culture of bacterial suspension then incubated at 37°C for 24 hours.

DISCUSSION

The growing concern about food safety has recently led to the development of natural antimicrobials to control food borne pathogens and spoilage bacteria. Spices are one of the most commonly used natural antibacterial agents in foods and have been used traditionally for thousands of years by many cultures for preserving foods and as food additives to enhance aroma and flavour (13).

Many of the spices and herbs used today have been valued for their antibacterial effects and medicinal powers in addition to their flavor and fragrance qualities (14).

Sterilizer solution	Source
Dettol Antiseptic (Disinfectant)*	India
Dettol Personal care Antiseptic (Antibacterial)**	Soap and Chemicals Industrial and Trading Co.- Sharjah
Sarttol Disinfectant Antiseptic	Ekal Industrial Co.-Jordan
Dettol Hand Sanitizer	Dubai
Hand Sanitizer Hi Geen	Jordan
Antibacterial Hand Gel	Alpha Al Bakkar Sirya

Sterilized hand	Sterilized hand + contaminated with meat	A		
		Sterilized hand + contaminated with meat + Dettol*	Sterilized hand + contaminated with meat + Dettol**	Sterilized hand + contaminated with meat + Sarttol
S. aureus	S. aureus and E. coli	Both E. coli and S. aureus was not inhibited	Both Inhibited completely	E. coli was not inhibited while S. aureus was inhibited
		B		
		Sterilized hand + contaminated with meat + Dettol Hand Sanitizer	Sterilized hand + contaminated with meat + Hand Sanitizer Hi Geen	Sterilized hand + contaminated with meat + Antibacterial Hand Gel
		E. coli was inhibited completely, the S. aureus present	E. coli was inhibited S. aureus was not inhibited	Large number of E. coli was inhibited and S. aureus was inhibited completely
		C		
		Sterilized hand + contaminated with meat + Dry black lime/ Senna	Sterilized hand + contaminated with meat + Nutmeg	Sterilized hand + contaminated with meat + Turmeric, Cubeb, Tea, Fennel, Ginger and Coriander
		Strong Inhibition for both bacteria	Weak Inhibition	No Inhibition for both bacteria

The results obtained and explained in Table 2, showed that the sterilizers used had variant effects against bacteria obtained from this design, and also showed the antibacterial effect of aqueous plant extracts against these bacteria, where Black dry lime and Senna extracts had a strong effect against both bacteria, while Nutmeg extract had a weak effect, the other extracts did not have any effect against these two bacteria.

Tables 3 and 4 and Figure 1 show the antibacterial activity of aqueous plant extracts used against E. coli and S. aureus respectively, and it appeared that Black dry lime inhibited E.

coli and S. aureus and the inhibition zones were (30, 29, 25, 15, and 9) mm and (33, 30, 30, 25 and 20) mm respectively at (100, 75, 50, 25 and 12.5)%, and the inhibition zone was 22 mm for Senna against E. coli at 100%, while Nutmeg inhibited S. aureus at the concentration 100% and the inhibition zone was 17 mm, while Table 5 shows the MIC, where in general S. aureus was inhibited at concentration less than E. coli.

In the current study the variation in antibacterial potentiality of examined plants could be attributed to their disparate contents of biocidal agents, and this is in accordance with

TABLE 3: Antibacterial activity of aqueous plant extracts against E. coli

Plant Extract	Scientific Name	Concentration				
		*Zone of inhibition/mm				
		100%	75%	50%	25%	12.5%
Coriander	Coriandrum sativum	-	-	-	-	-
Black Tea	Camellia sinensis	-	-	-	-	-
Bitter Fennel	Foeniculum vulgare	-	-	-	-	-
Cubeb	Piper cubeba	-	-	-	-	-
Dry Black Lime	Citrus aurantifolia	30	29	25	15	9
Ginger	Zingiber officinale	-	-	-	-	-
Nutmeg	Myristica fragrans	-	-	-	-	-
Turmeric	Curcuma longa	-	-	-	-	-
Senna	Cassia angustifolia	22	-	-	-	-

*: Values calculated as mean of triplicates. -: No inhibition zone or less than 8 mm.

TABLE 4: Antibacterial activity of aqueous plant extracts against S. aureus

Plant Extract	Scientific Name	Concentration				
		*Zone of inhibition/mm				
		100%	75%	50%	25%	12.5%
Coriander	Coriandrum sativum	-	-	-	-	-
Black Tea	Camellia sinensis	-	-	-	-	-
Bitter Fennel	Foeniculum vulgare	-	-	-	-	-
Cubeb	Piper cubeba	-	-	-	-	-
Dry Black Lime	Citrus aurantifolia	33	30	30	25	20
Ginger	Zingiber officinale	-	-	-	-	-
Nutmeg	Myristica fragrans	17	-	-	-	-
Turmeric	Curcuma longa	-	-	-	-	-
Senna	Cassia angustifolia	-	-	-	-	-

*: Values calculated as mean of triplicates. -: No inhibition zone or less than 8 mm.



Effect of Senna on E. coli



Effect of Black dry lime on E. coli



Effect of Nutmeg on S. aureus



Effect of Black dry lime on S. aureus

FIGURE 1: Antibacterial effect of aqueous plant extracts on E. coli and S. aureus.

TABLE 5: MIC determination of aqueous plant extracts ($\mu\text{g/ml}$) against *E. coli* and *S. aureus*

Plant extract	<i>E. coli</i>	<i>S. aureus</i>
Coriandrum sativum	7000	4000
Camellia sinensis	6000	3000
Foeniculum vulgare	8000	4000
Piper cubeba	8000	5000
Citrus aurantifolia	2000	1000
Zingiber officinale	6000	5000
Myristica fragrans	5000	3000
Curcuma longa	6000	6000
Cassia angustifolia	4000	4000

the results of different studies which provided evidence that some medicinal plants might indeed be potential sources of new antibacterial agents even against some antibiotic-resistant strains (14).

The results demonstrated that the *E. coli* was more resistant to the plant extract than *S. aureus*. Because lipopolysaccharide (LPS) layer of gram-negative bacteria in outer membrane have a high hydrophobicity which acts as a strong permeability barrier against hydrophobic molecules. Hydrophobic molecules can pass through cell wall of gram-positive bacteria easier than the gram-negative bacteria because cell wall of the gram-positive bacteria contained only peptidoglycan (15).

Similar findings were obtained from other researchers, as (15, 16 and 17), where they found that coriander, ginger, nutmeg and turmeric did not have antibacterial activity against the bacteria tested in their studies.

The mechanism of antibacterial action of spices and derivatives is not yet clear. Hypotheses have been proposed (different workers) which involve: hydrophobic and hydrogen bonding of phenolic compounds to membrane proteins, followed by partition in the lipid bilayer; perturbation of membrane permeability consequent to its expansion and increased fluidity causing the inhibition of membrane embedded enzymes; membrane disruption; destruction of electron transport systems and cell wall perturbation (13).

Other researchers found reversible results such as (18 and 19) and others. It was observed that the antimicrobial effect of plant extract varies from one plant to another in different researches carried out in different regions of the world. This may be due to many factors such as, the effect of climate, soil composition, age and vegetation cycle stage,

on the quality, quantity and composition of extracted product, different bacterial strains. Moreover, different studies found that the type of solvent has an important role in the process of extracting (The difference between their effects may be due to the quantity of the phenolic compounds).

CONCLUSION

It is concluded that the antibacterial effect of aqueous extract of black dry lime was the stronger in comparison, followed by senna, followed by nutmeg, and it is recommended using the plant extract to preserve the food, and prevent the contamination.

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