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, 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).
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 distill 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*10⁶ 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) µl 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 were 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) µg/ml (12). In addition to the control sample that consists of (10ml) of nutrient broth and (0.1ml 1*10⁶ 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).
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

<table>
<thead>
<tr>
<th>Plant Extract</th>
<th>Scientific Name</th>
<th>*Zone of inhibition/mm</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>100%</td>
</tr>
<tr>
<td>Coriander</td>
<td>Coriandrum sativum</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Black Tea</td>
<td>Camellia sinensis</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Bitter Fennel</td>
<td>Foeniculum vulgare</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Cubeb</td>
<td>Piper cubeba</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Dry Black Lime</td>
<td>Citrus aurantifolia</td>
<td>30</td>
<td>29</td>
</tr>
<tr>
<td>Ginger</td>
<td>Zingiber officinale</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Nutmeg</td>
<td>Myristica fragrans</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Turmeric</td>
<td>Curcuma longa</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Senna</td>
<td>Cassia angustifolia</td>
<td>22</td>
<td>-</td>
</tr>
</tbody>
</table>

*: 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

<table>
<thead>
<tr>
<th>Plant Extract</th>
<th>Scientific Name</th>
<th>*Zone of inhibition/mm</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>100%</td>
</tr>
<tr>
<td>Coriander</td>
<td>Coriandrum sativum</td>
<td></td>
<td>-</td>
</tr>
<tr>
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<td>Camellia sinensis</td>
<td></td>
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<td></td>
<td>-</td>
</tr>
<tr>
<td>Dry Black Lime</td>
<td>Citrus aurantifolia</td>
<td>33</td>
<td>30</td>
</tr>
<tr>
<td>Ginger</td>
<td>Zingiber officinale</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Nutmeg</td>
<td>Myristica fragrans</td>
<td>17</td>
<td>-</td>
</tr>
<tr>
<td>Turmeric</td>
<td>Curcuma longa</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Senna</td>
<td>Cassia angustifolia</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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

FIGURE 1: Antibacterial effect of aqueous plant extracts on E. coli and S. aureus.
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 finding 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. Hypothesis 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 electrons 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 the quantity of the phenolic compounds).

**CONCLUSION**

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

**REFERENCES**


