Chapter One

Introduction

Naturally occurring microbial inhibitors have been recovered from a wide variety of foods including fruits, vegetables, cereals and spices. Many of these antimicrobials contribute to the food stuff's natural resistance to deteriorations. The flavor components consist of such compounds as alcohols, aldehydes, esters, terpenes, phenols, organic acids and others, some of which have not yet been identified. Many of the plants used today were known to the people of ancient cultures throughout the world and they were valued their preservative and medicinal powers. Scientific experiments since the late 19th century have documented the antimicrobial properties of some spices, herbs, and their components (Shelef, L. A., 1983; Zaika, L. L., 1988).

Food infection and intoxication are considered as the most common causes of food borne diseases worldwide. Food borne pathogens causing these diseases find their way in foods through cross contamination, improper handling and temperature abuse. *Escherichia coli* O157:H7, *Staphylococcus aureus*, and *Bacillus sp.* are among the common food borne microorganisms that cause infection and intoxication.

Food spoilage occurs when the quality of food is damaged and it becomes poisonous and unfit for consumption. Foods can be spoiled by micro-organisms enzymes, insects and rodents. Besides, there are other factors which speed up the process of food spoilage; they are temperature, moisture, pH etc. Microbial spoilage changes the quality of the food stuff (Bryan, F. L., 1974). If this food is consumed, it could be bad to health hazards. In most cases, microorganisms grow on our food because food is a good source of nutrients for their own growth, which can result in deterioration of food by increase microbial numbers and utilizing nutrients, producing enzymatic changes and contributing off flavors by means of breakdown of a product and synthesis of new compounds, which cause food spoilage.
Food spoilage microorganisms on the other hand, cause products to lose their quality which renders them unacceptable to consumers. Short shelf life of food products is one of the major problems of the food industry. Prevention of pathogenic and spoilage microorganisms in foods is usually achieved by using chemical preservatives. These chemical preservatives act as antimicrobial compounds which inhibit the growth of undesirable microorganisms. Despite the use of wide range of food preservatives and post harvest technology, food poisoning is a major concern even today for both consumers and the food industry. Considerable amount of food is spoiled due to the non availability of effective preservatives and also due to increasing antibiotic resistance of some food borne pathogens. There has been increasing interest in the development of new types of effective natural and non-toxic plant based antimicrobial compounds. Spices and medicinal herbs were in use since ancient times, not only as flavoring agents, also as folk medicine and food preservatives. Plants are used medicinally in different countries and are a source of many potent and powerful drugs. Tayel (2009) studied antimicrobial potentialities of twenty five herbs and spices which are widely used in folk medicine by Egyptian housewives to treat gastrointestinal disorders against seven bacterial strains, mostly food borne including pathogens (Tayel, A. A. and EL-TRAS, W. F., 2009). Medicinal plants are used by 80% of the world population as the only available medicines especially in developing countries (Hashim, H., Kamali, E. L., Mohamed, Y., 2010). In addition to imparting characteristic flavors, certain spices and medicinal herbs prolong the shelf life of foods by preventing rancidity through their antioxidant activity. The extracts of many plants have become popular in recent years and attempt to characterize their bioactive principles have gained momentum for varied pharmaceutical and food processing applications. Plant oils and extracts have been used for a wide variety of purposes for many thousands of years (Jones, F. A., 1996). Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives (Geissman, T. A., 1963). Many studies have reported that phenolic compounds contribute to their antioxidant and pharmaceutical properties. Clinical microbiologists have great interest in screening of medicinal plants for new therapeutics (Periyasamy, A., Rajkumar, Mahalingam, K., 2010). The active principles of many drugs found in plants are secondary metabolites. The antimicrobial activities of plant extracts may reside in a variety of different
components, including aldehyde and phenolic compounds (Lai, P. K., and Roy, J., 2004). Phenolic compounds present in spices might also play a major role in their antimicrobial effects. Cinnamon, Bay leaf, Ajowan, Star-Anise and Tailed-Pepper are ancient spice and used in pharmaceutical preparations.

This study has evaluated the antimicrobial activity of some well known culinary spices (Cinnamon, Bay leaf, Ajowan, Star-Anise and Tailed-Pepper) with emphasis on their application as effective antimicrobial agents, thereby enhancing the extent of ethno-botanical medicine and their use in food preservation. It was observed that the active ingredients eugenol, cinnamaldehyde, thymol and anethole contained within the selected spices are effective antimicrobial agents that can inhibit and cause microbial death of various bacteria.

**List of plant material studied**

<table>
<thead>
<tr>
<th>Species</th>
<th>English name</th>
<th>Family</th>
<th>Part used</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cinnamomum zeylanicum</em></td>
<td>Cinnamon</td>
<td><em>Lauraceae</em></td>
<td>Stem bark</td>
</tr>
<tr>
<td><em>Laurus nobilis</em></td>
<td>Bay leaf</td>
<td><em>Lauraceae</em></td>
<td>Leaf</td>
</tr>
<tr>
<td><em>Illicium verum</em></td>
<td>Star Anise</td>
<td><em>Illiciaceae</em></td>
<td>Fruit</td>
</tr>
<tr>
<td><em>Piper cubeba</em></td>
<td>Tailed Pepper</td>
<td><em>Piperaceae</em></td>
<td>Fruit</td>
</tr>
<tr>
<td><em>Carum copticum</em></td>
<td>Ajowan</td>
<td><em>Apiaceae</em></td>
<td>Fruit</td>
</tr>
</tbody>
</table>

The phytochemical screening of the crude ethanol yields of chemical constituents of the spices studied showed that the bark leaf and fruits were rich in terpenes, flavonoids, phenolics, saponins, alkaloids and cardiac glycosides. The spices are rich in a wide variety of secondary metabolites which were found to have in vitro antimicrobial properties. The mechanisms of flavonoids that are antimicrobial can be classified as the inhibition of nucleic acid synthesis, cytoplasmic membrane function, and energy metabolism (Cushnie, T. P. T., and Lamb, A. J., 2005). The presence of phenolic compounds in the extract may attribute antibacterial activity. Phenolic compounds are thought to be toxic to micro organisms, inhibiting the enzymes which are essential for the growth of microorganism. The spices studied here can be seen as a potential source of useful preservatives and drugs. Other
researchers have also reported that phenolic compounds from different plant sources could inhibit various foodborne pathogens (Nychas, G. J. E., 1995; Smid, E. J. and Gorris, L. G. M., 1999; Prashanth, D., Asha, M. K., and Amt. A., 2001; Kim, Y. S., Hwang, C. S., Shin, D. H., 2005). Further studies are going on these spices in order to isolate, identify, characterize and elucidate the structure of the bioactive compounds for the preservation of food. The selection of crude spice extract for screening the phytochemicals has the potential of being more successful in the initial steps than screening of pure compounds.

The present study was aimed at investigating the potential of five extensively used culinary spices commonly known as Cinnamon, Bay leaf, Ajowan, Star-Anise and Tailed-Pepper as effective antimicrobial agents that could possibly act as a “natural antibiotic system”. Four food spoilage bacterial species including Gram-positive (B. subtilis ATCC 6633, B. cereus ATCC 11778, S. aureus ATCC 6538 and S. aureus ATCC 25923) and Gram-negative (E. coli ATCC 43888, E. coli ATCC 25922, E. coli ATCC 8739 and E. coli ATCC 43895) strains subjected to sensitivity testing, their average inhibition zone sizes determined when tested against various extracts of the test spices and the minimum inhibitory concentration (MIC) value of each extract was also determined.

Sensitivity and MIC testing indicated that all five spices possess a significant amount of compound shown antimicrobial activity against seven of the eight microbial strains tested with E. coli ATCC 25922 exhibiting the greatest amount of resistance against the different spice extracts.

The result of different studies provides evidence that some spices might indeed be potential source of new antimicrobial agents. Plants are important source of potentially useful structures for the development of new chemo-preventive agents; the first step towards this goal is in vitro antibacterial activity. The extract of higher plant and spices can be very good source of preservative against various bacterial pathogen. Plants based antimicrobial compound have enormous therapeutics potential as they can serve the purpose without any side effects that are often associated with synthetic antibacterial compounds.

From the total ANOVA results of the five spices, eight microbial species and the various spice extracts tested, the investigation concluded that Gram-positive and
Gram-negative bacteria exhibited successful inhibition during testing. The overall analysis clearly indicated that *E. coli* ATCC 25922 was the most resistant of the eight microbes with ATCC, *B. subtilis*, *E. coli* and *S. aureus* exhibiting greatest susceptibility with the overall conclusion that ethanol was determined as the most effective extracts followed by acetone, methanol, cold and then hot water.

However, the overall analysis of the mean resulted inhibition zones showed that the gram positive microbes were more susceptible to the various extracts of the different spices. These spices could be used for food preservation to extend shelf life of cooked food.