Chapter Two

Review of Literature

Food poisoning is still a concern for both consumers and the food industry despite the use of various preservation methods. Food processors, food safety researchers and regulatory agencies are continuously concerned with the high and growing number of illness outbreaks caused by some pathogenic and spoilage microorganisms in foods. Food poisoning bacteria survives in frozen foods also (Georgala, D. L. and Hurst, A., 1963). There is growing interest in using natural antibacterial compounds, such as extracts of spices and herbs, for food preservation (Desrosier, N. W., 1963; Smid, and Gorris, 1999). Consumers are also concerned about the safety of foods containing synthetic preservatives. Therefore, there has been increasing interest in the development of new types of effective and nontoxic antimicrobial compounds. Several studies (Shelef et al., 1980) showed that cinnamon had strong and consistent inhibitory effect against several pathogen and spoiling bacteria. Microbial control in foods could be assured by suppressing one or more essential factors for microbial survival (Horace, D. G., 1982). Antimicrobial activity of spices depend on several factors, which includes: i) kind of spice, ii) composition and concentration of spice, iii) microbial species and its occurrence level, iv) substrate composition, v) processing conditions and storage (Farag, R. S., Daw, Z. Y., Hewedi, F. M. and El-Baroty, G. S. A., 1989; Shelef, 1983). Spices have been defined as plant substances from indigenous or exotic origin, aromatic or with strong taste, used to enhance the taste of foods. Spices include leaves (bay, mint, rosemary, coriander, laurel, and oregano), flowers (clove), bulbs (garlic and onion), fruits (cumin, red chilli, and black pepper), stems (coriander, cinnamon), rhizomes (ginger) and other plant parts (Shelef, 1983). Although, spices have been well known for their medicinal, preservative and antioxidant properties, they have been currently used with primary purpose of enhancing the flavor of foods rather than extending shelf life (Aktug, S. E. and Karapinar, M., 1986). Aktug and Karapinar (1986) observed

Recently, there has been increasing interest in discovering new natural antimicrobials; this is also has been true in food microbiology (Jay, J. M., 1986). A review of the earlier literature (Janssen, A. M., Scheffer, J. J. and Baerheim Svendsen, A., 1987) reveals that the results reported for these different studies are difficult to compare, presumably because of the different test methods, bacterial strains, and sources of antimicrobial samples used. Several scientific reports describe the antimicrobial effectiveness of spices for use in food conservation systems and inhibitory effect of spices on a variety of microorganisms, although considerable variation for resistance of different microorganisms to a given spice and of the same microorganisms to different spices has been observed. It has been hypothesized that the inhibition involves phenolic compounds, because these compounds sensitize the phospholipids bilayer of the microbial cytoplasmic membrane causing increased permeability, unavailability of vital intracellular constituents and/or impairment of bacterial enzymes systems (Farag et al., 1989; Juven, B. J., Kanner, J., Sched, F. and Weisslowicz, H., 1994; Kim, J., Marshall, M. R., and Wei, C., 1995; Wendakoon, C. N. and Sakaguchi, M., 1995).

In addition to imparting characteristic flavors, certain spices and herbs prolong the storage life of foods by preventing rancidity through their antioxidant activity or through bacteriostatic or bactericidal activity (Beuchat, L. R. and Golden, D. A., 1989). Being plant natural foodstuffs, spices appeal to consumers who tend to question the safety of synthetic additives (Farag et al., 1989; Sagdic, O., 2003). The “Spice” is a culinary term, not a botanical category it does not refer to a specific kind of plant or plant part (Farrell, K. T., 1990). Generally, gram-negative bacteria have been reported to be more resistant than Gram-positive to essential oils antimicrobial effect because of their cell wall lipopolysaccharide (Russel, A. D., 1991). The International Commission on Microbiological Specifications for Foods (1974) has set up maximum limit of $10^6$, $10^4$ and $10^3$ CFU of total aerobic mesophilic bacteria (TAMB), fungi, coli forms and *E. coli*, respectively, per gram spice. Naturally occurring compounds in spices such as, sulphur compounds, terpenes and terpene derivatives, phenols, esters, aldehydes, alcohols and glycosides have shown antimicrobial functions (Davidson, P. M. and Baren, A. L.,
The successful story of microbial chemo-control lies in the continuous search for new antimicrobial substances to control the challenge posed by resistant strains (Notermans, S. and Hoogenboom-Verdgaal, A., 1992). The agents, which have the capacity to kill the microbes or arrest the multiplication, are called the antimicrobial agents or drugs (Lawrence, B. M., 1993; Prashanth et al. 2001).

Therefore, there has been increasing interest in the development of new types of effective and nontoxic antimicrobial compounds. However, the onset of increased demand for minimally-processed extended shelf-life foods and reports of chemical preservatives as having potential toxicity demand food manufacturers to find alternative sources of antimicrobial compounds (Daeschel, M. A., 1992; Conner, D. E., 1993; Nychas, G. J. E., 1995; Kasesart, J., 2006). These procedures could kill or make unviable some microorganisms. Scientists are now forming an impressive body of data that suggests a successful story of microbial chemo-control, which lies in the continuous search for antimicrobial agents of spices (Notermans, S. and Hoogenboom-Verdgaal, A., 1992).

Use of spices as microbial growth inhibitor in foods is often limited because of flavor considerations as effective antimicrobial dose may exceed the organoleptically accepted level (Pandit, V. A. and Shelef, L. A., 1994; Brull, S. and Coote, P., 1999). Nonetheless, combinations of spices and other antimicrobial barriers could enhance the food shelf stability and microbial safety even in moderated levels. Due to this and due to the fact that spices are as GRAS, the antimicrobial properties of spices continue to be of interest (Pandit and Shelef, 1994). Although some hypothesis have been given, which involve: i) hydrophobic and hydrogen bonding of phenolic compounds to membrane proteins, followed by partition in the lipid bilayer ii) perturbation of membrane permeability consequent to its expansion and increased fluidity causing the inhibition of membrane embedded enzymes iii) membrane disruption (iv) destruction of electrons transport systems cell wall perturbation. (Juven et al., 1994; Cox, S. D., Mann, C. M. and Markham, J. L., 2000; Caccioni, D. L. R., Guizzardi, M., Biondi, D. M., Renda, A. and Ruberto, G., 2000; Tassou, C. C., Koutsoumanis, K. and Nychas, G. J. E., 2000; Odhav, B., Juglal, S., and Givinden, R., 2002).
Spices and herbs have been added to foods since ancient times, not only as flavoring agents, but also as folk medicine and food preservatives (Beuchat, L. R., 1994; Nakatani, N., 1994; Cutler, H. G., 1995). Numerous studies have been published on the antimicrobial activities of plant extracts against different types of microbes, including food borne pathogens (Beuchat, 1994; Smith-Palmer, A., Stewart, J. and Fyfe, L., 1998; Hara-Kudo, Y., Kobayashi, A., Sugita-Konishi, Y., and Kondo, K., 2004). These chemicals evolved in plants to protect them against herbivorous insects, vertebrates, fungi, pathogens, and parasites (Walker, J. R. L., 1994). Plant essential oils are a potentially useful source of antimicrobial compounds. Reports have indicated that essential oils containing carvacrol, eugenol and thymol (phenolic compounds) had highest antibacterial performances (Lattaoui and Tantaoui-Elaraki, 1994; Kim et al., 1995; Hamilton-Miller, J. M., 1995; Meng, J. H., Zhao, S. H., Doyle, M. P., and Joseph, S. W., 1998). Each spice has a unique aroma and flavor, which derive from compounds known as phytochemicals or secondary compounds. These chemicals evolved in plants to protect them against herbivorous insects, vertebrates, fungi, pathogens, and parasites (Walker, 1994). For thousands of years, aromatic plant materials have been used in food preparation and preservation, as well as for embalming, in areas where the plants are native, such as Hindustan and the Spice Islands (Govindarajan, V. S., 1985; Dillon, V. M. and Board, R. G., 1994). Cell wall lip polysaccharides may prevent that essential oils active compounds reach the cytoplasm membrane of gram-negative bacteria (Chanegriha, N., Sabaou, N., Baaliouamer, A., and Meklati, B. Y., 1994).

Spices and herbs used today have been valued for their antimicrobial effects and medicinal powers in addition to their flavour and fragrance qualities (Ceylan, E. and Fung, D. Y. C., 2004). The antimicrobial compounds in spices and herbs are mostly in the essential oil fraction. Zaika (1988) proposed that Gram-positive bacteria are more resistant then Gram-negative bacteria to the antibacterial properties of plant volatile oils which is in contrast to the hypothesis proposed by Deans that the susceptibility of bacteria to plant volatile oils and the Gram reaction appears to have little influence on growth inhibition (Deans, S. G. and Ritchie, G., 1987). The extent of sensitivity varied with the strain and environmental conditions imposed. Phenols, alcohols, aldehydes, ketones, ethers and hydrocarbons have been recognized as major antimicrobial components in spices.
Several reports have shown the antimicrobial effect of eugenol against several species of bacteria, i.e. *E. coli* O157:H7 (Kim et al., 1995; Wendakoon and Sakaguchi, 1995; Hao et al., 1998; Friedman, M., Henika, P. R. and Mandrell, R. E., 2002). Scientists found that eugenol, an active component of allspice, “significantly” reduced *E. coli* O157 toxin growth. Gould (1995) has emphasized the possible use of spices and derivatives like alternatives for inclusion in a new perspective of food conservation called “natural antimicrobial system”, which could use the synergistic effect of antimicrobials. Antibacterial activity of spices has been reported by several researchers.

Food conservation has been characterized for nutritious and microbiologically stable foods and it has been archived by controlling the growth of spoiling and pathogenic food-related microorganisms. Microbial control could be possible by adding suitable substances (weak organic acids, hydrogen peroxide, chelators, organic biomolecules) and applying physical (temperature, packaging) and/or chemical procedures (pH, oxide-reduction potential, osmotic pressure) (Ray, B., 1996; Brull and Coote, 1999). Many essential oils are already used in the food industry as flavoring agents and some are known to exert antimicrobial activity, but the mechanism of action is often not entirely understood. Cinnamon, bay leaf, ajowan, star anise and tailed pepper are well known herb widely used as a spice, in folk medicine and in the pharmacy and food industries (Bauer, A. W., Kirby, W. M., Sherris, J. C., and Turck, M., 1966). Spices are used as substances that increase the taste and variation of food (Ceylan, A., 1997; Bulduk, S., 2004). Hao et al (1998a, 1998b) reported inhibitory effect of eugenol, active principle of clove. Spices active compounds have been included in class of naturally occurring food preservatives and have their inclusion in foods allowed by food production regulator offices (Brull and Coote, 1999).

Food processors, food safety researchers, and regulatory agencies have been increasingly concerned with the growing number of food borne illness outbreaks caused by some pathogens (Hancock, D. L., 1997; Castro-Rosas, J., and Escartin, E. F., 2000; Wilson, C. L., and Droby, S., 2000).

The antimicrobial activities of plant extracts form the basis for many applications, including raw and processed food preservation, pharmaceuticals, alternative medicines and natural therapies (Lis-Balchin, M. and Deans, S. G., 1997). Strains
of resistant food borne pathogens to a variety of antimicrobials have become a major health concern (Levy, S. W., 1997; Kiessling et al, 2002) and it could decrease the successful application of control measures on spoilage and pathogen microorganisms, many times leading for use of less safe, ineffective or expensive alternatives.

However, today consumers demand favor consumption of natural foods which contain fewer synthetic additives, this made the use of natural antimicrobials in food popular (Davidson, P. M., 1997). Outara et al. (1997) reported antimicrobial activity of many spices and classified their activities as strong, medium, or weak. Uncontrolled use of chemical antimicrobial preservatives has been inducing factor for appearance of microbial strains more and more resistant to classic antimicrobial agents. Fifty years of increasing use of chemicals antimicrobials have created a situation leading to an ecological imbalance and enrichment of multiples multi resistant pathogenic microorganisms (Levy, 1997).

Eugenol is the major essential oil component in Cinnamon (Davidson, 1997). Increasing antibiotic resistance of some pathogens that are associated with food borne illness is another concern (Meng et al., 1998; Perreten, V., Giampa, N., Schuler-Schmid, U. and Teuber, M., 1998; Stermitz, F. R., Lorenz, P., Tawara, J. N., Zenewicz, L. A. and Lewis, K., 2000). Spices and herbs and their constituents are generally recognized to be safe, either because of their traditional use without any documented detrimental impact or because of dedicated toxicological studies (Smid and Gorris, 1999). Being natural foodstuffs, spices and herbs appeal to many consumers who question the safety of synthetic food additives. Some spices and herbs used today are valued for their antimicrobial activities and medicinal effects in addition to their flavor and fragrance qualities. The extracts of many plant species have become popular in recent years and attempts to characterize their bioactive principles have gained momentum for varied pharmaceutical and food processing applications. There has been increasing concern of the consumers about foods free or with lower level of chemical preservatives because these could be toxic for humans (Bedin, C., Gutkoski, S. B. and Wiest, J. M., 1999). Concomitantly, consumers have also demanded for foods with long shelf-life and absence of risk of causing food borne diseases. This perspective has put pressure on the food industry for progressive removal of chemical preservatives and adoption of natural alternatives to obtain its goals concerning microbial safety.
This resulted in increasing search for new technologies for use in food conservation systems, which include: modified atmosphere packaging, combined effect of under lethal procedures, alternative antimicrobial compounds (ecstatic or cidal effect), combination of conventional (used in low levels) and alternatives antimicrobials (Brull and Coote, 1999).

Natural preservatives are the chemical agents derived from plants that prevent the decomposition of products by any means. The mode of action of these natural preservatives is inhibition of microbial growth, oxidation and certain enzymatic reactions occurring in the food stuffs. Spices offer a promising alternative for food safety. Current research on natural molecules and products primarily focuses on plants since they can be sourced more easily and be selected based on their ethnomedicinal uses (Arora, D. S., and Kaur, G. J., 2007). Naturally derived constituents of spice extracts contained high levels of phenolics and exhibited antibacterial activity against food borne pathogens. Some studies claim that the phenolic compounds present in spices and herbs might also play a major role in their antimicrobial effects. Food conservation for nutrition and superior shelf life can be obtained by controlling the growth of food borne pathogenic microorganisms and food spoilage. This could be achieved by suppressing one or more factors that are essential for microbial survival. Suppression might be possible by adding suitable chemical substances and by controlling physical factors for the growth of microbes (Brull and Coote, 1999). Still little information is available emphasizing the preservative and antimicrobial role of spices in the prevention of foods of the microbial action (Arora, D. S. and Kaur, G. J., 1999). The inhibitory effects of spices are mostly due to the volatile oils present in their composition (Arora and Kaur, 1999).

Antibiotic resistance in food borne pathogens is a reality, though substantial qualitative and quantitative differences have been observed (Teuber, M., 1999). Systematic screening for biological interactions between microorganisms and plant products has been valuable source of new and effective antimicrobial substances, which could have different action ways on the microbial cell when compared to other conventional antimicrobials. Plants synthesize by a secondary metabolism many compounds with complex molecular structures and some of them have been related with antimicrobial properties found in plant and their derivatives. Among these secondary metabolites are found alkaloids, flavonoids,
isoflavonoids, tannins, glycosides, terpenes and phenolic compounds. Spices active compounds have been included in class of naturally occurring food preservatives and have their inclusion in foods allowed by food production regulator offices (Brull and Coote, 1999).

Arora and Kaur (1999) analyzed the antimicrobial activity of various spice aqueous extracts on human pathogenic bacteria including Bacillus sp, Staphylococcus aureus and Escherichia coli, and found that all tested bacteria were sensitive to spice extract. Moreover, these bacteria present various undesirable attributes of virulence that in combination make them some of the most serious threats for food safety (Proctor, M. E. and Davis, J. P., 2000). In Turkish, food codex (Anonim, 2000), a spice is defined as a natural compound, or a mixture of natural compounds that is extracted from the seeds, fruits, flowers, or trunks (skins, roots, leaves) of several plants, and added to food in order to provide color, taste, smell, or flavor. Moreover, plant volatiles have been generally recognized as safe (GRAS) (Newberne, P., Smith, R. L., Doull, J. and Feron, V. J., 2000). Antimicrobial properties of spices have been documented in recent years and interest continues to the present (Domans, H. J. D. and Deans, S. G., 2000). Food antimicrobials are the added compounds in foods that hinder microbial growth or kill microorganisms. The functions of food antimicrobials are to inhibit or inactivate spoilage and pathogenic microorganisms. These functions have increased in importance in the past 10–15 years as food processors search for more and better tools to improve food safety especially those depending on the use of natural derivatives as antimicrobial agents (Davidson, P. M., 2001)

Changes in the antimicrobial target, inactivation by enzymes, changes in cellular permeability, antimicrobial active efflux, and overproduction of target enzymes and bypass of the antimicrobial have been common mechanisms of antimicrobial resistance (McKeegan, K. S., Borges-Walmsley, M. I. and Walmsley, A. R., 2002). Plant products are characterized for a wide range of volatile compounds, some of which are important flavor quality factors (Utama, J. M. S., Wills, R. B. H., Ben-Yehoshua, S. and Kuesk, C., 2002). Spices are recognized to stabilize the foods from the microbial deterioration the microbial growth become progressively slower or it is eventually totally suppressed (Kizil, S. and Sogut, T., 2003).
The main inducement to search for effective antimicrobials among naturally occurring compounds is to expand the spectrum of antimicrobial activity over that of the regulatory-approved substances; most of currently approved and traditional food antimicrobials have limited application due to pH or food component interactions (Davidson, P. M. and Zivanovic, S., 2003). The main factors that determine the antimicrobial activity are the type and composition of the spice, amount used, type of microorganism, composition of the food, pH value, temperature of the environment, and proteins, lipids, salts, and phenolic substances present in the food environment (Sagdic, O., 2003).

Presence of pathogenic and spoiling microorganisms in spices could act as vehicles for microorganisms to enter in foods. Frequently, spices are grown and harvested in warm and humid areas where the growth of wide variety of microorganisms is readily supported (Mousuymi, B. and Sarkat, P. K., 2003). As many other agricultural commodities, spices are exposed to a wide range of environmental microbial contamination during harvest, processing, and in retail markets by dust, waste water, and animal and even human excreta (Freire, F. C. O. and Offord, L., 2003). Antimicrobial activity of spices could be recognized as important factor for providing their inclusion in food conservation systems when pertinent measures are taken to assure their satisfactory microbiological quality. These measures must include action to control the moisture, good sanitary conditions in the processing, workers training, satisfactory transport conditions, proper storage, microbiological quality monitoring and actions of sanitary mark applied since harvest until insertion in foods.

In food industry, synthetic antioxidants are mostly used. However, modern consumers are commonly afraid of any synthetic chemicals. They feel that natural antimicrobials and antioxidants are safer and more acceptable to the human body. Therefore, food producers try to add natural antioxidants when possible (Pokorny, J., 2003). Common kitchen spices may reduce the deadliness of the E. coli O157 toxin, according to a new study in the Journal of Food Science. Sagdic et al. (2003) assayed the inhibitory effect of methanol extracts of seven Turkish spices on E. coli O157:H7 and showed prominent results as bactericidal in both paper disc and agitated liquid culture assay. However, it was also found that laurel (0.5, 1.0, 1.5 and 2.0 % v/v) stimulated the growth of E. coli O157:H7. Exact
mechanism of antibacterial action of spices and derivatives is not yet clear (Lanciotti et al., 2004).

However, the results reported for these different studies are difficult to compare directly, usually because of the low number of plant samples tested, different test methods and diverse bacterial strains and sources of antimicrobial samples used. Many studies have reported that phenolic compounds in spices and herbs significantly contributed to their antioxidant and pharmaceutical properties (Cai, Y. Z., Luo, Q., Sun, M., and Corke, H., 2004; Shan, B., Cai, Y. Z., Brooks, J. D. and Corke, H., 2007). Some studies claim that the phenolic compounds present in spices and herbs might also play a major role in their antimicrobial effects (Hara-Kudo et al., 2004). There has been no large scale systematic investigation of the relationship between bacterial inhibition and total phenolic content of spices and herbs. Previous studies (Cai et al., 2004; Shan et al., 2007) showed that a highly positive linear relationship exists between antioxidant activity and total phenolic content in some spices and herbs. However, there is no reported data on the relationship between antibacterial activity and antioxidant capacity of spices and herbs.

Plant products with antimicrobial properties notably have obtained emphasis for a possible application in food production in order to prevent bacterial and fungal growth (Lanciotti et al., 2004). Exact mechanism of antibacterial action of spices and derivatives is not yet clear (Lanciotti et al., 2004). Spices are used as substances that increase the taste and variation of food (Bulduk, S., 2004). Many authors have emphasized that the antimicrobial effect of essential oil constituents has been dependent on their hydrophobicity and partition in the microbial plasmatic membrane. Effect of specific ions due to their addition in/on plasmatic membrane had great effect on the protons motive force, intracellular ATP content and overall activity of microbial cells, including turgor pressure control, solutes transport and metabolism regulation (Lanciotti et al., 2004). Essential oils of herbs and their components, which are products from the secondary metabolism of plants, have many applications in ethno-medicine food, flavoring and preservation as well as in the fragrance and pharmaceuticals industries (Edris, A. E., 2007). The effectiveness of inhibitory compounds such as extracts from Bio-molecules of
plant origin appears to be one of the alternatives for the control of these microfloras.

Although numerous studies have been published on the antimicrobial activities of plant compounds against many different types of microbes, including food borne pathogens, the consequences of quality loss of food product caused by microorganisms (Kasetsart, J., 2007) are consumer’s risk, due to the presence of microbial toxins and pathogens. Food antimicrobials are compounds (www.sciencedirect.com) added to or present in foods to retard microbial growth or kill microorganisms. Plant essential oils are a potentially useful source of antimicrobial compounds that have been shown to possess distinct antimicrobial activities against many food borne pathogens.

Thymol and Eugenol are both natural preservative substances that are not toxic when applied at an appropriate amount in food products. The antimicrobial properties of essential oils have been of the growing demand on antimicrobials for preventing microbial food spoilage and bacterial infections; there is an increasing interest in medicinal plants as an alternative to synthetic preservatives and antibiotics (Burdock, G. A. and Carabin, I. G., 2009).

Thymol is the major essential oil component in *Carum copticum* and is also active against several species of bacteria such as *Staphylococcus aureus* and *Escherichia coli*.

There are a lot of antimicrobial agents of which some are discovered and some are hidden in the nature. Hence, the last decade witnessed an increase in the investigations on plants as a source of human disease management and more natural antimicrobials have driven scientist to investigate.

Microorganisms are the concealed enemies to the mankind (Suganya et al., 2012). They are small but cause a very profound damage in human body as well as other living organism.

It is established that spices and their derivatives could be suitable alternatives for inclusion in food conservation systems and could act sometimes as main or adjuvant antimicrobial compounds. Before including spices and/or their
derivatives in food conservation systems, some evaluations about microbiological quality, economic feasibility, and antimicrobial effect for a long time and toxicity should be carried out.

This system expanded on the synergistic effect of antimicrobial compounds extracted from plants and spices that was expressed in physical testing procedures in the attempt to create inhospitable living conditions for bacteria.

2.1 *Cinnamomum zeylanicum* (Cinnamon)

2.1.1 General characteristics of Cinnamon

Cinnamon is a spice obtained from the inner bark of trees from the genus *Cinnamomum* that is used in both sweet and savory foods. Cinnamon trees are native to South East Asia. Cinnamon comes in ‘quills’, strips of bark rolled one in another.

Cinnamon is the brown bark of the cinnamon tree, which when dried, rolls into a tubular form known as a quill. Cinnamon is available in either its whole quill form (cinnamon sticks) or as ground powder.

While there are approximately one hundred varieties of *Cinnamomum verum* (the scientific name for cinnamon), *Cinnamomum zeylanicum* (Ceylon cinnamon) and *Cinnamomum aromaticum* (Chinese cinnamon) are the leading varieties consumed.

*Cinnamomum zeylanicum* is a small, tropical, evergreen tree most noted for its bark, which provides the world with the commonly known spice, cinnamon. *Cinnamomum zeylanicum* is the scientific name, which refers to the specific species of tree that cinnamon is harvested from.
Fig 2.1.1 Cinnamon Tree and its Bark
2.1.2 Classification

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Laurales
Family: Lauraceae
Genus: Cinnamomum
Species: zeylanicum
2.1.3 Chemistry of Cinnamon

The primary constituents of the essential oil are 65% to 80% cinnamaldehyde and lesser percentages of other phenols and terpenes, including eugenol, trans-cinnamic acid, hydroxycinnamaldehyde, o-methoxycinnamaldehyde, cinnamyl alcohol and its acetate, limonene, alpha-terpineol, tannins, mucilage, oligomeric procyanidins, and trace amounts of coumarin.

Cinnamaldehyde is the organic compound that gives cinnamon its flavor and odor. This pale yellow viscous liquid occurs naturally in the bark of cinnamon trees.

Fig 2.1.3: Cinnamaldehyde
2.1.4 Economic and medicinal importance of Cinnamon

- It is the most common baking spice.
- Cinnamon is used more in dessert dishes. It is commonly used in cakes and other baked goods, milk and rice puddings, chocolate dishes and fruit desserts, particularly apples and pears.
- It is common in many Middle Eastern and North African dishes, in flavoring lamb tagines or stuffed aubergines.
- Stick Cinnamon is used in pickling and for flavoring hot beverages.
- It is used in curries and pilaus and in garam masala.
- It may be used to spice mulled wines, creams and syrups.
- The largest importer of Sri Lankan cinnamon is Mexico, where it is drunk with coffee and chocolate and brewed as a tea.
- Cinnamon is carminative, astringent, stimulant, antiseptic.
- It is more powerful as a local than as a general stimulant.
- It stops vomiting, relieves flatulence, and given with chalk and astringents is useful for diarrhea and hemorrhage of the womb.
- Because of its mild astringency, it is particularly useful in infantile diarrhea.
- Recent studies have determined that consuming as little as one-half teaspoon of Cinnamon each day may reduce blood sugar, cholesterol, and triglyceride levels by as much as 20% in Type II diabetes patients.
- It is used to treat nausea and flatulence.
- Cinnamon is a great remedy for people with cold feet and hands, especially at night.
- The cinnamaldehyde component is hypotensive and spasmolytic, and increases peripheral blood flow.
2.2 *Laurus nobilis* (Bay Leaf)

2.2.1 General characteristics of bay leaf

The bay leaf is oval, pointed and smooth, 2.5 - 8 cm (1 to 3 in) long. When fresh, the leaves are shiny and dark green on top with lighter undersides. When dried the bay leaf is a matte olive green. Warm and quite pungent when broken and the aromatic oils are released. Flavor is slightly bitter and strongly aromatic.

If eaten whole, bay leaves are pungent and have a sharp, bitter taste. As with many spices and flavorings, the fragrance of the bay leaf is more noticeable than its taste. When dried, the fragrance is herbal, slightly floral, and somewhat similar to oregano and thyme. Myrcene, which is a component of many essential oils used in perfumery, can be extracted from the bay leaf. Bay leaves also contain the essential oil eugenol.

![Bay Leaf Tree](image)

**Fig 2.2.1 Bay Leaf Tree**
Fig 2.2.2 Dried Bay Leaves (Indian: Tej Patta)

2.2.2 Classification

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Laurales
Family: Lauraceae
Genus: Laurus
Species: nobilis
2.2.3 Chemistry of Bay leaf

Fig 2.2.3: Eugenol
2.2.4 Economic and medicinal importance of Bay leaf

- Bay leaves are slightly bitter in taste, so they are taken out of the dish. It is added when cooking, just to add aromatic flavor.
- It is a traditional ingredient of French cuisines. It is used in the preparations of chicken, sauces, for garnishing, added to stews and bland vegetables.
- They are also characteristic of the cuisines of India, Brazil, Greece, Germany, Eastern Europe, Jamaica and Scandinavia.
- It is said to have many properties to treat high blood sugar, migraine, headache, bacterial and fungal infections and gastric ulcers.
- Bay oils are used for bruising and sprain.
- Bay leaves are reported to be anti-inflammatory, anti-oxidant, anti-fungal and anti-bacterial.
2.3 *Carum copticum* (Ajowan)

### 2.3.1 General characteristics of Ajowan

Ajowan (Ajwain) is very popular old spice used in different types of cuisines and highly valued for its medicinal uses. Different genres of medicines such as ayurveda and unani make an extensive use of this spice in medicines. Ajowan (*Carum copticum* Benth. & Hook.) is an annual herbaceous essential oil bearing plant belonging to the Apiaceae family, which grows in India, Iran, and Egypt (Zargari, A., 1996)

Its plant has similarity to parsley and its pods are egg shaped, very small in shape sometimes looks like other version of cumin seeds. It is originated in Egypt and the eastern Mediterranean area. Its plant is a small, erect, annual shrub with soft fine hairs. It has many branches of leafy stem which has 4-6 rays of flowers head, each bearing 6-16 flowers. The fruits are small egg-shaped pods, brown-gray in color.

![Fig 2.3.1 Ajowan Plant and its flowers](image-url)
2.3.2 Classification

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Apiales
Family: Apiaceae
Genus: Carum
Species: copticum
2.3.3 Chemistry of Ajowan

The essential constituents of ajwain are thymol, which constitutes 35-60% of the essential oil. It also contains alpha-pinene, p-cymene, and limonene and terpinene. Thymol is a natural monoterpenic phenol derivative of cymene found in oil of thyme. It has pleasant aromatic odor and strong antiseptic properties. Thymol also provides the distinctive, strong flavor of the culinary herb thyme. Thymol has microbial activity because of its phenolic structure, and has shown antibacterial activity against bacterial strains including *Staphylococcus aureus*.

![Thymol molecule](image)

**Fig 2.3.3 Thymol**
2.3.4 Economic and medicinal importance of Ajowan

- Ajowan seeds are used in different dishes and added to different pickles.
- Its powder is also used to add in many cooking dishes.
- It is said to have a valuable spice which appears with many properties such as spasmodic, germicidal, digestive, antiseptic and expectorant.
- It is said to have healing powers and curative properties, as its water is distilled to cure flatulence, indigestion and low appetite.
- Its oil is used to relieve various joint pains, and to cure wounds.
- It is taken to relieve diseases like asthma and bronchitis. It is a good medicine for toothache, headache, earache, influenza, cold and cough, rheumatism and mouth disorders.
- Ajowan oil is anti-infectious, antibacterial, antiviral, anti-fungal, anti-parasitic, antiseptic, anti-nausea, and a tonic.
- Ajowan oil is used as a sedative or for whooping cough and toothaches in India.
- Ajowan oil assists bronchitis, skin disease, gas, digestive weakness, cholera, & diarrhea.
- Ajowan oil is also used as a circulatory stimulant.
2.4 *Illicium verum* (Star-Anise)

2.4.1 General characteristics of Star-Anise

*Illicium verum*, commonly called Star anise is a spice that closely resembles anise in flavor, obtained from the star-shaped pericarp of *Illicium verum*, a small native evergreen tree of northeast Vietnam and southwest China. The fruit is brown and woody, and is shaped like a star with eight rays, or a flower with eight petals. Each “petal” or seed pod contains a brown seed. The fruit is aromatic and has a strong, pungent and mildly sweet taste.

![Star Anise Plant and its Leaves](image_url)

**Fig 2.4.1 Star Anise Plant and its Leaves**
2.4.2 Classification

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Austrobaileyales
Family: Illiciaceae
Genus: Illicium
Species: verum

Fig 2.4.2 Star Anise Fruits (Indian: Dagad Phool)
2.4.3 Chemistry of Star Anise

Star anise primarily contains anethole and fatty oil. Essential star anise oil has a sweetish, burning flavor and a highly aromatic odor. It is primarily located in the woody shell, to a lesser extent in the seed. It contributes a large component of the distinctive flavors of star anise (Illiciaceae). Anethole is only slightly soluble in water but exhibits high solubility in ethanol. It is distinctly sweet, measuring 13 times sweeter than sugar.

![Anethole](image)

**Fig 2.4.3 Anethole**
2.4.4 Economic and medicinal importance of Star-Anise

- The anti-bacterial and anti-fungal properties of star anise are useful in the treatment of asthma, bronchitis and dry cough. For this reason, some cough mixtures contain star anise extract.
- Star anise can also be used as for its sedating properties to ensure a good sleep.
- The oil of star anise is useful in providing relief from rheumatism and lower back pain.
- Star anise can also be used as a natural breath freshener.
- Shikimic acid, a compound present in star anise is used for preparing drug for curing influenza or the flu virus.
- Linalool, a compound present in star anise contains anti-oxidants properties that are very good for one's overall health.
- This exotic seed spice contains many plant derived chemical compounds that are known to have anti-oxidant, disease preventing and health promoting properties.
- The main constituent that gives characteristic sweet, aromatic flavor to anise is anethole, the essential volatile oil present in the seeds. Other important compounds found in these seeds include estragol, \( p \)-anisaldehyde, anise alcohol, acetophenone, pinene and limonene.
- Anise seed oil obtained from extraction of the seeds is found application in many traditional medicines as stomachic, anti-septic, anti-spasmodic, carminative, digestive, expectorant, stimulant and tonic.
- The seeds are an excellent source of many essential B-complex vitamins such as pyridoxine, niacin, riboflavin, and thiamin. Pyridoxine (vitamin B-6) helps increase GABA neuro-chemical levels in the brain.
- The spicy seeds are great source of minerals like calcium, iron, copper, potassium, manganese zinc and magnesium. Potassium in an important component of cell and body fluids that helps control heart rate and blood pressure.
- Copper is a cofactor for many vital enzymes, including cytochrome \( C \)-oxidase and superoxide dismutase (other minerals function as cofactors for this enzyme are manganese and zinc). Copper is also required in the production of red blood cells.
- The spice is also contain good amounts of anti-oxidant vitamins such as vitamin-C and vitamin-A.
2.5 *Piper cubeba* (Tailed-Pepper)

2.5.1 General characteristics of Tailed-Pepper

Cubeb (*Piper cubeba*), or tailed pepper, is a plant in genus *Piper*, cultivated for its fruit and essential oil. The genus *Piper* belongs to the family Piperaceae and has over 1000 species distributed in hemispheres, where they grow in the form of erect or scadent (climbing) herbs, shrubs, or, less frequently, trees (Junqueira, A. P. F., Perazzo, F. F., Souza, G. H. B., and Maistro, E. L., 2007). It is mostly grown in Java and Sumatra, hence sometimes called Java pepper. The fruits are gathered before they are ripe, and carefully dried. Commercial cubebs consist of the dried berries, similar in appearance to black pepper, but with stalks attached — the "tails" in "tailed pepper". The dried pericarp is wrinkled; its color ranges from grayish-brown to black. The seed is hard, white and oily. The odor of cubebs is described as agreeable and aromatic; the taste, pungent, acrid, slightly bitter and persistent. It has been described as tasting like all spice, or like a cross between all spice and black pepper.

A climbing perennial plant with dioecious flowers in spikes. The fruit is a globose, pedicelled drupe. It is extensively grown in the coffee plantations, well shaded and supported by the coffee trees. Odor aromatic and characteristic taste strongly aromatic and pungent and somewhat bitter. Commercial Cubebs are often adulterated with other fruits containing a volatile oil, but with very different properties. There is no evidence that the plant was known to the ancients, though it was probably brought into Europe by the Arabians, who doubtless employed the fruit as pepper.
Fig 2.5.1 Tailed-Pepper Plant

Fig 2.5.2 Tailed-Pepper Fruits (Indian: Kabab chini)
2.5.2 Classification

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Piperales
Family: *Piperaceae*
Genus: *Piper*
Species: *cubeba*
2.5.3 Chemistry of Tailed-Pepper

It contains 10 to 18 percent of volatile oil, also resins, amorphous cubic acid and colorless crystalline cubebin. By extraction with ether, it yields about 22 percent of oleoresin. **Cubebin** \((C_{10}H_{10}O_3)\) is a crystalline substance existing in cubebs, discovered by Eugene Soubeiran and Capitaine in 1839. It may be prepared from cubebene, or from the pulp left after the distillation of the oil.

![Cubebin](image)

**Fig 2.5.3 Cubebin**

2.5.4 Economic and medicinal importance of Tailed-Pepper

- Agri - horticulture: ornamental, cultivated or partially tended.
- Medicines: genital stimulants/depressants; malnutrition, debility; stomach troubles; tumors, cancers; venereal diseases.
- Phytochemistry: antibiotic, bacteristatic, fungistatic; aromatic substances.
- The fruits of this plant are used as a spice and have medicinal value, being often used for the treatment of abdominal pain, asthma, chronic bronchitis, diarrhea, dysentery, gonorrhea, enteritis and syphilis and reported to have an inhibitory effect on hepatitis C virus protease.
- Sanskrit texts included cubeb in various remedies. Charaka and Sushruta prescribed a cubeb paste as a mouthwash, and the use of dried cubebs internally for oral and dental diseases, loss of voice, halitosis, fevers, and cough.

- Tailed Pepper is considered a carminative, diuretic, expectorant, stimulant, and antiseptic.

- Tailed Pepper has been shown to be effective in easing the symptoms of chronic bronchitis.

- It is also used for digestive ailments and is effective in treating dysentery.

- Cubeb oil is effective against influenza virus and Bacillus typhosus.
2.6 Description of bacteria

2.6.1 *Bacillus subtilis*

2.6.1.1 Description and significance

*Bacillus subtilis* is a gram-positive, rod-shaped, endospore forming bacteria. It is regarded as an aerobe; however, it is also capable of growing and sporulating under anaerobic conditions when necessary. *B. subtilis* resides predominantly in soil, including low-nutrient soil. Due to its association with soil particles, it is also inevitably transferred to plants, foods, animals and even marine and freshwater habitats.

*B. subtilis* was one of the first bacteria studied by scientists. It was originally named *Vibrio subtilis* in 1835 by Christian Gottfried Ehrenberg and later renamed *Bacillus subtilis* by Ferdinand Cohn in 1872.

![Bacillus subtilis bacteria under microscope](image)

*Fig 2.6.1.1 Bacillus subtilis bacteria under microscope*
2.6.1.2 Classification

Kingdom: Eubacteria
Phylum: Firmicutes
Class: Bacilli
Order: Bacillales
Family: Bacillaceae
Genus: Bacillus
Species: subtilis
2.6.2 *Bacillus cereus*

2.6.2.1 Description and significance

*Bacillus cereus* is an endemic, soil-dwelling, Gram-positive, rod-shaped, beta hemolytic bacterium. Some strains are harmful to humans and cause food borne illness, while other strains can be beneficial as probiotics for animals. It is the cause of "Fried Rice Syndrome", as the bacteria is classically contracted from fried rice dishes that have been sitting at room temperature for hours. *B. cereus* bacteria are aerobes, and like other members of the genus *Bacillus* can produce protective endospores. Its virulence factors include cereolysin and phospholipase C.

*Bacillus cereus* causes a toxin-mediated food poisoning. *Bacillus cereus* is an aerobic and facultative anaerobic, spore-forming, gram-positive bacillus. *B. cereus* is responsible for a minority of food borne illnesses (2–5%), causing severe nausea, vomiting and diarrhea. *Bacillus* food borne illnesses occur due to survival of the bacterial endospores when food is improperly cooked. Cooking temperatures less than or equal to 100°C (212°F) allows some *B. cereus* spores to survive. This problem is compounded when food is then improperly refrigerated, allowing the endospores to germinate. Cooked foods not meant for either immediate consumption or rapid cooling and refrigeration should be kept at temperatures above 60°C (140°F). Germination and growth generally occurs between 10–50 °C (50–122 °F), though some strains are psychrotrophic. Bacterial growth results in production of enterotoxins, one of which is highly resistant to heat and to pH between 2 and 11; ingestion leads to two types of illness, diarrheal and emetic (vomiting) syndrome.
Fig 2.6.2.1 *Bacillus cereus* bacteria under microscope

Fig 2.6.2.2 *Bacillus cereus* bacteria under electronic high-resolution microscope
2.6.2.2 Classification

Kingdom: Bacteria
Phylum: Firmicutes
Class: Bacilli
Order: Bacillales
Family: Bacillaceae
Genus: Bacillus
Species: cereus

2.6.3 Staphylococcus aureus

2.6.3.1 Description and significance

*S. aureus* is a spherical bacterium (*coccus*) which on microscopic examination appears in pairs, short chains, or bunched, grape-like clusters. These organisms are Gram-positive (a cell with a thick wall). Some strains are capable of producing a highly heat-stable protein toxin (a poisonous substance) that causes illness in humans. *Staphylococcus aureus* is recognized as a cause of food poisoning which occurs after an initial contamination of food with a toxigenic strain. The growth may occur in the absence of enterotoxin synthesis and could have a role in the pathogenicity of some other staphylococcal diseases.

Foods that are frequently incriminated in *staphylococcal* food poisoning include meat and meat products; poultry and egg products; salads such as egg, tuna, chicken, potato, and macaroni; bakery products such as cream-filled pastries, cream pies, and chocolate eclairs; sandwich fillings; and milk and dairy products. Foods that require considerable handling during preparation and that are kept at slightly elevated temperatures after preparation are frequently involved in staphylococcal food poisoning. Human intoxication is caused by ingesting enterotoxins produced in food by some strains of *S. aureus*, usually because the
food has not been kept hot enough (60°C, 140°F, or above) or cold enough (7.2°C, 45°F, or below).

Fig 2.6.3.1 *Staphylococcus aureus* bacteria under microscope

Fig 2.6.3.2 *Staphylococcus aureus* bacteria under high resolution microscope
2.6.3.2 Classification

Kingdom: Bacteria
Phylum: Firmicutes
Class: Bacilli
Order: Bacillales
Family: Staphylococcaceae
Genus: Staphylococcus
Species: aureus

2.6.4 *Escherichia coli* O157:H7

2.6.4.1 Description and significance

*Escherichia coli* O157:H7 is an enterohemorrhagic strain of the bacterium *Escherichia coli* and a cause of food borne illness. Infection often leads to hemorrhagic diarrhea, and occasionally to kidney failure, especially in young children and elderly persons. Transmission is via the fecal-oral route, and most illness has been associated with eating undercooked, contaminated ground beef or ground pork, swimming in or drinking contaminated water, and eating contaminated vegetables.

*E. coli* serotype O157:H7 is a Gram-negative, rod-shaped bacterium. The "O" in the name refers to the cell wall (somatic) antigen number, whereas the "H" refers to the flagella antigen. *E. coli* serotype O157:H7 is a rare variety of *E. coli* that produces large quantities of one or more related, potent toxins that cause severe damage to the lining of the intestine. Also known as enterohaemorrhagic *Escherichia coli* (EHEC)

*E. coli* O157:H7 was first recognized as a food borne pathogen in 1982 during an investigation into an outbreak of hemorrhagic colitis (bloody diarrhea) associated with the consumption of contaminated hamburgers (Riley, et al., 1983). The
following year, Shiga toxin (Stx), produced by the then little-known *E. coli* O157:H7, was identified as the real culprit.

**Fig 2.6.4.1** *E. coli* O157:H7 bacteria under microscope

**Fig 2.6.4.2** *E. coli* O157:H7 bacteria under high resolution microscope
2.6.4.2 Classification

Kingdom: Bacteria
Phylum: Proteobacteria
Class: Gamma Proteobacteria
Order: Enterobacterales
Family: Enterobacteriaceae
Genus: Escherichia
Species: coli
2.6.5 Cell Wall Structure of Gram-positive and Gram-negative Bacteria

Single-celled organisms were the first life on the planet. Bacteria have adapted and evolved over millions of years, resulting in the numerous varieties of bacteria that exist on the planet today. Most bacteria can be divided into two classes, gram-negative and gram-positive, based on a differential staining process called the Gram stain. The Gram stain separates bacteria based on the ability of the cell wall to retain crystal violet stain when decolorized by an organic solvent like ethanol. The differences in the cell wall also play an important role in the types of antibiotics that will be effective against them.

The ability to retain Gram stain is based on the structure of the bacterial cell membrane. Gram-positive bacteria, which retain the Gram stain, have a membrane which is composed of two parts, the cell wall and the cytoplasmic membrane (Fig. 2.6.5.1). The cell wall is composed primarily of peptidoglycan, a complex of linked polysaccharide chains which provide strength and rigidity. This peptidoglycan layer also is responsible for the ability of the cell to retain the gram stain.

Gram-negative bacteria have a cell wall which consists of an outer membrane, a periplasmic space and a cytoplasmic membrane. The peptidoglycan layer, found in the periplasmic space, is much smaller, and there is no teichoic acid present. The outer membrane and cytoplasmic membrane are comprised of phospholipids, lipopolysaccharides (LPS) and proteins. The proteins allow small molecules, like glucose, to diffuse through the outer membrane. A cell wall of this type does not retain the gram stain. The resistance of gram negative bacteria towards antibacterial substances is related to the hydrophilic surface of their outer membrane which is rich in lipopolysaccharide molecules, presenting a barrier to the penetration of numerous antibiotic molecules. However, the gram positive bacteria do not possess such outer membrane and cell wall structures (Kalamb, D and Kanicka, A., 2003).
Fig 2.6.5.1 Cell Wall of Gram Positive and Gram Negative Bacterium
Fig 2.6.5.2 Cell Wall Structure of a Gram Positive Bacterium

Fig 2.6.5.3 Cell Wall Structure of a Gram Negative Bacterium