

## Biological Significance of Spicy Essential Oils

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### Abstract

Spices are used as food additives since ancient times, as flavouring agents but also as natural food preservatives. Spice essential oils are complex mixtures of volatile substances, ordinarily terpenes, sesquiterpenes and oxygenated derivatives. They have been largely employed for their properties already observed in nature i.e., for their antibacterial, antifungal and insecticidal properties. At present, approximately 3000 essential oils are known, 300 of which are commercially important especially for pharmaceutical, agronomic, food, sanitary, cosmetic and perfume industries. It is important to develop a better understanding of their mode of action for new applications in human health, agriculture and environment. Some of them constitute effective alternatives or complements to synthetic compounds of chemical industry.

**Key words:** Spice essential oil; Antibacterial; Antifungal; Insecticidal; Food additives

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### INTRODUCTION

Spices, which are important part of human diets, have been used for thousands of years to enhance the flavour, colour and aroma of food. In addition to boosting flavour,

herbs and spices are also known for their preservative (Nielsen & Rios, 2000), antioxidative (Shobana & Naidu, 2000), antimicrobial and various other medicinal values (Wood et al., 2001), which forms one of the oldest sciences. Scientific experiments since 19<sup>th</sup> century have documented the antioxidative and antimicrobial properties of spices and their components. Essential oils of spices are volatile, natural complex compounds, characterized by a strong odour and are found in aromatic plant as secondary metabolites for which aromatic plants are used in the pharmaceutical, food and fragrance industries. They comprise as several hundred constituents, especially hydrocarbon (terpenes and sesquiterpenes) and oxygenated compound (aldehydes, ketones, alcohols, acids, phenols, oxides, lactones, acetate, ethers and esters) (Anitescu et al., 1997).

The extraction of essential oil components using solvents at high pressure, or supercritical fluids, has received much attention in the past several years, especially in food, pharmaceutical and cosmetic industries, because it presents an alternative to conventional processes such as organic extraction and steam distillation (Eikani et al., 1999). The extraction product can vary in quality, quantity and in composition according to climate, soil composition, plant organ, age and vegetative cycle stage (Masotti et al., 2003; Angioni et al., 2006). A lot of data of spice essential oils are reported in the literature but they are not systematical and concise. Therefore, this review is written in keeping in view to provide a better understanding of methods of extraction of spices essential oils and their mode of biological action for new applications in human health, agriculture and environment. Some of them constitute effective alternatives or complements to synthetic compounds of chemical industry.

## 1. BOTANICAL ASPECTS

Essential oils may be found throughout the plant cellular tissue or in special cells, glands or ducts located in several parts of the plants, i.e., in the leaves, barks and roots, flowers, fruits and seeds, sometimes confined to special structures and in others not localized. The type of structure of the secretory tissues is one of the characteristics of the botanical family. With few exceptions (e.g., clove), the essential oil is present at a low percentage and constituents only a small fraction of the total plant weight. The quantity and composition of the essential oil varies not only with the type of plant but also in particular, with the conditions prevailing during its growth i.e. climate, soil, altitude etc. The function of essential oils in the living plant tissue is far being completely understood. Odours of flowers, for instance, may be directly associated with insect attraction or repulsion and so influences pollination and some to act as a form of protection against parasites and others have such a repulsive odour which affords the plant protection from animal depredation (Heath, 1978). Plant produces volatile oils because of the flowing biological activity (Hay & Waterman, 1993).

a. Attractants: Volatile oils associated with flowers can play a significant role in attracting pollinators.

b. Feeding deterrents: Mono and sesquiterpenes have both been widely implicated in the defence of plants against herbivores (e.g., Leaf cutter ants). Some simple acyclic sesquiterpenes appear to act as insect juvenile hormones; for example, juvabione, which occurs in basil (*Ocimum basilicum*), has the ability to arrest the development of some insects, leading imperfect adult forms or the complete failure to produce adults. Some components of volatile oils, notably alcohols such as linalool and  $\alpha$ -terpineol are able to reduce digestive efficiency in ruminants by their antibacterial activity.

c. Monoterpenes as natural products: When oils glands are fractured, the monoterpenes flow rapidly over the leaf surface carrying with them the less-volatile germacrene. They then evaporate to leave the germacrene more widely distributed.

## 2. CHEMICAL ASPECTS

Volatile oils are chemically complex mixture, often containing in the excess of 100 individual components. The chemical components present in the essential oils may be classified as follows:

a. Hydrocarbons of the general formula:  $(C_5H_8)_n$ —the terpenes;

b. Oxygenated derivatives of these hydrocarbons;

c. Aromatic compounds having a benzenoid structure;

d. Compounds containing nitrogen or sulphur.

Volatile oils used for culinary, pharmaceutical and perfumery purposes are composed almost entirely of two classes of compounds, terpenes and phenylpropenes.

The terpenes are abundant but where they occur, phenylpropenes are usually the major flavour/odour factors (Hay & Waterman, 1993) (e.g., anise, fennel, clove, cinnamon and basil). Most of the oils only slightly soluble in water but more soluble in organic solvents, such as petroleum ether, benzene and alcohols. They have characteristic odours and flammable. Most essential oils are mixtures of constituents compounds. However, a few consists of virtually a single compound. For example, oil of wintergreen consists of methyl salicylate and cassia oil of cinnamaldehyde. Some essential oils particularly those from citrus fruits and coniferous or eucalyptus trees, contain high proportion of organic compounds known as terpenes and sesquiterpenes. Other components of essential oils include alcohols, aldehydes, acids, esters, ketones, phenols, lactones and non-terpenes hydrocarbons. Some of these have open chain, many cyclic and bicyclic. Members of the aromatic series are also present (e.g., phenyl ethyl alcohol and benzyl acetate) and from recent investigations, it appears that the azulenes (Gessner, 1973) play an important role in essential oils.

## 3. METHODS OF EXTRACTION OF ESSENTIAL OILS

Essential oils are obtained from plants by various methods. Steam distillation has traditionally been applied for essential oils recovery from plant material. One of the disadvantages of the hydrodistillation methods is that essential oils undergo chemical alteration and the heat-sensitive compounds can easily be destroyed. Therefore, the quality of the essential oil extracts is extremely impaired (Illes et al., 2000). Earlier used methods such as distillation, extraction with fat and solvents are extensively described in books. Some of the recently developed techniques are described here

a. Extraction with liquefied  $CO_2$  gas.

b. Solvent free microwave extraction (SFME)

### 3.1 Extraction With Liquefied $CO_2$ Gas

The extraction of essential oil using liquefied like  $CO_2$  gas is of great importance. Pressure of solvent residues in the final product has always been a problem with the conventional solvent extraction processes. The process essentially involves circulation of liquefied gas near its critical point through a high pressure extraction vessel. The solute is separated from a solvent gas by change of pressure or by complete evaporation. The gas can be compressed for use again. The use of supercritical fluids, especially carbon dioxide, in the extraction of plant volatile components has increased during last two decades due to the expected advantages of the supercritical extraction process. Supercritical fluid extraction (SFE) is a rapid, selective and convenient method for sample preparation prior to the analysis of the compounds in the

volatile product of plant matrices. Also, SFE is a simple, inexpensive, fast, effective and virtually solvent-free sample pretreatment technique. This review provides a detailed and updated discussion of the developments, modes and applications of SFE in the isolation of essential oils from plant matrices. SFE is usually performed with pure or modified carbon dioxide, which facilitates off-line collection of extracts and on-line coupling with other analytical methods such as gas, liquid and supercritical fluid-chromatography. In this review, we showed that a number of factors influence extraction yields, these being solubility of the solute in the fluid, diffusion through the matrix and collection process. Finally, SFE has been compared with conventional extraction methods in terms of selectivity, rapidity, cleanliness and possibility of composition of extract.

### 3.2 Solvent Free Microwave Extraction (SFME)

Recently, an original method for extracting natural products by using microwave energy has been developed (Chen & Spiro, 1994; Pare & Belanger, 1997; Lucchesi et al., 2004). One of the advantages of the SFME is rapidity and high yield. Based on relatively simple principle, this method involves placing spice material in a microwave reactor without any added solvent or water. The internal heating of the in situ water within the plant material distends it and makes the glands. This process thus free essential oils, which is evaporated by the in situ water of the plant by azeotropic distillation. The vapours then pass through a condenser outside the microwave cavity, where it is condensed. The distillate is collected continuously in the receiving flask. The excess of water is refluxed through the extraction vessel. The essential oil is collected directly and dried without any added solvent extraction. SFME is based on the combination of microwave heating and dry distillation, and is performed at atmospheric pressure. The isolation and concentrations of essential oils are performed in a single stage. The obtained solution can be analysed directly without any preliminary clean-up or solvent exchange steps by GC-MS. SFME extraction can be continued until no more essential oil is extracted. However, extraction time of SFME must be lower than that of hydrodistillation to be interesting in terms of time and energy savings. The irradiation power determines the rate of evaporation of water or the azeotropic mixture (water and essential oil) during SFME. The greater the rate of evaporation, the greater the yield the quantity of essential oil extracted. Solvent free microwave extraction has been used to obtain essential oils from different raw materials. The SFME is neither a modified microwave assisted extraction (MAE) which uses organic solvents, or a modified hydro-distillation which uses a large quantity of water (Chemat, 2003; 2004).

## 4. ANALYTICAL TECHNIQUES

The volatile oils are a complex class of natural substances. Generally, they contain a wide variety of components, although in some instances one or a few constituents make up the major portion of the oil. These aromatic materials are evaluated on the basis of their organoleptic properties, particularly their odour and flavour. In this regards, the roles of physico-chemical constants, introduced by Otto Wallach (Wallach, 1959; Teesserie, 1964; Wallach, 1961) are still considered important. These properties (Guenther, 1948; Teranishi, 1967) included boiling point, refractive index, density, optical rotation, solubility, acid value, saponification value, hydroxyl value etc. These methods proved to be of great value in the essential industry because of their simplicity, rapidity and reproducibility. However, these routine methods are insufficiently selective when it is desired to determine, the actual concentration of particular constituents in the essential oils. The techniques used to analyse the essential oils is summarized under two heads.

- a. Separation techniques
- b. Identification techniques

### 4.1 Separation Techniques

Resolution of different constituents of essential oils and their quantitative estimation can be carried out by following methods:

- a. Fractional distillation
- b. Chemical methods
- c. Chromatography

#### 4.1.1 Fractional Distillation

It is the classical methods for separating the components of the volatile oils and is the backbone of essential oil industry. Through it, the complete separation of all the components is not readily achieved because many terpenoids and other classes of compounds have a small range of boiling points. This method has not been standardized with regard to distillation equipment or fractionation schedule. Fractional distillation is always carried out under reduced pressure (commonly 20-25mm of mercury), since some polymerization occurs when the volatile oils are distillation at atmospheric pressure (Mirov, 1946). The elevated temperatures during fractionation make the products more difficult to purify because of degradation or loss of odour. The detailed procedure is well expressed in literature.

#### 4.1.2 Chemical Methods

Here the separation of the components of complex mixture (essential oils) is carried out on the basis of chemical reactions (Kurth, 1939). Separation of neutral and acidic constituents can be accomplished by the neutralization of the acidic components with dilute alkali and extraction with the ether. Hirose and Nakatsuka (1958) separated acidic constituents from neutrals by treating ethereal

solution of the essential oil with 5% NaOH. The alkaline solution was acidified by dilute  $H_2SO_4$  and extracted with ether. The formation of water-soluble crystalline addition compound of many aldehydes and some ketones with sodium bisulphite may transfer these components to aqueous layer. For example, piperitone may be separated by formation of its sodium bisulphite adduct (Shriner et al., 1966). For this purpose, the oil was shaken with sodium bisulphite solution, which causes precipitation of solid piperitone bisulphite. This adduct was separated by filtration and piperitone was regenerated by treating the adduct with conc. HCl.

Calcium chloride form addition compounds with some alcohols and may permit a partial separation of alcohols (e.g., geraniol). Other examples of crystalline adduct formations are the formation of addition products of carvone with hydrogen chloride, cineole with resorcinol and cinnamaldehyde with sodium bisulphite (Kirchner et al., 1951). The unsaturated hydrocarbons (terpenes) of volatile oils may be separated by the formation of bromo derivative followed by refluxing with Zn-dust. Thus limonene was separated by making its tetrabromoderivative (God leveski & Wagner, 1898). For this purpose the essential oil containing limonene as major fraction was dissolved in equal part of amyl alcohols and ether, and then the solution was added drop by drop to ice-cold solution of bromine in ether. The solid tetrabromolimonene was separated by filtration and then converted to limonene by reducing with zinc in alcohol.

#### 4.1.3 Gas Chromatography

It is widely used technique for the analysis of essential oils and aroma research. This method is being used for separating components with sufficient vapour pressure. Thus, it became one of most important tools in the analysis of essential oil. Separation of the components by these chromatographic technique is accomplished within minutes and it requires only very small quantity of sample (say a micrograms), which is the added advantage over other chromatographic techniques. The application of GC and GLC in analysis of essential oils is widely reported (Rice et al., 1951; Kayioni & Hayashi, 1956; Pohloudek & Beyrich, 1960; Schulte et al., 1955; Furest & Feustel, 1962; Forass et al., 1964; Suzuki et al., 1964; Masada, 1976 & 1968; Ettre & Zlatkis, 1967; Vernin, 1967; Aratani & Komoe, 1961; Kingston, 1964, Schratz & Wahlig, 1965) in literature. There are many modification of GC, which are used for specific purposes.

Preparative GC involves the use of columns with greater capacity and injection of large amount of samples for the isolation processes. The columns used in a preparative GC, required samples from few  $\mu L$  to few mL. However, large quantities of essential oils components could be handled by the use of adsorption column chromatography.

Enantioselective GC in all its different forms has found a wide variety of application, for instance studies into vitispiranes in grapes (Heron et al., 1993), bark beetle responses (Emura et al., 1997), piperitones (Hobson et al., 1993), borneol (Ravid et al., 1994), cironellol (Ravid et al., 1996) and  $\alpha$ -terpineol in a variety of species, verbenone in rosemary oils (Ravid et al., 1995) and linalool in ocimum species as well as basil oils. Other application include methodology of separating chiral monoterpenes (Ravid et al., 1995), linalool and linalyl acetate in a variety of plant species, (Konig et al., 1992a, 1992b; Cassabianca et al., 1998), extracts of angelica seeds and roots (Holm et al., 1907), the composition of rose oxide ketones (Konig et al., 1992), and other monoterpenoid in geranium oils (Wist et al., 1997; Doimo et al., 1999; Kreis & Mossandl, 1993). The majority of studies have used multidimensional gas chromatography (MDGC) to effect the separations. In MDGC, one column separates the peak and the peak of interest are cut out of the first dimension and passed on to the second chiral column.

#### 4.1.4 High Performance Liquid Chromatography (HPLC)

It is a useful technique for those essential oils, which are thermally unstable, and do not survive the high temperature of GC. It is a fast technique as compared to classical column chromatography and gives quantitative analysis of material. By selecting the proper stationary phase and solvent composition, one may employ normal phase (polar stationary phase and less polar solvent) or reverse phase chromatography for separation of essential oil components. Mostly methanol-water system as mobile phase allowed better evaluation of effluent odour without significant solvent interference. The use of HPLC for the analysis of essential oils is well reported (Mondello et al., 1994; Mondello et al., 1995; Dugo et al., 1994), in literature. HPLC is an excellent analytical method for quantitative analysis of various non volatile constituents of plant and animal product (Upadhyay, 1992), such as carbohydrates, proteins, amino acids, nucleotides, phenolics, Flavonoids, organic acids, lipid, drugs and pharmaceutical preparations. The application of HPLC in the analysis of essential oils, volatile flavours and aromas has so far been handicapped because of its limitation in the resolving power of multicomponent mixtures and the poor response of presently available detectors. The successful separation of terpenic essential oils into oxygenated terpenes, monoterpenes hydrocarbons and sesquiterpene hydrocarbons using octadecylsilane bonded silica column with low UV monitoring detector was shown by Kubeczka (Kubeczka, 1981). Newbery et al. (1983) separated a series of monoterpenoids by reverse phase HPLC. Low temperature HPLC separation of mono and sesquiterpenes hydrocarbons as well as oxygenated terpenoids has been well reported (Yamasaki et al., 1986).

## 4.2 Hyphenated Separation Techniques

Generally HPLC will effect a broad range separation of essential oils. The separated fraction may be introduced into a GC system for further high resolution separation based on boiling point/or polarity, which constitute a hyphenated HPLC-GC techniques. This system was fully automated but off-line sampling of HPLC fraction may also be introduced by the use of large volume injection. Large volume or complete fraction injection allow conservation of mass of sample analysed and will be important for low-abundance constituents. Mondello et al. (1998 & 1996) have summarized their approach to this innovation in industry magazines. An online SFE-GC system was developed by Yarita et al. (1994). The silica gel phase used in SFE elution gave separation based on solute polarity (hydrocarbons, aldehydes, esters and alcohols) and introduced separately into the GC system. If the GC analysis could be significantly improved to give much better resolution, the rotational for hyphenated HPLC-GC or SFE-GC methods may be lost.

## 4.3 Recently Developed Analytical Techniques

Spectral techniques play an important role for identification of essential oil constituents. Starting in the early 1940, first ultraviolet spectroscopy and then IR spectrophotometry were introduced in analytical laboratories. These techniques for the first time provided direct access to molecular information. In 1952, the discovery (James & Martin, 1952) of GC made easy to analyse volatile and non-volatile natural mixtures. This spectacular development was accompanied by the systematic use of mass spectrometry to detect and identify the volatile constituents by the increased performance of data processing computer systems and appropriate softwares. During the 1970, the possibility of direct coupling of a fast scanning quadrupole mass spectrometer with a capillary gas chromatography (GC-MS) has become a determining step towards the rapid identification of volatile constituents in natural mixtures (Joulain, 1994). Important spectroscopic techniques that may be used for identification of essential oil constituents are mostly coupled with chromatographic techniques which are as under.

## 5. RESULTS AND DISCUSSION

Essential oils are very complex natural mixtures which can contain 20 to 60 components. They are characterized by 2 or 3 major components (Table 1) at fairly high concentration, e.g., linalool and geranyl acetate are the major components of *Coriander sativum*. While *Zingiber officinale* contain camphene and  $\beta$ -phellandrene as the major components, these are terpenes besides this it contain aromatic compounds such as p-cymene, and terpenoids such as 1,8-cineole and camphor. The major components of *Curcuma longa* are Ar-turmerone and  $\alpha$ -turmerone. It contains aromatic compounds such as p-cymene, terpenoids 1,8-cineole and caryophyllene

oxide. *Foeniculum vulgare* contains Fenchone as a major component which is a terpenoids along with several terpenes such as  $\alpha$ -pinene, camphene, myrcene, linalool and aromatic compounds such as p-cymene, trans-anethole and cis-anethole. Moreover, *Illicium verum* essential oil contain trans-anethole and methyl-chavicol as major components. While *Anethum graveolens* L. contain carvone and limonene as major component and 1,8-cineole and Aromadendrene as terpenoids where as its bark oil contains (E)-cinnamaldehyde and benzyl-benzoate as major components. Black pepper contains  $\beta$ -caryophyllene as major component and caryophyllene oxide as terpenoides. Moreover *Pimpinella anisum* contain trans-anethole as major components. Cardamom (*A. Sublatum*) contain  $\alpha$ -terpenol as major component and p-cymene and phenanthrene as aromatic compound and 1,8-cineole as terpenoids where as *Ocimum basilicum* contain Linalool as a major component.

Baysal and Starmens (1999) studies supercritical carbon dioxide of carvone and limonene from caraway seed. Their results showed that pressure and temperature have main effects on the extraction efficiency. They showed that at moderated temperatures just above the critical temperature of CO<sub>2</sub> (73.8 bar). Below this value, hardly any limonene is obtained from the seed matrix, regardless, of the temperature applied during the extraction procedure. At elevated temperature, a pressure of up to 125 bar, is required to extract limonene is only small quantities further increasing of the pressure yield resembles those found for limonene.

Pourmortazavi et al., (2003) have repoted that addition of ethanol enhanced the concentration of  $\alpha$ -pinene, camphene,  $\beta$ -pinene, myrcene, delta-3-carvone and decrease the content of 1,8-cineole+limonene, camphor and  $\beta$ -caryophyllene in the plant oil. Moreover Diaz-Maroto et al., (2005) obtained volatile oil extracts of fennel seeds (*Foeniculum vulgare* mill) and thyme leaves (*Thymus vulgaris* L.) by simultaneous distillation extraction (SDE) and SFE. They showed that the fennel oil extracted by SDE and SFE has similar composition with trans-anethole, estragole and fenchone as the main components. Most of the fungi cause disease in animals and plants. Several strains of *Aspergillus niger* are known to cause many important animal diseases such as mycotic abortion of sheep and cattle, pulmonary infection in birds and ostomycosis, mycotic keratitis, allergy and rarely mycetoma commonly known as aspergillosis in humans.

### 5.1 Antifungal Activities of Spice Essential Oils

Many publications have documented the antifungal activity of cinnamon, clove, and oregano oils against different microbial species (Farag et al., 1989; Salmeron & Pizo, 1991; Patker et al., 1993; Sinha et al., 1993) as shown in Table 2. *In vivo* studies showed that oregano oil was highly effective in controlling internal wheat fungi (Patker et al., 1993). Essential oils containing aliphatic

alcohols and phenols exhibited significant action against *A.aegyptiacus*, *Penicillium cyclopium* and *Trichoderma viride* (Megalla et al., 1980). The inhibitory effects of clove and cinnamon oils on the growth and aflatoxin production by *Aspergillus flavus* have also been reported (Bullerman et al., 1977; Sinha et al., 1993; Montes-Belmont & Carvajal, 1998). A perusal of literature reveals that there are many reports (Bhargawa et al., 1981; Rao & Joseph, 1971) on the fungitoxic nature of the *O. canum* oil. The toxicity of the thermostable fungitoxic factor of this oil remained (Dubey et al., 1983) active during storage up to 390 days. Moreover, it successfully prevented fungal deterioration of *Foeniculum vulgare* and *Corianderum sativum* during storage without showing any phytotoxic effect on the treated commodities. Recently, Velluti et al. (2003) reported that cinnamon oil showed inhibition on growth and FB<sub>1</sub> production by three different isolates of *F. proliferatum* in irradiated maize grain. It was also found to be effective against aflatoxin formation in maize grain by *A. Flavus* after 10 days under favourable conditions for mycotoxin production (Sinha et al., 1993). The ability of oregano oil to inhibit the growth of *A.flavus*, *A. Ochraceus* and *A. niger* has been evaluated previously (Paster et al., 1990; Paster et al., 1995). Mimica-Dukie et al. (2003) reported that the method of distillation did slightly affect the antifungal activity of the volatile oils against fungi. According to Edris and Farrag 2003, individual constituents at different ratios inhibited the growth of fungi in a dose dependent manner. Menthol was found to be the individual aroma constituents responsible for the antifungal properties of peppermint essential oil, while menthone alone did not showed no activity at all. Mixing the two components in a ratio similar to their concentrations in the original oil was found to enhance the antifungal properties of basil oil indicating a synergistic effect. Moreover, Singh and co-workers (Singh et al., 2004; Singh et al., 2005) reported *piper nigrum* and *Myristica fragrans* Houtt. (aril part ) volatile oil as 100% effective for *Fusarium graminearum*. In addition, ajwain oil has also been reported (Sridhar et al., 2003) to possess a broad spectrum of fungitoxic behaviour against the pathogenic fungi isolated from food materials such as *A. niger*, *A.flavus*, *A. oryzae*, *A. Ochraceus*, *F. Monoliforme*, *F.graminearum*, *Penicillium citrium*, *P. Madriti* and *Curvularia lunata*.

## 5.2 Antibacterial Activities of Spice Essential Oils

A number of reports have been published, on the action of *Nigella sativa* oil on different bacterial isolates. The oil has been reported to have a broad spectrum of activity against a number of microbes (Table 3). For example, in vivo antibacterial effects of the essential oil showed pronounced activity even in 1:100 dilutions against several organism that included *Staphylococcus albus*, *Escherchia coli*, *Salmonella typhi*, *Shigella niger* and *Vibrio cholera* (El-kamali et al., 1998; Aggrawal et al., 1979). Generally

speaking, the oil was more effective against gram-positive than gram-negative organisms. The effect was maximal when *Bacillus subtilis* was used. Valeoro and Salmeron (Valero & Salmeron, 2003) have given a description on the effects of adding essential oils of nutmeg, mint, clove, cinnamon, sage and thyme to carrot broth (used as a growth substrate) on the growth of food borne pathogen *Bacillus cereus*. The essential oil from the rhizomes of turmeric (*Curcuma longa* Syn *domestica* Val.) exhibited (Garg & Jain, 2003) good to moderate activity against *B subtilis*, *S. aureus* and *C. diphtheria*. Moreover, Smith-Palmer et al. (1998) reported that these oils of bay clove, cinnamon and thyme can act as potent inhibitors of *Listeria monocytogenes* and *Salmonella enteritidis*. The results of bacteriocidal and bacteristatic concentrations showed that the two gram-positive bacteria, *S. aureus* and *E. coli*. were more sensitive than gram negative bacteria. This is in agreement with the results of Prasad et al. (1986). Thus, in general, lower bacterostatic and bactericidal concentrations are required for basil essential oil against *S. aureus* and *L. monocytogenes*. It is not completely clear why gram-negative bacteria should be less susceptible, but it may be associated with the outer memberne of gram-negative bacteria that endows the bacterial surface with strong hydrophilicity and act as a strong permeability barrier (Nikaido & Vaara, 1985). De et al. (1999) reported that clove, cinnamon, bishop's weed, chili, horse raddish, cumin, tamarind, black cumin, pomegranate seeds, nutmeg, garlic, onion, tejpat, cellary, cmbodge have potent antimicrobial activity against *Bacillus subtilis*, *Escherchia coli* and *Saccharomyces cerevisiae*, Further, Lis-Balchin and Deans (1998) studied six different combinations of these different essential oil mixtures for their possible synergistic activity against 13 food borne bacterias in vitro. Mixing of fractions resulted in additive, synergistic or antagonistic effects (Davidson & Parish, 1989). Didry et al. (1993) observed synergistic activity between carvacrol and thymol. Lachowicz et al. (1998) used an impedance method to determine the time required for *Lactobacillus curvatus* and *Saccharomyces cerevisiae* to initiate growth in the presence of linalool and methyl chavicol increased the time required to initiate growth, but there was no evidence of synergy between the compounds. Furthermore, the crude essential oil was significantly more effective than any of the mixtures, suggesting that other components of basil oil contribute to antimicrobial activity. Most of the antimicrobial activity in essential oils derived from spices and culinary herbs appears to derive from phenolic compounds, while other constituents are believed to contribute little to the antimicrobial effects (Shelef, 1983; Martini et al., 1996; Nychas, 1998) Purified compounds derived from essential oils, such as carvacrol, eugenol, linalool, cinnamic aldehyde and thymol inhibit a wide variety of microorganisms (Hulin et al., 1998). Individual essential oils may contain complex mixtures of such compounds however, and little is known about the

effect of interactions between individual constituents on antimicrobial activity.

Banerjee et al. (1978) studied the antimicrobial activity of the essential oils of *Curcuma* spp. Hili and co-workers (Hili et al., 1997; Smith-Palmer et al., 1998) have assayed the antimicrobial activity of the *Cinnamom zeylanicum* leaf oil against three bacteria *E. coli*, *S. aureus* and *P. aeruginosa*. Among the constituents of *Cinnamom zeylanicum*, cinnamaldehyde possessed (Chang et al., 2001), the best antibacterial activity followed by cinnamyl alcohol. Among all the oils, the essential oil of cinnamon was the most effective, followed by the essential oil of oregano and thyme. The citronella oil was found to be more effective than rose geranium oil (Aggarwal et al., 2000). Citronella and geranium oils as such were not effective against gram negative bacteria but their components viz. citronellol and d-citronellol of citronella geranium oil showed antibacterial activity against gram-negative bacteria, but much less than gram positive bacteria. Singh et al. (1998, 2000, & 2001) reported that the volatile oils of some spices such as *Mentha piperita*, *M. spicata*, *Carrum capticum*, *Cuminum cymium*, *Corianderum satium*, and *Apium graveolens* act as potent antimicrobial agents against human pathogenic bacteria.

### 5.3 Insecticidal Activities of Spice Essential Oils

Some of the essential oils tested against various insects, have shown promising results as reported in Table 4. The egg hatching of *Aedes aegyti* by constituents of essential oils were also observed and carvacrol, citral, citronellal, eugenol, farnesol, gernaol were found to be active principles. The oils of *Cymbopogon nardus*, *Cyathodia lyrata* and *Tridex procumbens* have also shown insecticidal activity. Many workers (Saraswati & Dixit, 1998; Singhmony et al., 1990; Dubey et al., 1991; Shukla et al., 1992; Dixit et al., 1992; Manuel et al., 1988; Tom & Lichtenstein, 1979) have reported the insecticidal activity of oils of *piper nigrum*, *Apium graveolens*, *Cuminum cyminum*. Though no substantial work seems to have been done on the structure activity relationship but a few available reports reveal that alkyl side chain and free phenolic, hydroxyl and methylene dioxy groups (as in saffrole) are significant for showing ovicidal activity. The sweet smelling terpenoids attract the insects on one hand and are ovicidal on the other. Maini and Rajesis 1998 reported that the oils of star anise and oregano showed 100% mortality at 10 and 20 ppm against golden snails. The eggs and nymphs of White flies were also found to be effected (Pant et al., 1993) by neem and citronella oils. The essential oil of *Santolina chamaecypriissus* reduced the survival of *Nibparvatha lugens* after 72 hours and it was found to be a good pesticide. The insecticidal constituents of the *Illicium verum* fruits were identified (Chang & Ahn, 2001), as phenylpropene (*E*)-anethole. The insecticidal activity of the constituent was much more pronounced in males than females. Similar results

have been reported for the toxicity of b- asarone of adults of *S. oryzae* and *L. serricornis*. However, the adulticidal activity of the phenylpropenes (*E*)-anethole, estragole and the monerpene (+) fenchone against *S. oryzae* and *L. serricornis* is dependent upon both dose and exposure time. But according to Chang and Ahn (2001) the adulticidal activity of (*E*)-anethole against *B. germanica* varied according to the dose rather than the exposure time (Kim & Ahn, 2001). Chantraine et al. (1998) evaluated insecticidal activity of 52 Bolivian plant species has been evaluated on *Aedes aegypti* larvae. The monoterpenes E-anethol and E-nerolidol were found to be the active principles of the most toxic essential oils. Singh et al. (2000, 2002, & 2003) worked on *Odontotermes obesus*, Rhamb have reported that the volatile oils of *Curcuma zeadoaria*, *C. Aromatica*. Kanat et al. (2004) reported that essential oils of wood turpentine, thyme herb oil, cypress berry oil, styrax were found to be most effective in terms of mean mortality time against larvae of pine processionary moth (*Thaumetopoea pitycampia* Schiff).

Several factors may contribute to disparate observations on the antimicrobial potential of essential oils. Variability in qualitative and quantitative estimates of activity has been ascribed to differences in analytical techniques (Zaika, 1988). The volatility and poor solubility of most essential oils are problematic, particularly with methods that rely on diffusion or dilution of the test substance in a microbiological medium. Generally the extent of inhibition of the oils could be attributed to the presence of an aromatic nucleus containing polar functional groups. In addition, differences in composition related to variety, agronomic practice and processing are also likely to influence antimicrobial properties, since these factors contribute to both the profile and relative concentrations of active ingredients.

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