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2.1 Introduction

The mere mention of a natural antioxidant brings about an association with spices and herbs, in that product developer utilize spice and herb extracts as replacements for synthetic antioxidants. This chapter will provide information regarding the geographical distribution and description of rosemary, ginger, pepper, and clove; the extraction methods of the active components and structural components of the active antioxidants.

2.2 Rosemary

Botanical name: *Rosemarinus officinalis*

2.2.1 Description and distribution:

It is a dense evergreen highly branched shrub growing up to one meter, with almost cylindrical leaves, two to four cm long by one to three mm thick, having inrolled margin, which are dark green in the upper side and silver stripped underneath. The plant blooms throughout the year and abundantly in spring. The flowers nestle in clusters at the terminal of the branches (Plate 2.1).

Rosemary grows wild and is also cultivated in Yugoslavia, Spain, Portugal, France and Europe as well as in California in U.S.A. It is a native of Southern Europe and grows wild on dry rocky hills in the Mediterranean region. It has been suggested as suitable for cultivation in temperate Himalayas and Nilgiri Hills with dry to moderately moist climates. Its lovely name 'Rosemary' joins two latin words meaning 'dew of the sea', because it thrives best where fog rolls in from the sea, as in the case along with its native Mediterranean region. The colour of the dried herb is brown green. The crushed rosemary however has an agreeable and fragrant, spicy aroma with a camphoraceous note. The taste has fragrant, spicy, pungent, bitter and camphoraceous notes.

2.2.2 Antioxidant constituents of Rosemary

From time to time studies have been undertaken to evaluate the antioxygenic properties of spices. However it was Chipault et al., (1956) who by a systematic investigation compiled the “Antioxidant index” of spices which led to the finding that rosemary, its petroleum extracts, tops the list in retarding lipid oxidation. The art of steam distilling the essential oil from the over ground parts of rosemary is known for centuries. All parts of the plant except the woody stem are exploited for oil of rosemary and is distilled in most countries from freshly harvested plants.
Plate 2.1 Rosemary (*Rosmarinus officinalis*)
(a) Rosemary flower (b) Rosemary leaves
Extensive research has been done to unmask the source of antioxidant activity of rosemary. A number of compounds from the herb have been identified as having this property and they include:

1) Camosic acid
2) Rosemanol
3) Epi Rosmanol
4) Iso Rosmanol
5) Rosmadiol
6) Rosmariquinone
7) Rosemaridiphenol
8) Rosemarinic acid
9) Camosol

The chemical structures of these compounds are given in Figure 2.1
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Carnosol

Rosmanol

Epirosmanol

Isorosmanol
Figure 2.1
Of these carnosol is an artifact derived from carnosic acid. From an oxidation inhibition point of view, some of these compounds equal or surpass Butylated Hydroxy Anisole (BHA), Butylated Hydroxy Toluene (BHT) and tocopherol. Of the spices, rosemary is outstanding as a rich source of tremendously valuable and versatile antioxidant. The antioxidant principles of rosemary have stolen the limelight and thrown a challenge to synthetic antioxidants, which are vigorously contested by numerous food laws.

The use of extract from rosemary spice as a natural antioxidant was first reported by Raﻞo€LQsmc-Matijasevic (1955). In 1973, a patent was issued to Bemer et al (1973), for the extraction of rosemary with oil. Later, chang et al. (1977) reported a patented process for the extraction of rosemary and sage, followed by a vacuum steam distillation of the extract in an edible oil or fat to obtain an odourless and flavourless natural antioxidant. Its antioxidant activity was demonstrated in both animal fats and vegetable oils. Further more, it was able to improve the flavour stability of soyabean oil, as well as potato chips.

Procedure for preparing an odorless and flavorless antioxidant as described by Chang et al. (1977) is epitomized in the flow chart (Figure 2.2).
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Rosemary (100gm)
(ground to a fine powder)

1) Extracted with 240ml of ethylether under reflux - 2 hrs
2) Filtered

Residue

3) Extracted with ethylether
4) Desolventised

crude antioxidant
(26 gm)

1) washed with 100ml cold H₂O (several times)
2) 100ml of 80°C H₂O (several times)
3) Dissolved in methanol 10% solution for 15 min
4) Bleached with active carbon
   (20% by weight of crude antioxidant) by stirring at 60°C for 15 min

Bleached solution

Desolventised

10gm of purified A.O.

Figure 2.8 Flow chart for the preparation of rosemary antioxidant (Chang et al 1977)
100gm of rosemary that had been ground to a fine powder were extracted with 240 ml of diethyl ether under refluxing conditions for 2 hrs. The mixture was filtered and the residue could be extracted again with fresh solvent. The combined filtrate was freed of solvent to yield up to 26 g of crude antioxidant depending on the number of extractions.

The crude antioxidant was washed with 100ml of cold water several times, and then with 100ml of 80°C water several times. It was then dissolved in methanol (10% solution) and bleached with active carbon by stirring at 60°C for 15min. up to 20% wt of the crude antioxidant may be used. The bleached solution was freed of solvent to yield approximately 10g of purified antioxidant.

Bracco et al., (1981) also reported the use of double step, falling film molecular distillation to obtain an active antioxidant from rosemary extract.

This involves the microionisation of the herb in edible oil. e.g., ground nut, peanut oil. The antioxidants are transferred to the liquid phase. This is followed by a cleaning operation, either by filtration or centrifuging and molecular distillation on falling film or centrifugal system to collect the low molecular weight, active components, which deodorises and partially bleaches them. The antioxidants thus retrieved are subjected to column chromatography using solvents of increasing polarity. The fractions obtained were further investigated by mass spectrometry and UV absorption. The various stages are epitomized in the flowchart (Figure 2.3).
Figure 2.3: Flow chart for the recovery of Active antioxidant of Rosemary (Bracco et al., 1981).
Wu et al., (1982) has also reported the fractionation and identification of carnosol one of the active antioxidant components in the extract of rosemary. The scheme of preparation is shown in flow chart (Figure 2.4).

Figure 2.4: Flow chart for the preparation of Rosemary antioxidant.
Three kg of rosemary leaves that had been ground to a fine powder was extracted with 18L of methanol at 60°C for 2 hr. The mixture was filtered and the residue was extracted with 12L of fresh methanol. The combined filtrate was bleached with 600g of active carbon and then filtered to yield a light brown filtrate. The methanol solution was then concentrated to about 2L by rotary evaporation and then filtered to remove the precipitates (A). The filtrate was freed of solvent to yield 3.5% of rosemary antioxidant (B).

### 2.2.3 Fractionation of Rosemary Antioxidant (RA)

RA (10g) was first separated into 7 fractions using glass column (id, 1.75 inches length, 23 inches) packed in the activated silicic acid. The column was eluted by stepwise gradient elution, using 5% ether in hexane, 10% ether in hexane, 25% ether in hexane, 50% ether in hexane, 75% ether in hexane, pure ether, and pure methanol.

Each fraction was then rechromatographed on the same silicic acid column to yield a total of 16 fractions (Figure 2.5).

![Figure 2.5 Chromatographic fractionation of rosemary antioxidant.](image-url)
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In order to elucidate the chemical structure of the components responsible for the antioxidant properties of RM, it was fractionated by repeated column chromatography with silicic acid using stepwise gradient elution. 7 primary fraction and 16 sub fractions was obtained. After further crystallization and spectral analysis (IR and NMR) the structure of carnosol was confirmed.

2.3 Ginger

Botanical name: Zingiber officinale

2.3.1 Description and distribution

It is a tropical perennial herb of the Zingiberacea family. Elongated, multibranched, irregular fleshy and pungent, its rhizome is prized for its healing properties. Though originated in tropical Asia it is widely grown in India, Jamaica, China, Hawaii, Australia, and Nigeria. (Plate 2.2).

2.3.2 Active constituents of Ginger

Ginger contains 1.5% - 3% essential oil, fixed oil 2-12%, starch 40-70%, protein 6-20%, fibre 3-8%, ash up to 8%, water 9-12%, pungent principles and other saccharides, cellulose colouring matter and trace minerals (Ridley, 1912; Govindrajan, 1982; Purseglove et al., 1981; Weiss, 1997; Langner et al., 1998).

The essential oil is composed mainly of sesquiterpene hydrocarbons. This group compounds to about 50%-66% of the volatile oil. Oxygenated sesquiterpene are present up to 17%, and the remainder is composed of monoterpen hydrocarbons and oxygenated monoterpenes. Of the sesquiterpenes hydrocarbons, about 20% - 30% is (-)α-Zingiberene, up to 12% (-)α bisabolene, up to 19% (+)-ar-curcumene and up to 10% is farnesene (Weiss, 1997). A sensory study showed that α sesquiiphellandrens and ar-curcumene were the major contributors to the "ginger flavour", whereas α - terpieol and citral contributed a limony flavour. The high lemon flavour and high citral content is apparent is Australian ginger. This product contains up to 19.3% citral versus up to 4% in other sources.

There have been numerous studies concerning the pungent components of ginger and what contribute to that pungency (Purseglove et al., 1981). Fresh ginger contains gingerol, which can be described as a series of compounds with the general structure \( \text{C}_\alpha \text{H}_\beta \text{O}_\gamma (1-4 \text{ hydroxy} - 3 - \text{ methoxy} \)
Plate 2.2  Ginger (Zingiber officinale)

(a) Ginger plant  
(b) Ginger Rhizome
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phenyl)-5 hydroxyl alkan-3-one. They are mainly condensation products of Zingerone with saturated straight chain aldehydes of chain length, 6, 8, and 10. These are described as (6) (8) and (10) gingerols. The structure of the pungent principles derived from ginger rhizome are as shown in Figure 2.6

![Diagram of chemical structures]

Figure 2.6: Structure of the pungent principles of ginger.
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Gingerols I  Shogoals II

\[ \begin{align*}
    n = 1, (3) & \quad - \quad \text{gingerol} & \quad n = 2, (4) & \quad - \quad \text{Shogaol} \\
    n = 2, (4) & \quad - \quad \text{gingerol} & \quad n = 4, (6) & \quad - \quad \text{Shogaol} \\
    n = 3, (5) & \quad - \quad \text{gingerol} & \quad n = 6, (8) & \quad - \quad \text{Shogaol} \\
    n = 4, (6) & \quad - \quad \text{gingerol} & \quad n = 8, (10) & \quad - \quad \text{Shogaol} \\
    n = 6, (8) & \quad - \quad \text{gingerol} \nonumber \\
    n = 8, (10) & \quad - \quad \text{gingerol} \nonumber \\
    n = 10, (12) & \quad - \quad \text{gingerol} \nonumber
\end{align*} \]

Ginger has been reported to exert antioxidant activity (Hirahira et al., 1974; Hirosue et al., 1978; Lee et al., 1982; Jitoe et al., 1992) but there have been few published reports on its active components. Fujio et al. (1969) reported that the antioxidant activity of the pungent principle, zingerone and shogoal, in dehydrated pork. Lee and Ahn (1985) examined the effectiveness of gingerol in a α-carotene - linoleic acid - water emulsion system.

2.3.3 Extraction and fractionation

Dried steamed rhizomes of ginger (995 g) were ground and extracted five times with CH\(_2\)Cl\(_2\) (Dichloro methane) (2 L each) and subsequently thrice with 2 litres of methyl alcohol at room temperature (Kikuzaki and Nakatani, 1993). The combined CH\(_2\)Cl\(_2\) extract concentrated to yield a brown viscous residue. This extract was separated by steam distillation to obtain a volatile and non volatile oil. The latter was subjected to column chromatography on silica gel to give 11 eluted fractions using the benzene - acetone solvent system. The combined, methanol extract was freed from the solvent. The steps in extraction and fractionation are shown in Fig. 2.7
Dried rhizome of ginger

$\text{CH}_2\text{Cl}_2$ extract

Steam distillation

Volatile fraction

non volatile fraction

$\text{CH}_2\text{Cl}_2$ extract

Residue

Extrated with MeOH

MeOH extract

Figure 2.7: Flowchart for the extraction and fractionation of Ginger rhizome.

The fractions with positive activities (fraction 1-10) were purified by column chromatography on silica gel and sephadex LH - 20 and by preparative HPLC. Gingerol (1) was isolated from fraction 6 and 7 and - shogal (2) mainly from fractions 2 and 3 Gingerdiol analogues (compounds 3 and 5) were obtained from fraction 8 and 4 respectively, and dehydrogingerone (6) was isolated from fraction 1. Seven diaryl heptanoids (7-13) were obtained from the more polar fraction, 7 to 10; and curcumin (14) the first known isolated compound from *Zingiber officinale* was obtained from fraction 8.

The compound tested and isolated for antioxidant activity was structurally classified in to five types, according to the substitution pattern of the side chains as follows (Figure 2.8).
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(1)

(2)

3; 3R 5S  
4; 3S 5R

(5)

(6)
Figure 2.8: Structure of compounds isolated from ginger.
Gingerol related compounds (1-6) delayed oxidation of linoleic acid, and based on low absorbance values their activities were higher than that of α-tocopherol. Efficiency tended to increase in the order 6<4< (6) - gingerol (1) < (6) shogaol (2) < (6) - gingerdiol (3) < 5. The antioxidant activity of the diaryl heptanoids with two 4-hydroxy-3 methoxy phenyl groups (7-11 and 14) at the same concentration was compared. They appeared to exert greater antioxidant potential than tocopherol, and showed a tendency to be more active than gingerol related compounds (1-6) in each of the corresponding five stages. The antioxidant effect increased in the order 14<10<7<8<11, which suggested that the substitution pattern of side chain was important in antioxidant activity. The difference in activity among these compounds increased as concentration decreased. We could summarize the activity increases in the order 6<1d"4<2d"3<5, that is for the side chains, 1-ene, 3, 5 Dione<5 dihydroxy -3one d"3S, 5S-diol<4-en-3 one <3R, 5S-diol< 3R, 5S-diacetate, together with results for the diaryl heptanoids.

Comparisons of the activity of 11 with that of 12 or 13 indicated that a compound with a 4-hydroxy -3 methoxy phenyl group appeared more active than those with a 4- hydroxy -3, 5-dimethoxyphenyl group; or 3,4 dihydroxy phenyl group. This suggests that the efficiency was also dependent upon substituents on the benzene ring.

2.3.4 Extraction of Ginger Oleoresin

Ginger oleoresin is a dark brown viscous liquid that has a warm, spicy, sweet, and very rich odour and sharp pungent flavour. On dilution, the oleoresin, affords a characteristic ginger, fresh, sweet, aromatic, rooty spicy and warm note and with a strong pungent sensation (Govindarajan, 1982).

The oleoresin is obtained from dry ginger rhizome by solvent extraction. For the successful recovery of oleoresin with acceptable physico chemical organoleptic properties, the integrity of the raw material, its technique of drying and physical modification prior to extraction are important.

With commercial grade ginger as substrate, yields of oleoresin ranged from 3.5-11% with 15-30% volatile oil have been realized using solvents which include methanol ethanol, isopropanol, acetone, ethyl acetate, methylene chloride, ethylene dichloride, mixed solvents, acetone-H₂O combination and supercritical CO₂.
Kinetics of the extraction with different solvents has been examined. Fast reaction rates are realised by solvents of low viscosity. Ethanol retrieves from the rhizome upto 20 percent of oleoresin, relatively low in volatile oil and pungent principles admixed with other extractives. The ethanolic extract is called as gingerine (Ridley, 1912).

2.3.5 Pharmacological cum healing profile.

Kirtikar et al., (1984) have capsuled the herbal remedies of ginger. The rhizome is an appetiser, useful in diseases of heart and throat, indigestion, asthma, bronchitis, dyspepsia and inflammations. Ginger provides relief in piles, rheumatism, headache, and lumbago pain. For the eyes, the rhizome gives luster, remedies the opacity of the cornea. The beneficial pharmacological applications are due to the cumulative contribution of the individual properties of action of the constituents present in ginger. In this respect, the pungent principles play a notable role (Yamahara et al., 1992) in which (6) gingerol and (6) - paradol are most potent.

Ginger is a warming herb with powerful ability to stimulate heart muscle, which accelerates blood supply. Consequently, cellular metabolic activity is improved and this contributes to the relief of cramps and tension. Ginger acts directly on the digestive tract and releases constipation cramps and flatulence (Yamahara et al., 1990; Kikuzaki, 1993). Ginger can reduce cholesterol concentration in a cholesterol rich diet (Tanabe et al., 1993). Lowering of cholesterol may be attributed to the antioxidant potential of some of the constituents in the spice (Jitoe et al., 1992). Ginger is endowed with antioxidant activity that enables it to preserve lipids and reduce lipid peroxidation in biological system. For this reason, ginger in addition to imparting flavor is competent to offer health benefits by inhibiting lipid per oxidation.

Antioxidants are increasingly linked to the prevention of certain cancers and coronary heart disease, as well as their more established role in preserving lipid based food. Studies include role of components such as gingerol inhibiting linoleic acid oxidation (Kikuzaki and Natakani, 1993); extending the shelf life of meat (Ziauadin et al., 1995), dehydrated pork (Fujio et al., 1969) and fermented meat sausage (Al-Jaley et al., 1987).

Ginger has antimicrobial activity due to the presence of gingerols, e.g. in relation to Bacillus subtilis and Escherichia coli (Yamada et al., 1992) and Mycobacterium (Galal, 1996).
Ginger has a known influence on the eicosanoid cascade which influences such functions as wound healing, inflammation and platelet aggregation and is involved in conditions such as arteriosclerosis (Srivasta, 1986; Sakawa, 1987; Kiuchi et al., 1992).

Ginger has beneficial effect on the digestive system enhancing gastro intestinal motility and is used traditionally for the treatment of stomach ache, vomiting and indigestion (Yamahara et al., 1990). It has also been investigated for its gastro protectant and anti ulcer activity (Yamahara et al., 1988; Yamada et al., 1992; Yoshikawa et al., 1994).

2.4 Pepper - White and Black

Botanical name: *Piper nigrum*

2.4.1 Description & Distribution

White and Black pepper are both from the same plant- *Piper nigrum*, which is indigenous to the Malabar coast of Southern India.

Black Pepper is the unripe, dried fruit, while white pepper is the mature berry in which the hull is removed. Green pepper corns are immature berries. These are usually freeze dried or mechanically air dried. Pepper is grown on vines, which are usually supported by trees in the wild or wooden stakes if they are cultivated.

Malabar black pepper is generally an aromatic pepper with a high piperine level. Sarwak pepper is from Malaysia, along the north western coast of Borneo. Brazilian pepper is lower quality than Indonesian and Indian varieties.

Black pepper is harvested by hand, when the berry is green and dried in the sun on the spikes. They are heaped in piles to promote a browning reaction caused by fermentation, turning the berries dark, and then raked to allow uniform drying (Plate 2.3).

White pepper is harvested differently. The bright red berries are picked and removed from the spikes and packed in to bags and soaked in slow running water. This loosens the pericarps or hull from the core of the berry. After 2 weeks of soaking, the berries are crampled to remove the rest of the hull and the cores are washed and sun dried.
Plate 2.3 Pepper (Piper nigrum)

(a) Pepper vine
(b) Ripe berry
(c) Dried pepper corns
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2.4.2 Chemical and physical specification.

There are two main components of black and white pepper, the volatile oil and the pungent components commonly known as piperine. The volatile oil level in black pepper is usually higher than in white pepper. The hull of the pepper contains fibre and some essential oil. This essential oil is removed during processing into white pepper. Black pepper contains about 0.6%-2.6% volatile oil, depending on the source, but usually contains 2%-5% in good quality pepper berries. White pepper contains 1.0%-3% volatile oil. The maturity of the berry can influence volatile oil content.

The volatile oil content increases up to the level in a green pepper corn, and then decreases with maturity. The essential oil contains a large number of compounds. The main compounds present are α-pinene, β-pinene 1-α-phellandrene, δ-caryophyllene, limonene, sabines—delta-3-carene.

The main pungency component of pepper is piperine. There has been some debate over the years as to whether piperine was the component, which caused pungency, or not.

Piperine is the trans, transforms of 1-piperoyl piperidine. Other minor pungent alkaloids present are piperidine, piperyline, piperolein A and B and piperamine. Piperine content increases with the maturity of the berry.

Piperine can occur in four isomers when synthesized. The structure of piperine is shown in Fig. 2.9.

![Structure of Piperine](image)

Figure 2.9: Structure of Piperine.
The isomers have different configuration at the double bonds as follows.

**Piperene** - Trans – trans

**Iso piperine** - Cis-trans

**Iso chavicine** - Trans-cis

**Chavicine** - Cis-cis.

None of the isomers have the high pungency level of piperine. It has been suggested that there is a slow photo isomerisation of piperine to its isomers during storage, thus decreasing its pungency.

Black pepper oleoresin and essential oil are both available. The oleoresin varies from source and is available in a variety of strengths with regard to volatile oil and piperine content.

### 2.4.3 Isolation of oleoresin of black pepper

The manufacturing of black pepper oleoresin through homogenization of pepper oil obtained by steam distillation and the solvent extraction of the distillation residue is a versatile procedure.

One of the pre-requisites for successful oleoresin production is the availability of pepper oil. By steam distilling coarse, ground or flaked pepper berries, the oil is collected. The yield composition and aroma profile of the oil vary depending on the spice. Berries of 4 ½ to 5 months maturity are rich in piperine and essential oil.

The distilled spice oil has about twice the amount of low boiling terpenes and only two thirds the amount of sesquiterpenes as compared to their amounts in the extracted oil. For an acceptable flavour, partial elimination of monoterpenes is necessary. During distillation, the oil may be divided into three parts (a) initial fraction mainly of C_{10}H_{16} hydrocarbons, (b) intermediate with minimum amount of sesquiterpenes, (c) heavy oil rich in sesquiterpenes.

Certain proportion of the three fractions is pooled in a manner to give upgraded oil with acceptable aroma profile. When steam distillation of the spice is not pushed deliberately to the end, a good portion of the heavy oil is retained in the residue from which it is recovered by solvent extraction. A finished oleoresin can be built up from the three distillation fractions, individually or collectively together with the extracted oil.
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Piperine is in the ‘solubles’ extracted from the residue after distillation. Piperine constitutes the overwhelming proportion of the alkaloid mixture that gives the pungent flavour to black pepper. It is sensitive to heat, so that steam distillation provokes its decomposition and additional degradation occurs during desolventisation.

Steam distillation cum-extraction is a volatile route for the manufacture of standard resins. This is accomplished by mixing the essential oil and solvent extractive in the required proportion. Piperine is not very soluble in oleoresin of whole pepper. By centrifugation, substantially all of the undissolved piperine is retrievable as a dry solid residue, leaving a dark oily fluid, referred to as supernatant fraction containing some piperine. The liquid oil fraction consists essentially of liquid volatile oil, liquid non volatile oil and also dissolved piperines.

2.4.4 Antimicrobial activity

Pepper is known to be antibacterial. Two new phenolic compounds reported to be present in green pepper but absent in black, were tested for their antibacterial activity against the foodborne pathogens, Salmonella typhimurium, Staphylococcus aureus, Bacillus cereus and Escherichia coli. The compounds 3,4-dihydroxyphenyl ethanol glucoside (A) and 3,4-dihydroxy-6-(N-ethylamino) benzamide (B) were found to inhibit the growth of all of the four bacteria. (Pradhan et al., 1999). Dorman and Deans (2000) assessed the antimicrobial activity of volatile oils of black pepper (Piper nigrum) against 25 different genera of bacteria. These included animal and plant pathogens, food poisoning and spoilage bacteria. The volatile oils exhibited considerable inhibitory effects against all the organisms, while their major components demonstrated various degrees of growth inhibition. Ejechi and Akpomedaye (2005) studied the activity of essential oil and phenolic acid extracts of pepper fruit against some food-borne microorganisms Staphylococcus aureus, Salmonella sp., Pseudomonas aeruginosa, Proteus sp., Escherichia coli., Enterococcus faecalis., Serratia sp., Bacillus sp., Clostridium sp., Penicillium sp., Aspergillus flavus, which were susceptible to the extracts with a minimum inhibitory concentration (MIC) range of 1.0-4.0 mg/ml. The essential oil inhibited the foodborne organisms better than the phenolic acid

Yamazaki et al. (2007) studied and characterized the antioxidant activity of extracts from Japanese pepper fruit. The antioxidant activity of the methanol extract from Japanese pepper fruit was found to be equal to that of α-tocopherol and stable under heat treatment. The main compounds
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that gave a significant antioxidant activity from the methanol extract were identified to be hyperoside (quercetin-3-O-galactoside) and quercitrin (quercetin-3-O-rhamnoside) as determined by HPLC, mass spectrometry, UV/VIS spectroscopy, and TLC. Evaluation of radical-scavenging activities of hyperoxide and quercitrin from Japanese pepper fruit using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method revealed that hyperoxide and quercitrin scavenged DPPH radical strongly. Hence they concluded that Japanese pepper fruit showed the presence of strong antioxidants, namely hyperoside and quercitrin.

Oboh et al (2007) studied invitro the ability of aqueous extracts of ripe (red) and unripe (green) hot peppers to prevent 25 µM Fe²⁺ induced lipid peroxidation in rat’s brain; assessed using TBARS (Thiobarbituric acid reactive species). They found that the inhibitory effect of pepper on lipid peroxidation of both basal and Fe²⁺ induced lipid peroxidation and Fe²⁺ chelating effect of the extracts were dose dependent.

2.5 Clove

Botanical Name: *Eugenia caryophyllus*

2.5.1 Description, Distribution and Economic importance

Clove is one of the most ancient and valuable spices of the Orient, known as far as 100 B.C. This spice was later known to the Chinese. Clove was imported into Europe in 1265.

In India, clove was introduced in 1800 AD by the East India Company. By far the biggest clove producing region in the world today in Zanzibar, followed by Pemba, Madagascar and Indonesia clove is also produced in Malaysia, Srilanka and Haiti but not in commercially significant quantities.

A tropical plant, the life zone of clove falls between 20° North to 20° south of the equator, with even distribution of rainfall from 150-200 cm and tolerates acid soils to pH 4.5.

The clove leaf is nearly elliptical in shape 7-13 cm long and from 3.6 cm wide, smooth with dark green upper surface. The leaves are very aromatic, long full of minute oil glands, just visible with an ordinary lens as green dots on lower surface (Plate 2.4).
Plate 2.4 Clove (*Eugenia Caryophyllus*)
(a) Branch of a clove tree  (Dried clove bud)
2.5.2 Processed products

2.5.2.1 Clove Bud oil

This is derived from the dried buds by steam distillation, (yield 16%) contains as its main constituents, free Eugenol (70-90%), Eugenol acetate and Caryophyllene. Although these substances amount to some 99% of the oil, they are not responsible for the characteristic fresh and almost fruity note of the pure clove oil.

2.5.2.2 Clove stem oil

The chemical composition of the oil derived from clove stem (yield 4-5%) has not been investigated as thoroughly as that of commercially much more important clove bud oil, which is used widely in food products and in pharmaceuticals. The percentage of free eugenol present in clove – bud oil occurs also in the stem oil, but in somewhat different proportions (Fig.2.10)

2.5.2.3 Clove leaf oil

Clove leaf oil (yield 1-2%) usually contains a somewhat lower percentage of total Eugenol to that present in clove bud oil. The trace substance methyl –n- amyl ketone for example, which imparts characteristic, almost fruity odour to the bud oil, occurs in leaf oil, probably in even more minute quantities than in the stem oil. Quality or chemical composition of clove bud stem and leaf oil with particular reference to their aroma quality, has been studied and the found antioxidant activity in clove and thyme. Kramer (1985), through the use of thin layer chromatography, ultraviolet (UV), Infrared (IR), mass spectrometry (MS) and HPLC, determined quantitatively that gallic acid and Eugenol were 1.26g and 3.03g respectively in 100gm of clove.
2.5.3 Extraction procedure

150gm of ground clove was packed in to a glass chromatography column (500mm×35mm). Two litres of petroleum ether were percolated through the column from rotary evaporator reservoir above, to remove fat and much of the colour pigment. This was followed by 2litres of 80% ethanol to extract the phenolic compounds and sugars. The ethanol extract was concentrated on a rotary evaporator and extracted three times with ethyl acetate to remove the phenolic compounds and polar organics. Finally the remaining ethanol solution was extracted three times with ethyl ether to remove the nonpolar organic compounds. The steps in extraction are shown in Fig. 2.11.

Figure 2.11: Flowchart for the extraction of clove.