CHAPTER -2

Screening of spices for antioxidant activity
Introduction

Reactive oxygen species (ROS) are highly reactive and potentially damaging chemical species (Frankel and Meyer, 2000, Carpenter et al., 2007, Suk Kim et al., 2011). The oxidative damages caused by ROS Oxidative stress is one of the major etiological factors for diseases like Cataract, Cancer, Heart ailments, Arthritis, Alzheimer’s disease, nutritional deficiencies, bacterial, viral infections (Halliwell B., 1996). Antioxidants can prevent the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions (Tachakittirungrod et al., 2007). Spices are a strong source of natural antioxidants which known to protect tissues / cells from oxidative stress, which is generally considered to be a cause of mutation and leads to cancer (Ringman et al., 2005).

Generally, Spices, like turmeric, fenugreek, mustard, ginger, etc. may offer many health benefits and have been proven to counteract oxidative stress in vitro and in vivo (Tachakittirungrod et al., 2007). Most of these spices have been intensely studied only for their components like phenolic compounds, beta carotene, curcuminoids and flavonoids (Manda and Adams, 2010, Suk Kim et al., 2011), but when these so called active components are subjected to thermal stability tests, it is observed that, their antioxidant ability is considerably reduced. Only few spices have shown their stability against temperature and retain most of antioxidant activity. Herein efforts were directed to screen different spices for their best possible antioxidant activity and also to unravel the underlying mechanism of this protection, various approaches have been taken because there is a need to identify non toxic, inexpensive, easily available powerful antioxidant from dietary sources. The above was the aim to screen different spices in search of source of antioxidants.

**Star Anise (Illicium verum),** commonly used as spice, obtained from the star-shaped pericarp of Illicium verum. The star shaped fruits are harvested just before ripening. It is a major source of the chemical compound shikimic acid. Shikimic acid is a primary precursor in the pharmaceutical synthesis of anti-influenza drug (Wang et al., 2011). Star anise has been used in a tea as a traditional remedy for rheumatism, and the seeds are sometimes chewed after meals for better digestion. Star Anise is used to relieve cold-stagnation in traditional Chinese medicine (Divya Chouksey et al., 2010, Cheng HongYang et al., 2012).
Cloves (Syzygium aromaticum) are the aromatic dried flower buds of a tree in the family Myrtaceae. Cloves are used in Ayurvedic medicine, Chinese medicine, and western herbalism and dentistry. Clove oil, applied to a cavity in a decayed tooth, also relieves toothache (Alqareer et al., 2006). The clove oil is used in aroma therapy and as painkiller in dental emergencies. Cloves are used to increase hydrochloric acid in the stomach and to improve absorption. Cloves are also said to be a natural anthelmintic. Topical application over the stomach or abdomen are said to warm the digestive tract (Balch et al., 2000). Research results show that, extracts of clove buds inhibits carbohydrate hydrolyzing enzymes in case of type 2 diabetes (Stephen and Ganiyu Oboh, 2012, Pradeep Kumar et al. 2011).

Cinnamon (Cinnamomum verum) is obtained from the inner bark of trees of Cinnamomum that is used in both sweet and savoury foods. Cinnamon is one the most popular spices used in food preparation (Wu et al., 2013). Cinnamon bark is used widely as a spice for its attractive flavour. The bark oil used in the manufacture of perfumes, soaps, toothpastes and flavouring agent for liquors and in dentifrices. Besides these, Cinnamon and Cassia have a wide range of medicinal and pharmacological application like appetite stimulation, treatment for arthritis (Denys J. Charles, 2013).

Cumin (Cuminum cyminum) is of the family Apiaceae, has been used as a spice and is native to the eastern Mediterranean, extending to East India (Sowbhagya et al., 2008). Cumin seeds are used for their unique aroma and its medicinal properties. Cumin is used in foods, beverages, liquors and in medicines (Sowbhagya et al., 2007). Literature shows that, Cumin rich in vitamin A, beta carotene, calcium and magnesium. In traditional medicine, cumin is used to treat jaundice, dyspepsia and diarrhea (Muhammad Namdeem and Riaz, 2012, Thippswamy and Naidu, 2005, Ani et al., 2006).

Fenugreek or methi seeds (Trigonella foenum) belong to the subfamily Papilionacae of the family Leguminosae. Fenugreek is used both as an herb (the leaves) and a spice (the seed). It is reported that, Fenugreek seeds rich with protein, crude fibre and crude fat (Nazar and Tinay 2007, Shakuntala et al, 2011). As mentioned in Ayurveda and Unani, the seeds are bitter, mucilaginous, aromatic, carminative, tonic, thermogenic, galactogogue, astringent, emollient and an aphrodisiac. It is used to treat for fever, vomiting, anorexia, cough, bronchitis and callosities (Anon., 1976, Kirtikar and Basu, 1984, Xue et al. 2007). In Ayurveda, the
seeds are used to treat smallpox, enlargement of liver and spleen and rickets. The Fenugreek seeds contain steroidal substance, which is used as a starting material in the production of sex hormones and oral contraceptives (Sharma, 1986, Narender et al. 2006, Acharya et al., 2011).

**Black pepper (Piper nigrum)** belongs to the family of Piperaceae. It is cultivated for its fruit, dried and used as a spice. It is widely consumed spice throughout the world. It is rich with carbohydrates, protein, vitamin A, phosphorus and Potassium. It increases villi surface and so it used to adjunct to increase absorption of other compounds (Ernest Hogson, 2004, Zachariah et al. 2005, Meghwal and Goswami, 2012). It is used as medicines for analgesic, antiseptic, antispasmodic, antitoxic, aphrodisiac, diaphoretic, digestive, diuretic, febrifuge, laxative and tonic for spleen (Gulcin, I., 2005, Nooman et al., 2008, Meghwal and Goswami, 2012).

**Turmeric (Curcuma longa)** belongs to Zingiberaceae family has been attributed a number of medicinal properties in the traditional system of medicine for treating several common ailments (Sivananda, 1958; Nadkarni and Nadkarni, 1976; Parastoo et al., 2012). It belongs to the genus Curcuma, which consists of several plant species with underground rhizomes and roots. About 40 species of the genus are indigenous to India, indicating the Indian origin (Velayudhan et al., 1999, El-Masry, A.A., 2012). Originally, it had been used as a food additive to improve the palatability, storage and preservation of food. Literature shows that, it is rich with Carbohydrates, proteins, Curcumin I, II, III, curcuminoids etc. It has a wide range of medicinal property like antimutagenic, anticarcinogen, antioxidant, antiviral, antibacterial, antiparasitic, antiinflammant etc. (Mithra et al., 2012, Ammon and Wahl, 1991, Araujo and Leon, 2001, Meera and Divya, 2012, Leela Srinivas et al., 1992, Trinidad, 2012).

**Wild Turmeric (Curcuma aromatica)** is belongs to the Zingiberaceae family. In Ayurveda and Unani, it is mentioned that, it has strong antibiotic properties. It is believed to play a role in preventing and curing cancer in Chinese medicine, in an effort to remove cell accumulations such as tumors (Lee et al., 2007, Angel et al., 2012). *Curcuma aromatica* possesses curcuminoids, which give it natural antibiotic powers. It is used as anti-inflammant (Amit Kumar et al, 2009, Ahmed et al., 2005), anti-allergic (Ram et al., 2003) and anti-dementia (Lim et al., 2001, Shahwar et al., 2012, Srividya et al., 2012).
Star Anise (*Illicium verum*)

Cinnamon (*Cinnamomum verum*)

Fenugreek or methi seeds (*Trigonella foenum*)

Cumin (*Cuminum cyminum*)

Black pepper (*Piper nigrum*)

Turmeric (*Curcuma longa*)

Wild Turmeric (*Curcuma aromatica*)
Materials

Selected spices were obtained from authentic sources, cleaned thoroughly, dried in shade, powdered and sieved through B.P.100 mesh. Butylated hydroxyl anisole, α-tocopherol, thiobarbituric acid, ferrous sulphate, ascorbic acid, 2-deoxy ribose, ferric chloride, sodium carbonate, sodium tartarate, linoleic acid were purchased from Sigma, US. Sephadex G 10 and G 25, DEAE were from Pharmacia, Sweden. All other chemical unless otherwise mentioned were purchased from E. Merck, Germany. The 0.45μm and 0.22μm filter were purchased from Sartorius, India. The spectro-photometric analysis was done using Shimadzu UV-1601 Spectrophotometer. All organic solvents were of analytical grade and were distilled prior to use if needed.

Methods

2.1.1 Aqueous extract of spices: Flow Chart – 1

500 mg of spice powder + 50 ml distilled / distilled boiling water

Stirred for two hours

Centrifuged at 10000 rpm for 20 min at 4°C

Supernatant

Residue

Filtered through Whatman No. 1 filter paper and 0.22 μm filter (Sartorius)

Freezed and lyophilized
2.1.2 Solvent extract of spices: Flow Chart - 2

- 500 mg of spice powder + 50 ml Organic solvent
- Stirred for two hours
- Filtered through glass wool
- Supernatant
- Concentrated using flash evaporator
- Make the final volume to 2 ml with D.D. water
- Residue

2.3. Thermal stability of spice extracts

The stability of the spice extracts were tested against heat treatment at 100°C up to 60 minutes. The solubility was tested in water, tris buffer (10mM, pH 7.4), phosphate buffer (10mM, pH 7.4) and saline.

2.4 Biological characterization

2.4.1 DPPH radical scavenging activity of the spice extracts

DPPH radical scavenging activity was assessed according to the method of Shimada et al. (1992) with minor modifications. The spice extracts at a concentration of 25 µg was mixed in 1 ml of freshly prepared 0.5 mM DPPH ethanolic solution and 2 ml of 0.1 M acetate buffer pH 5.5. The resulting solutions were then incubated at 37°C for 30 min and measured colorimetrically at 517 nm. Ascorbic acid, BHA and Curcumin were used as positive control under the same assay conditions. Negative control was without any inhibitor or spice extracts. Lower absorbance at 517 nm represents higher DPPH scavenging activity. The % DPPH radical scavenging activity of spice extracts was calculated from the decrease in absorbance at 517 nm in comparison with negative control.
2.4.2 Superoxide radical scavenging activity

The Superoxide radical (\(O_2^-\)) scavenging activity of spice extracts were measured according to the method of Lee et al. (Lee et al., 2002) with minor modifications. The reaction mixture containing 100\(\mu\)l of 30mM EDTA (pH 7.4), 10\(\mu\)l of 30mM hypoxanthine in 50mM NaOH, and 200\(\mu\)l of 1.42mM nitro blue tetrazolium with or without spice extracts and SOD serving as positive control at various concentrations ranging from 50-300\(\mu\)g. After the solution was pre-incubated at ambient temperature for 3 min, 100\(\mu\)l of xanthine oxidase solution (0.5U/ml) was added to the mixture and incubated for one hour at 37°C, and the volume was made up to 3ml with 20mM phosphate buffer (pH 7.4). The solution was incubated at room temperature for 20 min; absorbance was measured at 560 nm. Appropriate controls were included to rule out the artifacts induced reaction. The control was without any inhibitor. Inhibitory effect of spice extracts on superoxide radicals were calculated as

\[
\text{% Superoxide radical scavenging activity} = \left(\frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}}\right) \times 100
\]

2.4.3 Lipid peroxidation inhibition activity

The evaluation of antioxidant activity of spice extracts based on the inhibition of peroxidation according to Shimazaki et al., 1984 with minor modifications. The evaluation of oxidation was done by measuring the TBA reactive substances (Dahle et al, 1962). In another model like linolenic acid micelles, 100 \(\mu\)l of linoleic acid was subjected to peroxidation by ferrous sulphate and ascorbic acid (10:100 \(\mu\)mol) (Gutteridge, 1984) in final volume of 1 ml of Tris buffered saline (20 mM, pH 7.4, 150 mM NaCl). The reaction mixture was treated with or without spice extracts (25\(\mu\)g), BHA, \(\alpha\)-tocopherol and Curcumin (400\(\mu\)M) were used as positive control. The contents were incubated for 1 h at 37°C. The reaction was terminated by the addition of 10 \(\mu\)l of 5% phenol and 1 ml of 1% TCA. To each system 1 ml of 1% TBA was added, the contents were kept in a boiling water bath for 15 min, cooled and centrifuged at 6000 rpm for 10 min. The absorbance of supernatants was measured colorimetrically at 535 nm. Appropriate blanks were included for each measurement. The negative control without any test sample was considered as 100% peroxidation.
The % inhibition of lipid peroxidation was determined accordingly by comparing the absorbance of the test samples with negative control.

2.4.5. Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of spice extracts were done according to the method of Halliwell et al., 1981 with minor modifications. The reaction mixture containing FeCl₃ (100 μM), EDTA (104 μM), H₂O₂ (1 mM) and 2-deoxy- D-ribose (2.8 mM) were mixed with or without spice extracts (25μg) in 1 ml final reaction volume made with potassium phosphate buffer (20 mM pH 7.4) and incubated for one hour at 37°C. BHA and Curcumin (400μM) were used as positive control. The mixture was heated at 95°C in water bath for 15 min followed by the addition of 1 ml each of TCA (2.8%) and TBA (0.5% TBA in 0.025 M NaOH containing 0.02% BHA). Finally the reaction mixture was cooled on ice and centrifuged at 5000 rpm for 15 min. Absorbance of supernatant was measured at 532 nm using the negative control without any antioxidant was considered 100% oxidation. The percentage hydroxyl radical scavenging activity of spice extracts was determined.

2.5. Statistical Analysis

Results are expressed as Means ± S.D of five experiments. Student’s t-test was used for statistical significance using SPSS (statistical presentation system software) for windows version 10.0.1 software, Inc. New York. A probability level of P < 0.05 was considered as statistical significance in comparing with relevant controls.
Fig. 2.1  Inhibition of Lipid peroxidation and scavenging of hydroxyl radicals, DPPH radical and superoxide radicals by Star anise (*Illicium verum*) extracts

% inhibition of Lipid peroxidation and scavenging of hydroxyl radicals, DPPH radical and superoxide radicals was calculated as described in methods.

The control was without any antioxidant and the results are mean ± SD (n = 5).

Standard antioxidants BHA, α-tocopherol and Curcumin used at a dosage of 400µM and Star anise (*Illicium verum*) extracts used at a dosage of 25 µg.
Fig. 2.2  Inhibition of Lipid peroxidation and scavenging of hydroxyl radicals, DPPH radical and superoxide radicals by Clove (*Syzygium aromaticum*) extracts

% inhibition of Lipid peroxidation and scavenging of hydroxyl radicals, DPPH radical and superoxide radicals was calculated as described in methods.  
The control was without any antioxidant and the results are mean ± SD (n = 5).  
Standard antioxidants BHA, α-tocopherol and Curcumin used at a dosage of 400µM and Clove (*Syzygium aromaticum*) extracts used at a dosage of 25 µg.
Fig. 2.3  Inhibition of Lipid peroxidation and scavenging of hydroxyl radicals, DPPH radical and superoxide radicals by Cinnamon (*Cinnamomum verum*) extracts

% inhibition of Lipid peroxidation and scavenging of hydroxyl radicals, DPPH radical and superoxide radicals was calculated as described in methods.

The control was without any antioxidant and the results are mean ± SD (n = 5).

Standard antioxidants BHA, α-tocopherol and Curcumin used at a dosage of 400µM and Cinnamon (*Cinnamomum verum*) extracts used at a dosage of 25 µg.
Fig. 2.4  Inhibition of Lipid peroxidation and scavenging of hydroxyl radicals, DPPH radical and superoxide radicals by Cumin (*Cuminum Cyminum*) extracts

% inhibition of Lipid peroxidation and scavenging of hydroxyl radicals, DPPH radical and superoxide radicals was calculated as described in methods.

The control was without any antioxidant and the results are mean ± SD (n = 5). Standard antioxidants BHA, α-tocopherol and Curcumin used at a dosage of 400µM and Cumin (*Cuminum Cyminum*) extracts used at a dosage of 25 µg.
In Fig. 2.5, the inhibition of lipid peroxidation and scavenging of hydroxyl radicals, DPPH radical and superoxide radicals by Methi (*Trigonella foenum graecum*) seeds extracts is shown.

The graph compares different extracts of Methi seeds, including water extract, boiling water extract, ethanol extract, methanol extract, hexane extract, and petroleum ether extract, against standard antioxidants like BHA, α-tocopherol, and Curcumin. The results are mean ± SD (n = 5).

% inhibition of lipid peroxidation and scavenging of hydroxyl radicals, DPPH radical and superoxide radicals was calculated as described in methods.

The control was without any antioxidant and the results are mean ± SD (n = 5).

Standard antioxidants BHA, α-tocopherol and Curcumin used at a dosage of 400µM and Fenugreek or methi (*Trigonella foenum*) seeds extracts used at a dosage of 25 µg.
Fig. 2.6  Inhibition of Lipid peroxidation and scavenging of hydroxyl radicals, DPPH radical and superoxide radicals by Black pepper (*Piper nigrum*) extracts

% inhibition of Lipid peroxidation and scavenging of hydroxyl radicals, DPPH radical and superoxide radicals was calculated as described in methods.

The control was without any antioxidant and the results are mean ± SD (n = 5).

Standard antioxidants BHA, α-tocopherol and Curcumin used at a dosage of 400µM and Black pepper (*Piper nigrum*) extracts used at a dosage of 25 µg.
Fig. 2.7  Inhibition of Lipid peroxidation and scavenging of hydroxyl radicals, DPPH radical and superoxide radicals by Turmeric (*Curcuma longa* L) extracts

% inhibition of Lipid peroxidation and scavenging of hydroxyl radicals, DPPH radical and superoxide radicals was calculated as described in methods.

The control was without any antioxidant and the results are mean ± SD (n = 5).

Standard antioxidants BHA, α-tocopherol and Curcumin used at a dosage of 400µM and Turmeric (*Curcuma longa* L) extracts used at a dosage of 25 µg.
% inhibition of Lipid peroxidation and scavenging of hydroxyl radicals, DPPH radical and superoxide radicals was calculated as described in methods.

The control was without any antioxidant and the results are mean ± SD (n = 5).

Standard antioxidants BHA, α-tocopherol and Curcumin used at a dosage of 400µM and Wild Turmeric (Curcuma aromatica) extracts used at a dosage of 25 µg.

Different extracts of Wild Turmeric (Curcuma aromatica)
Table-2.01  Heat stability nature of Star Anise, Clove, Cinnamon and Cumin extracts : A comparison

<table>
<thead>
<tr>
<th>Spices</th>
<th>Extracts</th>
<th>% inhibition of lipid peroxidation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Minutes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Star Anise</td>
<td>Water</td>
<td>55±2</td>
</tr>
<tr>
<td>(Illicium verum)</td>
<td>Boiling water</td>
<td>52±1</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>45±2</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>49±2</td>
</tr>
<tr>
<td></td>
<td>Hexane</td>
<td>36±3</td>
</tr>
<tr>
<td></td>
<td>Petroleum ether</td>
<td>25±1</td>
</tr>
<tr>
<td>Clove</td>
<td>Water</td>
<td>67±1</td>
</tr>
<tr>
<td>(Syzygium aromaticum)</td>
<td>Boiling water</td>
<td>69±1</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>64±2</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>64±2</td>
</tr>
<tr>
<td></td>
<td>Hexane</td>
<td>55±2</td>
</tr>
<tr>
<td></td>
<td>Petroleum ether</td>
<td>46±3</td>
</tr>
<tr>
<td>Cinnamon</td>
<td>Water</td>
<td>58±1</td>
</tr>
<tr>
<td>(Cinnamomum verum)</td>
<td>Boiling water</td>
<td>52±2</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>61±1</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
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<tr>
<td></td>
<td>Hexane</td>
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<tr>
<td></td>
<td>Petroleum ether</td>
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</tr>
<tr>
<td>Cumin</td>
<td>Water</td>
<td>54±1</td>
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<tr>
<td>(Cuminum Cyminum)</td>
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<td></td>
<td>Ethanol</td>
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<td></td>
<td>Methanol</td>
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<td></td>
<td>Hexane</td>
<td>48±2</td>
</tr>
<tr>
<td></td>
<td>Petroleum ether</td>
<td>34±2</td>
</tr>
</tbody>
</table>

Spice extracts were heated in boiling water bath, 60 minutes, the antioxidant activity was estimated by TBARS method. The antioxidant activity is expressed as % inhibition.

The results are mean ± SD (n = 5).
Table-2.02  Heat stability nature of Fenugreek, Black Pepper, turmeric and wild turmeric extracts : A comparison

<table>
<thead>
<tr>
<th>Spices</th>
<th>Extracts</th>
<th>% inhibition of lipid peroxidation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Minutes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Fenugreek ((Trigonella foenum graecum))</td>
<td>Water</td>
<td>63±1</td>
</tr>
<tr>
<td></td>
<td>Boiling water</td>
<td>69±2</td>
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<tr>
<td></td>
<td>Ethanol</td>
<td>63±2</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>60±1</td>
</tr>
<tr>
<td></td>
<td>Hexane</td>
<td>55±2</td>
</tr>
<tr>
<td></td>
<td>Petroleum ether</td>
<td>44±3</td>
</tr>
<tr>
<td>Black Pepper ((Piper nigrum))</td>
<td>Water</td>
<td>52±1</td>
</tr>
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<td></td>
<td>Boiling water</td>
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<td></td>
<td>Petroleum ether</td>
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<td>Turmeric ((Curcuma longa))</td>
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<td>Boiling water</td>
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</tr>
<tr>
<td></td>
<td>Petroleum ether</td>
<td>55±2</td>
</tr>
<tr>
<td>Wild Turmeric ((Curcuma aromatica))</td>
<td>Water</td>
<td>51±2</td>
</tr>
<tr>
<td></td>
<td>Boiling water</td>
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<td>Ethanol</td>
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<td></td>
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<td></td>
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<tr>
<td></td>
<td>Petroleum ether</td>
<td>31±2</td>
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</table>

Spice extracts were heated in boiling water bath, at the end of 60 minutes, the antioxidant activity was estimated by TBARS method. The antioxidant activity is expressed as % inhibition.

The results are mean ± SD (n = 5).
**Discussion**

In the present chapter, ambient temperature water, boiling water, ethanol, methanol, hexane and petroleum ether extracts of eight spices was done to evaluate their antioxidant activity against lipid peroxidation inhibition and scavenging of hydroxyl, DPPH and superoxide radicals.

Linoleic acid micelles were subjected to peroxidation with ferrous sulphate - ascorbic acid and the extent of lipid peroxidation was estimated by measurement of TBARS. In the lipid peroxidation inhibition studies, antioxidants like BHA, Curcumin and alpha tocopherol used as standard antioxidants.

Hydroxyl radicals are known to be the most reactive of all reduced forms of dioxygen and are thought to initiate cell damage *in vivo* (Rollet-Labelle et al., 1998). The hydroxyl radical scavenging method is an assay to determine the rate constants for reactions of hydroxyl radical. The extracts on hydroxyl radicals generated by Fe$^{3+}$ ions were measured by determining the degree of deoxyribose degradation, an indicator of (TBA-MDA) adducts formation. The appropriate controls like BHA, Curcumin and α-tocopherol at 400µM dose were used.

1,1-Diphenyl-2-picrylhydrazyl, (DPPH) is a cell-permeable, stable free radical that acts as a hydrogen radical scavenger. It is useful in inducing free-radical injury to tissues and as a screening tool for detecting free radical scavenging capacity of other antioxidants.

The superoxide radical is a free radical form of superoxide dismutase (SOD). The two oxygen molecules are connected together and one oxygen has an extra electron. This free radical targets cell membranes, the barrier protecting the inside of cells. The presence of copper and zinc are six histidine and one aspartate side-chains; one histidine is shared between the two metals can help SOD from forming into a superoxide free radical and the manganese SOD present in mitochondria.

Herein we have used different extracts of spices **Star anise (Illicium verum)**, **Clove (Syzygium aromaticum)**, **Cinnamon (Cinnamomum verum)**, **Cumin (Cuminum cyminum)**, **Methi (Trigonell foenum graecum)**, **Black pepper (Piper nigrum)**, **Turmeric (Curcuma longa)** and **Wild turmeric (Curcuma aromatica)** to check its inhibitory activity.
The water and boiling water extracts of selected eight spices was done as shown in Flow chart -1. Different solvent extracts of spices was done as shown in Flow chart-2. As shown in Figure 2.1, the lipid peroxidation inhibition capacity and hydroxyl radical, DPPH radical and superoxide radical scavenging activity of different extracts of Star Anise (*Illicium verum*) was done using standard antioxidants BHA, α-tocopherol and Curcumin. The ambient temperature water extract, the boiling water extract, ethanol and methanol extract of Star Anise showed more inhibition of lipid peroxidation when compared to other extracts. The hydroxyl radical scavenging activity of methanol extract of Star Anise was more than other extracts. The boiling water and ambient temperature water extract showed more DPPH radical scavenging activity. In superoxide radical scavenging activity, the boiling water, ethanol and methanol extracts are more active. The above results indicate that, the ambient temperature water extract, boiling water extract, ethanol extract and methanol extracts of Star Anise are effective towards inhibiting lipid peroxidation and scavenging hydroxyl, DPPH and Superoxide radical effectively. Figure No. 2.2 shows that, inhibition of lipid peroxidation, scavenging of hydroxyl radicals, DPPH radicals and superoxide radicals by aqueous and solvent extracts of Clove (*Syzygium aromaticum*). The ability of inhibition of lipid peroxidation by all the extracts of clove is promising when compared to standard antioxidants. In hydroxyl radical scavenging activity, the ambient temperature water, boiling water, water and ethanol extracts are more when compared to hexane and petroleum ether extracts. In DPPH radical scavenging activity, the methanol extract, boiling water extract and ambient temperature water extract showed more activity. In the superoxide radical scavenging studies, all the extracts showed the inhibitory activity. From the above results, it is clear that, except petroleum ether extract, all other extracts acts as effective antioxidants.

The lipid peroxidation inhibitory activity and hydroxyl radicals, DPPH radicals and superoxide radicals scavenging activity results of Cinnamon (*Cinnamomum verum*) are as shown in Figure No. 2.3. The ethanol and methanol extracts of Cinnamon showed inhibition of lipid peroxidation where as the inhibition shown by Hexane and petroleum ether was negligible. The hydroxyl radical scavenging activities of ethanol and methanol extracts are more. The DPPH radical scavenging activity of methanol extract was more. The superoxide radical scavenging activity of methanol, ethanol and boiling water extract are more. The
above results show that, only the ethanol and methanol extract of Cinnamon are acts as effective antioxidants.

Figure 2.4 shows that, the lipid peroxidation inhibitory activity and hydroxyl radicals, DPPH radicals and superoxide radicals scavenging activity results of Cumin (Cuminum Cyminum). Except Petroleum ether extract, all other extracts are showing good peroxidation inhibitory activity. In hydroxyl radical scavenging activity, both ambient temperature water extract and boiling water extract are showing more scavenging activity. But in the DPPH radical scavenging activity, all the extracts shows comparatively equal activity. The ethanol, boiling water extract, ambient temperature water extract of Cumin shows good amount of superoxide radical scavenging activity whereas, petroleum ether extract shows very less. The above results confirm that, all the extracts of Cumin effective against DPPH radicals than standard antioxidants like BHA and Curcumin.

As shown in Figure 2.5, the ambient temperature water extract, boiling water extract, ethanol, methanol, hexane and petroleum extracts of Fenugreek or Methi (Trigonella foenum graecum) showed very good antioxidant activity by inhibiting lipid peroxidation and by scavenging hydroxyl radicals. But the DPPH and Superoxide radical scavenging activity of all the extracts are very less and negligible. The above results confirm that, all the extracts of Fenugreek are inhibitors of lipid peroxidation and scavenge hydroxyl radicals.

The figure 2.6 showed that, the ethanol and boiling water extract of Black pepper (Piper nigrum) showed lipid peroxide inhibitory activity and very less activity reported by Hexane and petroleum ether extracts. The ambient water extract, boiling water extract, ethanol extract are showing highest hydroxyl radical scavenging activity and the extract of petroleum ether was very less. The DPPH radical scavenging activity of all the extracts are almost equal and having antioxidant activity. In superoxide radical scavenging activity of black pepper, very less was reported by petroleum ether and methanol extract and others are negligible. It is confirmed that, all extracts of Pepper effective in scavenging hydroxyl radicals, DPPH radicals and inhibits lipid peroxidation.

The Figure 2.7 shows that, among Turmeric (Curcuma longa L) extracts, the boiling water extract, ethanol extract and methanol extracts showed lipid peroxidation
inhibitory activity when compared to standards whereas, the ambient temperature water extract showed comparatively less activity. Except petroleum ether extract, all the other extracts are showed hydroxyl radical scavenging activity. In DPPH radical scavenging activity, only ambient temperature water extract and boiling water extract are showed radical scavenging activity. The boiling water extract and ambient temperature water extract are showing superoxide radical scavenging activity. Above results indicates that, only ambient temperature water extract and boiling water extracts are effective antioxidants in all model systems.

In *Curcuma aromatica* extracts, the ambient temperature water extract, the boiling water extract, ethanol extract and methanol extracts showed good percentage of lipid peroxidation inhibitory activity when compared to standards as shown in Figure 2.8. The ambient temperature water extract, boiling water extract, ethanol extracts are showed hydroxyl radical scavenging activity when compared to other extracts. The boiling water, ethanol and methanol extracts are showed DPPH radical scavenging activity and petroleum ether extract showed very less. The ethanol and methanol extracts are showed superoxide radical scavenging activity when compared to hexane and petroleum ether extracts. The above result shows that, the ethanol and methanol extracts of *Curcuma aromatica* are effective when compared to other extracts.

Finally all eight spice extracts were subjected to thermal stability test by keeping all extracts in boiling water bath for 60 minutes; their ability towards inhibiting lipid peroxidation was examined at the end of 60 minutes as in shown in Table 2.01 and Table 2.02. At “zero” minute, all the extracts showed their highest lipid peroxidation inhibitory activity. At the end of 60 minutes of heat treatment, the boiling water extract of Clove (37 ± 1%), boiling water extract of black pepper (25 ± 1%), ambient temperature water extract of Turmeric (68 ± 3%), boiling water extract of Turmeric (71 ± 2%), ambient temperature extract of wild turmeric (35±2%) and boiling water extract of wild turmeric (36±1%) retained their antioxidant potential against lipid peroxidation inhibition. Among the above extract only boiling water extract of Turmeric (*Curcuma longa* L) was retained most of its antioxidant activity. Hence, Turmeric (*Curcuma longa* L) was selected for further studies to understand its antioxidant ability and other biological activities.
Reference


- Araujo, C.A.C. and Leon, L.L. (2001); Biological activities of *Curcuma longa* L. Memórias do Instituto Oswaldo Cruz 96, 723–728.


• Denys J. Charles, (2013); Cinnamon, Antioxidant Properties of Spices, Herbs and Other Sources, pp 231-243

• Divya Chouksey, Preeti Sharma, Pawar, R.S., (2010); Biological activities and chemical constituents of Illicium verum hook fruits (Chinese star anise), Pelagia Research Library Der Pharmacia Sinica, 1 (3): 1-10.


• Gulcin, I., (2005); The antioxidant and radical scavenging activities of black pepper (Piper nigrum) seeds, Int J Food Sci Nutr., Nov;56(7):491-9.


• Lee, Y.L., Weng, C.C. and Mau, J.L. (2007); Antioxidant properties of ethanolic and hot water extracts from the rhizome of Curcuma aromatica, Journal of Food Biochemistry, 31: 757–771


- Narender, T., Puri, A., Shweta Khaliq, T., Saxena, R., Bhatia, G. and Chandra, R. (2006); 4-Hydroxyisoleucine, an unusual amino acid as antidyslipidemic and antihyperglycemic agent. Bioorganic and Medicinal Chemistry 16(2), 293–296.


- Pradeep Kumar, Preetee Jaiswal, Vinay Kumar Singh and Dinesh Kumar Singh, (2011); Medicinal, therapeutic and pharmacological effects of Syzygium aromaticum (Laung), Pharmacologyonline; 1: 1044-1055.


- Sivananda, S. (1958); *Home Remedies*. The Yoga Vedanta University, Sivanad Nagar, India.


- Stephen Adeniyi Adefega and Ganiyu Oboh, (2012); In vitro inhibition activity of polyphenol-rich extracts from Syzygium aromaticum (L.) Merr. and Perry (Clove) buds against carbohydrate hydrolyzing enzymes linked to type 2 diabetes
and Fe$^{2+}$-induced lipid peroxidation in rat pancreas, Asian Pac J Trop Biomed, 2(10): 774-781

- Suk Kim I, Mi-Ra Yang, Ok-Hwan Lee, and Suk-Nam Kang, (2011); Antioxidant activities of Hot Water Extracts from Various Spices Int. J. Mol. Sci., 12.


