CHAPTER - 2

REVIEW OF LITERATURE
REVIEW OF LITERATURE

Yang et al. in 2010 have reported that oil from Australian Lavandula angustifelia have high efficacy against lipid peroxidation in comparison to oil extracted from other plants. The Lavandula angustifelia oil constitute linalool and linalyl acetate.

Wei et al. in 2010 have reported the antioxidant property in the oil extracted from Thymus vulgaris, Eugenia caryophyllus and Ocimum basilicum. It is notable that ρ-cymene and thymol are the main constituents of Thymus vulgaris, eugenol and β-caryophyllene of Eugenia caryophyllus, and linalool, isoanethole and eugenol form the major components of Ocimum basilicum.

The antioxidant activity of essential oils of five spice plants used in the Mediterranean diet was evaluated by Viuda-Martos et al. in 2010. The thiobarbituric acid reactive species (TBARS) test using egg yolk as substrate was used. The essential oils of five plants, Thymus vulgaris, Eugenia caryophyllus, Origanum vulgare, Salvia officinalis and Rosmarinus officinalis were tested and found to have antioxidant activity, Thymus vulgaris oil presented the best activity, close to that verified for BHT. Terpinen-4-ol, γ-terpinene, cis-sabinene hydrate, linalool and ρ-cymene are its main constituents. Carvacrol was reported to constitute the main component of Origanum vulgare oil, as well as richly present were the phenolic components, oregano and thyme. Thyme was found to possess the best activity (Viuda-Martos et al., 2010).

The aerial parts (leaves, flowers and stem) of Thymus vulgaris were used for supercritical fluid extraction of its volatile oil, and was compared to the isolated
essential oils separated by hydrodistillation. The main components in both the cases were ρ-cymene and γ-terpinene, linalool, thymol and carvacrol. ρ-cymene and thymol dominated in both cases. The great difference was the presence of thymoquinone in the volatile oil. The presence of this component is reported to be responsible for the highest antioxidant activity (Grosso et al., 2010).

The antioxidant activity of the extracts of Artemisia herba-alba, cultivated in Southern Tunisia was reported by Mighri et al. in 2010. One of the diverse methods used was the β-bleaching test. Four types of oils were detected: β-thujone, α-thujone, thujones (α + β), and 1, 8-cineole / camphor / thujones (α + β) all of which exhibited weak antioxidant abilities for preventing the linoleic acid oxidation. The best inhibition percentage (12.5%) was showed by β-thujone-rich oil. Such results were attributed to the absence of non-phenolic compounds.

The chemical evaluation of the extracts of different parts of Myrtus communis, viz., leaf, stem and flower showed that α-pinene and 1, 8-cineole dominated the leaf oil; 1, 8-cineole, α-pinene, transcaryophyllene and linalool as the main components of the stem oil, and besides these four main components, flower oil constituted of α-terpineol and eugenol. Leaf and flower oils had the best antioxidant activities, but inferior to those of BHT and BHA. Such weak activities are attributed to the low level of phenolic compounds such as eugenol in flowers, or their absence as in stem and leaf (Wannes et al., 2010; Jia et al., 2010).

Hymenocrater longiflorus from Iran was evaluated for its antioxidant activity by Ahmadi et al. in 2010. The main constituents of oil include α-pinene, 1, 8-cineole, β-eudesmol, spathulenol, hedycaryol, δ-cadinene, and it was found that
oxygenated sesquiterpenoids (47.4%) predominated amongst other components. The essential oils of *Hymenocrater longiflorus* were able to inhibit the bleaching of $\beta$-carotene. The percentage of inhibition was even close (66.4%) to those found for the non-polar-sub-fraction (chloroformic 69.1%), which presented the best activity.

Many other oils isolated from diverse Iranian plants are also known for their antioxidant properties as reported by Ebrahimabadi *et al.* in 2010; Gholivand *et al.* in 2010. The activities were found weak. The percentage inhibition of *Salvia eremophila* was of about one third of that of BHT, in contrast to the methanolic extracts of the same plant. The absence of phenolic compounds in the extraction was held responsible by the authors for such weak activity. $\alpha$-Pinene, borneol, camphene and transcaryophyllene were the main components of the oil. Gholivand *et al.* in 2010 attributed the weak activity of the vatke oils from *Psammogeton canescens*, to the presence of $\beta$-bisabolene, apiole, $\alpha$-pinene and dill apiole, all of them being non-phenolic compounds.

The antioxidant, antimicrobial and antispasmodic activities of *Origanum acutidens* from the Turkish flora were evaluated by Goze *et al.* in 2010. The major component of the extract was reported as carvacrol. This extract oil presented anti-oxidant activity, but inferior to that of the reference substance, BHT. In spite of the high percentage of carvacrol present in the oil, the inhibition percentage only reached 65%, in contrast to the 100% of BHT.

The extract of *Ageratum conyzoides*, constitute precocene I and caryophyllene that possess good capacity for preventing lipid peroxidation, using lipid substrate as liver homogenate (Patil *et al.*, 2010). The methanolic extracts of the same
plants have also been tested but the activities were about 100 times lower than those of the essential oils. According to the authors, the antiflatoxigenic activity found for the essential oils may be partly due to their antioxidant activity.

The widely distributed *Zingiberaceaeous* plant in the South-West of China is *Amomum tsao-ko* having antioxidant property, reported by Yang *et al.* in 2010 after TBARS evaluation. A weak antioxidant activity was reported by the author and was concluded that it was due to the low phenolic content of the plant. The main component reported was 1, 8-cineole (Joshi *et al.*, 2010; Kulisic-Biluscis *et al.*, 2010).

The anti-hyperlipidemic and antioxidant activities of essential oils extracted from *Ocimum sanctum* leaves have been investigated by Suanarunsawat, *et al.* in 2010, in rats fed with cholesterol diet Eugenol and methyl-eugenol were found as the most important components of essential oil. The high levels of TBARS, either in cardiac or liver tissues were decreased by the extract oils, protecting them against stress induced oxidation. Thus, it was proved that the plant have potent anti-hyperlipidemic and antioxidant activity.

The extracts of *Majorana hortensis* have appreciable antioxidant activity probably due to the presence of carvacrol with positive synergism with other components (Martino, *et al.* 2010).

*Citrus maxima* and *Citrus sinesis* have high antioxidant activities, despite their essential oils with difference in their chemical compositions. *Citrus sinesis* extract constitutes of limonene, whereas *Citrus maxima* oil consisted of significant amounts of limonene, E-citral, Z-citral and 3, 3-dimethyl-1-hexene (Singh *et al.* 2010).
The moderate antioxidant activity have been reported in *Heracleum pastinacifolium* and *Heracleum persicum*, the main constituents being myristicin and *trans*-amethole respectively (Firuzi *et al.*, 2010).

Three Mexican oils of *Lippia graveolens* with different chemical compositions along with their microcapsules were evaluated in terms of antiradical activities. The authors concluded that microcapsulation increased the anti-radical activity (Sanchez-Arana *et al.*, 2010).

The flower oils of *Retama raetam*, the plant cultivated in Tunisia is considered to possess good antioxidant activity, as measured through the DPPH method. The appreciable activity was reported to attribute to the high percentage of monoterpenes present in the essential oils (Edziri *et al.*, 2010).

The root oil of *Ridolfia sagetum* from Tunisia, has been also reported for good activity (Saei-Dehkordi *et al.* 2010), and its antiradical activity was attributed to the high amounts of two phenyl-propanoids dillapiole and myristicin (Grosso *et al.* 2010).

The moderate antioxidant activity of extract of *Myrtus communis* separated by TLC coupled with DPPH method was attributed to the presence of the components, 1, 8-cineole and methyl eugenol (Mimika-Dukie *et al.*, 2010).

The extracts of *Aniba panurensis* and *Aniba rosaeodora* from Brazil exhibited low antioxidant activity. The IC50 value was found greater than 1,000 mg/mL superior to that of quercetin, the reference used by the authors (Alcantara *et al.*, 2010). Such low activity was reported due to the absence or to the very low
concentrations of some compounds responsible for anti-oxidant activity like β-caryophyllene and phenolic compounds.

The extracts of about 17 species from Lamiaceae family have been found to possess effective antioxidant activity. The compounds, actively known for this effectiveness, detected by DPPH/TLC method included phenols, non-phenols, oxygenated or non-oxygenated compounds (Saleh et al., 2010).

*Lindera pulcherrima, Dodecadenia grandiflora* and *Dodecadenia gamblei* presented the best inhibition of, lipid peroxidations as determined through the TBARS method and β-carotene bleaching test, but the DPPH assay revealed that only *Dodecadenia grandiflora* was the powerful radical scavenger (Joshi et al., 2010).

The antioxidant activity of *Juniperus phoeniceae* was evaluated by Ghazghazi *et al.* in 2010. They reported that the antioxidant activity was due to the presence of the main components in varied proportion, these being α-pinene, δ-3-carene, α-terpineol, β-myrcene.

The scavenging ABTS capacity was found in *Rosa canina*, although the harvesting place was the determinant in such activity, which seemed to be related to the presence of high percentages of vitispirane (Ghazghazi *et al.*, 2010).

The scavenging ability was possessed for *Satureja intricata*, whereas *Satureja obovata* did not possess such ability (Jordan *et al.*, 2010). This behaviour was attributed to the presence of thymil and its precursors ρ-cymene and γ-terpinene, along with borneol in *Satureja intricata*. 
Artemisia herba-alba have been reported to possess little ability for inhibiting lipid peroxidation (Mighri et al., 2010), and when evaluated in terms of free radical scavenging, it continued to have weak activity. This contrasted the work of Joshi et al. and Patil et al. in 2010, with the fact that the essential oil of Artemisia herba-alba was not effective either against lipid oxidation nor as free radical scavenger.

In the ABTS-scavenging activity, the extratracts of Mentha x piperita and Boswellia carteri were the best. The major components of the essential oils were reported to be linalool and linalyl acetate. However, limonene predominated in the Citrus x limon that did not exhibit the best scavenging activity (Yang et al., 2010).

The leaf extracts of Pistacia atlantica from Alergia showed weak antioxidant activity with the inefficient ability for scavenging DPPH free radicals, at least when compared to those of references of BHT and BHA. In contrast, the same oils had a higher antioxidant capacity relative to the antioxidant of reference ascorbic acid when measured through the FRAP assay (Gourine et al., 2010). The best activity was reported due to the presence of the main components, α-pinene + α-thujene, camphene and spanthulenol.

The male and female leaf essential oils of Pistacia atlantica remarkably showed seasonal qualitative difference in composition and antioxidant activities. The main components of male leaf oil were α-pinene / α-thujene, spathulenol and bicyclogermacrene, and the female leaf oil consisted of δ-3-carene as the main constituents. The seasonal variation showed that most of the main components of the leaf oils reached their highest values in September. The highest antioxidant
capacity to scavenge free radicals reached in the month of June for male leaf and September to October for the female leaf (Gourine et al. 2010).

The antioxidant activity measured by DPPH radical scavenging and ferric reducing power (FRAP) of *Zingiber officinale* and *Cuminum cyminum* was compared, and similar trends in both methods were reported by El-Ghorab et al. in 2010. The best activity was revealed by *Cuminum cyminum*, its constituents mainly were cuminal, γ-terpinene, pinocarveol, carotal, α-pinene, sabinene, β-terpineol and linalool.

*Eugenia caryophyllus* (clove) showed the highest ferric reducing capacity in terms of Trolox concentrations. The antioxidant measurement by DPPH and FRAP methods showed that *Eugenia caryophyllus* exhibited higher activity. Clove oil showed higher antioxidant activity that the volatile aglycone fraction, although both samples have eugenol content. In assay, the reference eugenol showed weak ability, whereas exhibited considerable activity in the DPPH method (Politeo et al., 2010).

The reducing power of the essential oils of the Himalayan Lauraceae species, *Psammogeton conescens* were tested by Gholivand et al. in 2010, and the antioxidant activity measured by the β-carotene bleaching and DPPH tests showed weak activity. This was attributed to the low concentration of phenolic compounds present in the oil.

The safranine method was used to evaluate the ability to scavenge hydroxyl radicals of oils of *Thymus marschallianus* and *Thymus proximus*, and the latter was found to be more effective. The antioxidant activity of both were mainly attributed to the presence of thmol, ρ-cymene and γ-terpinene (Jia et al., 2010).
Thymus camphorates, Thymus caespititius and Thymus capitellatus are some of the important Portugese Thymus species which were reported to be significantly more effective in scavenging hydroxyl radicals. The major constituents, borneol, camphor, α-terpineol, 1,8-cineole, camphene, α-pinene and ρ-cymene were present in different relative amounts. The oils in which thymol or carvacrol predominated did not present significant activities (Dandlen et al., 2010).

The young and mature leaves of Ageratum scoparia exhibited best of hydroxyl radical scavenging activity. However, the scavenging activity of principle constituents, β-myrcene and ρ-cymene was found to be very less. The antioxidant activity of the samples was also evaluated by the DPPH method, and the less activity of the main components was reported, although the mature leaf oils were more effective as antioxidants than young leaf oils (Singh et al., 2010).

The scavenging hydroxyl radical activity of the extracts from the aerial parts (leaves, flowers, stem) or from the fruits of Foeniculum vulgare was always found less than 50%, at the concentrations assayed (100-2000 mg/mL). Higher concentrations, however presented percentages greater than 50%. (Miguel et al., 2010).

The extracts of Aloe barbadensis, Citrus aurantium, Cinnamomum zeylanicum, Eucalyptus globules, Juniperus communis and Cananga odonta were found to exhibit strong lipoxygenase inhibitory effects (Wei et al., 2010). Such activities are attributed to the presence of 1, 8-cineole, α-pinene and β-caryophyllene in the their essential oils (Kamatou et al., 2006).

Eucalyptus camaldulensis, growing wildly in different localities of Sardinia (Italia) to possessed high antioxidant activities, that were attributed to the
presence of the components, $\rho$-cymene, 1, 8-cineole, $\beta$-phellandrene, spathulenol and cryptone in different proportions (Barra et al., 2010).

The major components present in the essential oils of four indigenous South African Helichrysum species were reported to be 1, 8-cineole, $\alpha$-pinene and $\beta$-caryophyllene which were found to inhibit 5-lipoxygenase (Laurens et al., 2004).

Chamazulene and $\alpha$-bisabolol are the main components present in Chamomile that showed anti-inflammatory activities, partly due to the inhibition of leukotriene synthesis as they appear to be good 5-lipoxygenase inhibitors (Kamatou et al., 2006).

The oil extracts of leaves and rhizomes of Alpinia murdochii, Alpinia scabra and Alpinia pahangensis showed lypoxygenase inhibitory effects. $\beta$-pinene and sabinene as the main components of leaf oils, and sesquiterpenes, $\gamma$-selinene, $\alpha$-selinene and $\alpha$-panasinsen as those of rhizome oils attributed to the antioxidant activities (Syamsir, 2009).

The antioxidant and cytotoxic activities of Thai medicinal plants, Arcangelisia flava, Coscinium blumeanum and Fibraurea tinctoria have been assessed against DPPH radicals. Methanol extract of Arcangelisia flava, and methanol and chloroform extracts of Coscinium blumeanum exhibited moderate antioxidant activity with EC$_{50}$ values of 25-55 Mg/ml. Chloroform extracts of Arcangelisia flava and Fibraurea tinctoria showed cytotoxic activity. Triacontanyl caffeate was mainly responsible for antioxidant activity of Coscinium blumeanum. Jatororrhizine, another component possessed moderate antioxidant activity, whereas palmatine and berberine were less active (Keawpradub et al., 2005).
The plant extracts of *Centella asiatica* possess antioxidant property by showing significant PI value. The presence of the compounds, madecassoside in the extract was attributed to the antioxidant activity (Gajera *et al.*, 2005). Another compound present in *Centella asiatica* was Kaemferol; an alkaloid, which also possessed antioxidant property, and this is also present in *Kaemferia galangal* (Rastogi *et al.*, 1991).

The highest PI value of *Leuces aspera* when compared with other plant extracts was due to its high activity. Methanol extract of *Leucas aspera* possessed antioxidant properties, and isolation guided by dual assay methods showed the presence of flavonoids and eight lignans. These compounds included nectandrin B, meso-dihydropaviretic acid, macelignan, chianine, licarin A, erythro-2-1-propane-1-OL, myristegenol B and machitinc which were found to exhibit high antioxidant efficacy (Sadhu *et al.*, 2003).

*Plantago major* possessed antioxidant activity despite its lowest PI value. The presence of caffeic acid, cinnamic acid, ferulic acid and plantagoside in the extracts of *Plantago major* mainly attributed for its antioxidant activity. (Prajapati *et al.*, 2003).

Methanolic extracts of leaves of *Artemisia vulgaris*, one of the medicinal plants of Nepal, is reported to possess the highest DPPH scavenging activity. The higher antioxidant activity of *Artemisia vulgaris* was due to the high concentration of total phenolic compounds such as caffeic acid, neochlorogenic acid and ferulic acid, as well as flavonoids in the plants, and predominantly eriodictyol and luteolin (Wojdylo *et al.*, 2007).
High antioxidant activity was found in the leaves of *Ficus lacor*, which had low phenolic content, indicating that the major antioxidant components may not be phenolics. Dey *et al.*, 2008 reported that the leaves of *Ficus lacor* consisted of high tannin content, which contributed to the high antioxidant properties.

The presence of known bioactive compounds such as tannins, dimeric chalcone derivatives, triterpenoids and steroids in the leaf extract of *Mallotus philippensis* was reported to be responsible for its high antioxidant activity. Phenolic compounds are also present, but the weak linear correlation between total phenolic and antioxidant activity was found, indicating that the major antioxidant components might not be phenolics (Tanaka *et al.*, 1998; Arfan *et al.*, 2009).

*Acorus calamus* exhibited higher antioxidant activity with low phenolics and flavonoids, indicating that active compounds of different polarity could be present in this plant. The presence of bioactive compounds such as lignans, epieudesmin, terpenes and quinine in the extracts of *Acorus calamus* contributed to its high antioxidant properties (Weber *et al.*, 2007).

The stem extracts of *Aconkanthera oppositifolia* and *Adenia gummifera* possessed antioxidant properties, as well as they served as free radical inhibitors or scavengers. The method extracts of stem of *Aconkanthera oppositifolia* and *Adenia gummifera* were evaluated by DPPH and FRAP methods, and the high activity was attributed to the presence of phenols and flavonoids. The stem extract od *Adenia gummifera* had higher levels of total flavonoids and pro-anthocyanidins (Adeolu *et al.*, 2008).
Methanolic extracts of the bark of *Terminalia arjuna* was reported to be effective to prevent against lipid damage in terms of TBARS and LOOH formation. It could scavenge free radicals produced by $\gamma$-radiation and other sources of oxidative stress. It was reported that the cardioprotective effects produced by *Terminalia arjuna* was due to its antioxidant properties. The flavonoids in *Terminalia arjuna* including baicalein, soluble in methanol, were capable of protecting against oxidative damage by scavenging free radicals (Gao *et al.*, 1996). The active constituents were reported to include tannins, triterpenoid saponins, gallic acid, ellagic acid, oligomeric proanthocyanidines, phytoserols, Ca, Mg, Zn, Cu, besides flavonoids such as arjunone, arjunolone, luteolin and baicalein (Ratty *et al.*, 1988; Shieh *et al.*, 2000).

*Adhatoda vasica*, an evergreen shrub, is the important medicinal plant native to India. The leaves and roots showed hypoglycemic and antiulcer activities owing to the presence of alkaloids like vasicine, 1-vasicinone, maiontone, and vasicinol (Modak *et al.*, 1982; Shrivastava *et al.*, 2006). Flavonoids like apigenin, astragalin and kaempferol as well as triterpenes like daucosterol from flowers showed high antioxidant activity. The crude extract of *Adhatoda vasica* was thus protective against oxidative stress (Sen *et al.*, 1924; Jain *et al.*, 1982; Muller *et al.*, 1993).

The evaluation of antioxidant potential of leaf extracts of *Adiantum capillus veneris* was carried out by Kumar in 2009 against hydrogen peroxide induced oxidative damage in peripheral blood lymphocytes; and significantly reported its efficacy to inhibit lipid peroxidation and enhance the activities of antioxidant enzymes and glutathione content.
Adeolu et al. in 2008 reported the remarkable antibacterial and antioxidant activities in the methanol extracts of the leaves and stems of *Calpurnia aurea*, attributable to the presence of phenols, flavonoids, flavonols, tannins, quercetin and proanthocyanidins. The studies revealed that the level of phenolic compounds in the methanol extracts of the leaves and stem of *Calpurnia aurea* were considerable. The stem extracts consisted of higher levels of total phenol and flavonoids than the leaf extract. On the other hand, the leaf extract possessed higher levels of pro-anthocyanidins and total flavonols. DPPH radical scavenging activity of the methanolic extracts of leaves and stem, compared with BHT and ascorbic acid, revealed that the leaves exhibited higher activity than that of the stem. These extracts were found fast and effective scavengers of the ABTS radical.

The evaluation of some of the medicinal herbal extracts, used as folk medicines in Taiwan have been made for their antioxidant activities by DPPH radical scavenging, such as *Ludwigia octovalvis*, *Vitis thunbergii*, *Zanthoxylum nitidium*, *Lindernia anagallis* and *Rubus parvifolius*, and were reported to exhibit strong activities. The plant extracts of *Ludwigia octovalvis* and *vitis thunbergii* exhibited potent antioxidant effect, and served as good factors for further evaluation of their bio-efficacies, active constituents, and molecular and biological mechanisms in vitro as well as in vivo on antioxidation or cancer chemoprevention effects (Lie-Fen Shyur et al., 2005).

The investigation of the antioxidant effect of the different crude extracts (Petroleum ether, ethanolic and aqueous) of the plant. *Glycosmis pentaphylla* was done by Gupta et al. in 2011, and evaluation was done as well by various antioxidant assays, such as DPPH, ABTS Nitric oxide and hydrogen peroxide
scavenging method. It was reported that the plant extracts of *Glycosmis pentaphylla* showed the presence of steroids, alkaloids, glycosides saponins, flavonoids, tannins and carbohydrates that was attributed to their good antioxidant and scavenging ability. The ethanolic extract showed the maximum antioxidant activity which was significantly attributed to triterpenoid and polyphenolic as well as phytochemical constituents.

The important medicinal plant, *Olea europaea* (syn. Olive, Zaytoon) possessed high antioxidant activity, as investigated in the methanolic extracts of olive pulp / fruit, due to the presence of phenolic compounds; also it was reported to be rich in unsaturated fatty acids, oleic acid, palmitic acid and arachidic acids. Thus the olives are considered as good sources of natural antioxidants for medicinal and commercial uses (Kaskoos et al., 2009).

*Chrozophora tinctoria*, a native medicinal plant of Africa & Asia is reported for its efficacy in antioxidant activities as shown by the inhibition of lipid peroxidation and DPPH assay. Its high activities were due to the presence of flavonoids, alkaloids, coumarins etc. in its extracts (Delazar et al., 2006).

Silymarin, a purified extract from the seeds of *Silybum marianum*, also called ‘milk thistle’ consisted of most active components, isosilybin, silydianin and sily chrestin, and widely used as hepatoprotectant and in therapy of liver disorders. It is related to the antioxidant and free radical scavenging activity of silymarin enzymes. FRAP assay showed high antioxidant capacity of silymarin compared with other standard and green tea (Zarban et al., 2006).

The Iranian medicinal plant, *Achillea wilhelmsii* full of flavonoids and sesquiterpene lactones have been reported to possess good antioxidant property;
screening of the activity was done by lioleic acid peroxidation test. The plant extract was found effective in lowering blood lipids and hypertension (Sharififar et al., 2009; Souri et al., 2000).

Green tea made solely with the leaves of Camellia sinensis contains caffeine and two caffeine metabolites, theophylline and theobromine. It was tested for antioxidant activity with comparison with sodium metabisulfite and butilated hydroxyl toluene, and the green tea extract was found effectively antioxidant. (http://www.medicinal food news.com, 2006; Semnani et al., 2006).

The extract of Zea-mays, traditionally known as ‘corn silk’, is known for its efficient antioxidant activity. The ‘corn silk’ extract contained significantly phenol and flavonoids. The nitric oxide scavenging effect was reported to be less, and the reducing ability was found high. FTC (ferric thiocyanate) method showed the extract to exhibit 88% inhibition of linoleic acid peroxidation (Ebrahimzadeh et al., 2008).

Five plants from the rich Herbal state of India, Chhattisgarh, were evaluated by Jain et al. in 2011 for their bioactive phytochemical constituents and their antioxidant properties. The stem bark extracts showed the highest phenolic content in Buchanania lazan, followed by Sesbania grandiflora, Wrightia tinctoria, Alangium savifolium, Artocarpus heterophyllus respectively. Buchanania lazan exhibited the strongest antioxidant activity through all concentration of DPPH assay and reducing power method. Since the antioxidant activity of the plant extract is reported to often originate from phenolic compounds (Velioglu et al., 1998; Amarowicz et al., 2000), a correlation
between the total phenolic content and antioxidant activity in the plant samples is known.

The antioxidant activity of *Anethum graveolons* was investigated with DPPH free radical scavenging and β-carotene / linoleic acid. The presence of Carven and limoneme attributed to the antioxidant properties of *Anethum graveolons* and due to its high activity it could be used as a natural antioxidant in food stuffs (Sabeti, 1976).

Considerable antioxidant effect was seen in the extract of *Nepeta ispahanica*, the member of Lamiaceae and endemic to Iran. The antioxidant activity was attributed to the presence of 1,8-cineol, β-pinene, germacrene-D and α-pinene (Farag *et al.*, 1989).

The ethanolic extracts of *Smyrnium cordifolium* were investigated for antioxidant activity using 2,2-diphenyl, 1-picryl hydrazil for measurement of free radical scavenging activity. These assays showed high antioxidant activity of the plant extract (Khanahmadi *et al.*, 2010).

In 1976, the antioxidant activity of *Curcumin* was reported by Sharma *et al.* and its scavenger property against oxygen free radicals was reported by Ruby *et al.*, (1995) and by Subramaniam *et al.*, (1994). Its protective role for haemoglobin against oxygen free radical was also reported by UnniKrishnan *et al.*, (1995).

Manoharan *et al.* in 2009, have reported protective effect on *Jasminium grandiflorum* flower extract on cell surface abnormalities during DMBA-induced mammary carcinogenesis. The aim of the experiment was to investigate the protective effect of ethanolic extract of *Jasminium grandiflorum* flower (JgE
Fet) on cell surface integrity by measuring the status of lipids and glycoconjugates during DMBA induced mammary carcinogenesis. The result of their experiment showed that IgE Fet significantly protect the cell surface and membrane abnormalities during DMBA induced carcinogenesis.

Kaleena et al. in 2006 have evaluated the antioxidant potential of extract of *Hydrastis canadensis* (HC) against hydrogen peroxide induced oxidative damage in peripheral blood lymphocytes. They have incubated PBLs with 100µM H$_2$O$_2$ for 2 hours and found significantly increased lipid peroxidation and decreased level of glutathione and the antioxidant enzymes. But after treatment with extract of *Hydrastis canadensis* they have found inhibited lipid peroxidation and enhanced activities of antioxidant enzymes and glutathione content significantly. They concluded that the cytoprotection due to direct action in scavenging free radicals and thereby modulating the antioxidant defense system.

Free radical scavenging activity of *Lantana aculeate* root extract in hyperlipidemic rat was studied by Vinoth in 2009. In this study the root was studied for their free radical scavenging potential in hyperlipidemic animal by administering the alcoholic extract (LAR) in doses of 25, 50 and 100 mg/kg for 30 days. The level of LPO, non enzymatic antioxidant (TRG) and enzymatic antioxidants viz. SOD, CAT and GPx that showed changes in diseased condition were reverted back to near normal values by LAR extract treatment of plasma, liver and heart tissue. The presence of flavonoids besides Oleanolic acid in large amounts might have caused the observed effect.
Gajare et al. (2006) studied the effect of *Bacopa monniera* extract of lysosomal membrane integrity in the brain and heart of D-galatose induced oxidative stressed mice. *Bacopa monniera* is a medicinal plant used as memory tonic in Ayurvedic medicines. They studied antioxidant property of *Bacopa monniera* with respect to protection of the lysosomal membrane from the oxidative damage induced by D-galactose. For this purpose the female albino mice were treated with D-galactose and D-galactose in combination with *Bacopa monniera*. The lysosomal enzyme, acid phosphatase was measured in both lysosomal and post lysosomal fractions and ratio of lysosomal and post lysosomal acid phosphatase activity was studied. The ratio was more than one control group and less than one in D-galactose treated group. In *Bacopa monniera* co treated group the ratio was also more than one in various brain regions (cerebral cortex, hippocampus, corpora quadrigemina, cerebellum) as well as in heart (auricle and ventricle). This indicates that *Bacopa monniera* extract provides protection against oxidative stress and prevents lysosomal membrane leakage, thus acting as a potent membrane stabilizer.

*Echium amoneum* plants extract was found rich in flavonoids, saponins, unsaturated tepenoids and sterols attributing to its high activity. Flavonoids, linolenics acid and some amount of alkaloids present in the extracts establish the plants antioxidant properties, and help to prevent oxidative stress in humans (Ranjbar et al., 2006).

Patil et al. in 2009 investigated the antioxidant effects of methanol extract of seeds of *Phaseolous trilobus* from the forests of Satpura hills of Maharashtra, in their experiments in rats. The seed extract was reported efficient enough in reducing the number of free radicals, decreasing lipid peroxidation ad
maintaining the levels of GSH. The antioxidative and protective action of methanol extract of seeds of *Phaseolous trilobus* attributed to the presence of the flavonoids (Pourmorad *et al.*, 2006).

The extracts of leaves and seeds of *Coriandrum sativum* were evaluated by Wangensteen *et al.* in 2004 for their antioxidant property and found that their phenolic content attributed to the activity. They also reported that the coriander leaves showed stronger antioxidant activity than the seeds. The ethyl acetate extract of both the leaves and seeds contributed to the strongest activity, and showed that coriander have a potential natural antioxidant, that could inhibit unwanted oxidation process. In the carotenoid fractions, b-carotene has been identified as the principal antioxidant (Guerra *et al.*, 2005), reported that the leaves and stem of coriander extracts contain phenolic acids, the principle contents responsible for the antioxidant activity.

The plant of *Sphagneticola trilobata* is rich in bioactive sesquiterpene lactones, trilobolid-6-O-isobutyrates A and B. The flower extracts attributed their antioxidant activity to the presence of trilobolide-6-O-isobutyrate that shows a eudesmanolide sesquiterpene skelton (Validation of plants, 2007). It comprises of two cyclohexane rings and a lactone ring (Maldini *et al.*, 2009). Flower is reported to contain other bioactive compounds such as grandiflorenic acid, 1β-Acetoxy-4α, 9α-dihydroxy-6c-isobutyroxy-prostatalide and β-daucosterol (Melian *et al.*, 2009).

Brito *et al.* in 2006 reported the presence of Kaurenolic acid or diterpene besides eudesmanolide lactones and luteolin in the extracts of leaves and stems of *Sphagneticola trilobata* responsible for anti-leishmanial activity as well as
larvicidal activity. Luteolin showed efficacy in mutagenic and anti-oxidant properties with depressant action on smooth muscles (Block et al., 1998).

The antimicrobial and antioxidant activity of *Sphagnoticola trilobata* is mainly attributed to the presence of terpenoids and tannins (Singh et al., 2003). The abundance of tannins in all extracts is responsible for the potent bioactivities of the plant (Kaur et al., 2009).

The whole plant of *Sphagnoticola trilobata* and its iso-flavonoids rich in antioxidant activity are used for the treatment of liver disorders like hepatitis and cirrhosis (Subramonium et al., 1999).

The lipophilic extracts of *Sphagnoticola trilobata* are known to be the important sources of anti-inflammatory activity. The protein denaturation was inhibited by ethanol extracts of leaf (87.14%), followed by stem (86.76%) and flower (61.63%) (Govindappa et al., 2011). The antioxidant activity of ethanol extract of leaf, stem and flower of *Sphagnoticola trilobata* was also reported. The water extracts of fresh parts of stem and flowers have been reported to show high scavenging activity than the respective dry part extracts. It was also reported that the extracts showed the proton donating ability and thus could serve as free radical inhibitors or scavengers, possibly acting as primary antioxidants (Govindappa et al., 2011).

The plant extracts of *Sphagnoticola* have been reported to contain natural products such as flavonoids, terpenoids and steroids which owe to the various pharmacological and antioxidant properties. These are also responsible for the analgesic and anti-inflammatory activity (Bhargava et al., 1974).
Achyranthes aspera remarkably exhibit the phytochemical and pharmalogical properties. Various phytochemical constituents have been reported and isolated from the plant which possessed antiperiodic, antiallergic, diuretic, laxative and hepatoprotective activities alongwith the unique antioxidant and medicinal properties (Tijani et al., 2008).

The seeds of Achyranthes aspera were chemically investigated by Hariharan et al., 1970; Ali et al., 1993 and constituents like saponins A and B alongwith oleanolic acid, hentri acontane and aminoacids were isolated. Saponin A was identified as D-Glucuronic Acid and saponin β-D-galactopyranosul ester of D-Glucuronic Acid. Also three oleanolic acid glycosides were identified from the seeds of Achyranthes aspera (Rameshwar et al., 2007).

Alongwith the oleanolic acids from the seeds of the plant of Achyranthes aspera, sapogenin was also separated as reported by Khastgir et al., 1958.

Betanine, a water soluble alkaloid was isolated from the plant of Achyranthes aspera that was reported to affect the dilation of bold vessels and blood pressure (Kapoor et al., 1966).

The water soluble alkaloid, Achyranthine, was isolated from Achyranthes aspera which exhibited pharmacological activities like lowering of blood pressure, and increasing the rate and amplitude of respiration (Neogi et al., 1970).

Ecdysterone was isolated from the methanolic extract of roots of Achyranthes aspera exhibiting the high antioxidant activity (Banerji et al., 1970; Ikan et al., 1971) Oleanolic acid was also isolated from glycosidic fraction of the roots (Ram et al., 2004).
New aliphatic acid was isolated from the ethanolic extracts of the roots of *Achyranthes aspera* and found of phytochemical significance. It was identified as n-hexacos-14-enoic acid which responded positively to Liebermann Burchard test for sterols (Sharma *et al.*, 2009).

The constituents isolated from the methanol extract of the aerial parts like shoots and leaves of *Achyranthes aspera* were reported as three bisdesmosidic saponins (I-III), 20-hydroxyecdosone and quercetin-3-O-β—galactoside, flavonoids and triterpenoids that attributed to the antioxidant properties of the leaves (Kunert *et al.*, 2000; Gayathri *et al.*, 2009).

The rich presence of phytoactive constituents in the seeds of *Achyranthes aspera*, such as saponins and oleanolic acids, and other polyphenolic compounds were reported and found highly antioxidanty active by Malarvili *et al.*, 2009. These constituents attributed to the reduction in rate of lipid peroxidation and enhancement in free radical scavenging activity of the herbal seed powder.

The ethanolic and queuous extracts of *Achyranthes aspera* have been reported to exhibit free radical scavenging activity by Edwin *et al.* in 2008. Two methods, DPPH radical scavenging activity and superoxide scavenging activity were used to assess both the extracts, and thus the plant was found to exhibit good antioxidant effect, by preventing the formation of free radicals.

The aqueous and ethanol extracts of leaves of *Achyranthes aspera* when used for wounds showed free radical scavenging activity in erradicting the released free radicals that were responsible for damage of various cells structures and membrane. The antioxidant activity of the plat extract increased antioxidant
enzymes (SOD and catalase) levels, that are known to quench radicals and thus prevent damage of cell and inhibit lipid peroxidation (Singh et al., 2006).

The phytochemical investigation revealed that aqueous and ethanol extracts of leaves of *Achyranthes aspera* contain tannins, flavonoids, glycosides and alkaloids. Mainly flavonoids and also other phytochemical compounds present, account for antioxidant activity, free radicals scavenging action and immune-enhancing property of the plant (Vasudeva et al., 2006).

Jayakumar et al. in 2010 reported that the chloroform extract of stem of *Achyranthes aspera* showed high radicals scavenging potential. The inflorescence also exhibited higher activity that was found less than that of stem. Chloroform root extract, however, did not show any antioxidant activity, that was, most probably thought to be due to the interference of some chemical components present in the chloroform extract.

The antioxidant activity of ethyl acetate extract of *Achyranthes aspera* of root was reported to be higher than that of stem. The concentration and time-dependant evaluation showed that the ethyl acetate inflorescence extract exhibited the best antioxidant activity, followed by that of leaf, which was higher in activity than that of root and stem extract. The methanolic extract of all parts of the plant exhibited very high antioxidant activity which was closer to standard L-Ascorbic acid (Jaya Santhi et al., 2011).

Antioxidant potential of the methanol and aqueous extract of stem of *Achyranthes aspera* where measured by DPPH radical scavenging activity. It was found that methanol extract showed high antioxidant activity than that of aqueous extract (Jitendra et al., 2009).
Edwin et al. in 2008 reported the wound healing and antioxidant activity of *Achyranthes aspera* and their correlation was also evaluated. The tissue injury in the wounds, aggravates the production of reactive oxygen species (Lopes et al., 2005).

*Chrysanthemum* is the genus well known for synthesizing and accumulating a variety of secondary metabolites (Kumar et al., 2005). Several biological active natural products have been isolated from the different parts of the plant such as pyrethroids, sesquiterpenoids, flavonoids, monoterpenoids, coumarins, steroids, phenolics, purines and lipids. (Uchio et al., 1978 & 1981; Stoianova-Ivanova et al., 1983). Such phytochemicals are responsible for the various medicinal and antioxidant values of *Chrysanthemum* and such natural products are used in pharmaceuticals (Vantu et al., 2009).

Twelve bioactive compounds were identified in the *Chrysanthemum cinerariaefolium* by GC and GC-MS, the major compounds being Trans-β-farnesene (41.36%), β-cubebene (17.27%) and δ-nerolidol (14.23%) and the monoterpenoids were reported to be the important constituents (Saggar et al., 1997).

The essential oils / extracts of *Chrysanthemum coronarium* and *Chrysanthemum sibiricum* showed the presence of many biologically active compounds; significantly the petroleum ether diethyl ether compound that showed high cytotoxic properties, and mild antioxidant property (IC₅₀ = 97.2 Mg/ml) Flavonoids & luteolin isolated from the flowers of *Chrysanthemum morifolium* also exhibited cytotoxic activity against human colon cancer cells (Xie et al., 2009).
The pharmacological activities of the aqueous extract of leaves and stem of *Chrysanthemum indicum* have been reported owing to the presence of glycosides, flavonoids, tannins and alkaloids in the extracts of *Chrysanthemum* showed the presence of 63 phenolic compounds making this herb an important herbal medicinal plant exhibiting wide pharmacological effects and antioxidant activities (Lin *et al.*, 2010). Thus, the aerial parts such as stems, leaves and flowers are taken orally or used externally to treat vertigo, and various infectious diseases and dermatosis (Shunying *et al.*, 2005).

The inflorescence or bud of *Chrysanthemum indicum* constitute of high percentage of 1,8-cineole, camphor, flavones, flavone-glycosides, sesqiterpenes, chrysanthemol, due to which it forms traditional medicine used mainly for the treatment of inflammation, hypersensitive symptoms, respiratory diseases and in prevention of thrombosis (Cheng *et al.*, 2005; Levy *et al.*, 1988).

The extracts of *Chrysanthemum* are known to be the electron donor with reducing power like other antioxidants. Flavonoids, a subgroup of phenolic compounds present in *Chrysanthemum* extract contribute chiefly to its antioxidant activity, as the flavonoids can scavenge reactive oxygen radicals such as hydroxyl and superoxide radicals (Levy *et al.*, 1988).

The *Chrysanthemum* extracts also have been reported to exhibit significant inhibitory activity against peroxidation of lecithin, thereby reducing lipid peroxidation and playing an important role in protecting the cell membrane against any damage caused. The inhibitory activity against nitric oxide production was seen in the methanolic extract and ethyl-soluble portion of
flowers due to the presence of acetylene compounds and flavonoids, (Yoshikawa et al., 2000).

The presence of antioxidants and anti-carcinogenic properties have been reported from the extracts of *Chrysanthemum* plants by Hanen et al. in 2009 and Ding et al. in 2010, that attribute to the presence of the component, quercitrin. Quercitrin is reported to affect inhibition of neoplastic transformation by blocking activation of the MAPK pathway and stimulation of cellular protection signally.

Melatonin, along with adenosine have been reported as the effective dangerous substances within the brain, with free radical scavenging properties. Their concentrations were found to increase after seizures and altered in neurological conditions (During et al., 1992; Bazil et al., 2000).

The deficient oxidative defence mechanisms in the brain and the oxidative stress developed has been implicated in the development of various neurogenerative diseases. However, effectiveness of melatonin with its antioxidant properties have been reported by Antolin et al. in 2002 for the treatment of Parkinsonism and by Mevissen et al. in 1998 for epilepsy.

Melatonin has been reported as the most potential endogenous substance for the therapeutical exploitation in neural diseases because of it being a good antioxidant. The free radical scavenging ability facilitated melatonin to cross orthophysiological barriers like blood-brain barrier, intracellular and subcellular barriers with ease (Reiter et al., 1999).
It was first suggested by Ianas et al. in 1991, that melatonin played an important role in scavenging free radicals, and likely worked via electron donation to directly detoxify free radicals.

Reiter in 1995 reported melatonin as an antioxidant, which is most effective in protecting nuclear DNA, membrane lipids, as well as cytosolic proteins from oxidative damage. Also, it has been reported to alter the activities of enzymes that improve the antioxidative defense system of the organism.

Melatonin has been known to scavenge the hydroxyl ions, more than any other antioxidants which usually have less ability to quench the hydroxyl radicals as compared to melatonin. Melatonin’s property to scavenge \( \cdot \)OH resulted in the formation of cyclic 3-hydroxy-melatonin, a harmless product that is excreted in the urine (Tan et al., 1998; Poeggeler et al., 2002).

The ability of melatonin to detoxify \( \cdot \)OH radicals has been quite remarkable. Unlike other antioxidants, melatonin is known to directly neutralize the precursor of \( \cdot \)OH, i.e., hydrogen peroxide (H\(_2\)O\(_2\)) (Bromme et al., 2000; Tan et al., 2000).

Peroxyl radical (LOO\(^{\cdot}\)) generated during the process of lipid peroxidation, has been claimed by Pieri et al. in 1994 of being scavenged by melatonin.

Melatonin is reported to inactivate HOCl, i.e., Hypochlorous acid which has been known as the oxidizing agent with the capability of damaging a variety of molecules (Dellagar et al., 1999).
The comparison of melatonin’s efficacy with the classical antioxidants in terms of pharmacologically protecting against free radical damage, has been found better at a lower dose than other antioxidants (Montilla et al., 2001).

Melatonin is reported to stimulate many antioxidant enzymes including SOD, glutathione peroxidase and glutathione reductase. Melatonin is also known to promote the activity of GPx, thereby maintaining high levels of reduced glutathione (Albarran et al., 2001; Wakatsuki et al., 2001; Pablos et al., 1997).

The importance of melatonin as an antioxidant is reported due to its lipophilic and hydrophilic nature, its ability to cross the blood-brain barriers with ease, and its availability to all tissues and cells. Also, the antioxidant nature of melatonin is attributed to its protective effects against metal induced oxidative damage (Omurtag et al., 2008; Escames et al., 2009).

The accumulation of hippocampal cadmium and also modulation of other trace elements that lead to increased lipid peroxidation and disrupted antioxidant defence system can be prevented by melatonin. This unique ability of melatonin is attributed to its role in stabilizing and maintaining the blood-brain barriers (BBB). The trace elements and cadmium, otherwise serve as cofactors of antioxidant enzymes (Kostial et al., 1978).

The dopamine metabolism in dopaminergic neurons give rise to oxidative stress due to the generation of ROS. No generation contribute to neuronal cell death in Parkinson’s disease. Melatonin is reported to detoxify NO and NOS as well, and thus attribute to its potential use in Parkinson’s disease because of its free radical scavenging properties. Its synergistic effects have also been reported (Antolin et al., 2002; Khaldy et al., 2003).
Mukherjee et al. in 2010 reported through their studies of cadmium-induced oxidative stress in hippocampus, that melatonin effectively protected against the adverse effects of cadmium on endogenous antioxidant status including the depletion of GSH as well. The state of lipid peroxidation and free radical generation were reported to overcome by the antioxidant action of melatonin.

The administration of melatonin in pregnant rats resulted in the increase in the activities of GPx and SOD (antioxidant enzymes) in the fetal brain, as was reported by Baydas et al. in 2007.

Anaerobic metabolism and accumulation of lactic acid result in DNA fragmentation and cell death due to oxidative stress in many mammalian species, leading to ischemic brain injury or stroke. Melatonin, in such cases, have showed a remarkable capacity to reduce or inhibit neuronal cell death owing to its antioxidant ability (Manev et al., 1996; Cuzzocrea et al., 2000; Koh, 2008; Alonso-Alconada, 2012).

Neonatal asphyxia and reperfusion in case of hypoxic-ischemic brain lesions in newborns leads to overproduction of free radicals, oxygenated compounds such as MDA and increased lipid peroxidation. This is reported to be abolished my melatonin in late-gestation fetal sheep (Miller et al., 2005) and decrease MDA induced by hypoxia in rat pups (Tutunculer et al., 2005), attributed to the antioxidant property of melatonin.

AFMK and AMK are the metabolites of melatonin, formed by enzymatic / non-enzymatic metabolic pathways, and by deformylation respectively. They have been found to exhibit protective effects against oxidative stress, and are reported to reduce lipid peroxidation and oxidative DNA damage. They are able to
deactivate a wide variety of ROS and RNS, and known to scavenge ‘OH radicals (Burkhardt et al., 2001; Manda et al., 2007; Maharaj et al., 2002; Schaefer et al., 2009).

AFMK, the melatonin metabolite, has been reported to be less effective protector than AMK and melatonin regarding their relative antioxidant capacity (Hardeland, 2005; Silvo et al., 2004). AMK was found to be a potent singlet oxygen scavenger while AFMK is insert in this regard. AMK was reported as better NO and ROS scavenger than melatonin or AFMK, as well as efficient in preventing protein oxidation (Leon et al., 2006; Ressmeyer et al., 2003).

Hiroshi et al. in 2012 highlighted the role of melatonin as an antioxidant in reproductive physiology, where it functions as a free radical scavenger. High concentrations of melatonin present in human prevulatory follicular fluid, and its efficacy have been reported in oocyte maturation and embryo development as the antioxidant to reduce oxidative stress induced by ROS produced during ovulation process.

The lipophilic and hydrophilic properties of melatonin enable it to easily pass through cell membranes, and thus, act as cell protectors and detoxific agents of hydroxyl radicals (‘OH) owing to its antioxidant properties. Such high activities of melatonin attribute to its ability to suppress the oxidative effects of ROS indirectly by enhancing the production of endogenous antioxidants (Hardeland, 2005; Allegra et al., 2003.)

Melatonin has been known to stimulate activities and m-RNA levels of antioxidative enzymes including SOD, GPx and catalase (Mayo et al., 2002; Rodriguez et al., 2004). Thus, the multiple actions of melatonin protect cells from ROS-mediated lipid peroxidation, protein destruction and nuclear DNA...
damage (Chen et al., 2005; Melchiorri et al., 1995; Ortego-Guitierraz et al., 2007).

Melatonin concentrations were reported to be highest in proestrus, i.e., when the ovary had preovulatory follicles, during the estrus cycle, as in the serum of hamsters. The oxidative stress due to the generation of ROS during the process is neutralized / reduced by the high antioxidant activities of melatonin. Thus, melatonin levels in ovarian follicles increase depending on follicular growth (Tamura et al., 1998).

High quality oocytes produce well developed embryos. But during oocyte maturation and its meiotic division, oxidative stress is developed due to the release of free radicals. The melatonin incubated oocytes showed decrease in oxidative stress as well as in blocking of inhibitory effects of H₂O₂ (Shi et al., 2009).

Erten et al. in 2003 reported in the studies of melatonin effects on spinal cord ischemia, where the presence of melatonin activated the levels of antioxidant enzymes, that were otherwise decreased due to free radical generation in the damaged tissues.

Roth et al. in 2001 demonstrated melatonin’s ability for directly promoting oesteoblast maturation in preoesteoblast and rat oesteoblast-like oesteosarcoma cells. In these cells, low concentrations of melatonin increased the mRNA levels of several genes expressed in oesteoblast. Melatonin is also known to prevent bone deterioration (Satomura et al., 2007).
Papis et al. in 2007, demonstrated the beneficial effects of melatonin on bovine embryo development which was observed in high oxygen environment where free radicals are easily produced.

The recent studies on ‘effects of topical melatonin and vitamin E in a rat ischemic wound’ showed that the administration of melatonin was not effective in healing of wounds as compared to vitamin E. Although topically applied melatonin was found to stimulate the antioxidant enzymes and decrease MDA levels, it decreased the collagen synthesis and thus delaying the wound healing process (Mehmet et al., 2011).