Review of Literature
REVIEW OF LITERATURE

TISSUE CULTURE STUDIES IN LAMIACEAE

In vitro cell and tissue culture methodology is envisaged as a means for germplasm conservation to ensure the survival of endangered plant species, rapid mass propagation for large scale revegetation and for genetic manipulation studies. In addition, tissue culture techniques are widely used for the commercial propagation of a large number of plant species, including many medicinal plants (George and Sherrington, 1984; Rout et al., 2000). Lamiaceae is a large family that occurs worldwide and has species that are adapted to almost all habitats and altitudes. The family is well known for its medicinal, food and ornamental plants. Wang and Staba (1963) attempted callus formation and cell suspension culture studies in Mentha species.

In vitro plant regeneration from excised nodal segments and subsequent establishment of plantlets in soil has been reported in Ocimum gratissimum and O. viride. BA in combination with NAA or IAA was more effective for inducing axillary bud break, multiple shoot formation and complete plantlet regeneration. In O. viride complete plantlets were recovered on a medium containing BA (1.1 mg/l) and NAA (1.8 mg/l), whereas in O. gratissimum BA (1.1 mg/l) in combination with IAA (0.17 mg/l) favoured complete plantlet regeneration (Ahuja et al., 1982).

Lavandula latifolia leaf explants were cultured on MS medium supplemented with different concentrations and combinations of IAA and NAA with BA. Bud regeneration was achieved in media containing BA (0.6µM) and IAA/NAA (0.6µM). MS medium supplemented with coconut milk (20%), IAA (0.57µM) and (8.88µM) BA was effective for elongation of shoots derived from hypocotyl explants (Calvo and Segura, 1989). Cell culture and secondary metabolite production was reported in different species of Lavender by Segura and Calvo (1991).

Funk et al. (1992), Hippolyte et al. (1992) and Tawfic et al. (1992) established the callus, cell suspension, immobilized cell and hairy root cultures in Salvia officinalis and S. canariensis (Luis et al., 1992). Hu and Alfermann (1993) and Morimoto et al. (1994)
reported diterpenoid production in hairy root culture and lithospermic acid B and rosmarinic acid in cell suspension culture of *S. miltiorrhiza*.

Natural compounds like anthocyanins and rosmarinic acids were produced in cell and hairy root culture of *Ocimum basilicum* by Madhavi *et al.* (1995) and Tada *et al.* (1996). Multiple shoot formation was induced from shoot bud explants of *O. americanum* and *O. sanctum* on MS medium supplemented with BA (0.25 and 1.0 mg/l) (Sitakanta Pattnaik and Pradeep Chand, 1996). Micropropagation of *Lavandula latifolia* was achieved from axillary buds of mature field grown plants when cultured on MS medium supplemented BA (5μM). Shoots were rooted on MS medium with macronutrients at half strength (Carlos and Carmen, 1996).

Adventitious roots of *Ajuga reptans* were cultured in half strength MS liquid medium supplemented with NAA (1mg/l) produced high content lithospermic acid B. Calli cultured on half strength MS solid medium containing 2, 4-D (1mg/l) produced large amount of rosmarinic acid (Yoshie Murakami *et al.*, 1997). Carvone, rosmarinic acid and caffeic acid derivatives were detected in *Mentha piperita* shoots cultured on MS solid medium (Toshio Omoto *et al.*, 1998). Internodal region of *M. arvensis* cultured on MS medium supplemented with NAA (0.5-2 μg/ml) induced callusing. NAA (1μg/ml) in combination with BA (5μg/ml) produced multiple shoots from the callus tissue (Ajit Kumar Shasany *et al.*, 1998).

Singh and Sehgal (1999) obtained an actively growing whitish compact callus on MS + 2, 4-D (0.5 mg/l) from inflorescence explant of *Ocimum sanctum*. The callus subcultured on MS medium fortified with BA (1mg/l) induced multiple shoots. IAA (0.05mg/l) along with BAP (1.0mg/l) enhanced the number of shoots. Callus was induced from the leaf explants of *Salvia officinalis* and *S. fruticosa* on MS medium supplemented with 2, 4-D (1.8-18 μM). NAA (10.5 and 10μM) in combination with BA (10.5 and 21μM) induced somatic embryos (Kintzios *et al.*, 1999).

Cuenca and Amomarco (2000) reported shoot proliferation from the nodal explants of *Salvia valentina* on MS+Kin (4.6-9.3 μM). In *Rosmarinus officinalis*, zeatin
(1mg/l) and IAA (0.1mg/l) proved to be the best combination for the production of green callus followed by the formation of shoots (Caruso et al., 2000). Nodal explants of Mentha arvensis cultured on MS medium supplemented with TDZ (10 μM) and inter nodal explants cultured in modified MS medium supplemented with BA (40μM) NAA (0.5 μM) produced multiple shoots (Savithri Bhatt et al., 2001).

Sairam Reddy et al. (2001) developed a protocol for high frequency shoot organogenesis and plant establishment in Coleus forskohlii from leaf derived callus. Callus developed from mature leaves on MS medium supplemented with kinetin (2.4 μM) alone. Shoots were regenerated from the callus on MS medium supplemented with kinetin (4.6 μM) and NAA (0.54 μM). In Cunila galioides BA at 17.6 μM induced callus formation and BA at 8.8 μM produced multiple shoots from the axillary bud (Fracaro and Echeverrigaray, 2001).

In vitro clonal propagation of native Mediterranean Lavandula viridis was obtained using nodal explants on half strength MS medium supplemented with BA (0.67 μM). Shoots were easily rooted on GD (Gresshoff and Doy) medium and increasing sucrose concentration from 58.4 to 87.6 μM resulted in a significant increase in rooting frequency (Dias et al., 2002). Multiple shoots were obtained on MS medium containing BA (2.22μM) from the nodal explants of Orthosiphon spiralis. Half strength MS medium supplemented with IBA (4.9 μM) produced roots from micro shoots (Elangomathavan et al., 2003). MS medium supplemented with BA (1.5 mg/l) and 2, 4-D (0.05 mg/l) produced highest number of shoots from the nodal segments of Salvia officinalis (Paula Santos and Manuel Fernandes, 2003).

In Salvia nemorosa shoot proliferation from shoot tip explant was obtained on MS medium supplemented with BA (8.9 μM) and IAA (2.9 μM). Multiple shoot induction was achieved through callusing on MS+ BA (0.9 μM) and IAA (2.9 μM) from leaf lamina. MS medium fortified with IAA (0.6μM) showed root induction (Ewa Skala and Halina Wysokinska, 2004). Callusing was obtained from stem and petiole explant of S.canariensis on MS + BA (4.44 μM) and NAA (10.74 μM). Multiple shoots were
obtained in the same medium when the concentration of NAA was reduced to 2.69 μM. (Mederos Molina, 2004).

Axillary buds of *Lavandula dentata* cultured on MS medium supplemented with BA (2.2 μM) and IBA (2.5μM) showed shoot multiplication and elongation (Echeverrigaray *et al.*, 2005). Rani *et al.* (2006) developed a rapid and highly effective method for micropropagation from nodal and shoot tip explants in *Coleus blumei*. Nodal segments and shoot tips cultured on MS medium containing BA (2 mg/l) and NAA (1 mg/l) showed multiple shoot induction.

Nodal explants of *Ocimum gratissimum* cultured on MS medium fortified with BAP (0.5 mg/l) and IAA (0.25 mg/l) showed maximum number of multiple shoots (Gopi *et al.*, 2006). *In vitro* seed germination was observed in *Salvia brachyodon* on MS +GA3 (1mM). The nodal segments excised from the seedling produced multiple shoots on half strength MS+BA (0.01 to 30.0 μM) and IAA (0.57 μM) (Misic *et al.*, 2006).

*In vitro* seed germination of *Hoslundia opposita* was achieved on MS basal medium with sucrose and agar. Shoot multiplication was observed from the seedling nodal explants when cultured on MS medium containing BA (2.2-11μM). *In vitro* regenerated shoots developed roots on MS medium supplemented with IAA (3.6 μM) (Prakash and Van Staden, 2007). Hypocotyl explants of *Savia africana-lutea* cultured on MS medium supplemented with BA (4.4 μM) and NAA (2.7 μM) formed adventitious shoots and continuous subculture in the medium fortified with BA and IAA resulted in shoot multiplication (Makunga and Van Staden, 2008).

**PHYTOCHEMISTRY**

Plants are the traditional source of many chemicals used as pharmaceuticals. Most valuable phytochemicals are products of plant secondary metabolism. The production of secondary metabolites *in vitro* can be possible through plant cell culture (Barz and Ellis, 1981; Deus and Zenk, 1982). Lamiaceae is characterized by the presence of essential oils. Many biologically active essential oils have been isolated from various members of this family. An unusually large number of useful secondary metabolites belonging to various chemical groups such as terpenoids, phenolic compounds and flavonoids have been
isolated from several members of this family (Richardson, 1992; Lu and Yeap Foo, 2002; Zegorka and Glowniak, 2001).

The genus *Isodon* is rich in diterpenoids. The phytochemical studies on *Isodon* diterpenoids can be traced back to 1910. Enmein a major bitter principle was isolated from *I. japonica* by three Japanese research group in 1958. Natsume and Iitaka (1966) first elucidated the structure of enmein. During the mid 1970s, some diterpenoids isolated from *I. japonica* were found to possess highly selective antibacterial and anticancer activities (Kubo *et al.*, 1974).

Sun *et al.* (2001) isolated and characterized about 500 new diterpenoids (mainly ent-kaurenoids) with different oxygenations and cleavage patterns. Most importantly, a number of those isolated diterpenoids have been found to have potent antitumor and anticancer activities with low toxicity. Sun *et al.* (2006) made a thorough phytochemical and pharmacological investigation of diterpenoids in *Isodon* and reported C-20 non-oxygenated ent-kaurenes, C-20 oxygenated ent-kaurenes, 6,7-seco-ent-kaurenes, 8,9-Seco-ent-kaurenes, 8,15-Seco-ent-kaurenes, 15,16-seco-ent-kaurenes, 7,20-cyclo-ent-kaurenes, ent-kaurene dimmers, miscellaneous ent-kaurenes, ent-gibberellane, abietanes and ent-abietanes, tricyclic and bicyclic diterpenes.

A new diterpenoid, megathyrin A, together with three known compounds, rabdocoetsins B, C, and D were isolated from the leaves of *Isodon megathyrsus* (Sun *et al.*, 1994). Abietane diterpenoids namely 16-acetoxy-7α, 12-dihydroxy-8, 12-abietadiene-11, 14-dione has been isolated from the acetone extract of the root of *Plectranthus hereroensis* and this compound showed antibacterial activity against *Vibrio cholerae*, and antiviral activity against *Herpes simplex* type II (Olga Batista *et al.*, 1995).

Sun *et al.* (1995a) isolated one new diterpenoid loxothyrin A and one known diterpenoid, adenolin B, from *Isodon pleiophyllus* and coetsoidins A, B, G, and one known diterpenoid, longikaurin F from *I. adenoloma*. Three new cytotoxic diterpenoids, gesneroidins A, B and C, together with one known diterpenoid, dawoensin A, were isolated from *I.gesneroides* and the structures were determined by combination of one and two dimensional NMR techniques (Sun *et al.*, 1995b).
Acetone extract of the whole plant of *Plectranthus grandidentatus* yielded abietanes royleanone, 6,7-dehydroroyleanone, horminone, 6β-hydroxyroyleanone, 7α-acetoxy-6β hydroxyroyleanone and the abietane dimmers such as grandidone C, grandidone D and 7-epigrandidone D, together with a mixture of fatty acid esters of 7α-acyloxy-6β,12-dihydroxy-abieta-8,12-diene-11,14-dione (Antonio Teixeira *et al.*, 1997). Six new *ent*-kaurene diterpenoids, lungshengenins B-G together with three known diterpenoids, lungshengenin A, inflexin and lushanrubescinsin C were isolated from the leaves and tender branches of *Isodon lungshengensis*. Their structures were elucidated by means of spectroscopy, mainly 1D and 2D NMR techniques by Bei Jiang *et al.* (1999).

A new *ent*-kaurene diterpenoid, phyllostachysin C together with five known compounds, sculponeatins B and C, nodosin, ursolic acid and 2α-hydroxyursolic acid were isolated from the leaves of *Isodon phillostachys* (Hou *et al.*, 2000). Bei Jiang *et al.* (2000) isolated a new diterpenoid called 11β-hydroxyisopimara-8, 15-diene-3-one from the leaves of *Lophanthoides*, in addition to lophanic acid and 8(17), 12, 14-labdatriene-19-oic acid. Three new eudesmane sesquiterpenes, plectranthone, desacetylplectranthone, isodeacetylplectranthone and the three known flavonols such as pachypodol, casticin, and chrysosplenol D were isolated from the aerial parts of *Plectranthus ciliatus* (Khaled Orabi *et al.*, 2000).

Baolin Li and Xianhua Tian (2001) isolated the enmein type diterpenoids, taibaijaponicains A and B from the ethanol extract of the leaves and branches of *Isodon japonica* and their structures were designated as 6β,11α-dihydroxy-16 α -methoxymethyl-6,20-epoxy-6,7-seco-ent-kaur-15-one-1,7- olide and 3β -acetoxy-6α,11α -dihydroxy-16α -methoxymethyl-6,20-epoxy-6,7-seco-ent-kaur-15-one-1,7-olide, respectively, on the basis of detailed spectroscopic analyses. 7, 20-epoxy *ent*-kaurene diterpenoids like xerophilusins G and I-K were isolated from the leaves of *I. xerophilus* along with four known enanderianin C, rosthorian A, longikaurin B and rabdoterin D were isolated. The structure and stereochemistry were confirmed by X-ray crystallography (Hou *et al.*, 2001).

Zhi Na *et al.* (2002) isolated 11 known *ent*-kaurene diterpenoids such as macrocalin B, xerophilusin A, trichorabdal A, trichorabdal B, effusin, angustifolin,
longikaurin D, longikaurin F, enanderinanin B, xerophilusin G and shikokianin from the aerial parts of *Isodon enanderianus*. In addition, 6-epiangustifolin (11α-hydroxy-6α-methoxy-6, 19-epoxy-6, 7-seco-ent-kaur-16-en-15-one-7, 20-olide) and enanderinanins F-H were also isolated. Methanolic extract of the leaves of *I. rubescens* var. *lushanensis* was found to contain four new ent-kaurene diterpenoids lushanrubescensins F-I together with 11 known ones, lasiodonin, oridonin, ponicidin, isodonoiol, isodonal, rabdosin B, rabdoternins A and B, enmenol, epinodosin and inflexusin and their structures were elucidated by spectroscopic analysis (Han *et al.*, 2003).

Three new ent-kaurene diterpenoids, oreskaurins A-C together with ten known ent-kaurene diterpenoids, enmenin monoacetate, effusanin E, adenolin B, maocryystal G, enmelol, trichokaurin, sodoponin, trichorabdal A, nodosin, enmein, and a flavonoid, vitexin were isolated from *Isodon oresbius*. Their structures were determined by spectroscopy (Wei Xiang *et al.*, 2004). From the leaves of *I. rubescens* var. *rubescens*, five new ent-kaurene diterpenoids xindongnins H-L together with five known ones, xindongnins A and B, dawoensin A and glabcensin V were isolated (Han *et al.*, 2004).

Isolation and structure elucidation of nine new ent-kaurene diterpenoids, albopilosins B-J, together with six known analogues, albopiliosin A, macrocalyxin C, rabdokunmin C, excisanin, amethystonoic acid and coetsanoic acid were reported from the aerial parts of *Isodon albopilosus*. Their structures were established using 1D and 2D NMR analysis (Huang *et al.*, 2005). A trihomoabietane diterpenoid and an unusual addition of acetone to the ortho-quinone system of cryptotanshinone have been isolated from an acetone extract of *Plectranthus grandidentatus* (Marques *et al.*, 2005).

Three labdane diterpenoids, 6-O-acetylforskolin, 1, 6-di-O-acetylforskolin and 1, 6-di-O-acetyl-9-deoxyforskolin were isolated from acetone extract of *P.ornatus*. Their structures were closely related to that of forskolin which was determined by NMR studies (Patricia Rijo *et al.*, 2005). Several diterpenoids such as oridonin, ponicidin, xindongnin A, and xindongnin B were isolated from *Isodon rubescens*. These compounds were found to be potent inhibitors of NF-κB transcription activity and the expression of its downstream targets cyclooxygenase-2 and inducible nitric-oxide synthase (Leung *et al.*, 2005).
Phytochemical studies on the secondary metabolites of *Isodon rubescens* has led to the isolation of three new dimeric *ent*-kaurenoids, bisrubescensin A, bisrubescensin B and bisrubescensin C and two known *ent*-kaurenoids, oridonin and rabdotemin (Huang et al., 2006). Bioactivity guided isolation of methanol extract of *Lexicus* yielded five new diterpenoids, excisusin A-E along with seven known compounds, inflexarabdonin I, inflexarabdonin G, inflexin, inflexanin A, inflexanin B, inflexinol and inflexarabdonin A. The structures were determined by 2D NMR (Hong et al., 2007).

**ANTIOXIDANT STUDIES**

Production of free radicals and other reactive species in cells and body tissues has been linked to aging and more than one hundred diseases (Maxwell, 1995). Antioxidant treatment may terminate the attack of reactive species and reduce the risk of coronary heart diseases, cancers and other aging associated diseases (Rice-Evans et al., 1996). Dietary antioxidants serves as one of the source of protection that the body needs to protect against the damaging effects of reactive species (Prior et al., 1998). Several studies have demonstrated that plants produce potent antioxidants and represent an important source of natural antioxidants (Santos Gomes et al., 2003; Couladis et al., 2003 and Es-Safi Ne et al., 2005).

The Lamiaceae family includes a large number of plants that are well known for their antioxidant properties. Among these, rosemary and sage have been widely used and most of their antioxidant components have been identified. It has been established that the antioxidant effects are mainly due to phenolic compounds (Das and Pereira, 1990; Pokorny, 1991; Schwarz and Ternes, 1992). Cuvelier et al. (1994) reported antioxidant, antiinflammatory, hypoglycemic and antimutagenic activities of *Salvia officinalis*. Essential oils, flavones, and rosmarinic acid isolated from the ethanolic extract of *S.lavandulaefolia* were reported to be antioxidants (Dorman et al., 1995).

Antioxidant activity of sage and rosemary had been attributed mainly due to the presence of carnosic acid and rosmarinic acid (Cuvelier et al., 1996). Abietane diterpenoids such as carnosic acid, royleanonic acid, carnosol and rosmanol have isolated from sage and rosemary plants showed good antioxidant abilities (Gu and Weng, 1997).
Many herb species, such as oregano and thyme showed strong antioxidant activity (Hirasa and Takemasa, 1998). The antioxidant activity of acetone extract of thyme was evaluated in sunflower oil and its 20% oil in water emulsion. The thyme extract inhibited generation of hexanal and pentanal in both the oil and in the emulsion (Abdalla and Roozen, 1999).

In *Salvia officinalis*, polyphenols including flavone glycosides and some rosmarinic acid derivatives such as salvianolic acid K, salvianolic acid I, sagecoumarin and sagerinic acid were found to display potent antioxidant activity against DPPH and superoxide anion radicals (Lu and Yeap Foo, 2001). Thin layer chromatographic examination of aqueous extracts of sweet marjoram, sage and *origanum* extracts showed that the extracts were rich in bound forms of phenolic compounds such as hydroxycinnamic acids, flavonoids, rosmarinic and caffeic acids to which have a remarkable capacity in retarding lipid oxidation (Triantaphyllou et al., 2001).

The essential oils from some *Mentha* species including *M. spicata*, *M. piperita*, *M. arvensis*, *M. rotundifolia*, *M. suaveolens* and *M. pulegium* showed wide range of antioxidant and other biological activities (Kaur and Kapoor, 2002). Deodorized aqueous extracts of *Origanum vulgare*, *Rosmarinus officinalis*, *Salvia officinalis* and *Thymus vulgaris* showed iron reduction capacity, DPPH, ABTS$^+$ and OH radical scavenging activities (Dorman et al., 2003).

Koleva et al. (2003) reported the antioxidant activity of *Sideritis scardica*, *S. montana* and *S. syriaca* methanolic extracts in the range of 48.2 to 72.5%. Acetone extracts of Iranian *Ocimum* accessions (*O.basilicum*) showed total antioxidant activity ranging from 10.8 to 35.7µM. The observed antioxidant activity was attributed to the phenolic compounds (Javanmardi et al., 2003). DPPH radical and hydroxyl radical scavenging activity as well as the inhibitory activity of the superoxide anion of both rosmarinic acid and rosmarinic acid methylester of *Lepechinia graveolens* were evaluated by Irene Parejo et al. (2004). Iron induced lipid peroxidation inhibiting activity of *Teucrium polium* was most effective and the IC$_{50}$ value was calculated as 16µg/ml (Predrag Ljubuncic et al., 2005).
The free radical scavenging activities of *Salvia condensata* and *S. erythrantha* extracts were found as 72.01±1.93 and 71.48±1.95% respectively. The antioxidant activity by phosphomolybdenum method of both extracts was observed as 279.37±3.61 and 146.11±3.11 mg/g respectively (Gulcan Ozkan *et al*., 2005). Bektas Tepe *et al.* (2005) studied the free radical scavenging activity and inhibition of linoleic acid peroxidation by aqueous methanol extract of *S.tomentosa*.

Polar fractions of *Melissa officinalis*, *Mentha suaveolens*, *Salvia officinalis*, *Lavandula angustifolia* and *L.pedunculata* showed appreciable DPPH radical scavenging and prevention of β-carotene bleaching activities (Ferreira *et al*., 2006). The hydromethanolic extract of *Salvia verbenaca* showed a significant antioxidant effect at 100μg/ml and strong inhibition of oxygen consumption and conjugated dienes formation of low density lipoprotein peroxidation as well as linolenic acid oxidation (Khlifi *et al*., 2006).

Methanol extracts of six *Salvia* species such as *S. caespitosa*, *S. hypargeia*, *S. euphratica*, *S. sclarea*, *S. candidissima* and *S.aethiopis* showed their possible antioxidant activities by two complementary test systems, namely DPPH free radical scavenging and β-carotene/linoleic acid systems (Bektas Tepe *et al*., 2006). Freeze dried aqueous extract of *Coleus aromaticus* showed notable inhibitory activity in the β-carotene-linoleate model system, moderate concentration dependent inhibition of the DPPH radical, significant superoxide and nitric oxide scavenging activity and also ferrous ion chelating potency (Kumaran and Joel Karunakaran, 2006).

The methanolic extract of *Ocimum gratissimum* had a significant DPPH radical scavenging activity of 84.6% at 250μg/ml and a reductive potential of 0.77 at 100μg/ml (Akinnmoladun *et al*., 2007). Methanol extract of *Sideritis sipylea* and acetone extract of *Origanum sipyleum* showed hydroxyl radical scavenging activity with an IC<sub>50</sub> value of 1.1μg/ml and 3.6μg/ml respectively. DPPH radical scavenging activity of different solvent extracts of *S. sipylea* and *O.sipyleum* ranged an IC<sub>50</sub> value from 0.05-1.05 mg/ml (Nakiboglu *et al*., 2007).
The main components of the essential oils of Tunisian *Thymus capitatus* were carvacrol, p-cymene, γ-terpinene and β-caryophyllene. The essential oils were showed positive reductive potential, DPPH radical scavenging and inhibition lipid peroxidation activities (Bounatirou *et al*., 2007). Dimethyl lithospermate isolated from *Salvia miltiorrhiza* scavenged peroxynitrite formed from superoxide and nitricoxide and to protect cells against reactive species (Kim *et al*., 2008).

**ANTIBACTERIAL AND ANTICANCER STUDIES**

Renewed interest in plant antimicrobials has emerged during the last 20 years (De Smet, 1997), probably due to the increasing development of drug resistance to human pathogenic organisms, as well as the appearance of undesirable side effects of certain antibiotics and the emergence of previously uncommon infections (Davies, 1994).

It is well-known that most spices, especially those belonging to the Lamiaceae family possess a wide range of biological and pharmacological activities. Since ancient times they have been used to improve the flavor and the organoleptic properties of different types of food. The antimicrobial activity of *Salvia officinalis* was attributed to the presence of 1, 8-cineole, thujone and camphor (Jalsenjak *et al*., 1987; Sur *et al*., 1991). Ulubelen *et al.* (1994) studied the antimicrobial properties of caryophyllene and caryophyllene oxide from *Salvia sclarea* and *Satuleja coerulea*. The essential oil of *Origanum vulgare* ssp, *hirtum* was extremely bactericidal against gram positive and gram negative strains at 1/4000 dilution and even at dilutions as high as 1/50000 caused considerable decrease in growth rates (Sivropoulou *et al*., 1996).

Dorman and Deans (2000) reported the antibacterial activity of α-pinene and borneol from the essential oil of *Salvia officinalis* and *S. triloba*. The essential oils obtained from *Mentha piperita* showed inhibitory effects against *Staphylococcus aureus*, *S.epidermidis*, *Listeria monocytogenes*, *Pseudomonas syringae*, *P. syringae* pv. *syringae*, *P. syringae* pv. *phaseolicola*, *Xanthomonas campestris* pv. *campestris*, and *X. campestris* pv. *phaseoli*. All peppermint oils showed stronger inhibition with an MIC value of 0.07-1.25 mg/ml (Iscan *et al*., 2002).
Chloroform extract of *Salvia hortensis* was found to be effective against *Bacillus subtilis*, *Enterococcus fecalis*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, and *Streptococcus pyogenes*. The essential oil had great potential of antimicrobial activities against 23 bacterial and 15 fungal species (Gulluce et al., 2003). The essential oil obtained from *Ocimum acutidens* exhibited strong antimicrobial activity with a significant inhibitory effect against 77% of the 35 bacterial species (Munevver Sokmen et al., 2004).

Ethanol extracts of *Mentha longifolia* and *Melissa officinalis* were effective on methicillin resistant *Staphylococcus aureus*. The minimum inhibitory concentration and minimum bactericidal concentration values of the ethanolic extract of *Mentha longifolia* and *Melissa officinalis* were in the range of 3.125 to 12.50 mg/ml and 12.50 to 25.00 mg/ml, respectively (Bassam et al., 2006). Abietane diterpenoids such as salvipisone, aethiopinone, 1-oxoaethiopinone and ferruginol isolated from the hairy root cultures of *Salvia sclarea* showed antibacterial activity against *Staphylococcus aureus*, *S.epidermidis* and *Enterococcus faecalis* at the concentrations of 37.5-75.0μg/ml (Kuzma et al., 2007). Essential extracted by hydrodistillation and solvent free microwave methods from *Origanum glandulosum* were more active against *Escherichia coli*, *Staphylococcus aureus* and *Salmonella thyphimurium* with inhibition zones of 24-27, 23-24 and 25-26 mm respectively (Bendahou et al., 2008).

The use of Lamiaceae species in treatment of cancer has been reported by Nago and Itoz (1982) and (Qian) 1987. Ethanol extract of *Isodon rubescens* was anti-leukemic due to the presence of several isoprenoids including ponicidine, oridonin and other terpenes (Fuji et al., 1988). Abietane diterpenes of the royleanone and coleon types from *Plectranthus hereroensis* and *P.grandidentatus* showed antitumor and antimicrobial activities (Olga Batista et al., 1994).

Baicalin from *Scutellaria baicalensis* have been shown to block cell proliferation and induce apoptosis in several *in vitro* models of human cancer, including human bladder cells (Ikemoto et al., 2000), prostate cell lines (Chen et al., 2001) and hepatoma (Chang et al., 2002a). Aqueous extract of *Clinopodium vulgare* showed strong antitumor activity when tested *in vitro* on human metastatic melanoma, epidermoid carcinoma and
mouse lymphoma cell lines. The concentrations of aqueous extract inhibiting the growth of the cells by 50% were calculated to be 20, 10 and 17.8μg/ml respectively (Balik Dzhambazov et al., 2002).

Abietane diterpenes of the royleanone and coleon type, namely fatty acid esters of 7α-acyloxy-6β-hydroxyroyleanone, grandidone A, 7α-acetoxy-6β-hydroxyroyleanone and coleon U isolated from Plectranthus grandidentatus showed their inhibition on the proliferation of human lymphocytes induced by phytohaemagglutinin (Cerqueira et al., 2004). According to Karl Franek et al. (2005), the purified plant flavins scutellarin, baicalin and extracts purified from Salvia miltiorrhiza showed their antiproliferation effects on the human breast cancer, mammary ductal carcinoma (T-47D) and the human head and neck cancer epithelial cell lines (CAL-27). Oridonin isolated from Isodon rubescens exhibited antiproliferative activity against prostate, breast and ovarian cancer cell lines with an IC_{50} ranging from 5.8±2.3 to 11.72±4.8 μM (Sophie Chen et al., 2005). Ethanol extract of Salvia mirzayani showed protective effect on H_{2}O_{2} induced cytotoxicity in human non-immortalized fibroblasts (Soheila Moein et al., 2007). Ent-kaurene diterpenoid nervonin A-J isolated from I.nervosus showed cytotoxic activity against K562 (Human leukemia) and HepG2 cells (Hepatoma) (Li et al., 2008).

ANTICARCINOGENIC STUDIES

Studies of genotoxicity and antigenotoxicity of natural plant extracts can help us to evaluate the safety and effectiveness of herbal health products (Bast et al., 2002). DNA damage and oxidative stresses are accepted to be major factors in many degenerative diseases and in the aging process (Ceruti, 1985). Accumulating data from in vitro and short term in vivo studies as well as long term carcinogenicity studies with chemically treated animals continue to show that phytochemicals could possess anticarcinogenic and antimitagenic effects (Mitscher et al., 1992).

Tanshinones from Salvia miltiorrhiza enhanced the mutageniticity of Trp-P-1 (3-amino-1, 4-dimethyl-5-H-pyridol [4, 3-b] indole) and B (a) P (benzo[a]pyrene) in Ames test using Salmonella typhimurium TA98 (Sato et al., 1992). Quercetin is important phenolic compound abundantly present in Lamiaceae. A diet rich in quercetin
has been reported to inhibit the development of carcinogen induced oral cancer (Makita et al., 1996). Quercetin may account for the beneficial effects of dietary fruits and vegetables on mutagens and carcinogens including metals.

Duthie et al. (1997) used the comet assay to observe the protective action of quercetin against hydrogen peroxide-induced DNA damage in human lymphocytes. *Mentha piperita* and *M. pulegium* infusions showed similar levels of antigenotoxicity of 141 and 134% respectively against $\text{H}_2\text{O}_2$ induced genotoxicity (Yildirim et al., 2000). The essential oil of *M. chamomilla* plant was shown to be antigenotoxic in the sister chromatid exchange assay (Harbone and Williams, 2000). Ng et al. (2000) demonstrated that tanshinones isolated from *Salvia miltiorrhiza* were effective antioxidants that inhibit the association of lipid peroxidation products with DNA.

Phenols present in *Mentha piperita* infusions such as limonene could relate to the antigenotoxic effects (Grassmann et al., 2002). Herbal infusions of *Mentha piperita* and *M. pulegium* assayed for antigenotoxicity using the somatic mutation and recombination test (SMART) showed a significant genotoxicity, quite the reverse they were able to behave as desmutagens, detoxifying the mutagen hydrogen peroxide (Romero Jimenez et al., 2005).

Rosmarinic acid and luteolin-7-glucoside from *Salvia officinalis* showed cytoprotective effects against tert-butyl hydroperoxide (t-BHP)-induced oxidative damages in HepG2 cells (Cristovao et al., 2007). Two tanshinones isolated from roots of *Hyptis martiussi* at concentrations of 1, 3, 6 and 12µg/ml for 3 h was shown to be quite strongly genotoxic against V79 cells (Chinese hamster lung) by the alkaline comet assay (Cavalcanti et al., 2008).