REVIEW OF LITERATURE

Jose Mary Das “Cytotoxic, antitumor, antioxidant and phytochemical assays in some species of Alpinia Roxb. ”, Department of Botany, University of Calicut, 2007
REVIEW OF LITERATURE

CYTOTOXIC ASSAYS

Cytotoxic studies of plant extracts on *Allium cepa*

Natural products are a source of therapeutic drugs and are used by physicians of indigenous systems of medicine for over hundreds of years (Houdret, 2000). The standard herbal preparations mostly consist of complex mixtures of one or more plants which are used in most countries (Calixio, 2000). In order to initiate the search for drugs from plants, the antimitotic activity of the extracts were tested by *Allium cepa* assay (Levan, 1949). The *Allium cepa* root meristem assay is considered widely as a practical and reliable system for the screening of environmental mutagens and carcinogens (Fiskejo, 1985; Stich et al., 1975).

As the patterns of divisions in onion cells and animal somatic cells are similar, an extract which is able to inhibit the cell division in *Allium cepa* root cells, will be effective in human/animal cells. Thus it is possible that chemicals that affect plant chromosomes will also affect the chromosomes of animals. Hence these meristematic cells of plants can be used for preliminary screening of antimitotic / anticancer activity of extracts / drugs (Williams, 1996). The onion root tip assay is used by many researchers to screen several plant extracts to evaluate their antimitotic activity.

Cytotoxic assays were conducted on a wide spectrum of plants by several earlier workers using different test materials. Shehab (1979) studied the cytological effect of water extract of *Pulicaria crispa* on *Allium cepa*. The extract affected the mitotic index and percentage of the mitotic
stages in treated roots. The percentage of anomalies increases with increase of concentration and duration of treatment. The abnormalities were spindle disturbances, stickiness, bridges and laggards.

In 1980, Shehab reported the antimitotic effect of water extract from *Teucrium pilosum* on *Allium cepa*. The induced cytotoxic effects include stickiness, C-mitosis, laggards, bridges, polyploidy and chromosomal breaks.

*Cata edulis* extract showed a decrease in the rate of cell division in *Allium cepa* root tip meristem (Kabarity and Mallalah, 1980). The leaves and buds of *Cata edulis* were said to contain alkaloids similar to caffeine. The mitodepressive effect increased as the time of exposure increased. In the short period of treatment with the same extract, the root tip showed a slight increase in the mitotic index after a period of recovery in water. The harmful effect of *Cata edulis* extract affected the rate of cell division of *Allium cepa* root tip cells and caused an imbalance in the frequency of mitotic phases.

The cytological effect of *Anastatica heirochuntica* extract on *Allium cepa* root meristem was studied by Shehab and Adams (1983). Mitotic depression occurred after direct and recovery treatments. The abnormalities observed were despiralization, spindle disturbances, chromosome stickiness, lagging, bridges, etc.

The antimitotic activity of the aqueous extract from *Erica undevalensis* on the root tip of *Allium cepa* was investigated. The role of the phenolic acid components in this activity was demonstrated (Pascual, 1987).

Ayuso (1988) reported the antimitotic activity of the aqueous extract of *Quercus rotundifolia* leaves on the root tips of *Allium cepa*.
Inhibition increased as a result of treatment duration. The importance of tannin content on inducing mitotic inhibition was demonstrated.

Mitotic effects of aqueous leaf extract of *Cymbopogon citratus* were demonstrated on *Allium cepa* root tips (Williams, 1996). A steroidal drug, sarsapogenin was tested for its cytotoxicity and antimitotic activity on root tip meristematic cells of *Allium cepa*. The drug was found to possess profound effect on mitotic spindle inhibition and chromosomal abnormalities during prolonged treatment (Sinha, 1996).

Minija *et al.* (1999) reported the mitoclastic properties of *Mentha rotundifolia* L. The abnormalities observed were clumped metaphase, scattered metaphase, polyploidy, diagonal anaphase, asynchronous movement of chromosomes, ball metaphase, etc.

Veronica *et al.* (2001) reported the effect of medicinal tea prepared from *Averrhoa carambola*, *Syzygium cuminum* and *Cissus sicyoides*. The results showed that tea did not alter the cell cycle of *Allium cepa*. But lower concentrations after 24 hr. treatment showed decrease in the mitotic index.

The genotoxic effect of an aqueous extract of neem was evaluated using *Allium cepa* chromosome aberration assay. Neem extracts suppressed the mitotic activity of *Allium* root meristems after 24-48 hr. treatment with all concentrations. The extracts caused different kinds of chromosome aberrations in dividing and non dividing cells of *Allium cepa* such as micronucleus, multinucleated cells in the interphase stage, bridges, stickiness, non-congression metaphase, laggards, polyploidy and disturbed anaphase (Soliman, 2001).

The cytotoxic potential of tomato fruit extract on onion root meristem was investigated by Yadav *et al.* (2001). The results showed that
tomato fruit extract induced various types of nuclear and chromosomal abnormalities such as chromosome breakage, scattered metaphase, disturbed polarity, lagging chromosomes, ring formation, extrusion, bridge formation and binucleate cells.

Cytotoxic effect of extract of castor seed was reported by Borah et al. (2002). The chromotoxic effects included fragments, ring chromosomes, c-mitosis and end to end attachment of chromosomes at metaphase, laggards, multipolar cells, etc. The most significant observations were the denaturation of chromatin fibre and somatic reduction that are encountered during treatment with 50 and 100% concentration of seed extract.

The cytotoxic potential of *Artemisia nilgirica* extract comprising both polar and non-polar fractions and plant extract having polar chemical compounds alone were evaluated on the meristematic root tip cells of *Allium cepa* (Leeja et al., 2004). The abnormalities noticed were of both clastogenic and non-clastogenic types. Chromosome stickiness, bridges, binucleate cells, etc. were the most frequent abnormalities noticed. Mitotic index reduced considerably in a gradual manner as the concentration of the extract and duration of treatment increased.

**Cytotoxicity of plant extracts on in vitro cell lines**

Cytotoxic components of *Zingiber zerumbet*, *Curcuma zeodaria* and *C. domestica* were studied by Matthes et al. (1980). Root extracts of the three species of Zingiberaceae showed marked cytotoxicity against neoplastic cells. One new compound (3",4"-o-diacetyl afzelin) and five known compounds (zerumbone, zerumbone epoxide and the curcuminoids, diferuloyl methane, feruloyl-p-coumaryl methane and di-p-coumaryl-methane) were isolated and all these were found to be cytotoxic.
Toshihiko (1987) found that the growth of V-79 cells (lung fibroblast of Chinese hamster) were completely inhibited on treatment with camphor, present in *Bosenbergia pandurata* (Zingiberaceae) volatile oil at 0.3% for 24 - 48 hrs.

The sesquiterpene, β-sesquiphellandrene, one of the minor compounds detected in *Curcuma longa* volatile oil was reported to be cytotoxic against mouse lymphocytic leukaemia cells – L 1210 (Ahn and Lee, 1989).

Qureshi *et al.* (1994) studied the effects of *Alpinia galanga* (Zingiberaceae) on cytological and biochemical changes induced by cyclophosphamidem in mice. The administration of the extract of *A. galanga* rhizomes caused mitodepression effects.

Zhao *et al.* (1995) have found the cytotoxic activity of *Hedychium forestii* (Zingiberaceae) rhizome extracts against KB cells. The diterpenoid hedychenone, T-hydroxyl hedychenone and coronarin were found to be the cytotoxic compounds.

The cytotoxic effect of eight synthetic curcuminoids on L929 cells were reported by John *et al.* (1996). All synthetic curcuminoids used in the study were found to be cytotoxic against L929 cancer cells.

Dubey *et al.* (1997) reported that citral, one of the major compounds of *Zingiber officinale* volatile oil was found to be cytotoxic against P388 mouse leukaemia cells.

The bioactive labdane diterpenoids isolated from *Renealmia alpinia* of Zingiberaceae collected from Surinam rain-forest was found to be cytotoxic against M109 cancer cells (Zhou-Bing Nan, 1997).
Ethanolic extracts of forty three Jordanian medicinal plants were examined for cytotoxicity. Among them, *Curcuma longa* and *Zingiber officinale* showed cytotoxicity against A549 cancer cells, MCF-7 female breast carcinoma and HT 29 colon adenocarcinoma cell lines (Alkofahi, 1997).

Cytotoxicity of curcuminoids and some novel compounds from *Curcuma zeodaria* were reported earlier by Syu et al. (1998). Extracts of roots of *C. zeodaria* led to the isolation of the curcuminoid, identified as dimethoxy curcumin. Cytotoxicity was exhibited against human ovarian cancer cells.

*Curcuma domestica, C. xanthorrhiza, Kaempferia galanga, Zingiber casummar, Z. officinale* and *Z. zerumbet* exhibited cytotoxicity against 12-o-tetradecanoyl phorbol-13 acetate induced Epstein Barr virus early antigen. The results showed that Zingiberaceae rhizome extracts used in Malaysian traditional medicine contain naturally occurring non-toxic components that inhibits EBV activation (Vimala et al., 1999).

The pharmacological effects of elemene isolated from the roots of *Curcuma zeodaria* were investigated in human leukaemia K562 cells (Yuan-Jing et al., 1999). Inhibition of cell proliferation was measured using calorimetric MTT assay. Elemene induces apoptosis and regulates expression of bcl-2 protein in human leukaemia K562 cells.

Mackeen et al. (1999) reported the antimicrobial and cytotoxic properties of some Malaysian traditional vegetables (locally known as Ulam) belonging to 15 plant families. Among them *Kaempferia galanga* of Zingiberaceae was found to be cytotoxic against HeLa (Human Cervical Carcinoma) cell line. Cytotoxic, antioxidant and antiinflammatory activities of curcumin I-III from *Curcuma longa* were studied by Ramsewak
et al. (2000). The compounds showed cytotoxic activity against melanoma, renal and breast cancer cell lines.

Minor cytotoxic and antibacterial compounds were isolated from the rhizomes of *Amomum aculeatum* of Zingiberaceae. Aculeatin-D isolated from *A. aculeatum* showed high cytotoxicity against KB and other cell lines (Heilmann et al., 2001).

Tezuka et al. (2001) reported eleven diarylheptanoids and two unusual diarylheptanoid derivatives from the seeds of *Alpinia blepharocalyx*. All compounds were examined for cytotoxicity against murine colon carcinoma 26-L5, human HT-1080 fibrosarcoma cells and showed cytotoxicity against both cell lines.

Moon et al. (2001) reported the antigenotoxicity of galangin as a cancer chemopreventive agent. Flavanoids extracted from *Alpinia officinarum* (galangin) was capable of modulating enzyme activities and suppressing cytotoxicity of chemicals.

Carnesecchi et al. (2001) studied the presence of geraniol as one of the minor compounds of *Bosenbergia pandurata* of Zingiberaceae and it was found to be cytotoxic against Caco-2 – human colon cancer cells.

Evaluation of cytotoxic potential of Indonesian medicinal plants in cultured human cancer cells was carried out by Gowooni et al. (2002). Out of the different plants studied *Zingiber casumunar* showed moderate toxicity to A549 cancer cells.

7,8-dihydroxy flavanone isolated from the seeds of *Alpinia kastumudai* was found to have an *in vitro* cytotoxic effect against A549 (human lung cancer cell line) and K562 (human leukemia cell line) cells (Ryeong et al., 2003).
Han-Ahrenm (2003) reported the potential cytotoxic principle of chloroform extracts of *Zingiber casumunar* rhizomes. The cytotoxic compound curcumin was isolated, which showed significant cytotoxicity to human cancer cell lines like A549 cells.

Protective effects of *Alpinia oxyphylla* water extracts on neurons from ischemia damage and neuronal cell toxicity was studied by Koo-Bynug Soo *et al.* (2004). The results indicated that *Alpinia oxyphylla* protects neurons against ischemia induced cell death. *A. oxyphylla* may exert its neuroprotective effect by reducing the nitric oxide mediated formation of free radicals or antagonizing their toxicity.

Thippeswamy *et al.* (2006) reported that *Curcuma aromatica* extract induces apoptosis and inhibits angiogenesis in Ehrlich Ascites Carcinoma cells *in vivo*. The results showed that the ethanolic extract of *C. aromatica* has potent antiangiogenic and pro-apoptotic properties that can be further developed into potential anticancer drugs.

Twelve Thai medicinal plants including *Curcuma zeodaria* as the ingredients of a traditional formula for cancer treatment were selected to test cytotoxic activity against human cancer cell lines, *viz.*, large cell lung carcinoma and prostate cancer cell lines and one type of normal human cell line fibroblast cells. Ethanolic extracts of six plants including *Curcuma zeodaria* showed cytotoxicity against lung and prostate cancer cell lines. The water extract of these plants exhibited no activities against all types of human cells (Saetung *et al.*, 2005).

Toxicity of crude extract of *Kaempferia galanga* of Zingiberaceae was studied by Kanjana (2004). The ethanolic rhizome extract of *K. galanga* (Zingiberaceae) was studied by conventional pharmacological methods. In the acute toxicity test, oral administration of 5 gm/kg body weight of
the extract produced neither mortality nor significant differences in the body and organ weights between control and treated animals. Moreover, gross abnormalities and histopathological changes were not comparatively detectable. In subacute toxicity studies, no mortality was observed when varying doses of 25, 50, 100 mg/kg body weight of ethanolic extracts were administered orally.

Methanolic extracts, water extracts and volatile oils of fresh rhizomes of *Alpinia galanga*, *Bosenbergia pandurata*, *Curcuma longa* and *Zingiber officinale* have been assessed for cytotoxic activity against MCF-7 breast adeno-carcinoma and LS 174T – colon adeno-carcinoma cell lines by Zaeoung et al. (2005). The results showed that methanolic extract of *Curcuma longa* showed strong activity against MCF 7 and LS 174 T cell lines, whereas the water extracts of these plants exhibited slight cytotoxic activity. All volatile oils and methanol extracts were capable of inhibiting proliferation of the two cell lines. It is notable that volatile oils of these rhizomes were mainly composed of monoterpenes, sesquiterpenes and phenyl propanoids and could be responsible for the cytotoxic activity.

Lee et al. (2005) reviewed the cytotoxic activity of extracts of *Alpinia galanga*, *A. officinarum*, *Cayratia japonica*, *Physalis minima* and *Tabernaemontana divaricola* against human cancer cell lines, viz., CoRL 23 – lung cancer cell line, MCF-7 breast cancer cell lines and a non cancer MCF-5 cell line. The results indicated that the extracts exhibited cytotoxicity. 1'-acetoxy chavicol acetate was isolated as the major cytotoxic component of both *Alpinia* species and physalin as the cytotoxic component of *Physalis minima*. The Malaysian *Alpinia galanga* showed weak activity when compared with Thai sample and this was due to the relatively high amounts of 1'-acetoxy chavicol acetate present in the Thai sample.
Biological effects of indigenous medicinal plants like *Curcuma longa* and *Alpinia galanga* were reported by Khattak *et al.* (2005).

The effect of turmeric extracts on inflammatory mediator production was reported by Lantz *et al.* (2005). Water soluble extracts were not cytotoxic and did not exhibit biological activity. Organic extracts of turmeric were found to be cytotoxic. Crude organic extract of turmeric were found to be capable of inhibiting lipopolysaccharide induced tumor necrosis factor (TNF-x) production. Purified curcumin was found to be more active. Fractions and subfractions of turmeric extracts obtained via preparative HPLC resulted in a loss of activity, indicating interactions of the compounds within the fraction to produce antiinflammatory effects.

Photo-induced cytotoxicity of curcumin in selected aqueous preparations on the salivary gland acinar cells and difference in photo-toxic effects of natural and synthetic curcumin was investigated by Bruzell *et al.* (2005). The results indicated that photo-toxic effect on cells was dependent on curcumin concentration, the light dose and the type of preparation.

**ANTITUMOR ASSAYS**

Cancer research is developing into a logical science, where the complexities of the disease, described in the laboratory and clinic, will become understandable in terms of a small number of underlying principles. Several lines of evidences indicate that tumorigenesis is a multistep process and these steps reflect genetic alterations that drive the progressive transformation of normal cells to the highly malignant derivatives.
Cancer development is now commonly recognized as a micro-evolutionary process that requires the cumulative action of multiple events. These events may occur in a single cell clone and can be explained by a simplified three-stage model. These stages include (a) induction of DNA mutation in a somatic cell, known as initiation (b) stimulation of the initiated cell and its clonal expansion, referred to as promotion, and (c) malignant conversion of the benign tumor into cancer, termed as progression. Oxygen free radicals have been shown to stimulate cancer development by playing a role at all the three stages namely, initiation, promotion and progression.

Plants have always been a common ingredient in the traditional medicinal preparations. So plants are invaluable in the generation of new drugs. Some examples of plant derived drugs are taxol, camptothecin, vincristin and vinblastin (DeVita et al., 1993). Some of the indigenous plants have cytotoxic and antitumour property in experimental animal models (Shylesh and Padikala, 2000).

A survey of literature cited below depicts the antitumour effect of members of Zingiberaceae, spices and medicinal plants.

Anticancer activity of the rhizome of turmeric (Curcuma longa) was evaluated in vitro using tissue culture methods and in vivo in mice using Daltons lymphoma cells grown as ascites form. Turmeric extract inhibited the cell growth in Chinese hamster ovary (CHO) cells at a concentration of 0.4 mg/ml and was toxic to lymphocytes and Daltons lymphoma cells at the same concentration. Cytotoxic effect was found within 30 minutes at room temperature (30°C). The active constituent was found to be curcumin, which showed cytotoxicity to lymphocytes and Daltons lymphoma cells. Initial experiments indicated that turmeric extract and
curcumin reduced the development of animal tumours (Kuttan et al., 1985).

In the course of search for antitumor agents, Sang Hyun et al. (1996) found that the extract of Curcuma longa was effective in inducing apoptosis or programmed cell death (PCD) in human myeloid leukaemia cells. Active compounds for PCD were isolated and identified as ar-turmerone and β-atlantone. These findings suggest that isolated compounds may exert their antitumor activity through induction of apoptosis.

Antitumour activity of synthetic curcuminoids were studied by John et al. (1996). Eight synthetic curcuminoids were investigated for their cytotoxic and tumoricidal activities. All curcuminoids were found to be cytotoxic to cultured L929 cells. As antitumor agents, veratryl curcuminoids and salicyl curcuminoids increased life span of animals by 100% and 86.9% respectively.

Inhibition of tumor promotion in SENCAR mouse skin by ethanolic extract of Zingiber officinale rhizome was reported by Katiyar et al. (1996). The results showed that pre-application of ginger extract to mouse skin i.e., 30 min. prior to that of each 12-0-tetradecanoyl phorbol-13-acetate (TPA) application could reduce edema by 56% and hyperplasia by 44%.

Mehta et al. (1997) studied the antiproliferative effect of curcumin against human breast tumor cell lines. The results indicated that the growth inhibitory effects of curcumin was time and dose dependent and curcumin is a potent antiproliferative agent for breast tumor cells and may act as a potential anticancer agent.
Chemopreventive substances are capable of inhibiting, retarding or reversing the multistage carcinogenesis. A wide range of phenolics present in dietary and medicinal plants have been reported to possess substantial anticarcinogenic and antimutagenic activities. Curcumin, a yellow ingredient from *Curcuma longa* (Zingiberaceae) has been extensively investigated for its chemopreventive potential. Yakuchinone A and Yakuchinone D present in *Alpinia oxyphylla* (Zingiberaceae) have inhibitory effects on phorbol ester-induced inflammation and skin carcinogenesis in mice and oxidative stress *in vitro*. The results suggest that these compounds have the ability to suppress proliferation of human cancer cells via induction of apoptosis (Surh, 1999).

Zingiberaceae rhizomes commonly used in Malasian traditional medicine were screened by Vimala *et al.* (1999) for antitumor promotor activity. Using the short term assay of inhibition of 12-0-tetradecanoyl phorbol-13-acetate (TPA), they induced Epstein Barr virus early antigen (EBV-EA) in Raji cells. The rhizomes of *Curcuma domestica*, *Curcuma xanthorrhiza*, *Kaempferia galanga*, *Zingiber casunmar*, *Zingiber officinale*, *Zingiber zerumbet* and *Zingiber officinale* (red variety) were found to possess inhibitory activity towards EBV activation. The study suggests that the naturally occurring non-toxic compounds that inhibit the EBV activation, if further investigated could contribute in the development of cancer prevention methods at the tumoral promoting stage.

Inhibitory effects of curcumin and catechin on lung metastasis induced by B16 F-10 melanoma cells were studied in male mice by Menon (1999). Curcumin and catechin inhibited lung tumor formation 89.3% and 82.9% respectively and significantly increased life span 143.9% and 80.8% respectively. Curcumin and catechin treatment significantly inhibited the invasion of B16 F-10 melanoma cells. These findings suggest
that curcumin and catechin inhibit the invasion of B16 F-10 melanoma cells by inhibition of metallo proteinase, thereby inhibiting lung metastasis.

Curcumin, a dietary pigment in turmeric, possess anticarcinogenic and antimetastatic properties. The study conducted by Kim et al. (2001) revealed the in vitro chemopreventive effect of curcumin in transformed breast cells. The results indicate that curcumin inhibits invasion and induces apoptosis, proving the chemopreventive potential of curcumin.

Tanaka et al. (2001) revealed that dietary administration of zerumbone caused reduction in the frequency of azoxymethane-induced colonic abberant crypt foci (ACF) in rats. Further studies revealed that zerumbone feeding, significantly lowered the number of silver stained nucleolar organizer regions (AgNORs) in colonic cryptal cell nuclei and it reduced expression of cyclo oxygenase (COX) in colonic mucosa. These findings might suggest the possible chemopreventive ability of zerumbone.

Murakami et al. (2002) reported that zerumbone suppresses proinflammatory protein production and cancer cell proliferation accompanied by apoptosis. The results indicated that zerumbone, the food phytochemical has distinct potentials for use in antiinflammation, chemoprevention and chemotherapy strategies.

Redox regulated mechanism by which the zerumbone suppresses cancer cell proliferation was studied by Hoffman et al. (2002). The results showed that an appropriate dose of zerumbone can be made high enough to stop the proliferation of cancer cells, but not high enough to stop proliferation of normal cells.

Murakami et al. (2003) studied the suppression of dextran sodium sulfate induced colitis in mice by zerumbone, a subtropical ginger
sesquiterpene and nimesulide, separately and in combination. Ulcerative colitis and Crohn's disease are inflammatory disorders of unknown cause and difficult to treat, though some synthetic chemicals including ligands for peroxisome proliferator – activated receptors are anticipated to be useful drugs. A few food phytochemicals have been reported to suppress colitis in animal models. The results indicates that a food chemical, zerumbone has a marked suppression effect on dextran sodium sulfate induced colitis in mice.

Antitumor activity of the extract of *Zingiber aromaticum* and its bioactive sesquiterpenoid, zerumbone was studied by Kirana et al. (2003). The results indicated that zerumbone is effective as an anticancer agent, possibly by its apoptosis-inducing and antiproliferative activities.

A diarylheptanoid from *Alpinia officinarum* (Lesser galangal) inhibits proinflammatory mediator (via) inhibition of mitogen activated protein kinase and transcription factor, nuclear factor KB was reported by Yadav et al. (2003). The study also suggest the mode of action of dietary biologically active compounds in preventing inflammation, which may be helpful in the development of therapeutic treatment of chronic/acute inflammatory diseases.

Zerumbone, a sesquiterpenoid in subtropical ginger, suppresses skin tumor initiation and promotion stages in mice (Murakami et al., 2004). The results indicate that zerumbone acts as a promising agent for the prevention of both tumor initiating and promoting processes, through induction of antioxidative and phase II drug metabolizing enzymes as well as attenuation of proinflammatory signalling pathways.
Murakami et al. (2004) revealed that the antiinflammatory phytochemical, zerumbone induces expression of proinflammatory genes in human adenocarcinoma cell lines.

The effect of turmeric extract on inflammatory mediator production was investigated by Lantz et al. (2005). The results indicated that the water soluble extracts were not cytotoxic and did not exhibit biological activity. Organic extracts of turmeric were cytotoxic only at concentrations above 50 \( \mu g/ml \). Crude organic extracts were capable of inhibiting lipopolysaccharide induced necrosis factor. Purified curcumin was more active than either dimethoxy or bisdimethoxy curcumin.

Takada et al. (2005) reported that zerumbone found in subtropical ginger \textit{Zingiber zerumbet} abolishes nuclear factor kappa B and kinase activation leading to suppression of antiapoptotic and metastatic gene expression, upregulation of apoptosis and down regulation of invasion. The results indicated that zerumbone inhibits activation of nuclear factor Kappa B and NP Kappa B regulated gene expression induced by carcinogens and this inhibition may provide a molecular basis for the prevention and treatment of cancer by zerumbone.

Formation of new blood vessels is critical for the growth of the tumor. Angiogenic factor secreted by the tumor cells induces neoangiogenesis. Inhibition of tumor angiogenesis and activation of tumor cell apoptosis will inhibit the growth of tumor. Thippeswamy et al. (2006) reported that \textit{Curcuma aromatica} extract induces apoptosis and inhibits angiogenesis \textit{in vivo}. The growth of Ehrlich ascites tumor cells and formation of ascites in the peritoneum of Ehrlich ascites tumor bearing mice was inhibited by \textit{Curcuma aromatica} ethanolic extract. The decrease
in the peritoneal angiogenesis and microvessel density shows the antiangiogenic potential of *Curcuma aromatica* extract *in vivo*.

**ANTIOXIDANT ASSAYS**

Free radicals are unstable atoms or molecules with an unpaired electron that include hydrogen atoms, nitric oxide and molecular oxygen (O$_2$). These are naturally occurring in the body as a result of chemical reactions during normal cellular processes. Reactive oxygen species (ROS) are various forms of activated oxygen, which include free radicals such as super oxide ions, hydroxyl radicals and non free radical species such as hydrogen peroxide. In living organisms various forms of ROSs can form in different ways, including normal aerobic respiration, via stimulated polymorphonuclear leukocytes, macrophages and peroxisomes. These appear to be the main endogenous sources of most of the oxidants produced by cells. In an attempt for free radicals to stabilize, they attack other molecules in the body potentially leading to cell damage and triggering the formation of other free radicals resulting in a chain of reactions (Odukoya et al., 2005).

Excess production of reactive oxygen species generated from isolated leukocytes, under chronic inflammation may have an important role in tumor initiation and promotion. All aerobic organisms, including human beings have antioxidant defences that protect excess production of reactive oxygen species, generated from isolated leukocytes under chronic inflammation and may have an important role in checking tumor initiation and promotion.

Oxidative stress has been implicated in numerous pathological conditions including cancer due to genetic variations (Forsberg, 2001). The complex process of carcinogenesis involves oxidative stress.
Oxidative stress can come from both endogenous and exogenous sources and is ubiquitous to all aerobic organisms. It was demonstrated that oxidative stress could cause chromosomal damage or aberrations, induce many types of DNA-protein cross-links, depurination and depyramidination (Mates, 2000). Oxidative stress also damages mechanisms which include defence enzymes like catalase, superoxide dismutase, and cell cycle check point systems (Shackelford, 2000). Thus free radicals often attack DNA, protein molecules, enzymes and cells leading to alterations in genetic material and cell proliferation (tumor masses).

Antioxidants are compounds that help to inhibit the many oxidation reactions caused by free radicals such as singlet oxygen, superoxide, peroxyl radical, hydroxyl radicals and peroxo nitrite, thereby preventing or delaying damage to the cells and tissues. Their mechanism of action include scavenging reactive oxygen and nitrogen free radical species, decreasing the localized O₂ concentration thereby reducing molecular oxygen's oxidation potential, metabolizing lipid peroxides to non-radical products to prevent generation of free radicals. In this way antioxidants limit the free radical damage from oxidizing low density lipoprotein (LDL) cholesterol, which may increase the risk of various diseases (Lakenbrink, 2000).

However, this natural antioxidant mechanism can be inefficient and hence dietary intake of antioxidant compound is important. Recent reports indicated that there is an inverse relationship between dietary intake of antioxidant rich food and the incidence of human diseases (Ali et al., 2001) i.e., inhibition that block reactive oxygen and nitrogen species may inhibit or delay carcinogenic processes (Cross et al., 1987).
The strongest antioxidant and anticancer activity has been demonstrated by natural compounds having multifunctional ability. Suppressive effects of curcumin isolated from *Curcuma longa* of Zingiberaceae on lipid peroxidation induced in rats by carbon tetra chloride was reported by Ikuonishigaki (1992). The results indicated that administration of curcumin once a day for three successive days before the administration of carbon tetra chloride suppressed this increase in lipid peroxide level in the liver.

Free radical scavenging activity of synthetic curcuminoids were studied by John *et al.* (1996). Eight synthetic curcuminoids were screened for their free radical scavenging activity and found to inhibit *in vitro* lipid peroxidation and scavenged superoxides and hydroxyl radicals. The results showed that synthetic curcuminoids, like natural curcumin are potent antioxidants.

Sreejayan *et al.* (1996) reported the nitric oxide scavenging activity of curcuminoids. Curcumin being a compound with antiinflamatory and anticancer activity, inhibits induction of nitric oxide synthase in activated macrophages and has been shown to be a potent scavenger of free radicals. Curcumin reduced the amount of nitrite formed by the reaction between oxygen and nitric oxide generated from sodium nitroprusside. The result indicated curcumin to be a scavenger of nitric oxide.

Novel sesquiterpenes, *viz.*, oxyphyllenoidiol A and B, and tri noreudesmane type sesquiterpenes were isolated from the methanolic extract of *Alpinia oxyphylla* kernels and were found to inhibit the nitric oxide production in lipopolysaccharide activated macrophages (Muraoka *et al.*, 2001).
Antioxidant activity of extracts of *Alpinia kastumudai* seed was reported by Lee *et al.* (2003). The results indicated that high levels of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity were detected in the seed extract. The total seed extract of *A. kastumudai* shows dose dependently enhanced activities of superoxide dismutase, catalase and glutathione peroxidase in V79-4 cells *i.e.*, taken together, the findings showed that *A. kastumudai* contain significant antioxidant activity.

Nakamura *et al.* (2004) implicated zerumbone, a sesquiterpene compound from *Zingiber zerumbet* as one of the promising chemopreventive agents against colon and skin cancer. The result provide biological evidence that zerumbone has a significant ability to suppress oxidative stress possibly through induction of the phase II xenobiotic metabolizing enzymes. Considering the importance of oxidative damage in carcinogenesis, the antioxidant effect of zerumbone can be explored as a cancer chemopreventive agent targeted towards inflammation related carcinogenesis.

Kalpana *et al.* (2004) reported the modulating effects of curcumin on lipid peroxidation and antioxidant status during nicotine induced toxicity. The results indicated that administration of curcumin reversed the changes induced by nicotine, supporting the hypothesis that plant products are effective antioxidant agents. Curcumin significantly enhanced the antioxidant status in the liver, lung and kidney of nicotine treated rats. Thus curcumin exerts its protective effects against nicotine induced lung toxicity by modulating the extent of lipid peroxidation and augmenting antioxidant defence system.

Suppressive effects of Mioga ginger (*Zingiber mioga*) and ginger constituents on reactive oxygen and nitrogen species generation and the
expression of inducible proinflammatory genes in macrophages was studied by Kim et al. (2005). The results indicated that the constituents of *Zingiber mioga* have antioxidative and antiinflammatory potentials and may be considered as a promising candidate in prevention and or therapy for chronic inflammation associated carcinogenesis.

Antioxidant activity of Nigerian dietary plants including *Afiramomum danielli* (Zingiberaceae) was reported by Odukoya et al. (2005). The results suggest that reducing power does not fully characterize the antioxidant activity. Moreover, spices containing high phenolics provide a source of dietary antioxidant in addition to imparting flavours to the food. They possess potential benefits by inhibiting lipid peroxidation, justifies their traditional use in pepper soup as a cure all medicine for the sick and potential use as a value added ingredient for stabilizing food matrixes against lipid peroxidation reactions.

Antioxidant activities of rhizome extracts of six plants of Zingiberaceae family (*Zingiber casumunar, Alpinia galanga, A. allughas, Hedychium corancium, H. coccinum, Kaempferia galanga*) was measured by Padma et al. (2006). The results showed that the extracts from six species of Zingiberaceae family showed moderate to good antioxidant properties. *Alpinia galanga, A. allughas* and *Zingiber casumunar* were found to be more effective. Methanolic extracts of the plants show better results for DPPH analysis than dichloromethane extract.

Matsuda et al. (2006) suggested that 80% aqueous extract from the rhizome of *Alpinia officinarum*, a Chinese medicinal herb, were found to inhibit nitric oxide production in lipid polysaccharide activated mouse peritoneal macrophages.
PHYTOCHEMICAL ASSAYS

GC-MS analysis of essential oils

Essential oils are valuable natural products used as raw materials in perfumes, cosmetics, aromatherapy, phytotherapy, spices and nutraceuticals (Buchbauer, 2000). The volatile oils are complex mixtures comprising several compounds. Each of these constituents contribute to the beneficial or potential effects of these oils. Thus the intimate knowledge of essential oil composition allows for a better and specially directed application (Buchbauer, 2000). Considering all the above mentioned difference in essential oil composition it is clear that only a detailed knowledge of the constituents of the essential oil will lead to a proper use in cosmetics by perfumers and cosmetic chemists. Such a detailed knowledge can be obtained by means of carefully performed capillary – GC experiments (Buchbauer, 2000).

Panizzi (1993) reported that variation in the chemical composition of essential oil may be due to climatic, seasonal, geographic conditions, harvest period and distillation technique followed. The effect of plant maturity at the time of oil production and the existence of chemotype difference can also drastically affect the composition of essential oils (Lahlou and Berrada, 2003).

The previous works on the essential oil composition of different species of *Alpinia* is reviewed in the following table.
Table 1. Previous reports on the essential oil composition of different species of *Alpinia*

<table>
<thead>
<tr>
<th>Name of the plant</th>
<th>Parts used</th>
<th>Components</th>
<th>Authority</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Alpinia breviligulata</em></td>
<td>Seed</td>
<td>(E,E)-farnesol 65.0%, α-humulene 6.1%</td>
<td>Nguyen <em>et al.</em></td>
<td>1994</td>
</tr>
<tr>
<td><em>A. breviligulata</em></td>
<td>Fruit peel</td>
<td>β-pinene 22.9%, α-terpinene 7.3%, caryophyllene oxide 11.2%</td>
<td>Nguyen <em>et al.</em></td>
<td>1994</td>
</tr>
<tr>
<td><em>A. breviligulata</em></td>
<td>Leaf</td>
<td>Caryophyllene oxide 23.1%, α-pinene 17.7%</td>
<td>Nguyen <em>et al.</em></td>
<td>1994</td>
</tr>
<tr>
<td><em>A. chinensis</em></td>
<td>Flower</td>
<td>α-farnesene 26.5%, α-humulene 22.3%, β-bisabolene 17%, β-caryophyllene 13.1%</td>
<td>Nguyen <em>et al.</em></td>
<td>1994</td>
</tr>
<tr>
<td><em>A. chinensis</em></td>
<td>Leaf</td>
<td>β-bisabolene 47.9%</td>
<td>Nguyen <em>et al.</em></td>
<td>1994</td>
</tr>
<tr>
<td><em>A. conchigera</em></td>
<td>Rhizome</td>
<td>β-sesquiphellandrene 20.5%, β-bisabolene 12.1%, 1,8-cineole 11.6%</td>
<td>Sirat <em>et al.</em></td>
<td>1995</td>
</tr>
<tr>
<td><em>A. conchigera</em></td>
<td>Rhizome</td>
<td>β-bisabolene 28.9%, 1,8-cineole 15.3%, β-caryophyllene 10%</td>
<td>Wong <em>et al.</em></td>
<td>2005</td>
</tr>
<tr>
<td><em>A. galanga</em></td>
<td>Rhizome</td>
<td>Limonene 3.7%, 1,8-cineole 33%, camphor 5%, α-terpineol 9.3%, α-fenchyl acetate 12.7%, (E) methyl cinnamate 5.3%</td>
<td>Mallavarapu <em>et al.</em></td>
<td>2002</td>
</tr>
<tr>
<td><em>A. galanga</em></td>
<td>Leaf</td>
<td>α-pinene 6.6%, camphene 5%, β-pinene 21.5%, 1,8-cineole 34.4%, camphor 7.8%</td>
<td>Mallavarapu <em>et al.</em></td>
<td>2002</td>
</tr>
<tr>
<td>Name of the plant</td>
<td>Parts used</td>
<td>Components</td>
<td>Authority</td>
<td>Year</td>
</tr>
<tr>
<td>-------------------</td>
<td>------------</td>
<td>------------</td>
<td>-----------</td>
<td>------</td>
</tr>
<tr>
<td><em>A. galanga</em></td>
<td>Rhizome</td>
<td>1,8-cineole 39.4%, β-pinene 11.9%</td>
<td>Raina <em>et al.</em></td>
<td>2002</td>
</tr>
<tr>
<td><em>A. galanga</em></td>
<td>Leaf</td>
<td>1,8-cineole 32.5%, β-pinene 22.7%, camphor 12.8%</td>
<td>Raina <em>et al.</em></td>
<td>2002</td>
</tr>
<tr>
<td><em>A. galanga</em></td>
<td>Leaf</td>
<td>1,8-cineole 28.3%, camphor 15.6%, β-pinene 5% (E) methyl cinnamate 4.6%, bornyl acetate 4.3%, guaicol 3.5%</td>
<td>Leopold <em>et al.</em></td>
<td>2003</td>
</tr>
<tr>
<td><em>A. galanga</em></td>
<td>Stem</td>
<td>1,8-cineole 31.1%, camphor 11%, (E) methyl cinnamate 7.4%, guaicol 4.9%, bornyl acetate 3.6%, β-pinene 3.3%, α-terpineol 3.3%</td>
<td>Leopold <em>et al.</em></td>
<td>2003</td>
</tr>
<tr>
<td><em>A. galanga</em></td>
<td>Rhizome</td>
<td>1,8-cineole 28.4%, α-fenchyl acetate 18.4%, camphor 7.7%, (E) methyl cinnamate 4.2%, guaicol 3.3%</td>
<td>Leopold <em>et al.</em></td>
<td>2003</td>
</tr>
<tr>
<td><em>A. galanga</em></td>
<td>Root</td>
<td>α-fenchyl acetate 40.9%, 1,8-cineole 9.4%, borneol 6.3%, bornyl acetate 5.4%, elemol 3.1%</td>
<td>Leopold <em>et al.</em></td>
<td>2003</td>
</tr>
<tr>
<td><em>A. hainanensis</em></td>
<td>Leaf</td>
<td>Ocimene 27.4%, β-pinene 10%, 9-octadecanoic acid 6.5%, n-hexadecanoic acid 5.8%, 9,12-octadecadienoic acid 5.4%, terpinene 4.3%</td>
<td>Nan Peng <em>et al.</em></td>
<td>2004</td>
</tr>
<tr>
<td>Name of the plant</td>
<td>Parts used</td>
<td>Components</td>
<td>Authority</td>
<td>Year</td>
</tr>
<tr>
<td>-------------------</td>
<td>------------</td>
<td>------------</td>
<td>-----------</td>
<td>------</td>
</tr>
<tr>
<td><em>A. hainanensis</em></td>
<td>Flower</td>
<td>Ocimene 39.8%, β-pinene 17.7%, terpinene 5.5%, p-menth-1-en-ol 4.9%, phellandrene 4.4%</td>
<td>Nan Peng <em>et al.</em></td>
<td>2004</td>
</tr>
<tr>
<td><em>A. kastumudai</em></td>
<td>Leaf</td>
<td>p-menth-1-en-ol 22%, terpinene 19%, 4-carene 9.1%, 1,8-cineole 8.3%, camphor 5.6%</td>
<td>Nan Peng <em>et al.</em></td>
<td>2004</td>
</tr>
<tr>
<td><em>A. kastumudai</em></td>
<td>Flower</td>
<td>p-menth-1-enol-21.3%, 1,8-cineole 20.2%, terpinene 12.6%, phellandrene 7%, 4-carene 6.4%, β-pinene 5.2%</td>
<td>Nan Peng <em>et al.</em></td>
<td>2004</td>
</tr>
<tr>
<td><em>A. latilabris</em></td>
<td>Rhizome</td>
<td>Methyl cinnamate 89.5%</td>
<td>Wong <em>et al.</em></td>
<td>2005</td>
</tr>
<tr>
<td><em>A. purpurata</em></td>
<td>Rhizome</td>
<td>α-pinene 24.9%, β-pinene 65.8%</td>
<td>Sadaquat Ali <em>et al.</em></td>
<td>2002</td>
</tr>
<tr>
<td><em>A. purpurata</em></td>
<td>Leaf</td>
<td>α-pinene 79.6%, β-pinene 29.4%</td>
<td>Sadaquat Ali <em>et al.</em></td>
<td>2002</td>
</tr>
<tr>
<td><em>A. purpurata</em></td>
<td>Flower</td>
<td>α-pinene 81%, β-pinene 43%, β-caryophyllene 24.2%</td>
<td>Sadaquat Ali <em>et al.</em></td>
<td>2002</td>
</tr>
<tr>
<td><em>A. smithiae</em></td>
<td>Leaf</td>
<td>1,8-cineole 14.68%, β-caryophyllene 27.22%, geraniol 1.37%, camphor 6.3%</td>
<td>Roy <em>et al.</em></td>
<td>2001</td>
</tr>
<tr>
<td><em>A. speciosa</em></td>
<td>Flower</td>
<td>β-pinene 34%, α-pinene 14.8%, β-caryophyllene 10.8%</td>
<td>Nguyen <em>et al.</em></td>
<td>1994</td>
</tr>
<tr>
<td>Name of the plant</td>
<td>Parts used</td>
<td>Components</td>
<td>Authority</td>
<td>Year</td>
</tr>
<tr>
<td>-------------------</td>
<td>------------</td>
<td>------------</td>
<td>-----------------</td>
<td>------</td>
</tr>
<tr>
<td><em>A. speciosa</em></td>
<td>Rhizome</td>
<td>terpinen-4-ol 20.2%, 1,8-cineole 15.9%, sabinene 9.8%, γ-terpinene 9.3%</td>
<td>Pooter <em>et al.</em></td>
<td>1995</td>
</tr>
<tr>
<td><em>A. zerumbet</em></td>
<td>Rhizome</td>
<td>β-pinene 4.0%, 1,8-cineole 28%, terpinen-4-ol 41.9%</td>
<td>Sadaquat Ali <em>et al.</em></td>
<td>2002</td>
</tr>
</tbody>
</table>