Chapter 2: Review of Literature

The present review deals with phytochemical evaluation, molecular characterization and *in vitro* studies of some zingiberaceous taxa. Zingiberaceae family consists of rhizomatous medicinal and aromatic plants. The rhizomes are aromatic, tonic and stimulant; occasionally they are nutritive. An important distinguishing characteristic is the presence of essential oils in their tissues. Some of the plants are used as food as they contain starch in large amounts while others used as ornamental species, cultivated for their showy flowers. It comprises of 52 genera and about 1400 species as reported by Burtt and Smith, (1972). Plants of this family are found in the tropics of Africa, Asia and America with the greatest number in South-east Asia. The family is represented by 178 species under 22 genera in India (Jain and Prakash 1995). The important genera coming under Zingiberaceae are *Curcuma*, *Zingiber*, *Kaempferia*, *Alpinia*, *Hedychium*, *Amomum*, *Costus* etc.

2.1. Phytochemical evaluation

In recent times, secondary metabolites of plants are important source of current pharmaceutical drug, and they are becoming valuable products for herbal remedies. Research on the chemical and biological activities of plants during the past two centuries have derived various compounds for the emergence of medicinal chemistry as a foremost direction towards the discovery of novel and more efficient therapeutic agents (Roja and Rao, 2000). To date, primary focus of research on medicinal plants has been in the area of phytochemistry and pharmacognosy. The identification of bioactive compounds is a necessary requirement for quality control and dose determination of plant based drugs. Research in the pharmacognosy of medicinal plants has also involved assays of bio-activity, determination of potential mode of action, and target site for active phytomedicinal compounds as stated by Briskin, (2000).

2.1.1. Phytoconstituent analysis

Because of the infusions and the tinctures of various aromatic species, the Zingiberaceae family has held a place of importance for hundreds of years. They have been and are still used as components of herbal remedies for a variety of ailments. Economically many member of Zingiberaceae are of marvellous importance since their volatile essential oils form vital ingredient of flavour, fragrance, perfumery and
pharmaceutical industries. Phytoconstituent analysis of various members of Zingiberaceae is discussed below.

Parveen et al., (2013) investigated the leave essential oil of Curcuma longa L. by GC-MS analysis for the identification of chemical constituents of the essential oil which showed eucalyptol (10.27%) as the major constituent and other components were α-pinene (1.50%), β-phellandrene (2.49%), β-pinene (3.57%), limonene (2.73%) etc.

Kuanar et al., (2009) compared micropropagated turmeric (Curcuma longa L.) oil with that of field grown turmeric. In vitro-cultured turmeric leaves yielded 0.2% essential oil and GLC analysis showed the presence of main constituents similar to that of the mother plant, i.e. alpha-phellandrene (38.5%), (1,8-cineole (8.2%), alphapinene (2.4%), geraniol (1.5%), myrcene (1.0%), linalool (0.4%). Chemical analysis of the rhizome essential oil from the Roma cultivar of turmeric (Curcuma longa) was done by using GC-MS which revealed turmerone (49.76%) as major constituent (Singh et al., 2011a).

GC-MS analysis of the essential oil and oleoresins of Zingiber officinale was done by Singh et al., (2008). Geranial (25.9%) and eugenol (49.8%) were found to be the major component in essential oil and ethanol oleoresin respectively, while zingerone was the major constituent in tother 3 oleoresins. The chemical composition of the rhizome essential oils of the Zingiber officinale and three ginger-lilies like Hedychium flavescens, Hedychium coccineum and Hedychium coronarium were investigated by GC and GC-MS (Gurib et al., 2002). Z. officinale oil was found to contain geranial (16.3%), neral (10.3%), zingiberene (9.5%), beta-sesquiphellandrene (6.3%) and ar-curcumene (5.1%). The constituents of essential oils were as follows: H. flavescens: linalool, 1, 8-cineole, beta-pinene, alpha-terpineol and alpha-pinene; H. coccineum: (E)-nerolidol, trans-sesquisabinene hydrate; H. coronarium: alpha-muurolol, alpha-terpineol,1, 8-cineole, an unknown sesquiterpene alcohol, alpha-fenchyl acetate, citronellal and (E)-methyl cinnamate.

The essential oils of Zingiber zerumbet and Zingiber ottensii fresh rhizomes were analysed by capillary GC and GC-MS analysis (Sri-Nurestri et al., 2005). Zerumbone, representing 73% and 37% of the total oil, was the most abundant component of Z. zerumbet and Z. ottensii rhizome oil respectively. Other compounds found in Z. ottensii were terpinen-4-ol (16.8%), alpha-humulene (10.9%) and sabinene (7.2%), and in case of Z. zerumbet alpha-humulene (5.9%), camphene (2.8%) and caryophyllene oxide (2.7%) were found.
A combination of capillary GC and GC-MS was used to analyse the essential oil obtained from fresh rhizomes of *Curcuma zedoaria* which detect 12 components (79.41% of the total). The components were identified by comparing with Kovats Indices and their mass spectral data. The essential oil contained monoterpenoids and sesquiterpenoids, with furanogermenone (45.23%) as the major component (Malek *et al*., 2004). Mau *et al*., (2003) studied the essential oil isolated from the dried rhizome of *Curcuma zedoaria* using simultaneous steam-distillation and identified a total of 36 compounds in the essential oil.

The rhizome and leave essential oils of *Amomum pterocarpum* were analysed by Sabulal *et al*., (2007). 36 constituents each, from the rhizome and leaf oils was identified GC-MS and GC-FID analysis. The most abundant constituent was beta-pinene in both the rhizome oil (65.5%) and the leaf oil (41.7%). Phytol (26.5%) was one of the constituent in the leaf oil. Beena *et al*., (2006) reported that 1, 8-cineole (41.42%, 37.44%), beta-pinene (10.39%, 17.4%) and alpha-terpineol (8.8%, 6.7%) were found as the main constituents in fresh and dried *Hedychium coronarium* oil after GC-MS analysis.

Ali *et al*., (2002) analysed the essential oils of the leaves, flowers and rhizomes of *Alpinia zerumbet*, *Alpinia purpurata*, *Hedychium coronarium* and *Hedychium gardnerianum* using GC-MS. The rhizome and leaf oils of *A. zerumbet* were found to be rich in beta-pinene (4.0%, 10.0%), 1, 8-cineole (28.1%, 13.2%) and terpinen-4-ol (41.4%, 40.9%), respectively. The rhizome oils of *A. purpurata* contained alpha-pinene (24.9-36.1%) and beta-pinene (65.8-71.3%), while the leaf and flower oils contained alpha-pinene (79.6-81.0%), beta-pinene (29.4-43.0%) and beta-caryophyllene (0-24.2%) respectively.

GC-MS analysis of *Alpinia hainanensis* and *Alpinia katsumadai* essential oils was carried out by Nan *et al*., (2004). The leaf and flower oil of *A. hainanensis* was rich in ocimene (27.4% and 39.8%). In *A. katsumadai*, the most abundant constituent was p-menth-1-en-ol (22.0% and 21.3%) in leaf and flower oils.

The rhizome essential oils of *Alpinia aquatica* Rosc. and *Alpinia malaccensis* Roscoe were analysed by capillary GC and GC-MS (Sirat *et al*., 2011). *A. aquatica* rhizome oil was found to contain 18 compounds, representing 98.4% of the essential oil, with the major constituent β-sesquiphellandrene (36.5%), while rhizome oil of *A. malaccensis* contained methyl (E)-cinnamate (78.2%) as the highest component.
Joseph et al., (2001) investigated the composition of leaf and rhizome essential oils of *Alpinia smithiae* and was analysed by gas chromatography. The major components identified in both the samples were β-caryophyllene, sabinene, myrcene and 1,8-cineole. The essential oils of the aerial parts and flowers of *Alpinia nutans* were analyzed through GC and GC-MS by Joshi et al., (2010) and they found that Sabinene (27.8%), 1,8-cineole (17.4%), terpinen-4-ol (14.9%), p-cymene (5.2%), γ-terpinene (5.1%) β-caryophyllene (3.9%) and caryophyllene oxide (1.3%) were found in the oil of aerial part and the flower oil was characterized by the presence of terpinen-4-ol (25.1%), γ-terpinene (19.4%), sabinene (14.2%), 1,8-cineole (10.8%), linalool (1.6%) and caryophyllene oxide (1.0%).

The essential oils of the rhizomes and the leaves of *Alpinia galanga* were also analysed by capillary GC and GC-MS (Mallavarapu et al., 2002; Raina et al., 2002). The rhizome oils contained Eucalyptol, β-caryophyllene, alpha-turmerone, limonene, alpha-fenchyl acetate, camphor, alpha-terpineol, and (E)-methyl cinnamate as the major constituents while the major constituents found in the leaf oils were β-pinene, terpinolene, 1, 8-cineole, alpha-pinene, alpha-phellandrene, camphene and camphor. Ibrahim et al., (2004) examined the rhizome and seed oils of *Alpinia galanga* by capillary GC and GC-MS which revealed the presence of 1, 8-cineole (40.5%), β-bisabolene (8.4%), (Z,E)-farnesol (3.8%), β-caryophyllene (3.6%) and (E)-β-farnesene (3.2%) in rhizome oil whereas the seed oil was found to contain β-bisabolene (37.6%), (E)-β-farnesene (22.7%), (E, E)-farnesyl acetate (7.9%), (Z,E)-farnesol (3.9%) and β-caryophyllene (3.0%) as major compounds.

The chemical compositions of volatile oils of the leaves, stem, rhizomes and roots of *Alpinia galanga* from South Kerala, were analysed by capillary GC and GC-MS (Menon, 2006). The leaf oil was characterized by the presence of fenchyl acetate (20.7% leaf oil-1) and β-caryophyllene (40.5% leaf oil-2) along with β-farnesene, caryophyllene oxide, and 1, 8-cineole. The major compounds of the stem oil-1 were cubenol, humulene, germacrene and cadinene. Galangal rhizome oil contained carotol (26.7%) with 1-8, cineole, fenchyl acetate, β-caryophyllene and rhizome oil (2) contained 1, 8-cineole along with β-pinene, limonene, fenchyl acetate, camphor, methyl cinnammate, alpha-terpineol, and cubenol. The root oil (1) was rich in fenchyl acetate (30.5%) along with 1, 8 cineole and limonene.
The essential oils of different parts of *Alpinia calcarata* were analyzed by GC-MS (Bhuiyan et al., 2011). The leaf oil was found to have 1, 8-cineole (28.48%) and camphor (21.40%) as the major constituents while the stem sheath oil contained fenchyl acetate (19.16%) and carotol (16.77%) as the major compounds. On the other hand, fenchyl acetate (51.34%) and borneol (11.44%) were found as the main constituents in the root oil. The leaf or rhizome essential oils of *Alpinia zerumbet* were investigated for their chemical composition by Elzaawely *et al.*, (2007b). The leaf oil was found to have 1,8-cineol, camphor and methyl cinnamate whereas rhizome oil rich in dihydro-5,6-dehydrokawain (DDK) and methyl cinnamate.

Faridah *et al.*, (2010) studied the essential oils from the leaves and rhizomes of *Alpinia conchigera* Griff. dried for different times (0 (fresh), 1, 2, 3 and 7 days of drying, respectively). GC and GC-MS analysis revealed the major constituents in fresh, 1, 2 and 3 days dried leaves were cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl) while for leaves dried for 7 days were ‘1, 6, 10-dodecatriene, 7 and 11-dimethyl-3-methylene.

Ibrahim *et al.*, (2009) investigated *Alpinia conchigera* essential oils from the dried leaves, pseudostems and rhizomes by capillary GC and GC-MS and found that beta-bisabolene (15.3%), beta-pinene (8.2%), beta-sesquiphellandrene (7.6%), chavicol (7.5%) and beta-elemene (6.0%) were found in the leaf oil, while beta-bisabolene (19.9%), beta-sesquiphellandrene (11.3%), beta-caryophyllene (8.8%) and beta-elemene (4.7%) were present in the pseudostem. 1, 8-cineole (17.9%), beta-bisabolene (13.9%), beta-sesquiphellandrene (6.8%) and beta -elemene (4.0%) were found in the rhizome as main components.

The leaf and rhizome essential oils of *Alpinia nigra* were studied by Kanjilal *et al.*, (2010). 18 compounds were identified out of which 1,8-cineole was the most abundant constituent in both leaf (25.4%) and rhizome (34%) oils. β-pinene (15.1%), camphor (15.3%), α-pinene (7.8%), carotol (7.3%) and camphene (7.0%) were also present in leaf oil, whereas α-fenchyl acetate (13.1%), α-terpineol (9.6%), β-pinene (8.1%) and camphene (7.0%) were the other constituents found in the rhizome oil. As reported by Ghosh *et al.*, (2014), GC-MS/GC-FID analysis of the essential oils of *Alpinia nigra* which revealed the presence of 63 chemical constituents including β-caryophyllene as major component. Padalia *et al.*, (2010) studied the composition of essential oil of *Alpinia galanga, Alpinia calcarata, Alpinia speciosa*, and *Alpinia allughas* by capillary
GC and GC-MS. 1, 8-Cineole, alpha-terpineol, (E)-methyl cinnamate, camphor, terpinen-4-ol, and alpha- and beta-pinenes were found to be the major constituents in leaf and flower essential oils.

Woerdenbag et al., (2004) investigated the volatile components of rhizomes of *Kaempferia rotunda* L. and *Kaempferia angustifolia* Roscoe, by GC and GC-MS (EI) analysis. The most abundant constituents were benzyl benzoate, n-pentadecane and camphene in *K. rotunda* and n-pentadecane, camphene, camphor and bornyl formate in *K. angustifolia*.

Wong et al. (1992) identified fifty four components in the rhizome essential oil of *Kaempferia galanga*. The major constituents were ethyl trans-p-methoxy cinnamate (51.6%), ethyl cinnamate (16.5%), pentadecane (9.0%), 1,8-cineole (5.7%), delta-car-3-ene (3.3%) and borneol (2.7%). The volatile oil content was higher in rhizome than in root as revealed by the study of Arambewela et al., (2000).

Ravindran and Balachandran (2005) found that *Kaempferia galanga* rhizome contains 2.5 to 4% essential oil. The major components found in the oil were ethyl-p-methoxy cinnamate (30%), ethyl cinnamate (25%), p-methoxy cinnamic acid, 3-carene-5-one, Kaempferol, quercetin, cyanidin and delphinidin were found in the leaves.

The major chemical components present in the volatile oil of *Kaempferia galanga* were identified by Tewtrakul et al. (2005) as ethyl-p-methoxy-cinnamate (31.77%), methyl cinnamate (23.23%), carvone (11.13%), eucalyptol (9.59%), and pentadecane (6.41%).

According to Khare, (2007), the essential oils from rhizomes of *Kaempferia galanga* contain n-pentadecane, ethyl-p-methoxy cinnamate, ethyl cinnamate, carene, camphene, borneol etc.

Reports on phytoconstituent analysis of plant extracts of zingiberaceous taxa are relatively less as compared to that of essential oil.

Nayak et al., (2014) investigated phytochemical constituents of methanol fractions of the crude extracts of *Zingiber roseum*, *Curcuma angustifolia*, *Globba marantina* using GC-MS technique. It reveals the presence of seventeen compounds in *Zingiber roseum*, fifteen in *Curcuma angustifolia*, and nine in *Globba marantina*. *Zingiber roseum* has Cyclopentane, 1-methyl-2-(2-propyl) trans-(2.76%), Estazolam (3.20%), 2-hexane (20.00%), 3-Heptanone (29.29%), Iso-propyl benzene (Cumene) (4.47%), Pentadecenol (18.08%), n-Amylmercaptan (8.65%), Methyl cyclopentane (9.20%), 2,7-naphthalenedirol (12.44%), Camphor (15.38%), Trans-nerolidol (12.02%), Humulen-6,7-epoxide
(9.21%), Octadecanoic acid, butyl ester (11.56%), α-amorphene (23.02%), Aristoloshene (3.80%) were found in *Curcuma angustifolia* whereas *Globba marantina* contain Heptadecane (6.19%), Pinocarvone (54.27%), L-linalool (2.54%) etc. Phytochemical investigation on rhizomes of *Alpinia mutica* was done by Mustahil *et al.*, (2013) which identified 5 compounds namely 5,6-dehydrokawain, flavokawin B, pinostrobin and pinocembrin together with β-sitosterol. Chemical investigation of the chloroform extract of *Alpinia galanga* rhizomes gave p-hydroxycinnamaldehyde and [di-(p-hydroxy-cis-styryl)] methane (Barik *et al*., 1987). Chemical constituents of the rhizomes of *Alpinia malaccensis* were analysed by Nuntawong & Suksamrarn, (2008) which resulted in the isolation of 8 compounds like 5, 6-dehydrokawain, coronarin E, coronarin A, alpinetin, cardamonin etc. Othman *et al.* (2006) isolated a vasorelaxant active compound ethyl cinnamate as colourless oil and Ethyl-p-methoxycinnamic acid as white needles from the crude extract of *Kaempferia galanga*.

### 2.1.2. Bioactivity study

India is largely using medicinal plant to generate potential drug. Regardless of knowing the biologically active compounds herbal drugs are widely used due to their effectiveness, less side effects, and low cost. Although medicinal plants have been utilised for curing of ailments for several years by traditional medicinal healers, there has always been a persistent query in scientific world about their remedial efficiency. As a result, numerous pharmacological activities of various medicinal plants have been studied.

#### 2.1.2.1. Antioxidant activity

Free radicals and other reactive oxygen species (ROS) make oxidation of proteins, amino acids, DNA and unsaturated lipids. ROS produce molecular changings connected to different diseases like aging, cancer, Alzheimer, Parkinson, diabetes etc. (Butterfield & Lauderback, 2002; Zarkovic, 2003). When unevenness occurred between free radical generation and their elimination by the body’s defence system it leads to a phenomenon well-known as ‘oxidative stress’ (Abdollahi *et al*., 2004; McCord, 2000). Balance between free radicals and antioxidants can be recovered from an external supply of antioxidants.

In couple of years back, muchmore concentration has been committed to natural antioxidants and their alliance with our health. Antioxidant potential of Zingiberaceous plants are discussed below.
Many rhizomes of Zingiberaceous species have potential antioxidant activity which was determined by DPPH and β-carotene-linoleic acid method (Vankar et al., 2006). As reported by Habsah et al., (2000), methanol and dichloromethane extracts of Alpinia, Zingiber and Costus genera have revealed strong antioxidant properties comparable with or higher that of α-tocopherol. Antioxidant activity of callus extract of Zingiber zerumbet has been evaluated by using DPPH assay and Total Phenolic Content (TPC) assay (Stanly et al., 2011). Antioxidant potential of essential oil of Curcuma zedoaria was determined by Mau et al., (2003). Similarly Phang et al., (2011) evaluated the antioxidant activity of crude as well as fractionated methanol extracts) of the rhizomes of Alpinia mutica. The highest antioxidant property was seen in the ethyl acetate fraction in comparison to the other extracts against all assays conducted. The ethyl acetate fraction was also found to have the highest phenolic content among the extract and fractions. Zhang et al., (2010) also reported better antioxidant activity from essential oil and crude methanolic extracts of rhizome of Alpinia officinarum by DPPH assay. Another researcher Elzaawely et al., (2007) also examined the antioxidant properties of the oils of different parts of Alpinia zerumbet. Antioxidant activity of rhizome essential oil of Alpinia calcarata has been done through DPPH assay by Arambewela et al., (2010).

Other species of Alpinia like Alpinia nutans Rosc also studied by same DPPH assay and flower oil found to be more effective than aerial parts (Joshi et al., 2010). Similarly Kaempferia rotunda has also examined for its antioxidant properties and the chemical constituents of the plant were qualitatively analyzed to confirm the presence of flavonoids and phenolic derivatives (Mohanty et al., 2008b).

2.1.2.2. Antimicrobial activity

In the current years, infectious diseases have increased to a large extent and antibiotic resistances have become an ever increasing therapeutic dilemma (Sekhri, 2013). Growing bacterial resistivity is recently becoming a challenging research towards identification and application of herbal drug against multidrug resistant strains (Alviano and Alviano, 2009). The utilization of plant products with potential antimicrobial molecules, are of enough implication to curative treatments (Nagesh and Shanthamma, 2009).

In the last two decades, there has been a significant increase in the prevalence of life-threatening universal fungal infections. Development of strong strategies for treating
fungal diseases is a challenge now (Pfaller et al., 2004; Singh et al., 2000). The various drawbacks of most clinically used antifungal drugs include- they are pretty toxic, they have a low efficacy and high cost, furthermore, resistant strains are produced by their frequent use (Fridkin et al., 2005); therefore, there is a great need for new antifungals that concern to a broad array of structural classes, that can selectively work on new targets with less side effects (Angelini et al., 2012; Pagiotti et al., 2011).

In view of the enormous potentiality of plants as sources for antimicrobial agents, antibacterial and antifungal activities from different medicinal plants of Zingiberaceae family are discussed below.

Different solvent mediated extracts of Zingiberaceae species like Alpinia, Zingiber and Costus genera have been scrutinized for antimicrobial properties by Habash et al., (2000). Most of the extracts were found to have antibacterial activities but the methanol solvent extract of Costus discolor showed comparatively more antifungal activity.

Antifungal activity of extracts of 11 Zingiberaceaeous species was tested using disc diffusion bioassay. Some of the species like Alpinia galanga, Curcuma zedoaria and Zingiber purpureum extracts were analysed and exhibit varied inhibitory activities to several highly human pathogenic fungus (Ficker et al., 2003). The antimicrobial properties of Zingiber officinale were studied by Singh et al., (2008) using various food-borne pathogenic microorganisms.

In Zingiberaceae family, many members are aromatic due to the presence of essential oils that are located in highly specialized secretory structures. The antimicrobial properties of the volatile oils extracted from different plant taxa of Zingiberaceae family have also reported (Sasidharan, 2010, Kader et al., 2011, Singh et al., 2002).

A diverse antimicrobial properties study was carried out in Alpinia species having varied compound summary. To date maximum study has been focused on Alpinia galanga which hold more active constituents in comparison to other species in the genera (Weerakkody et al., 2011). Rhizome oils of Alpinia galanga have potential inhibitory effect against a wide range of microorganisms (Oonmetta-aree et al., 2006). Ethanol extract of Alpinia galanga and aqueous, methanol, ethanol extract of Alpinia calcarata possess potential antimicrobial activities against different microbes (Bhunia et al., 2012).

Arambewela et al., (2010) determined antifungal activity of Alpinia calcarata rhizome essential oil against crop pathogens Curvularia spp. and Colletorichum spp. using the
agar plate method. Oil from the red cultivar showed significant inhibition of microbes. The volatile oils isolated from different parts of the plant *Alpinia conchigera* was investigated for antimicrobial properties and found to be poor inhibition against the microorganisms examined (Ibrahim *et al.*, 2009).

### 2.1.2.3. Anticancer activity

A huge reservoir of bioactive compounds exists in many plant species of this Earth, but only a few percentage of such plants have been investigated and continued to be an important source of anticancer agents. Efforts are ongoing worldwide for identification of new anticancer compounds from plants. Due to present turn down in the number of new compounds from the pharmaceutical industry, novel anticancer agents are required from traditional medicines. Due to lack of efficient drugs, cancer is a fatal disease rating the top 3 cause of death. The chemotherapeutic agents for cancer treatment are highly expensive, mutagenic, carcinogenic and teratogenic and marrow inhibition restricts their applications (Kumarappan *et al.*, 2007). Therefore the quest for effective plant based anti-cancer drug is an active research field.

The Zingiberaceous plants contain a number of volatile chemical compounds including terpenoids, phenyl propanoids, flavonoids, and sesquiterpenes, which have reported to have antitumor activity. The Zingiberaceaeous plants are excellent candidates for development of novel chemotherapeutics because they considered safe for human consumption.

Curcumin of *Curcuma longa* inhibits the growth of cancer by preventing production of dangerous eicosanoid such as PGE-2. The anticancer potential of curcumin has been confirmed in initiation, promotion and progression stages of cancer development (Suran *et al.*, 2013).

Anti-proliferative and cytotoxic activity of the ethanol extract of *Costus pictus* was demonstrated at lower concentrations and it induced cell death in HT-1080 fibrosarcoma cells. The ethanol extract of *Costus pictus* had no cytotoxicity on normal lymphocytes. Aqueous and methanol extracts were less effective as compared to ethanol extracts. Isa *et al.*, (2012) investigated the cytotoxicity of boesenbergin A, isolated from *Boesenbergia rotunda*, using Human hepatocellular carcinoma (HepG2), colon adenocarcinoma (HT-29), non-small cell lung cancer (A549), prostate adenocarcinoma (PC3), and normal hepatic cells (WRL-68) by MTT assay.
Cytotoxicity of pure methoxyflavone components from *Kaempferia parviflora* rhizome extracts was evaluated by Hossain *et al.*, (2012). The cytotoxicity of 3,5,7,4′-tetramethoxyflavone (TeMF), 5,7,4′-trimethoxyflavone (TMF), and 5-hydroxy-3,7,3′,4′-tetramethoxyflavone (5-H-TeMF) purified from its rhizome extracts on human colorectal carcinoma (HCT-15) cells was evaluated. All the three compounds exhibited dose-dependent inhibitory effect on HCT-15 cells. All these compounds have the potentiality to be the novel anti-cancer drugs.

Many *in vitro* studies that have been carried out in various cancer cell lines prove the potential of *Alpinia* species as anticancerous plant. A novel compound, Pinostrobin chalcone, isolated from *Alpinia mutica*, showed remarkable cytotoxic activity to various human carcinoma cell lines (KB, MCF7 and Caski cells) (Malek *et al.*, 2011). Antiangiogenic activity of *Alpinia oxyphylla* fruits has been found in n-hexane and ethyl acetate fractions (He *et al.*, 2010). 4 compounds isolated from *Alpinia officinarum* has been screened for cytotoxic activity in some cancer cell lines and only 7-(3,4-dihydroxyphenyl)-1-(4-hydroxy-3-methoxyphenyl)-4-en-3-heptanone was found notable cytotoxic compound against HepG2, MCF-7 and SF-268 (An *et al.*, 2008). The role of flavonoids of *Alpinia officinarum* on whitening effects based on melanin biosynthesis have been studied by Lu *et al.*, (2007) and found that the flavonoid mixture and galangin could be a whitening agent and a capable candidate for prevention of skin cancer.

The anticancerous activities of the extracts and isolated major constituents of the *Alpinia* genus like 1′S-1′-Acetoxychavicol acetate and p-coumaryl alcohol γ-O-methyl ether in *Alpinia galanga* (Nam *et al.*, 2005), 7-(3,4-Dihydroxyphenyl)-1-(4-hydroxy-3-methoxyphenyl)-4-en-3-heptanone in *Alpinia officinarum* (An *et al.*, 2008), Oxyphyllone A and B in *Alpinia oxyphylla* (Xu *et al.*, 2009), Rubraine, isorubraine and sumadain in *Alpinia katsumadai* (Hua *et al.*, 2009), 1′S-1′-Acetoxychavicol acetate in *Alpinia conchigera* (Awang *et al.*, 2010), Hexane and dicholoromethane extract in *Alpinia scabra* (Ibrahim *et al.*, 2010), Pinostrobin in *Alpinia mutica* (Malek *et al.*, 2011), Galangin in *Alpinia officinarum* (Lu *et al.*, 2007) have been reported.

2.2. Molecular characterization

DNA-based genetic markers have enormous application in various areas of genomics, plant breeding, metabolomics, genetic engineering etc. These markers are efficiently used in DNA fingerprinting, genotyping study, determining individuals’ purity, in
phylogenetic testing etc. The development of DNA based markers become easier after the innovation of Polymerase Chain Reaction (PCR).

DNA markers are easy to handle, proficient and less time consuming. In recent times there is an increased emphasis in molecular markers for characterization of the genotypes, genetic fingerprinting, in identification and cloning of important genes, marker-assisted selection, and in understanding of interrelationships at the molecular level. The PCR based molecular markers like RAPD, ISSR, SSR and AFLP are widely acceptable for its genetic integrity as well as cost effectiveness and simplicity. The main PCR technique that has been applied to the study of molecular characterization is RAPD analysis (Williams et al. 1990). RAPD is a technique that generates random fragments and thereby known as multiple arbitrary amplicon profiling (MAAP). The methodology is easy and has been extensively utilized by several researchers for the estimation of genetic diversity, investigation of hybridization, genetic variation within species and cultivar identification (Minano et al., 2009).

Mandal et al., (2007) reported a great deal of polymorphism among the accession of Costus speciosus despite the morphological identity among them. RAPD polymorphism was used to differentiate within and among Curcuma wenyujin, Curcuma sichuanensis, and Curcuma aromatica (Chen et al., 1999). Pradhan et al., (2014) investigated the genetic diversity among five varieties of ginger through RAPD markers. Total 104 clear, reproducible and scorable fragments ranging from 150-13000 bp were generated from 21 primers and 99% were found polymorphic.

SSR and ISSR markers are also proved to be the potent marker for molecular characterization analysis. Inter simple sequence repeats (ISSR) is a molecular marker that can be carried out without prior knowledge of DNA sequence in the genome. Microsatellites (SSR) correspond to major source of polymorphism from repetitive sequences.

Sigrist et al., (2011) examined the genetic diversity among 39 accessions of turmeric (Curcuma longa) from a Brazilian germplasm collection. Genetic analysis was performed using 17 microsatellite markers. Jan et al., (2012) also studied genetic variability in turmeric germplasm using agro-morphological traits. Twenty one polymorphic microsatellite loci were isolated and characterized from turmeric (Curcuma longa L.). These markers were screened across thirty accessions. The number of alleles observed for each locus ranged from two to eight with an average of 4.7
alleles per locus. The simple sequence repeat (SSR) markers can complement the currently available SSR markers and would be useful for the genetic analysis of turmeric accessions (Senan et al., 2013).

Assessment of genetic variation and relationships between five varieties of curcuma (Curcuma alismatifolia) was done using PCR-based molecular markers by Taheri et al., (2012). 16 ISSR primers generated 139 amplified fragments, of which 77% had high polymorphism among these varieties. Genetic similarity among the varieties was estimated using Jaccard’s similarity coefficient. A principal component plot was developed to examine genetic relationships among varieties.

The genetic analysis of 18 ginger cultivars from Northwest Himalayan region was undertaken using ISSR and SSR by Pandotra et al., (2013). Both ISSR and SSR techniques were efficient in distinguishing all the 18 ginger cultivars, however, SSR markers were found suitable than ISSR.

Genetic fingerprints of many species of Zingiberaceae were examined by Mohanty et al., (2014) by RAPD, ISSR and SSR markers. The combined data reveals better distribution of individuals as per their similarity as well as dissimilarity with respect to above molecular markers.

Xia et al., (2005) undertook molecular genetics and chemical assessment of rhizome curcuma in China. The chemical fingerprint and the genetic characteristic could serve as markers for quality control of Curcuma species. Saritnum and Sruamsiri, (2003) used RAPD markers to assess the level of genetic diversity in 37 galanga (Alpinia spp.) accessions collected from different areas of Thailand. In this study out of 22 RAPD primers, only 8 primers produced a total of 73 polymorphic bands. The UPGMA cluster analysis of genetic similarity also separated the accessions into 5 major clusters. The origin and relationship of Alpinia galanga based on its molecular data was studied by Rangsrirui et al., (2000a).

Kress et al., (2002) reported the DNA fingerprinting study of gingers based on nuclear internal transcribed spacer (ITS) and plastid matK regions. He suggested that a number of morphological characters, based on which the ginger cultivars are catagorized as homoplasious and three of them are paraphyletic. Cao et al., (2001) and Sasaki et al., (2002) used sequence analysis of Curcuma drugs of Chinese and Japanese on the 18S rRNA gene and trnK gene for their authentication purpose. Cao et al., (2003) used trnK nucleotide sequencing-for proper identification of curcuma species. A phylogenetic
study was also carried out in Zingibereae by taking nuclear ribosomal DNA (ITS1, 5.8S, and ITS2) and chloroplast DNA (trnL [UAA] 5’ exon to trnF [GAA]) (Ngamriabsakul et al., 2003).
Zhao et al., (2001) investigated *Alpinia galanga* and its related species i.e. *A. conchigera*, *A. suishaensis*, *A. maclurei* and *A. polyantha* for authentication purpose. Sequence analysis of these well known conserved nuclear ribosomal DNA internal transcribed spacer (ITS) regions of the five taxa showed that they can easily be distinguished from each other. Consequently, evidence from nrDNA ITS sequence variation can identify the medicine at the DNA level.
Genetic diversity among 17 *Alpinia* species native to Taiwan representing four subsections of sect. *Alpinia* within genus *Alpinia* of family Zingiberaceae was evaluated individually using different marker systems by Lin, (2014). The applied marker systems potentially targeted different regions of the genome and included 15 ISSR markers and 10 sequence-related amplified polymorphism (SRAP) markers. *Alpinia* species were clustered into two groups using ISSR, SRAP, or combined data set demonstrating that the genetic diversity in different target regions of examined *Alpinia* genomes was alike.

2.3. *In vitro* studies

Plant tissue culture technology has emerged to be a versatile tool for propagating elite clones and for screening of useful variants. Plant propagation through culture technology i.e. the totipotency of cells has been established by the German scientist Haberlandt (1902). Contribution of Laibach (1929), White (1934), Loo (1945) and Murashige and Skoog (1962) led the way to lay a strong foundation, which brought the technology and its versatile application to the forefront especially in the field of agriculture and horticulture. The successful application of plant tissue culture technology for plant improvement is based upon the mass regeneration of plants from cultured cells or tissues.

2.3.1. Micropropagation

Micropropagation is a reliable methods of *in vitro* studies by which large number of disease free plants can be produced. Micropropagation can also be defined as the true-to-type propagation of a selected genotype using *in vitro* culture technique (Debergh and Read, 1991). Micropropagation has been recognized as a sound commercial proposition, especially in ornamentals and plantation crops to produce nuclear stock free from pathogens and viruses. To speed up the process of producing better varieties,
technologies like pollen, anther and protoplast culture are being used. All these developments have contributed to the acceptance of in vitro culture techniques as viable and valuable tools.

In vitro culture methods have been established in many species of Zingiber i.e., Z. wightianum, Z. montanum, and Z. zerumbet (Tyagi et al., 2006) and Z. officinale (Mohanty et al., 2008a). Micropropagated Curcuma species include C. longa (Panda et al., 2007), C. caesia (Mohanty et al., 2010a), C. aromatica (Mohanty et al., 2008c). Micropropagation has been reported in Alpinia galanga (Parida et al., 2011), Alpinia officinarum (Selvakkumar et al., 2007), Alpinia purpurata (Kochuthressia et al., 2010), Alpinia zerumbet (Rakkimuthu et al., 2011), Kaempferia galanga (Parida et al., 2010) and Kaempferia rotunda (Chirangini et al., 2005).

Protocols for micropropagation of many economically and medicinally important Zingiberaceous species like Curcuma aromatica, Amomum subulatum, Curcuma amada, Kaempferia galanga, Kaempferia rotunda, Alpinia spp. were developed. Most of the workers used MS basal media supplements with Benzyl Adenine or Kinetin (1 to 5 mg/l) for multiplication and around 1 mg/l IAA or NAA for rooting. An efficient protocol for mass propagation of Zingiber zerumbet Smith was developed by Faridah et al., (2011). The highest mean number of shoots (5.6) per explants was obtained on MS medium supplemented with a combination of 5.0 mg/l BAP and 2.0 mg/l IAA or 3.0 mg/l BAP and 0.5 mg/l IAA. The medium containing 1.0 mg/l of BAP and 2.0 mg/l IAA was optimum for best shoot length (9.44 cm). In vitro propagation of Etingera elatior from Axillary bud explants was reported by Abdelmageed et al., (2011).

An ideal micropropagation method of Boesenbergia rotunda (L.), an important medicinal plant has been developed by Yusuf et al., (2011). Numerous shoots were induced from young shoot bud of B. rotunda on Murashige and Skoog (1962) medium supplemented with 30.0 g/l sucrose, 2.0 g/l gelrite, different concentrations of 6-benzylaminopurine (BAP) and naphthaleneacetic acid (NAA). Multiple shoots were induced from 90% of the explants within 10 to 14 days of inoculation with 5 maximum numbers of shoots per explant. Rooting was spontaneous in almost all the treatments after 10 to 14 days of culture. Micropropagated plantlets were successfully acclimatized.

Mohanty et al., (2013) reported in vitro plant regeneration of a medicinally important herb Hedychium coronarium using sprouted buds of rhizomes. MS medium
supplemented with 2.0 mg/L BA and 0.5 mg/L NAA was most effective. Parida et al., (2013) also described an optimized protocol for large-scale in vitro propagation of *Hedychium coronarium*. Rhizome bud explants were inoculated on MS medium supplemented with 3 mg/l BA, 3 mg/l Kn, and 0.2 mg/l thidiazuron, yielded maximum 13.2 numbers of shoots. A consistent proliferation rate was seen after sub-culturing of shoots on fresh medium at an interval of 4 weeks. For rooting, Kn (3 mg/l) and IAA (0.5 mg/l) was appropriate to yield a maximum of 6.3 ± 0.5 number of roots. Chan and Thong, (2004) reported in vitro propagation of *Alpinia conchigera, Alpinia galanga, Curcuma longa, Curcuma zedoaria*, and *Kaempferia galanga*.

A successful protocol was developed by Rakkimuthu et al., (2011) for mass propagation of *Alpinia zerumbet*. MS medium supplemented with 3% sucrose (w/v) and the combination of 1.5 mg/L of BAP and 0.5 mg/L of kinetin was found appropriate for shoot induction and multiple shoot production. Jose et al., (2002) established in vitro culture method for rapid propagation of *Kaempferia galanga* on MS medium fortified with 4 mg BA, 1 mg Kn and 1mg NAA per litre. A protocol was developed for micropropagation of *Kaempferia galanga* and *Kaempferia rotunda* by Geetha et al., (1997). Development of microshoots from rhizomatous buds of *Kaempferia galanga* were observed by Chirangini et al., (2005) when cultured on MS medium supplemented with plant growth regulators. Leaf and rhizome explants of *Kaempferia galanga* were cultured aseptically on MS medium with various combinations and concentrations of IAA, BA, NAA, 2,4-D and Kn by Swapna et al., (2004).

### 2.3.2. Genetic stability of micropropagated plants

In any in vitro conservation effort, plant regeneration and successful propagation of genetically stable plantlets are the important prerequisites. Molecular markers have found to be the most advantageous tool for establishing genetic uniformity of the in vitro propagated plantlets. Different types of molecular markers like RAPD, ISSR, SSR, and AFLP are now routinely used in assessment of genetic stability of micropropagated plants. Genetic assessment of tissue culture derived plants by RAPD marker has been done by many workers. Genetic stability in micropropagated *Curcuma longa* was assessed by Panda et al., (2007) through RAPD analysis. Genetic stability of in vitro propagated plantlets from dormant axillary bud of *Zingiber officinale* has been reported by
Mohanty *et al.*, (2008a). They reported, the length of the culture period (more than two years) with regular subculture did not affect the genetic integrity of the micropropagated gingers. RAPD analysis has been carried out by Nayak *et al.*, (2005) in 16 promising cultivars of ginger (*Zingiber officinale*) revealing considerable genetic variability among them. RAPD profiling within the *in vitro* conserved lines of ginger replicates did not identify any polymorphism, demonstrating the genetic stability of the *in vitro* conserved lines. The ISSRs and SSRs are now proved to be much more effective in assessment of the genetic stability of micropropagated plants as reported by many workers in different species (Joshi and Dhawan, 2007; Bhatia *et al.*, 2009).

The genetic stability of *in vitro* conserved germplasm of *Curcuma* species as revealed by RAPD markers was reported by Hussain *et al.*, (2008), whereas the genetic stability of *in vitro* conserved *Curcuma longa* using RAPD markers was studied by Tyagi *et al.*, (2007). The genetic integrity of *Curcuma caesia* through RAPD and ISSR markers has been reported by Mohanty *et al.*, (2010a). Mohanty *et al.*, (2012a) determined the genetic stability of micropropagated *Curcuma amada* using RAPD and ISSR markers. Fifty regenerants were analyzed and all showed monomorphic bands thereby confirming their genetic uniformity.

The clonal fidelity of microrhizome induced varieties of turmeric (*Curcuma longa* L.) *viz.*, Alleppey Supreme, Prabha and Lakadong was checked by Archana *et al.*, (2013). The monomorphic pattern of RAPD profiles observed for the microrhizome derived plants in comparison with the mother plant confirmed the clonal fidelity. This recommends that planting material production through microrhizome technology is a safe method for multiplication of true-to-type plants in turmeric even after 30 subculture cycles.

Parida *et al.*, (2013) determined the genetic stability of micropropagated of *Hedychium coronarium* through RAPD and ISSR analysis. Genetic integrity of *in vitro* propagated clones of shampoo ginger (*Zingiber zerumbet*) were periodically evaluated using RAPD and ISSR analysis by Mohanty *et al.*, (2012b) and all regenerants was genetically uniform. Genetic fidelity of *in vitro* propagated *Alpinia galanga* was assessed by Parida *et al.*, (2011). RAPD and ISSR banding patterns of micropropagated plantlets were found monomorphic and similar to that of mother plant. The genetic stability of micropropagated *Kaempferia galanga* using RAPD and ISSR analysis was done by Mohanty *et al.*, (2010b) and Parida *et al.*, (2010).