Chapter 1: Introduction

India is one of the mega-diversity centres harbouring a multitude of medicinal plant species of economic importance. Zingiberaceae is a family of medicinal and economic significance. It occurs mostly in the tropics comprising of 53 genera with a total about 1200 species (Kress et al., 2002). The family is represented by twenty two genera and one hundred seventy eight species in the country India. (Jain and Prakash, 1995). There are nine genera and seventy species in South India (Sabu, 2006). The family is essentially tropical in distribution, with few species occurring in temperate climates, and is particularly richly represented in the Indo-Malaysian flora.

The family Zingiberaceae includes important genera such as Kaempferia, Alpinia, Curcuma, Hedychium, Zingiber, Elettaria and Costus comprising many species that provide useful products for food, medicine, spices, flavouring agents, fragrance, colouring or natural dyes, condiments as well as ornamentals (Burkill, 1966). Zingiberaceous species typically have thickened rhizomes with secretory cells producing essential oil (Mabberley, 1997). Alpinia and Kaempferia are two important genera under the family which possess medicinal and aromatic properties.

1.1. Importance of selected taxa of Alpinia and Kaempferia

1.1.1. Importance of Alpinia galanga

Alpinia galanga L. is a rhizomatous plant which is cultivated in many countries like India, Indonesia, Malaysia, Thailand, and China (Jatoi et al., 2006). Selected Alpinia species are extensively used in traditional medicines mostly in Asian countries due to their ethnomobotanical activities like anti-emetic, anti-oxidant, spasmolytic, anti-inflammatory, bacteriostatic. (Tachakittirungrod and Chowsananpohnpohn, 2007; Pompimon et al., 2009; Mahae and Chaiser, 2009). Its rhizomes are also used as flavouring agent. As its smells like cardamom it is used as spices and condiment. The rhizome product has enough importance to treat many diseases like rheumatism, ulcers, colds, whooping cough, vomiting, throat infections, stomach ache and indigestion. The essential oil present in this plant is also equally important for health care. Borthakur et al., (1999) has also reported that its phytoconstituents have anti-ulcer, antitumour, anticalculi properties along with anti-HIV agents (Ye and Li, 2006).
1.1.2. Importance of *Alpinia malaccensis*

*Alpinia malaccensis* is a perennial unexplored medicinal plant of family Zingiberaceae mostly growing in India, Indonesia and Malaysia. It is called “Kha Pa” in Thailand and “Ring Malacc” in Vietnam. *A. malaccensis* is cultivated as an ornamental in Bangladesh, Bhutan, India, Indonesia, Malaysia, Myanmar and Thailand. *A. malaccensis* is used to cure wounds and sores. Rhizome is chewed together with betel nut to make the voice strong and clear. It is applied on gastralgia with tympanites (decoction of fruit or the crushed seed) and it is used for bathing feverish people (Kress *et al*., 2005; Bhuiyan *et al*., 2010). Activities like antioxidant and antimicrobial of its essential oil as well as extract has been carried out by us (Sahoo *et al*., 2012, 2014).

1.1.3. Importance of *Alpinia nigra*

*Alpinia nigra* B.L. Burtt is an important medicinal herb available in India, China, Srilanka, Bhutan and Thiland. It is popularly used for spice and food flavouring agent as well as medicines. Peple used this plant parts as vegetable. It has also extensively used to treat many diseases like jaundice, dyspepsia, gastric ulcers, and bronchitis (Qiao *et al*., 2007). Its different plant parts have been used extensively in various traditional formulations. Tripura tribes consume the raw juice of green shoot for its presumed anthelmintic properties (Roy *et al*., 1999). *Alpinia nigra* has also potential effect on diseases like gastric diseases and stomachic um etc. (Habash *et al*., 2000). HPLC determination of *A. nigra* seed clusters revealed the presence of two bioactive flavones glycosides, astragalin and kaempferol-3-O-glucuronide (Qiao *et al*., 2007).

1.1.4. Importance of *Alpinia calcarata*

*Alpinia calcarata* is also one of the less explored species of Zingiberaceae. Its rhizomes exhibit antinociceptive activities (Arambewela *et al*., 2004). This medicinal plant has enormous significance to treat throat inflammation, purifying blood, stimulating digestion, rheumatism, bronchitis and asthma etc. Rhizome is the economically important part, which is a major constituent of many formulations of indigenous system of medicine. It is uslo useful in improving voice and marinating youthful vigour (Jayaweera, 1982).

1.1.5. Importance of *Kaempferia galanga*

*Kaempferia galanga* Linn. (aromatic or sand ginger) of family Zingiberaceae is an endangered rhizomatous plant with several medicinal properties. The plant stays close to the ground achieving a height of 3 inches, no central stem and its leaves just grow
right off the rhizomes up to 6 inches. The plant blossoms with small white fragrant flowers with a splash of purple at the centre but lasts only for a few hours. Many industries require its rhizomes for the essential oil for drug development as well as in preparation of perfumes (Rahman et al., 2004). Further, bulky leaves of the plant are used for flavouring foodstuffs, preparing mouthwashes and a hair tonic, locally; leaves are antinociceptive and anti-ulcerative (Sulaiman et al., 2008). Antioxidant activity of the plant has been reported using the ferric reducing antioxidant power, the β-carotene bleaching and the oxygen radical capacity assay by a Malaysian variety of the plant (Aziman et al., 2012). Rhizomes are used for curing bronchitis, asthma, malaria, skin disease, wounds and splenic disorders (Kochuthressia et al., 2012). The plant is reported to have antimicrobial, antimalarial, larvicidal properties, antioxidant, antinociceptive and anti-inflammatory activities (Hanumantharaju et al., 2010; Thiengsusuk et al., 2013; Satoto et al., 2013; Sumazian et al., 2010).

1.1.6. Importance of Kaempferia rotunda

*Kaempferia rotunda* L. is distributed mostly in India and in several parts of Bangladesh. In diabetic patients the plant is found to be quite effective for treatment of high blood sugar levels. This rhizome juice is also taken orally to relieve pain. The tubers of this plant are also one of the major components of an ayurvedic drug ‘Hallakam’ which is used to treat stomachic, mental disorders and insomnia (Sereena et al., 2011).

1.1.7. Importance of Kaempferia parishii

*Kaempferia parishii* belongs to family Zingiberaceae. The plant is characterized by shiny, ribbed green foliage having purple with pink flowers continuously all the way through summer. There is no published report available for this plant which indicates that the plant is in unexplored condition. This propels us to study this plant for evaluating its phytochemical contents and its molecular profiling.

1.2. Definition of the problem

In spite of the great economical potential of the species of *Kaempferia* and *Alpinia*, they have received much less attention from biotechnologists for their phytochemical profiling, molecular characterization and conservation. The increasing demand for medicinal plants has endangered several species. Some species of *Kaempferia* and *Alpinia* are in threatened condition. The plant extracts and essential oil of these species possessing myriad of therapeutic activities, are of high demand in pharmaceutical industries. Besides, the important bioactive constituents like ethyl –p-methoxy
cinnamate in *Kaempferia galanga*, 1,8-Cineol in *Alpinia galanga* have tremendous export potential. The cost of ethyl-p-methoxy cinnamate is $78 per 5 gms and that of 1,8-Cineol is $326 per 100mg. The price of the essential oil of the *Kaempferia galanga* varies from $600 - 700 per kg on the global marketplace, and rhizomes as well as leaves are well exploited through the local individuals and pharmaceutical industries (Chithra *et al.*, 2005). Ten *Alpinia malaccensis* seeds cost $2.70 indicating the value of the plant.

Due to overexploitation of these wild medicinal plants, there is rising alarm about declining populations, loss of genetic diversity and habitat deprivation. Therefore agencies concerned with conservation policies like BSI, ENVIS (MoEF) etc have recommended for domestic cultivation of these wild species as a viable alternative to rise above the problems that are inherent in herbal extract. Thus it is essential to develop phytochemical profiles which represent the bioactive constituents of the herbal drugs in order to find out the elite genotypes with high drug yielding potential.

In spite of vast economic potential and rich diversity among the species of *Kaempferia* and *Alpinia* in India, very little work has been done on their molecular characterization. Traditionally, the species characterization has been made on the source of the morphological markers. The accessibility of small figure of morphological markers, their weakly recognized genetic control and environmental influence on phenotypic expression at diverse stages of development are a few of the established impediment in using these as steady genetic markers. Moreover identification of taxa through herbarium is very difficult because of problem in preserving thick and fleshy rhizomes. The precise molecular characterization of available germplasm using molecular markers is one step ahead providing precise genetic information meant for proper identification and improvement of the desired taxa.

Some important wild species are being depleted due to commercial exploitation and deforestation. *Kaempferia galanga* and *Kaempferia rotunda* are saved by the Convention on International Trade in Endangered Species (CITIES) as a conservation measure. Conservations of germplasm of wild, endemic and high yielding elite genotypes in clonal repositories by conventional methods under field condition are very difficult due to greater disease susceptibility. Further, conventionally propagated plants grown in different agro-climatic regions are always not stable in content and quality of
useful secondary metabolites due to the effect of varying environmental and physiological factors thus necessitating their conservation through in vitro methods.

1.3. Necessity of phytochemical evaluation

Medicinal plants are very important for the treatment of many diseases in humans from ancient times and an vital part of the Indian traditional medicinal system, such as the Ayurveda and Siddha (Basu, 2002). Globally, an extensive activity of research is undergoing on diverse plant group and their remedial values. At present, about 25 percent of the active constituent has been identified from plants that are used as prescribed medicines (Gill et al., 2011). According to Prajapati et al., (2006), about 80,000 plant species are utilized by the different systems of Indian medicine. Scientifically, medicinal flora have proven to be an rich resource of biologically active compounds, many of which have already been formulated into valuable remedial substances or have provided a origin for the development of novel lead molecules for pharmaceuticals.

In this modern age of chemotherapeutics, the herbal medicinal system has drawn the attention of both academician and researchers for the development of novel drugs, use in cosmetic industries, food preservation etc. Among the plant products essential oils and extracts have been used for many thousands of years in foodstuff preservation, pharmaceuticals, alternative medicine, and innate therapies as well as in perfumes, aromatherapy, spices and nutrition (Buchbauer, 2000). Phytochemists take part in a fundamental role in the chemical assessment of these plants. Phytochemical screening can lead to identification of biologically active compounds from complex plant extracts through bioassay guided study (Harborne, 1998).

Zingiberaceae has a wealthy resource of bioactive compounds with diverse phytomedical attributes. As different parts of medicinal plants have significant amounts of secondary metabolites that inhibit the free radicals, so they can be used as antioxidant (Samatha et al., 2012). Some synthetic antioxidant molecules like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are globally existing, however, are reported to be toxic to animals together with human beings which have encouraged the curiosity of several researchers to explore natural antioxidants (Vinay et al., 2010). Antioxidants perform as radical scavengers, inhibit free radicals and are proficient to protect the human body as well as processed foods from oxidative damage.
attributed to the reaction of free radicals. Antioxidants are progressively more being recommended because they work directly on oxidative processes and may be a way to prevent diseases and health problems related to aging. Recently there has been a rise of attention in natural antioxidants present in medicinal and nutritional plants, which might assist to prevent oxidative harm. Natural antioxidants augment the antioxidant ability of the plasma and condense the threat of diseases (Shekhar et al., 2011).

As per the World Health Organization, the majority populations still depends on traditional medicines for their psychological and physical health needs (Rabe and Van Stoden, 2000), because of the cost of Western pharmaceutical products, side effects and poor medical facilities in rural areas (Griggs et al., 2001).

The utilization of foodstuff infected with food-borne microbes can cause a severe warning to human physical condition. The presence of microbes causes damage and reduces the nutritional quality of processed foods (Soliman et al., 2002). The inclination to exercise essential oils or plants bearing essential oils in foodstuff may serve as natural antimicrobials or antioxidant preservatives which may control the healthiness of consumers as well as long shelf-life of appropriate food stuffs (Burt, 2004; Svoboda et al., 2006).

Opposing to the artificial drugs, antibacterial activities of plant source are linked with lesser side effects and have a huge curative prospective to cure many communicable diseases. The potential for developing antibacterial from higher plants appears worthwhile as it will guide to the improvement of a phytomedicine to act in opposition to microorganisms. Nowadays numerals of clinically efficacious antibiotics are becoming less effectual due to increase of resistance. So, biomolecules of plant source come into sight to be one of the alternatives to manage these antibiotic resistant human pathogens (Kumaraswamy et al., 2008).

Universal special effects are ongoing for recognition of novel anticancer compounds from plants. By means of the present turn down in the figure of new molecular entities from the pharmaceutical manufacturing, novel anticancer molecules are being hunted from traditional medicines. Due to short of effectual drugs, cancer is a deadly disease ranking the top three reason of fatality. Numerous chemotherapeutic molecules those are available for the treatments of cancer are extremely high-priced, mutagenic,
carcinogenic and teratogenic (Kumarappan et al., 2007). Thus the search for effectual anti-cancer drug is a dynamic research field.

1.4. Significance of molecular characterization

Molecular methods are playing a significant role in management and use of plant genetic resources. Molecular markers are gradually more used for selection of germplasm to learn genetic diversity, recognize redundancies, and determine taxonomic associations, detection of genetic flaw and quantification of genetic drifts or shifts (Rao 2004). Molecular markers has a great role for assessing genetic relationships among different taxa, for assessing genetic stability of in vitro conserved plantlets, for detection of genetic changes caused due to mutation or genetic engineering and genetic characterization. It helps to indicate closeness of species and hybrids quickly and efficiently (Lim et al., 1999).

With the introduction of polymerase chain reaction (PCR) based techniques, a multitude of tools become available. These recently developed multi-loci marker techniques, which include Randomly Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Microsatelite (SSR), DNA Amplification Fingerprinting (DNAF) and Inter Simple Sequence Repeat ( ISSR) have proved especially important in diversity studies (Nebauer et al., 1999, 2000).

Molecular tools are supposed to be a trustworthy alternate means for the recognition of herbs (Kaplan et al 2004) and DNA bar-coding is the most recent step towards making of universal standard (Li et al 2011). The SSR and other sequence based markers like rbcl, matK, ITS can accurately characterize the germplasm and can be used as fast and reproducible identification tools.

Of several molecular markers available, random amplified polymorphic DNA (RAPD) seems to be one of the most popular techniques. The simplicity and cost effectiveness of RAPD and the number and speed of markers generated by this has encouraged the application of RAPD markers to several types of biodiversity-associated problems and fascinated several researchers, mainly those paying attention in either genetic fingerprinting or the patterns and levels of genetic diversity. The RAPD tool is mostly useful for population studies (Parker et al., 1998; Williams et al., 1990) as it surveys the whole genome, rather than certain fragments. RAP markers are also used for development of SCAR marker for species authentication, disease diagnostics etc.
Molecular markers have become an essential tool to analyse the extent of stability and variation present among tissue culture derived regenerants. These are now routinely used as because these markers are more reliable, less time consuming and easy to handle in comparison with morphological and biochemical markers. Random amplified polymorphic DNA (RAPD) and Inter simple sequence repeats (ISSR) seems to be the most popular techniques, used for measuring genetic stability in micropropagated plants (Panda et al., 2007; Venkatachalam et al., 2007; Mohanty et al., 2008a). The capability of ISSR in clonal reliability assessment on Allium and Aloe was established effectively by Gantait et al., (2010). Sreedhar et al., (2007) assessed the genetic fidelity of vanilla using RAPD and ISSR primers. Most recently Verma et al., (2009) used RAPD and ISSR markers in vanilla to gauge the genetic diversity. Debnath et al., (2008) used ISSR marker to differentiate the similarity of strawberry cultivars.

1.5. Significance of in vitro studies

As medicinal plants are constantly under threat because of over exploitation and depletion of natural habitats, the need for their rapid multiplication and conservation have gained importance. Due to the growing demand for plant parts like root, stem, leaf, rhizome and whole plant of most of the Zingiberaceous species, they are severely threatened. The indiscriminate collection of plants coupled with vanishing forests and grasslands has resulted the depletion of gingers in the wild. If major steps are not taken in favour of their farming, mass propagation and conservation, they possibly will be vanished from the natural foliage forever.

Selected plant species with important drug yielding traits need to be preserved using in vitro techniques for sustainable commercial utilization through stable supply of high quality drugs to market and to accelerate future improvement programme of these medicinally important plants. Conservation of germplasm in clonal repositories is difficult due to high occurrence of rhizome-rot disease. In view of this, conservation of germplasm in the in vitro gene banks is a viable method to supplement conventional conservation strategies. Most of the plants used as medicines in traditional practice are rare and not found commonly. Over exploitation of the natural herbs would soon result in depletion of the wild species and they would become extinct. Also, export potential of the medicinal herb is increasing, resulting in commercial cultivation of the medicinal herbs. The modern biotechnological tools help in the production of large number of
plants by tissue culture. Many private and government agencies produce plants of good qualities for supply to farmers for commercial cultivation (Leena and Jaindra, 2003).

Tissue culture is one of the techniques in biotechnology, which has brought about significant impact in the field of plant breeding and conservation of many endangered plants. This vital part of biotechnology can be used to progress the output of planting stuff through improved accessibility of recognized planting stockpile with required traits (Baskaran and Jayabal, 2005). Micropropagation is the true-to-type propagation of certain genotype by means of in vitro culture technique (Debergh and Read, 1991). One of the key applications of micropropagation is the bunch propagation of better-quality plants. In several instances, conventional propagation is a slow process throughout which diseases and pest trouble can limit the manufacture. Micropropagation offers the potential to make thousands or even billions of plants per year. Micropropagation allows the manufacture of huge number of plants from tiny pieces of supply plant in relatively short phase through tissue culture.

Tissue culture techniques have been routinely adapted for the propagation and effective multiplication of many medicinal plants to meet the demand of pharmaceutical firms and to protect the natural population of rare and endangered plant species (Chattopadhayay et al., 1995; Sudha and Seeni, 1996; Rani and Grover, 1999). Micropropagation techniques play a significant job for the conservation of vegetatively propagated Zingiberaceous species as a substitute to conventional field genebanks to protect against pests and ecological vagaries. Regeneration and flourishing propagation of genetically stable plantlets from culture are requisites for any in vitro management effort.

For mass propagation and rapid recovery of rare and endangered species of Zingiberaceae, tissue culture techniques have been effectively used worldwide (Tyagi et al., 2006; Mohanty et al., 2008a&b; Mohanty et al., 2010b; Parida et al., 2011). Several Zingiberaceae species are reported for its in vitro duplication which ar an uncomplicated and secure technique for manufacture of true-to-type plants within a small duration of instance (Selvakkumar et al., 2007). Tissue culture method is an efficient well recognized tool for rapid multiplication of true-to-type genotypes (Tyagi et al., 2004). The application of in vitro technology for induction of shoot culture and
micropropagation would be of huge importance and should be adopted for maintenance of elite and rare taxa of Zingiberaceae.

1.6. Importance of the present work in the context of current status

The present work deals with phytochemical evaluation, molecular characterization and *in vitro* studies of selected taxa of *Kaempferia* and *Alpinia*. Phytochemicals are major source of herbal medicines, dyes, flavors, sweetener, aromas, perfumes, insecticides, ant parasitic drugs and many other substances. Further research on plant will provide additional sources of these industrial raw materials, apart from drugs. *Kaempferia* and *Alpinia* have been in an important place since years due to the infusions and the tinctures of various aromatic species. All this potential justifies the broadest and most exhaustive phytochemical research. The individual chemicals isolated from essential oils are more often used than oils and the knowledge of essential oil composition helps to evaluate the quality of oils that allows a better and specially directed application of it (Buchbauer, 2000). Although some reports on the essential oils composition of *Kaempferia* and *Alpinia* species are available, investigations on the chemical constituents of their extract are still scarce (Nayak *et al*., 2014; George *et al*., 2007).

Although medicinal plants have been used for treatment of ailments for hundreds of years by traditional medicinal healers, there has always been a lingering question in scientific circles about their therapeutic efficacy. Modern day synthetic and chemical drugs often exhibit some side effects but traditional herbals are safer and easy to access. Nowadays, the interests in natural products are looking into sources of substitute, more natural and environment friendly antimicrobials, antioxidants, anticancer agents.

The importance of medicinal flora in disease prevention or control has been endorsed to a great extent to antioxidant properties of their constituents, generally linked to a broad choice of amphipathic molecules, generally termed polyphenolic compounds (Ivanova, 2005). Hence it is necessary to evaluate the polyphenols as well as antioxidant activity. The extracts of therapeutic plants and natural commodities have become a big resource of antioxidant and anti-ageing properties (Sumazian *et al*., 2010). The importance of the antioxidant components of plant resources in the maintenance of physical condition and protection from diseases has intrigued researchers for a lengthy point in time. Although *Kaempferia* and *Alpinia* species have been utilized for food flavouring and in traditional medicine, very little research has been conducted to investigate the antioxidant potential
of essential oil and extracts from a variety of species such as *Alpinia galanga* (Mahae et al., 2009), *Alpinia calcarata* (Arambewela et al., 2010), *Alpinia officinarum* (Zhang et al., 2010), *Alpinia zerumbet* (Elzaawely et al., 2007a&b), *Alpinia mutica* (Phang et al., 2011), *Kaempferia rotunda* (Lotulung et al., 2008), *Kaempferia parviflora* (Vichitphan et al., 2007). But there is still a demand to discover more information regarding the antioxidant potential of our target plant species of *Alpinia* and *Kaempferia*. To the best of our information, there is no previous literature on antioxidant activities of essential oils of *Alpinia galanga* and *Alpinia malaccensis*.

Plant based antimicrobials have enormous therapeutic potential and stand for an unexploited source for medicines. Few studies that have been conducted for antimicrobial activities of essential oils and extracts includes some species of *Alpinia* (Sunilson et al., 2009; Habsah et al., 2000; Das et al., 2012) and *Kaempferia galanga* (Parvez et al., 2005; Dash et al., 2014). However, to the best of our knowledge very little research has been done on the antimicrobial properties of oil and extracts of target taxa of *Kaempferia* and *Alpinia*.

A massive pool of bioactive compounds exists in various species of plants, but merely a small proportion of which have been examined and sustained to be and significant source of anticancer agents. Lots of of chemotherapeutic molecules which are available for the treatment of cancer are very much costly, mutagenic, carcinogenic and teratogenic which confines their applications (Kumarappan et al., 2007). Thus the search for effectual anti-cancer drug is a dynamic research pitch. Plants of Zingiberaceae family are safe for consumption and have proven record for having anticancerous effect, hence can be utilized as admirable candidates for development of novel chemotherapeutics. In spite of great medicinal and economic potential of *Kaempferia* and *Alpinia*, evaluation of its anticancer properties has been studied by few researchers (Suran et al., 2013; Isa et al., 2012; Hossain et al., 2012; Malek et al., 2011). Therefore studies are highly needed to evaluate the anticancer activity of the oils and extracts of target species of *Alpinia* and *Kaempferia* in order to set an appropriate formulation for novel drugs.

DNA-based molecular markers have a great usefulness for the characterization of medicinally important plant species. DNA based markers have their applications in fingerprinting genotypes and in phylogenetic analysis by which the conservation of the
plant can be made easy. The polymerase chain reaction (PCR) based technologies such as RAPD, ISSR, SSRs and other sequence based markers such as matK, rbcL etc are widely appreciated for its genetic integrity and simplicity. These molecular markers can provide a fast and reproducible identification tool for several taxa. Species identification through herbarium is very difficult because of problem in preserving fleshy and thick rhizomes of Zingiberaceaeous plants. Conventional method of identification of zingiberaceous species like Kaempferia and Alpinia by taxonomist are very difficult at non flowering stages. Molecular profiling reported in some zingiberaceous taxa (Mohanty et al., 2014; Pandotra et al., 2013; Nayak et al., 2006) does not include profiling of our target species using different molecular markers.

Tissue culture techniques contribute a vital role for the conservation of vegetatively propagated Zingiberaceous species as a substitute to conventional field genebanks to preserve against pests and environmental vagaries. Regeneration and successful propagation of genetically stable plantlets from culture are fundamentals for any in vitro conservation attempt. Though micropropagation method has been reported in few species of Kaempferia and Alpinia (Parida et al., 2010; Parida et al., 2011; Selvakkumar et al., 2007; Rakkimuthu et al., 2011) genetic stability assessment of in vitro generated plantlets has been made for a few genotypes. There is yet no report on micropropagation of Alpinia malaccensis and stability assessment of micropropagated plants through molecular markers based analysis. There are no reports so far available on genetic stability of in vitro propagated Kaempferia rotunda. The present work aims for an appropriate procedure for true-to-type in vitro production of selected plant species with high drug yielding potential and effort has been made to ensure desired genetic stability of in vitro propagated plantlets for more than two years by analyzing them with standardized molecular markers. This practice would facilitate an alternative method for large scale production of genetically identical clones and successful establishment of these medicinal species for steady supply of drug to the marketplace.

Thus keeping in view the importance of target species of Alpinia and Kaempferia, current status of research and existing lacunae in studies, the present work has been initiated to carry out phytochemical evaluation, molecular characterization and in vitro studies of selected species of Alpinia and Kaempferia.