CHAPTER 1

Introduction
The use of plant derived medicines has a long history. From the ancient time the ethno medicinally important plants are used as whole plant or the crude preparations are used for different therapeutic or experimental values. But, there are some drawbacks. The amounts of the active principles vary with variations in different agro-climatic zones, from one season to another, with different plant organs, with different plant clones, even with diurnal rhythms. Co-occurrence of undesirable principles may cause synergistic, antagonistic, or other undesirable, and possibly unpredictable, modulations of the bioactivity. Changes or losses of bioactivity due to variability in collection, storage, and preparation of the raw material also may impart confusion. To overcome these problems approaches to isolate the pure bioactive principles from these ethno medicinally important plants were formulated. Isolation, proper physicochemical characterisation of bioactive principles from plants help chemists to synthesize these compounds within the laboratory which may also lead to the cost effective production of these natural drugs. Several bioactive secondary metabolites have been enlisted as natural products that show various types of ecofriendly therapeutic effects. Under this category terpenoids, phenolics and nitrogen containing compounds, have secured successful positions.

This thesis primarily deals with the identification, chemical characterization and evaluation of some active principles from *Curcuma longa* L. (Turmeric) and *Curcuma caesia* Roxb. (Black turmeric). The present author of this thesis reports the presence of some bioactive terpenoids from the two plants. The compound isolated from *Curcuma longa* L. was identified as 2, 7, (14), 10 Bisabolatriene- 1,9,12 triol. Though, this was previously reported from *Curcuma xanthorhiza* (Siegfried *et al.*, 1986; Shin-ichi *et al.*, 1990), presence of this compound in *Curcuma longa* L. is being reported for the first time by us (Ghosh *et al.*, 2013a). Another three novel terpenoids
were also isolated and identified from *Curcuma caesia* Roxb. (Ghosh *et al.*, 2013a, Ghosh *et al.*, 2013b).

The bioactive potentialities of these isolated compounds were also evaluated and it was found that some of the isolated terpenoids showed antitumour activity, and more or less all the terpenoids had antimicrobial property.

Zingiberaceae family constitutes a vital group of rhizomatous medicinal and aromatic plants characterised by the presence of volatile oils and oleoresins of export value. Generally, the rhizomes and fruits are aromatic, tonic and stimulant; occasionally they are nutritive. Some are used as food as they contain starch in large quantities while others yield an astringent and diaphoretic juice. The important genera coming under Zingiberaceae are *Curcuma*, *Kaempferia*, *Hedychium*, *Amomum*, *Zingiber*, *Alpinia*, *Elettaria* and *Costus*. In the genus *Alpinia*, *A. galanga* is the most important one, which finds varying uses in ayurvedic preparations such as “Rasnani powder”. *Costus speciosus* is the only species in the genus *Costus* that is medicinally important. It is valued very much for its diosgenin content. In *Curcuma*, *C. longa* is the most popular one, which has been studied in greater depths already. *C. aromatic* is used in the treatment of skin diseases and is extensively used in vanishing creams. *Kaempferia galanga* has become very popular and is identified to have tremendous effect in curing bronchial and gastric diseases. Of late, it is being used in preparations of mouth washes and oral deodorants. *K. rotunda* is another related crop under this genus which has potential for great exploitation on commercial basis.

*Curcuma caesia* is a species with a blue rhizome (commonly called black turmeric), the color is much brighter and deeper blue than in *C. aeruginosa*. The leaves have a deep red-violet patch, which runs throughout the whole lamina. Some northeastern Indian tribes use this plant as a talisman to keep evil spirits away. *C.
caesia is known especially from Bengal, North East India, and Bangladesh. It is rarely found in other parts of India, such as Madhya Pradesh, Jharkhand, Chattisgarh, and Orissa. Its common names are usually derived from the deep color of the rhizome and thus this plant is called by following vernacular names: Kala haldi (Hindi) and Kalo halood (Bengali). It is used as local medicine in Bengal, and also in Madhya Pradesh and Chattisgarh.

The terpenoids constitute the largest class of natural products. The great structural diversity of the bioactive terpenoids has been a challenge to the synthetic chemist to synthesise them. In last twenty years there are some reports on the discovery of some novel terpenoids from plants due to advancement in the sophistication of separation and analytical techniques of phytochemicals. A dictionary of natural product gives data and references to a large proportion of known phytochemicals including terpenoids arranged in groups of related structural types. It helps to elucidate the structure of new terpenoids by providing an easy access to data on related compounds. The use of some physicochemical data like melting point, FT-I.R spectroscopic values, molecular mass, NMR etc. help to identify the compound of interest. This provides an efficient method of locating related or known phytochemicals/ terpenoids. The development of techniques for growing plant tissue cultures to generate terpenoids/ sterols/ sugars are in progress, however yields of secondary metabolites are normally disappointingly low.

Terpenoids have been identified to act as crop protectants in terms of their antifungal and antimicrobial actions. Biologically active secondary metabolites of spices, herbs and medicinal plants, due to their diverse range of antibacterial activities, are becoming increasingly important in food industry as potential food preservatives to control food borne pathogenic bacteria. Several terpenoid compounds
have been isolated from many plant species and reported to have various biological activities (Gao and Han 1997; Singh and Singh 2003). The rhizome extract of *C. longa* and *C. caesia* were effective against fungi like *Fusarium oxysporium*, *Aspergillus niger*, *A. nidulans*, *Alternaria solani* and bacteria like *Staphylococcus albus*, *E. coli*, and *Pseudomonas pyocyanea* etc. (Leal et al., 2003). Beside this *C. longa* also possess some antifungal activity against some more pathogenic fungi like *Botrytis cinerea*, *Erysiphe graminis*, *Phytophthora infestans*, *Puccinia recondita*, *Pyricularia oryzae*, and *Rhizoctonia solani* (Kim et al., 2003). The fungitoxic effects of alcoholic extracts of *C. longa* on *Fusarium udum*, which causes wilt disease of *Cajanus cajan* in *in vitro* and *in vivo*, were examined (Singh and Rai., 2000). Leaf extract of *Citrus medica*, root extract of *Asparagus adscendens*, rhizome extracts of *C. longa* and *Zingiber officinale*, and bulb extract of *Allium sativum* inhibited up to 100% growth of *Fusarium udum* at higher concentrations. (Allievi and Gualandris., 1984).

During the last few years, research on plant based antibacterial agents has produced a diverse range of products with novel modes of action, which are expected to have a significant impact on the control of food borne diseases in coming decade. Therefore, the demand for plant based antimicrobials as novel bactericide sources for controlling food borne pathogens is rapidly increasing (Ulubelen et al . 2000; Kubo et al . 2004; Giang et al . 2006). For example, The root extract of *Salvia hians* was reported to be rich in diterpenoids, quinnone, tanshinone and a new orthoquinoid diterpene (3a, 17-dihydro- Xytanshinone) (Khetwal K.S. et al., 1992). Methanolic extract of *C. longa* shows inhibition against *Helicobacter pylori*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* (Mahady et al., 2002; Singh et al., 2002). The essential oil of
the genus *Mentha* L. known as Pennyroyal is generally considered to be a rich source of pulegone, menthone, isomenthone as well as many other important terpenoidal compounds (Kokkini *et al*., 1991).

Derivatisation of phytochemicals or terpenoids helps to correlate structure activity relationship along with their modes of actions. Common derivatisation is done by acetylation, methylation and hydrogenation etc. The acetylation of an amino group or hydroxyl group is an important fundamental transformation in pharmaceutical chemistry (Greene and Wuts, 1999; Pearson and Roush, 1999) and this transformation leads to the development of new pharmaceutically active compounds. Some of the derivatives synthesized by the acetylation of amino group containing compounds, being presently marketed in different pharmaceutical preparations include acetyl cysteine, acetaminophen and phenacetin (Hoover, 1975; Gerbino, 2005). The, double-ended chelating compounds, synthesized by the acetylation of diamines, have previously been reported to be very effective for cancer chemotherapy, due to their chelating effect with the substrate (Breslow *et al*., 2000; Gershell, 2001). In our report also the acetylated derivative of 2, 7, (14), 10 Bisabolatriene- 1, 9, 12 triol shows greater antitumor activity than the parental compound (Ghosh *et al*., 2012). Although, the crystal structure studies of many of these compounds have previously been reported (Zhang *et al*., 1996; Aakeröy *et al*., 2010) and in some cases these compounds have been used in the synthesis of their metal complexes (Goodgame *et al*., 1999; Goodgame *et al*., 2000; Chatterton *et al*., 2001; Hussain, 2007; Hussain and Goodgame, 2007) but no systematic studies for the characterization of these compounds have been carried out and no report on the antibacterial and antifungal studies of these compounds exists.
Rational behind the choosing of the experimental plants

1. The two experimental plants incorporated in the thesis are ethnomedicinally important. Ethno medicinal values of both of the plants are very much common. Although, the phytochemical profiles of *C. longa* L. are well documented but that of *C. caesia* Roxb. is few, probably due to unavailability of this plant throughout the world.

2. Keeping in mind the ethnomedicinal importance of the two experimental plants, the present work was taken up to specifically identify the active principles that are responsible for the different types of activities. For this, analyses of the phytochemical profiles of the plants were given importance.

3. Furthermore, assessments of the bioactive potentialities of the isolated compounds were also taken under consideration.

4. However, *C. caesia* Roxb. is endemic, where as *C. longa* L. is restricted only in the Northeast Asian flora. So, for successful propagation of the two plants more pronouncely the attempt for micropropagation and production of *true to type* plants were also designed.
Systemic position of the two the experimental plants

The systematic position of two the experimental plants are as follows:

Kingdom : Plantae

Sub –kingdom : Phanerogamae

Division : Spermatophyta

Subdivision : Angiospermae

Class : Monocotyledonae

Series : Epigynae

Order : Scitaminales

Family : Zingiberaceae

Genus: *Curcuma*

Species 1. *longa* L.

Species 2. *caesia* Roxb.
Ex- situ conservation of Ethno medicinally important plant through 

in vitro technique

After isolation and physicochemical characterisation of the natural products, evaluation of their bioactive potentialities of these natural products become important. Initial screening for evaluation of the bioactive potentialities are usually done by different bioassay methods, that include antibacterial assay (Murray et al., 1995; Sokmen et al., 2004), antifungal assay (Perez et al., 1990; Alade and Irobi et al., 1993; Abioye et al., 2004) and antitumor assay (Meyer et al., 1982).

As described earlier the production and yield of natural products from plant itself varies from clone to clones, so formulation of some growth medium that can generate genetically true to type clone is essential. This objective meets the second aspects of this thesis where formulation of some growth mediums were raised through in vitro culture technique that can yield genetically true to type clones. The genetic fidelity was assessed by RAPD marker (Rani et al., 1994; Chaudhuri et al., 2007; Bhattacharya et al., 2010).

Today, plant biotechnology has emerged as an exciting area of research by creating unprecedented opportunities for the manipulation of biological systems of plants. It is a forward looking research area based on promising accomplishments in the past several decades. Plant biotechnology is changing plant science in three major areas: (1) growth and development control (vegetative, generative and propagative), (2) protection of plants against the environmental threats of abiotic or biotic stresses, and (3) expansion of ways by which specialty foods, biochemicals, and pharmaceuticals are produced. Early directions of plant biotechnology, which mostly focused on in vitro cell and tissue culture and their production of important products, are now advancing into new directions. The current state of plant biotechnology
research using a number of different approaches includes high throughput methodologies for functional analysis at the levels of transcripts, proteins, and metabolites, and methods for genome modification by both homologous and site specific recombination. Plant biotechnology allows for the transfer of a greater variety of genetic information in a more precise, controlled manner. The potential for improving plant productivity and their proper use in agriculture relies largely on newly developed DNA biotechnology and molecular markers. These techniques enable the selection of successful genotypes, better isolation and cloning of favorable traits, and the creation of transgenic organisms of importance to agriculture and industry. Regeneration of plants from callus issue is usually achieved either by organogenesis or by somatic embryogenesis. In the case of organogenesis, plant organs and tissues, such as shoots, roots, and vascular tissue connecting shoots and roots, are formed independently of each other. On the other hand, plant organs regenerated via somatic embryogenesis are thought to originate from a single cell in a callus or from suspension cultured cells. Due to this unique property of plant cells, in vitro cultures are now used to manipulate the biosynthetic potential of plants. In recent years, more plant cell culture studies utilized plant protoplasts. Isolated protoplasts from plant cells can now be micro-manipulated for somatic cell hybridization. However, the development of methods for protoplast isolation, culture, and subsequent plant regeneration are still critical for the successful somatic hybridization of higher plants. Therefore, in plant cell biotechnology, most emphasis is given to plant cells that are cultured in suspension. Cell suspension cultivation offers a unique possibility for the production of natural products on a large scale. However, plant cell suspension cultures, in general, are less productive due to the fact that undifferentiated cells are not able to produce a wide variety of secondary
metabolites. In addition, other problems arise in this kind of cultivation system that concern aseptic cultivation, specific design of bioreactors, stability of cell lines, and finally, differences in the cell cycle that are most efficient for optimizing cell suspension cultivation conditions. Plant cell suspension cultivation protocols still need to be very well designed using genetically stable cell lines with highest yields of desired secondary metabolites in order to elaborate conditions for long term cultivation. Several applications of in vitro cultures related to this thesis work have impact on

1. The production of natural products from plant cell cultures.
2. Biochemical studies of secondary metabolite pathways to answer fundamental questions of how and why these compounds synthesized.
3. Immobilization of plant cells for biotransformation of low value compounds into high value end products.
4. Micropropagation of plants that yield genetically stable fruits and vegetables, and those that produce valuable natural products.
5. Field testing of tissue culture derived transgenic plants.
7. Processes for scaling up and bioreactor design.

In continuation with these above advantages there are also some disadvantages of in vitro propagation regarding secondary metabolite production from plants which are comprised below.
### Table 1: Comparison of three different culture regimes for secondary metabolite production from plants

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<tr>
<th>Mode of cultivation</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<tbody>
<tr>
<td><strong>Green house cultivation</strong></td>
<td>Can use illumination and environmental parameters to control and regulate growth of plants and secondary metabolite production; growing of rare, endemic, or threatened plant species is possible.</td>
<td>Higher energy and labor costs than field cultivation, including automated greenhouse installation and operation.</td>
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<tr>
<td><strong>Field cultivation in a Nursery</strong></td>
<td>Can help to improve conservation of plant biodiversity; can use low winter temperatures to break seed dormancy; low cost of production of 1 year old plants.</td>
<td>Short growing season; problems of disease, insect attack, and herbivory; no control of weather conditions.</td>
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<td><strong>Cell/ tissue/ organ culture</strong></td>
<td>Can genetically modify culture to enhance metabolite biosynthesis; plant micropropagation is possible, resulting in genetic reproducibility; elicitation is more convenient; cultivation in bioreactors and enzyme-catalyzed modification of precursors into desired products are possible.</td>
<td>The process is labor intensive and is expensive due to the high cost of culture media constituents and the requirement for sterile culture conditions; stability problems of cell lines; low yield of end products in bioreactors.</td>
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Although there are some advantages and disadvantages of in vitro propagation, but it is the only way to conserve such ethnomedicinally important plants to grow throughout the year and for their scientific exploitation.