CHAPTER 2
2.1. Early history

Natural products (secondary metabolites) from different sources have been reported to be the most successful source of potential drug leads (Mishra et al., 2011; Rey-Ladino et al., 2011; Cragg et al., 2005; Hoefner, 2003; Butler, 2004). Clay tablets in cuneiform depicted some of the earliest records of natural products from Mesopotamia (2600 B.C.), containing documentation of oils from Commiphora species (myrrh) and Cupressus sempervirens (Cypress) which still finds use today in the treatment of coughs, colds and inflammation (Cragg et al., 2005). An Egyptian pharmaceutical record, the Ebers Papyrus (2900 B.C.), documents more than 700 drugs which are plant-based and includes different types of ointments, gargles, infusions, pills. Some other documented records of the uses of natural products for medicinal purpose include the Chinese Materia Medica (1100 B.C.) (Wu Shi Er Bing Fang, contains 52 prescriptions), Shennong Herbal (100 B.C., 365 drugs) and the Tang Herbal (659 A.D., 850 drugs) (Cragg et al., 2005). A record of the collection, storage and the uses of traditional medicinal herbs were also maintained by Dioscorides (100 A.D.), a Greek physician. The monasteries in France, Ireland, England, and Germany during the Dark and Middle Ages, were credited for preserving this Western knowledge whilst the Arabs were credited for preserving the Greco-Roman knowledge. The Arabs also expanded the uses of their own resources, along with the Indian and Chinese medicinal herbs which were unfamiliar to the Greco-Roman world (Cragg et al., 2005). Arabs were also the the first to own private pharmacies (8th century) with Avicenna, a Persian physician, pharmacist, philosopher and poet. Through their works such as the Canon Medicinae, they contributed a great deal to the sciences of medicine and pharmacy (Cragg et al., 2005). Throughout the history, the use of natural products as medicine has been described in several
forms such as traditional medicines, remedies, potions and oils and many of these biologically active natural products still remains to be identified. Traditional medicinal practices have played a crucial role in forming the basis of most of the early medicines which is subsequently followed by their chemical, clinical and pharmacological studies (Dias et al., 2012). The synthesis of acetylsalicylic acid (aspirin), an anti-inflammatory agent, is probably the most famous of all known examples to date. It is a derivative of the natural product, salicin, which has been isolated from the bark of willow tree *Salix alba* L (Der Marderosian, 2002). For thousands of years, different types of plants have been well documented as per their medicinal uses are concerned. These plants have undergone evolution and adaptation over millions of years in order to withstand the environmental factors including different types of insects, bacteria, fungi and weather to produce unique secondary metabolites of structurally diverse origin. The ethno-pharmacological properties of these plants have been the primary source of medicinal information for early drug discovery (Dias et al., 2012). Till date about 35,000-70,000 plants species have undergone screening for their medicinal use (Farnsworth et al., 1991). Their contribution to the world market for herbal remedies is shown in the graph below (Dev, 1999).

![Figure 2.1. World market for drugs from plant sources (Dev, 1999).](image-url)
About a hundred anticancer agents have been developed between the years 1981-2006, out of which, twenty five are derivatives of natural product, eighteen are natural product mimics, eleven are derived from a natural product pharmacophore and nine are pure natural products, thus showing a very significant contribution made by the natural sources towards the health care system (Newman, 2007). The most widely used drug for the treatment of breast cancer is paclitaxel (Taxol) which has been isolated from *Taxus brevifolia* (Pacific Yew) bark (Dias et al., 2012). Taxol, camptothecin, morphine and quinine amongst several other drugs have also been isolated from plant sources. The first two of these have been widely used as anticancer drugs, while the other two have found application as analgesic and antimalarial agents, respectively.

During 1998-2004, a total of 21 natural product and natural product derived drugs were launched in the United States, Europe and Japan, which can be classified as 3 natural products, 10 semi-synthetic natural products and 8 natural product derived drugs. Between the years 2005 to April 2010, a total of 19 natural product based drugs were approved for worldwide marketing, among which 7 have been classified as natural products, 10 as semi-synthetic natural products and 2 natural product derived drugs (Mishra et al., 2011). Review carried out on all the approved medicinal agents during the time frame of more than about twenty-five years, starting from 01/1981 – 06/2006, for all disease worldwide and from 1950 (earliest so far identified) to 06/2006, for all approved antitumor drugs worldwide, have revealed the utilisation of natural products as sources of novel structures, (Newman, 2007). According to WHO, the world’s population that has incorporated the utilization of medicinal plants into their primary health care system is almost about 65% (Farnsworth et al., 1985) and about 25% of all the drugs prescribed today come from plant sources (Farnsworth et al., 1976; Raskin et al., 2004). This estimate is sufficient enough for suggesting that plant-derived drugs make up a significant portion of natural product based pharmaceuticals.

Out of many different types of compounds or secondary metabolites on which the growth of a plant is not dependent, nitrogen containing alkaloids have
been found to have contributed the largest number of drugs to the modern pharmacopoeia which range in effects from anticholinergics (atropine) to analgesics (opium alkaloids) and from antiparasitics (quinine) to anticholinesterases (galantamine) to antineoplastics (vinblastine/vincristine) (Raskin et al., 2002) and as such the isolation of bioactive alkaloids has always been of great interest in natural product research.

2.2. Phytochemical investigation of Croton:

The genus Croton is considerably diverse in terms of its phytochemical content. The predominant secondary metabolites present in the genus are the terpenoids, chiefly diterpenoids (Block et al., 2004), belonging to the cembranoid, clerodane, neoclerodane, kaurane, secokaurane, labdane, halimane, isopimarane, phorbol and trachylobane skeletal types. Either pentacyclic or steroidal triterpenoids, have also been frequently reported from different Croton species. Presence of volatile oil constituents in several species of the genus is indicated by their aromatic nature (Oliveira et al., 2001; Lopes et al., 2003). Volatile oils containing mono and sesquiterpenoids and sometimes shikimate-derived compounds have also been reported from the genus. Croton is also quite rich in terms of the presence of active alkaloids (Amaral et al., 1998; Milanowski et al., 2002), as several members of the genus have been reported to possess different classes of alkaloids. This fact considerably enhances the importance of the genus from the medicinal point of view. As most Euphorbiaceae, the possession of latex by Croton species, which is red-colored in some species, is also a characteristic usually associated with medicinal properties (Sandoval et al., 2002; Risco et al., 2003). Phenolic substances, among which flavonoids, lignoids and proanthocyanidins predominate, have frequently been reported from different species of Croton (Salatino et al., 2007). Some of the phytochemicals reported from different species of Croton include 1-5% volatile oil including eugenol, crotsparine, crotfloatine, vanillin, oblongi-foliol, triterpenic acid, dotricontamol,
sparsiflorine, b-amyrin and b-sitosterol (zspdelhi.wordpress.com/2008/06/27/the-ethnomedicinal-use-of-Croton/).

**DITERPENOIDS**

**LABDANES**
- C. joufra
- C. oblogifolius
- C. zambesicus

**KAURANES**
- C. draco
- C. kongensis
- C. subyreatus
- C. tonkinensis

**TASPINES AND/OR BENZYLISOQUINOLINE-LIKE ALKALOIDS**
- C. Celtidifolius
- C. draco
- C. flavens
- C. hemyargyreus
- C. linears
- C. salutaris

**TRACHYLOBANES**
- C. insularis
- C. macroialectis
- C. nobustus
- C. zasbesicus

**AFRICA, ASIA, AUSTRALIA**
Except C. draco (America)

**VOLATILE OILS**
- C. arboreus
- C. caucara
- C. cuneatus
- C. draco
- C. hieronymi
- C. jimenezii
- C. malambo
- C. nepetaefolius
- C. ovalifolius
- C. sacaquinha
- C. sarcopetalus
- C. seelowi
- C. sonderianus
- C. zehntneri

**AMERICA**
- C. ollgandrum
- C. stellulifer
- C. zambesicus

**AFRICA**

Figure 2.2. Chemical and geographical affinities among *Croton* species (Salatino et al., 2007)

The diversified chemistry of the genus is probably the reason for possession of different bioactivities by the different species. A brief account of the different bioactivities (antimicrobial, anticancer, antioxidant and hepatoprotective activity) shown by different species of *Croton* are as follows.
2.3. Antimicrobial activity of different species of Croton:

The latex of *Croton laclleri* has been reported to show anti-bacterial properties (Salatino et al., 2007). Antibacterial activity of the aqueous-EtOH extract, some fractions of methanolic extract, catechin and acetyl aleuritolic acid isolated from *Croton urucurana* have been reported against *Staphylococcus aureus* and *Salmonella typhimurium* (Peres, et al., 1997). The crude essential oil of *C. urucurana* stem bark has been reported to inhibit the growth of *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Salmonella setutboul, Klebsiella pneumoniae, Saccharomyces cerevisiae, Candida albicans and Cryptococcus neoformans* (Simionatto et al., 2007). The red latex from *C. urucurana* has been reported to inhibit the growth of the fungi *Tricophyton tonsurans, Tricophyton mentagrophytes, Tricophyton rubrum, Microsporum canis and Epidermophyton floccossum*, thus showing a potential utility of the latex as an alternative treatment for dermatophytosis. Phytochemical analyses of the product demonstrated the presence of catechins, such as gallocatechin and epigallocatechin. Such substances were previously known to exert antifungal activity (Salatino et al., 2007).

The organic resins extracted from the roots of *Croton sonderianus* Muell. Arg. have been reported to show significant antimicrobial activity in qualitative biological assay. Both the ethanol (EtOH) and Hexane extract have shown biological activity in preliminary qualitative antimicrobial assay using the Gram positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*, the Gram negative bacteria *Pseudomonas aeruginosa* and *Escherichia coli*, the acid-fast bacterium *Mycobacterium smegmatis*, the yeast *Candida albicans* and *Saccharomyces cerevisiae*, the filamentous fungus *Aspergillus niger*, the dermatophyte *Trichophyton mentagrophytes* and the fungal plant pathogens *Polyporus sanguineus* and *Helminthosporium sp* as test microorganism (James et al., 1991). The essential oil of *Croton stellulifer* has been found to be active against both bacterial and fungal strain (Martins et al., 2000). Linalool-rich essential oil
isolated from *Croton cajucara* Benth is found to inhibit the growth of microbes associated with oral cavity disease such as, *Candida albicans*, *Lactobacillus casei*, *Staphylococcus aureus*, *Streptococcus sabrinus*, *Porphyromonas gingivalis* and *Streptococcus mutans* (Alviano et al., 2005). The stem bark of *Croton zambesicus* has shown significant antibacterial activity. Antibacterial activity of aqueous methanol extracts of *Croton zambesicus* on *Proteus mirabilis*, *Staphylococcus aureus*, *Bacillus megaterium* and *Bacillus subtilis* are comparable to that of ampicillin at 10μg/ml (Abo et al., 1999). 8, 9-Secokauranes from *C. kongensis* has been reported to exhibit anti-mycobacterial activity with a minimum inhibitory concentration of 6.25-25.0 μg mL⁻¹. Plaunotol, an acyclic diterpene isolated from the leaves of *Croton sublyratus* has been found to be active against fourteen methicillin-sensitive strains and twenty methicillin-resistant strains of *Staphylococcus aureus*. The phenylpropyl benzoates 3'-(4"'-hydroxy-3", 5"'-dimethoxyphenyl)-propyl benzoate, 3'-(4"'-hydroxyphenyl) propyl benzoate and 3'-(4"'-hydroxy-3"'-methoxyphenyl)-propyl benzoate, obtained from stems of *C. hutchinsonianus*, have been found to exert effect against *Candida albicans* (Salatino et al., 2007). Methanol extract of the root bark of *Croton caudatus* Geiseler has been reported to possess antibacterial and antifungal activity (Paul et al., 2012). Ethanol extract of the leaves of *Croton caudatus* Geiseler has been reported to show good activity against *Staphylococcus aureus* and *Pseudomonas putida* with zone of inhibition of 12 mm. Methanol extract of the leaves of *Croton caudatus* Geiseler has shown good activity against *Candida albicans*. Chloroform and ethanol extract of the leaves of *Croton caudatus* Geiseler have also been reported to show zone of inhibition of 10 mm and 12 mm against *Microphomina phaseolina* (Lokendrajit et al., 2012).

2.4. Anticancer activity of different species of *Croton*:

*C. hieronymi* shoots have been found to exhibit strong cytotoxic activity against lung A-549 carcinoma cells and mouse lymphoma and also moderate activity against human colon carcinoma (Catalan et al., 2003). The
dichloromethane extract of leaves of *C. zambesicus* has been reported to show *in vitro* cytotoxicity against human cervix carcinoma cells (Block et al., 2002). Root extract/fractions of *C. zambesicus* have been reported to show anticancer activity against HeLa cells (Okokon et al., 2013). The red latex of *C. lechleri* has shown anti-tumor activity (Salatino et al., 2007). TPA (12-O-tetradecanoylphorbol-13-acetate) obtained from the seed oil of *Croton tiglium* in synergy with ATRA (All-trans-Retinoic Acid) have been reported to induce apoptosis in LNCaP cells (Zheng et al., 2004). Isoguanosine isolated from *Croton tiglium* has shown an antitumor activity against implanted S-180 ascitic tumor mice (Kin et al., 1994). DHC (Dehydrocrotonin) and DCR (Dimethylamide-crotonin) obtained from the stem bark of *Croton cajucara* have been reported to induce apoptosis and cell differentiation in HL60 cells (Anazetti et al., 2003). DCTN, CTN, Transcajucarin A, Transcajucarin B, Cajucarinolide and Isoajucarinolide isolated from the mature stem bark of *Croton cajucara* have shown cytotoxic activity on cultured K562 leukemia cells (Maciel et al., 2007). Labdane diterpenoids and Croblongifolin isolated from the stem barks of *Croton oblongifolius* have shown cytotoxic activity against different tumor cell lines. Ent-16β-17α-dihydroxykaurane, a compound isolated from barks of *Croton malambo* has shown significant cytotoxic and proapoptotic activity on human mammary carcinoma cell line MCF-7 (Morales et al., 2005). Crude methanol extract of *Croton membranaceous* has shown cytotoxic activity against three human cancer cell lines, DLD-1, MCF-7 and M14 using MTT assay. Three compounds identified in the leaf essential oil (α-cadinol, β-elemene and α-humulene) of *Croton flavens* have shown cytotoxic activity against lung carcinoma cell line A549 and human adenocarcinoma cell line DLD-1 (Sylvestre et al., 2006). Red tree sap (Sangre-de-grado), obtained from the stem bark of *Croton palanostigma* has been found to induce apoptosis in human cancer cells, namely AGS (stomach), HT29 and T84 (colon) (Sandoval et al., 2002). The essential oil and one of its main constituent, ascaridole from the leaves of *Croton regelianus* has displayed cytotoxicity against HL-60 and SF-295 cell lines (Bezerra et al., 2009). The essential oils extracted from the leaves of *Croton matourensis* and flowers and
leaves of *Croton micans* have been found to exhibit moderate cytotoxicity against LoVo (colon carcinoma), X-17 (colon carcinoma), and HeLa (cervical cancer) cell lines (Compagnane et al., 2010). Methanol extract of the leaves/twigs, roots and stem bark of *Croton argyratus* has displayed toxicity to human lung cancer cell lines with an IC50 value of <5.0 μg/ml (Mohd Ali et al., 2012). In a primary screening using the murine lymphocytic leukemia P388 cell line, ethyl acetate extracts of *Croton barorum* and *C. goudotii* have been found to exhibit strong cytotoxic activity, with 100% inhibition at 10 μg/mL. Bioassay-guided fractionation led to the isolation of two new 3, 4-seco-atisane diterpenoids, crotobarin and crotogoudin. Both the compounds have been found to produce a net progression in the number of cells arrested at the G2/M growth stage in the cell cycle of the K562 human leukemia cell line at 4 μM (Rakotonandrasana et al., 2010). *Ent*-Kauranes from *C. tonkinensis* have also been found to be cytotoxic (Giang et al., 2005). Plaunotol, an acyclic diterpene present in *C. sublyratus* leaves, has recently been reported to have shown anti-cancer activity through inhibition of angiogenesis (Kawai et al., 2005). Anethole, a phenylpropanoid constituent of *C. zehntneri* volatile oil, has been reported to have anti-carcinogenic effect (Chainy et al., 2000). Taspine, an alkaloid obtained from *C. lechleri* red sap has been found to be active against KB and V-79 cells, a fact that is likely responsible for the purported anticancer activity of the sap (Chen et al., 1994). Glutarimide alkaloids, julocrotol, isojulocrotol and julocrotone isolated from the aerial parts of *C. cuneatus* have shown positive results in antitumor tests, using breast carcinoma and hepatoma cells (Suarez et al., 2004). Literature review revealed that *Croton bonplandianum* extracts possess appreciable cytotoxic activity (Qaisar et al., 2013).

### 2.5. Antioxidant activity of different species of *Croton*:

*C. celtidifolius* bark has been reported to show antioxidant activity, resulting from the direct action of constituents on specific targets, such as cyclooxygenase (Nardi et al., 2003). Red latex of *C. urucurana* has shown antioxidant effect against lipid peroxidation and free radical scavenging activity.
(Salatino et al., 2007), α-bisabolol, α-eudesmol and guaiol being the main components of the antioxidant fraction (Simionatto et al., 2007). *C. lechleri* sap has been found to possess significant antioxidant activity against the oxidative damages induced by apomorphine and hydrogen peroxide in *Saccharomyces cerevisiae* and maize plantlets (Lopes et al., 2004). Literature survey revealed that the essential oils from northeastern Brazilian *Croton* species; *Croton zenthmeri*, *Croton nepetaefolius* and *Croton argyrophylloides* exhibit good antioxidant activities (Morais de et al., 2006). Leaf extracts of *C. cajucara* have been found to exert antioxidant effects against the free radical DPPH and in paraquat treated yeast cells (Tieppo et al., 2006). Two of the aromatic acids, vanillic acid and 4-hydroxy-benzoic acid along with N-methyltyrosine have been isolated from *Croton cajucara*. These two aromatic acids have shown remarkable antioxidant activity in other species (Hung et al., 2002; Ohsuqi et al., 1999). Several kaempferol metabolites have proved to be antioxidant agents (Marfak et al., 2003; Jonson et al., 2003; Bonina et al., 2002) and *C. cajucara* leaves also contain two of them, e.g. kaempferol 3,4',7-trimethyl ether and 3,7-dimethyl ether (Maciel et al., 2000). Ethanol extract of the leaves of *C. argyratus* and *Croton caudatum* have shown effective antioxidant activity, thus indicating that the leaves of *C. argyratus* and *Croton caudatum* are a potential source of natural antioxidants (Mohd Ali et al., 2012; Deore et al., 2009). Root extract of *Croton zambesicus* has been reported to exhibit significant antioxidant activity (Okokon et al., 2013). Literature review revealed that *Croton bonplandianum* extracts possess appreciable antioxidant activity (Qaisar et al., 2013). Recently there has been a report showing the antioxidant potential of *Croton caudatus* Geisel leaves (Lokendrajit et al., 2012).

2.6. Hepatoprotective activity of different species of *Croton:*

Methanol extract of the aerial parts of *Croton oblongifolius* has been reported to show maximum hepatoprotective activity which might be related to the methanol soluble active principle like flavone and diterpene whereas petroleum ether and acetone extract have also exhibited a potent activity (Ahmed
et al., 2002). *Croton zehntneri* essential oil has been found to prevent acetaminophen-induced acute hepatotoxicity in mice (Lima et al., 2008). Leaves and stem bark of *Croton cajucara* Benth popularly known as "sacaca", a traditional medicinal plant in the Brazilian Amazonian region, have been reported to be used in the form of tea or pills for the treatment of hepatic disturbances and weight loss (Grassi-Kassisse et al., 2003).

Aforementioned review reflects the chemical diversity, antimicrobial activity, anticancer activity, antioxidant activity and hepatoprotective activity shown by different species of *Croton*. *Croton caudatus* Geisel has curative medicinal properties for malaria, ardent fever, convulsions, rheumatic arthritis, numbness and indigestion (Zou et al., 2010). As different species of *Croton* have shown potential antimicrobial activity, including the root bark of *Croton caudatus* Geisel, here it has been tried to see if the leaves of *Croton caudatus* Geisel show any antimicrobial activity. Despite the use of *Croton caudatus* Geisel for the relief of indigestion and liver ailments, there has been no available report in the literature describing the hepatic effects of the plant. Also the use of *Croton caudatus* Geisel leaves for the treatment of cancer has been reported from the Saikot area of Manipur. Keeping all these points in mind and as phytochemical study is necessary to uncover a novel bioactive compound; the “Phytochemical Screening and Bioactivity Evaluation of *Croton caudatus* Geisel (Euphorbiaceae)” was undertaken with the following objectives in mind:

- Collection of information about ethnomedicinal plant through literature survey and by field trips.
- Collection, identification and preservation of the plant in the form of herbarium sheets to be deposited to Assam University.
- Extraction of the plant parts (leaves) and bioactivity evaluation of the crude extract.
- Isolation and structure elucidation of the natural compound from the leaves of the plant.
- Bioactivity evaluation of the isolated natural compound.