Chapter 1

Introduction
INTRODUCTION

1.1: Overview

The plant kingdom represents an extraordinary reservoir of novel molecules. Of the estimated 400,000-500,000 plant species around the globe, only a small fraction has been investigated phyto-chemically and the numbers of species that are submitted to biological or pharmacological screening are even lower (Hostettmann et al. 1997). Human population, all over the world consume some form of plant parts as food supplements in their daily diet. The human diet is a highly complex and variable mixture of naturally occurring and synthetic chemicals. Of these, the naturally occurring chemicals far exceed the synthetic ones. In addition to the nutrients that are involved in normal metabolic activity, food contains components that may provide additional health benefits. These food supplements are derived from naturally occurring ingredients and are actively being investigated for their health promoting factors. In India, traditional medicinal practice always included such phytochemicals for improving ones well being whereas the western world has now started using the same. Currently there is a resurge of interest in the screening new lead compounds for the therapeutic use.

*Garcinia* is a relative new comer to the ranks of western herbalism, but was apparently used as a food supplement for thousands of years in the different regions of tropical Asia, Africa and Polynesia.

1.2: *Garcinia*: It's diverse role in modern herbalism

*Garcinia*, belonging to the family Clusiaceae (Syn - Guttiferae) is a large genus of polygamous trees or shrubs, distributed in tropical Asia, Africa and Polynesia. It consists of about 180 species, of which approximately 30 species are found in India (Jena et al., 2002). *Garcinia indica* (Syn - *Garcinia purpurea*) is the most common Indian species, popularly known as *kokam*. The tree is a native of the tropical rain forests of Western Ghats up to an elevation of 1800 m above the sea level, from Konkan southwards to Mysore, Coorg, and Wynnaad and also furnishes the vegetation of West Bengal and Assam. *Garcinia indica* is a slender evergreen tree with drooping branches. Leaves are ovate or oblong lanceolate, 2.5-3.5 inch long and 1-1.5 inch broad, dark green above and pale beneath. Fruits are globose or spherical, 1-1.5 inches in diameter, dark
purple when ripe and enclosing five to eight large seeds. The plant flowers in November-February, and fruits ripen in April-May. The root is astringent. The seeds of the fruit have edible fat, commercially known as kokam butter (Jena et al., 2002).

The fruit of *Garcinia indica* has an agreeable flavour and a sweet-acid taste. It is used as a garnish to give an acid flavour to curries and also for preparing syrups during summer months. Dried rinds of kokam are widely used all over South India for culinary purposes in place of tamarind or lemon and ‘Colombo curing’ of fish (Lewis and Neelakantan, 1965; Jena et al., 2002). The organic acid present in the fruit is responsible for the bacteriostatic effect of the pickling medium by lowering the pH (Sreenivasan et al., 1959).

Ayurveda and folkloric medication prescribe *Garcinia indica* for the treatment of oedema, delayed menstruation, constipation, intestinal parasites, rheumatism and bowel complaints and heart complaints (Wealth of India, 1976; Jena et al., 2002). Syrup from the fruit juice is given in bilious infections (Jena et al., 2002). Mainly the fruit rind is used (Jayaprakash et al., 2002), but leaves, roots and barks are reported to have the similar medicinal properties (Permana et al., 2001, Xu et al., 2001). Recent studies have focused on anti-HIV, anti-microbial, anti-inflammatory, anti-ulcerogenic, anti-tumor, anti-hepatotoxic, anti-obesity and antioxidative properties of the different species of *Garcinia*, particularly *G. mangostena*, *G. kola*, *G. atroviridis*, *G. cambogia* and *G. indica* (Grosvenor et al., 1995a,b; Inuma et al., 1996; Murakami et al., 1995; Mackeen et al., 2000; Permana et al., 2001; Terashima et al., 2002; Tamil Selvi et al., 2003).
The active principle of *Garcinia indica* fruit rind is (-) erythro hydroxy citric acid [(-) HCA], which comprises about 12.5-15.1% (w/w) of the fruit rind (Jayaprakash and Sakariah, 2002). Another important constituent is a polyisoprenylated benzophenone derivative, garcinol, which comprises about 2-3% of the fruit rind (w/w) (Krishnamurthy et al., 1981, 1982). The other constituents are different biflavonoids, xanthones, benzophenones, polyisoprenylated depsidones and heptacyclic xanthonoids. These properties have attracted the attention of biochemists and health practitioners with commercial and clinical applications (McCarty, 1995; Moffet et al., 1996).

1.3: (-) Erythro-Hydroxy Citric Acid

(-) Hydroxy citric acid [(-) HCA] is the principal acid of the fruit rinds of *Garcinia indica*. The acid content was found to be 12.5-15.1% (w/w) (Jayaprakash and Sakariah, 2002). The physiological and biochemical effects of (-)-HCA have been studied extensively for its unique regulatory effects on fatty acid synthesis, lipogenesis, appetite and weight loss. The derivatives of (-)-HCA have been incorporated into a wide range of pharmaceutical preparations in combination with other ingredients for the claimed purpose of enhancing weight loss, cardioprotection, correcting conditions of lipid abnormalities, and endurance in exercise (Jena et al., 2002; Lowenstein, 1973; Gutherie, 1976; Nishida, 1997; Policappelli et al., 1997; Hastings and Barnes, 1997; Wakat, 2000; Tomi et al., 1998; Majeed et al., 1998; Braswell et al., 1999; Okubo, 1999; Fushiki et al., 1998; Yamaguchi et al., 1999; Lewis and Neelakantan, 1965; Lewis, 1969; Lowenstein and Brunengraber, 1981).

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\begin{align*}
\text{COOH} \\
\text{HO—C—H} \\
\text{HO—C—COOH} \\
\text{H—C—COOH} \\
\text{H}
\end{align*}
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Structure of (-) Erythro-HCA

(-)-HCA is known to inhibit the activity of ATP: citrate lyase (ATP: citrate oxaloacetate lyase,) which catalyses the extra-mitochondrial cleavage of citrate to oxaloacetate and acetyl-CoA (Watson et al., 1969; Hoffman et al., 1980; Cheema-Dhadli et al., 1973; Szutowicz et al., 1976; Sullivan et al., 1977; Stallings et al., 1979). It is reported to be a potent inhibitor of lipogenesis (Sullivan et al., 1974, 1977, 1985) and ketogenesis (Brunengraber et al., 1978); promotes
glycogenesis, gluconeogenesis and lipid oxidation (Sullivan et al., 1974, 1983; and Sullivan and Green, 1985; Hellerstein and Xie, 1993; Jena et al., 2002). To our knowledge, reports on the genotoxicity of this compound are not available.

1.4: Garcinol

Another important constituent extracted from *Garcinia* is garcinol, a polyisoprenylated benzophenone derivative, comprising 2-3% of the fruit (Krishnamurthy et al., 1981, 1982). Garcinol, a yellow pigment (Krishnamurthy et al., 1987), has the property of free radical scavenging and anti-ulcer activities (Yamaguchi et al., 2000), can act as an anti-biotic reagent (Bakana et al., 1987; Iinuma et al., 1996) and has shown the property of apoptosis induction in different cancer cell lines (Pan et al., 2001; Matsumoto et al., 2003).

![Structure of Garcinol](image)

1.4.1: Free Radical scavenging property of Garcinol

Reactive oxygen species (ROS) have been shown to play a critical role in many diseases such as cancer (Muramatsu et al., 1995), arteriosclerosis (Steinberg et al., 1989), gastric ulcer (Das et al., 1997) and other conditions (Oliver et al., 1987; Babizhayav and Costa, 1994; Busciglio and Yankner, 1996; Smith et al., 1996). The intake of anti-oxidants such as polyphenols, have shown a positive trend to prevent these diseases (Vinson et al., 1995; Teissedre et al., 1996; Leanderson et al., 1997; Wiseman et al., 1997; Lotito and Fraga, 1997; Cohly et al., 1998; Cao et al, 1997). The superoxide anion-scavenging rate of garcinol was found to be moderately high. Garcinol can suppress the formation of all kinds of free radicals such as methyl radical, hydroxyl radical and superoxide anion. Among all the three groups, hydroxyl-scavenging activity is comparatively high (Yamaguchi et al., 2000). Iinuma et al., (1997) and Tamil Selvi et al., (2003), also reported the antioxidant activity of garcinol.
1.4.2: **Other properties**

Garcinol is known to have anti-ulcer property (Yamaguchi et al., 2000); inhibit development of preneoplastic lesions in the liver and colon (Hokaiwado et al., 2004); act as bacteriocidal (Inuma et al., 1996) and can strongly induce apoptosis in human leukaemia cells (Pan et al., 2001). Two other polyisoprenylated derivatives isogarcinol and xanthochymol isolated from *Garcinia* are found to induce apoptosis. Among them, isogarcinol and xanthochymol are found to be more effective when tested against four leukaemia cell lines U937, K562, NB4 and HL60 (Matsumoto et al., 2003).

In human leukaemia cells HL60 treatment with garcinol causes dose-dependent reduction in cell survival and induction of DNA fragmentation. Garcinol can stimulate Caspase-3 activity in a time- and dose-dependent manner by abolishing the Caspase-3 inhibitor, Z-DEVD-FMK and degrading PARP, an endogenous substrate of caspase-3, a hallmark of apoptosis.

1.5: Other species of *Garcinia*

1.5.1: **Garcinia mangostana**

*Garcinia mangostana* (G. mangostana), commonly known as mangosteen; is a native of Southeast Asia. It is used for the treatment of skin infection, wounds, abdominal pain, diarrhoea, astringent, dysentery, suppuration, chronic ulcer, leucorrhoea and gonorrhoea (Satyavati et al., 1976). *G. mangostana* has anti-inflammatory (Gopalakrishnan et al., 1980), antitumour, antioxidant (Williams et al., 1995) and antibacterial activities (Farnsworth and Bunyapraphatsara, 1992, Mahabusarakum et al., 1983). The pericarp (peel) of *G. mangostana* was reported to be the source of mangostin, tannin, xanthone, chrysanthenin, garcinone, gartanin, Vitamin B1, B2, C and other bioactive substances (Farnsworth and Bunyapraphatsara, 1992). Various xanthones isolated from the pericarp of mangosteen, were found to exhibit antiproliferative activity against human leukaemia HL60 cells and β-mangostin, the major xanthone derivative induce apoptosis in leukaemia cell lines (Matsumoto et al., 2003). Tannin from the fruit was found to be an inducer for apoptosis on human leukemia cells (Yang et al., 2000). Crude methanolic extract significantly inhibited the proliferation of breast cancer cells and induced morphological changes such as cytoplasmic membrane shrinkage, loss of contact with neighboring cells, membrane blebbing and formation of apoptotic body. In addition, oligonucleosomal DNA fragments (ladders) were exhibited in SKBR3 breast cancer cells (Moongkarndi et al., 2004). Other molecules such as mangostin, polysaccharides, isolated from the pericarps of mangosteen were found to inhibit the
oxidation of low-density lipoprotein (Mahausarakam et al., 2000; Williams et al., 1995) and were found to stimulate phagocytic cells and kill intracellular bacteria- *Salmonella enteritidis* (Chanarat et al., 1997).

1.5.2: *Garcinia kola*

*Garcinia kola* (*G. kola*) is a medium sized tree found in moist forest and widely distributed throughout west and central Africa. The nut is highly valued for its edible purposes particularly in these countries (Hutchinson and Dalziel, 1956). The seed commonly known as bitter kola is a masticatory and is a major kola substitute offered to guests at home and shared at social ceremonies. The seeds are used in folk medicines and in many herbal preparations for the treatment of ailments such as laryngitis, liver disorders, bronchitis (Iwu, 1982). *G. kola* contains a complex mixture of biflavonoids, prenylated benzophenones and xanthenes (Terashima., 1995; Terashima et al., 1999; Hussain et al., 1982). Recently, two new chromanols, garcinoic acid, garcinol, together with α-tocotrienol was reported (Terashima et al., 2002).

Antioxidant activities of kolaviron were reported in the scavenging of reactive oxygen species (Aruoma et al., 1990) in a dose dependent manner (Farombi, 2002b) and in the chemoprevention of chemically induced genotoxicity (Farombi, 2003). Another constituent, garcinoic acid was reported as a powerful antioxidative agent (Terashima et al., 2002).

1.5.3: *Garcinia cambogia*

Dried pericarp of the fruit of this species contains up to 30% by weight of (-)-hydroxycitric acid (HCA) (Lewis and Neelakantan, 1965). Thus *G. cambogia* is one of the prime sources of (-) HCA. The seeds of *G. cambogia* contain 31% edible fat. As cited earlier, (-)-hydroxycitric acid is found to inhibit the citrate cleavage enzyme, ATP: citrate lyase (ATP: citrate oxaloacetate lyase,) (Watson et al., 1969; Hoffman et al., 1980; Cheema-Dhadli et al., 1973; Szutowicz et al., 1976; Sullivan et al., 1977 and Stallings et al., 1979).

(-)-HCA is found to be a potent inhibitor of ketogenesis in liver (Brunengraber et al., 1978). The long-term oral administration of (-)-HCA to growing rats caused a reduction in body weight gain, food consumption and total body lipids (Jena et al., 2002; Sullivan et al., 1974). (-)-HCA may promote glycogenesis, gluconeogenesis and lipid oxidation (Sullivan et al., 1974; Sullivan et al., 1983; Sullivan and Green 1985; Hellerstein and Xie 1993; Jena et al., 2002).
1.5.4: *Garcinia parvifolia*

*Garcinia parvifolia* is a small or medium sized tree, native of the Island of Borneo, occurs naturally in Brunei and Malaysia. It is commonly known as Brunei cherry, and eaten fresh or as flavouring in other foods. Leaf extract of *G. parvifolia* shows cytotoxic activity against P-388 cell line (Xu et al., 2000) and a cytotoxic bixanthone have been isolated from the bark of *G. parvifolia* (Xu et al., 1998).

1.5.5: *Garcinia atroviridis*

*Garcinia atroviridis* is the source of garcinia acid, an identical synthetic (-)-hydroxycitric acid. *Garcinia atroviridis* has anti-bacterial activities (Grosvenor et al., 1995b; Mackeen, 1995; Murakami et al., 1995; Mackeen et al., 1997a, b). The extract of the trunk bark has a strong anti-oxidant, anti-tumour promoting activity (Murakami et al., 1995; Mackeen et al., 2000) and the fruit and leaf extracts can inhibit tumour-promotion in the Raji cells (Mackeen et al., 2000).
AIMS AND OBJECTIVES

In view of the ethno botanical importance of *Garcinia* coupled with the paucity of the genotoxicity studies, we thought it necessary to find if the fruit of *Garcinia* had any genotoxic effects, particularly at the doses being advocated for weight-loss in humans. Different studies have focused on the activities of individual ingredients of *Garcinia indica*. But the genotoxic or anti-genotoxic activities of the fruit rind-extract was our main concern. Our laboratory has been testing the genotoxic effect of different beneficial plant extracts *per se* (Mukherjee et al., 1991; Sen et al., 1996; De et al., 1995; Mukhopadhyay et al., 1998; Das et al., 2004) and considered important to carry out genotoxic testing of *Garcinia*.

Major Objectives:

1. Evaluation of the genotoxic potential of *Garcinia* fruit rind-extract.
2. Estimation of the threshold dose.
3. Evaluation of the modulatory effects of *Garcinia indica* against known mutagens.