Steroids are an important group of hormones which play a key role in the transmission of signals that mediate growth and physiological responses in most pluricellular organisms. In animal system, these steroids include androgens, estrogens, progestagens, gluco and mineralcorticoids and animal moulting hormones (ecdysteroids). Grove et al. (1979) reported a new steroidal lactone called brassinolide, an important member of brassinosteroid family from the bee collected pollen of *Brassica napus*. Since then, more than 70 different types of brassinosteroids (BRs) have been isolated from a number of plants and their plant parts (Bajguz and Tretyn, 2003).

BRs are derived from the 5α-cholestan skelton and their structural variations come from the type and position of functional groups in A/B rings and their side chain (Yokota, 1997). Plants can synthesise many brassinosteroids from a phytosterol named as campesterol (5-cholestan-24α-methyl-3β-ol). Castasterone and brassinolide are the most active brassinosteroids (Mussig and Altman, 2001). Brassinosteroids are found in gymnosperms, monocotyledonous and dicotyledonous plants, and in algae. New studies (Fujioka et al., 1998; Sasse 1997; Schmidt et al., 1998; Yokota et al., 1998) confirm that BRs are obligatory plant constituents, with the highest concentrations being found in the reproductive organs and in growing tissues (pollen, immature seeds, and shoots). Their biosynthetic pathway has been elucidated by a combination of metabolic studies with suitable radioactive precursors and the use of various *Arabidopsis* dwarf mutants (Clouse, 1996; Yokota, 1997). BRs are synthesized from phytosterols through multiple, oxidative reactions leading to the synthesis of brassinolide (BL). *In vivo* conversion studies revealed two alternative reaction routes, the early and late C-6 oxidation pathways that utilize 6-oxo or 6-deoxo intermediates, respectively.

The biological activity of brassinosteroids was initially evaluated by the bean second internode assay (Grove et al., 1979; Thompson et al., 1981, 1982; Mandava, 1988). BRs, when applied at nanomolar concentration to hypocotyl or epicotyl segments or intact plants elicit a wide range of responses such as stem elongation, pollen tube growth, leaf bending, root growth inhibition, synthesis of ethylene,
activation of proton pump, xylem differentiation, enhanced DNA, RNA and protein synthesis, increased enzyme activities (e.g. ATPase, invertase) stimulation of photosynthetic activity (Clouse and Sasse, 1998; Sasse, 2003; Yu et al., 2004) enhanced tolerance to biotic (pathogen infection) and abiotic (drought, temperature, salinity) stresses and increased crop yields (Clouse and Sasse, 1998). Like their animal counterparts, BRs regulate the expression of numerous genes, impart the activity of complex metabolic pathways and contribute to the regulation of cell division and differentiation and in the control of overall developmental programs including photomorphogenesis and cell expansion (Clouse, 2002).

Recent studies indicate antiviral activities of BRs against various viruses, like herpes simplex virus type I (HSV1), arena virus, measles virus and vesicular stomatitis virus (Wachsman et al., 2002; Wachsman et al., 2004; Romanutti et al., 2007). The treatment of BRs to these viruses was 10-18 folds more active than ribavirin towards HSV-I and arenavirus. It has further been reported that 24-epibrassinolide at subnanomolar concentration increased the mitochondrial membrane potential, reduced intracellular antibody levels, increased the proportion of cells in G0/G1 phase and reduced the population of cells in S-phase when tested in mammalian cell system (Franek et al., 2003). Medical applications of BRs were discussed by Malikova et al. (2008). The anticancerous activities of 28-homocastasterone and 24-epibrassinolide were studied in several normal and cancer cell lines. The BRs used showed high cytotoxic activity in breast (MCF-7/MDA-MB-468) and prostate cancer cell lines (LNCaP/DU-145). Recently Sondhi et al., 2008 explored the potential of BRs as antigenotoxic agents using *Allium cepa* chromosomal aberration assay. Cancer continues to be one of the major causes of death worldwide and only modest progress has been made in bringing down the morbidity and mortality of this dreadful disease. Extensive preclinical and clinical research has lead to the realization that most human malignancies should be fought on multiple fronts. Cancer prevention has become an important approach to control cancer (Hail, 2005; Sun et al., 2005). Some common prevention strategies include avoiding exposure to known cancer causing agents, enhancement of host defence mechanism against cancer, lifestyle modifications and chemoprevention. Chemoprevention utilizes non-toxic chemical substances (natural or
synthetic) to prevent the progress of cancers either by enhancing detoxification/elimination of carcinogens and their reactive metabolites or halting/reversing tumor promotion/progression (Surh, 2003; Bode and Dong, 2004; Chun and Surh, 2004). Data from both preclinical and clinical studies suggests that plant based diet rich in a wide variety of fruits and vegetables is effective in preventing cancer (Surh, 2003; Dorai and Aggarwal, 2004; Katiyar and Mukhtar, 1997). Frequently used plants in traditional medicine are assumed safe, due to their long term use (Elgorashi et al., 2002) and considered to have no side effects because they are natural (Popat et al., 2001). The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. The flora of Indian medicinal plants is potent source of bioactive principles. Because of these bioactive principles, plants have been the traditional source of raw materials for medicines. Our ancient rich heritage of knowledge on preventive and curative medicines was available in Atharva veda, Charaka, Sushruta, etc. An estimate suggests that about 13,000 plant species worldwide are used as drugs. The trend of using natural plant extracts has increased and the active plants extracts are frequently screened for new drug discoveries (Das et al., 1999). There is utmost need to search for novel phytochemicals as Brassinosteroids which can be useful in cancer chemoprevention. *Aegle marmelos* belongs to family Rutaceae and known as bael (Hindi) and golden apple (English), is an indigenous fruit of India. It grows throughout the Indian peninsula as well as in Sri Lanka, Pakistan, Bangladesh, Burma, Thailand and most of the Southeastern Asian countries. *Aegle marmelos* has an important place in indigenous systems of medicine. Leaf, root, bark, seed and fruits are valued highly in Ayurvedic medicine in India (Sharma et al., 1980). A number of phytochemicals have been reported from its various plant parts. It is claimed to be useful in treating pain, fever, inflammation, respiratory disorders, cardiac disorders, dysentery and diarrhoea (Dymock William et al., 1990; Kirtikar et al., 1935).

*Centella asiatica* (L.) Urban (Umbelliferae) is another important prostrate, perennial and aromatic medicinal herb. It is found in different parts of Asia, Sri Lanka, Madagascar, South Africa and Malaysia. *Centella asiatica* is a traditional medicinal plant frequently used in folk medicine practices of East Asia. It is placed at the interface between traditional and modern scientifically oriented medicine and regarded as one of
the best psychotropic drugs that has been used for centuries in Ayurvedic medicine to alleviate anxiety, and to promote a deep state of relaxation and mental calmness. It has been reported to have wound healing properties (Suguna et al., 1996), anticancer activity (Babu et al., 1995) and antioxidant activity (Zainol et al., 2003). Antitumor and cytotoxic properties of the crude extract and partially purified fractions of *C. asiatica* were reported by Babu et al. (1995).

The medicinal importance of *A. marmelos* and *C. asiatica* is well established and a number of bioactive compounds have been isolated. But no information is available as regards to presence of brassinosteroids in these plants. Keeping in mind, the pleiotropic role of BRs in a wide spectrum of plant responses and in medicinal applications, an attempt has been made to explore the presence of brassinosteroids in these two important medicinal plants and evaluate their antigenotoxic potential. The present work has been designed to meet the following objectives:

- Isolation of brassinosteroids from medicinal plants - *Aegle marmelos* (L.) Corr. and *Centella asiatica* (L.) Urban.
- Characterization of brassinosteroids by High performance liquid chromatography (HPLC) and Mass spectroscopy (GC-MS/QTOF- MS).
- Evaluation of the isolated brassinosteroids and their related fractions for their antigenotoxic potential if any, by employing *Allium cepa* chromosomal aberration assay and Comet assay.