1 INTRODUCTION

Plants have traditionally occupied an important position in the socio-cultural, spiritual and medicinal arena of rural and tribal living in India (Kumar *et al*., 2011). On an average natural product chemists are able to find one or two novel metabolites from investigating a single plant species. This means that plants are huge compound libraries which harbour exorbitant members of novel and potentially useful chemicals.

Plants subjected to adverse situations are able to display several mechanisms of defense or adaptations that allow them to survive. The regulation of such mechanisms is rather complex and requires the participation of a number of different molecules which are not primarily involved in housekeeping activities; and are collectively termed as secondary metabolites.

Plant-derived secondary metabolites are widely used as pharmaceuticals, food additives, nutraceuticals, etc. Many plant secondary metabolites have a similar ancestral pathway, and their enormous heterogeneity is usually derived from differential modification of common backbone structures. Secondary metabolites - include three main groups, terpenes, phenolics and nitrogen-containing compounds. These are produced by plants and used as taxonomic characters in classifying plants. From time immemorial human use some of these metabolites as medicines, flavorings, or recreational agents.

Flavonoids are a group secondary compounds exhibiting different characteristic pattern among different plant families. All these compounds play an important biochemical and physiological roles in various cell types or organs (leaves, seeds, roots, green parts, and fruits) where they accumulate. The chemical structures of this class of compounds are based on a C6-C3-C6 skeleton. They differ either in the saturation of the heteroatomic ring C, or in the placement of the aromatic ring B at the positions C-2 or C-3 of ring C, and in the overall hydroxylation patterns forming various flavonoids.

Quercetin is one of the most extensively studied flavonoids because of its biological properties (Hamamatsu *et al.*, 2004). Quercetin is commonly present as a glycoside and is converted to glucuronide or sulfate conjugates during intestinal absorption and only conjugated metabolites are found in circulating blood (Murakami *et al.*, 2008). Initially quercetin was extracted from red wine, which was subsequently found to exist widely in leaves (Lozoya *et al*., 1994; Alade *et al.*, 2012), fruits (Nielsen *et al*., 2002; Materska, 2012) and vegetables (Beninger and Hosfield, 1999; Abdallah *et al.*, 2012).

The technique of flavonoid isolation from a plant material, including the type of extracting solvent, depends generally on the type of flavonoid compound and the quantity of plant material. Different extraction techniques, such as hydrodistillation, maceration, soxhlet extraction, ultrasonic extraction, *etc.*, are widely used for obtaining extractable substances from different parts of a number of plants (Velickovic *et al*., 2007). Acid hydrolysis of flavonol glycosides improves the separation and estimation of quercetin and other flavonols from plant extracts (Marshall *et al.*, 2012). The combinative approach of qualitative (Thin layer chromatography, TLC) and quantitative (High performance liquid chromatography, HPLC) chromatographic techniques helps in evaluating the quality consistency (Sumathy *et al.*, 2011).

Quercetin is one of the most powerful and effective herbal anti-inflammatory, and antioxidant supplements on the market today. Quercetin content in plant extracts vary based on plant parts used (Feng and Liu, 2011) and season during which it has been collected for extraction (Jalili and Sadeghzade, 2012). Currently, only 10% of medicinal plant species are cultivated, and the remaining 90% are harvested from wild populations (Julsing *et al.*, 2007). Excessive harvesting can diminish native populations and erode genetic diversity while skewing the survivors toward accelerated development and reproduction.

Cost of raw materials for preparation of quercetin supplements is increasing in the world market, which necessiates further improvement of quercetin production for viable commercial exploitation. Continuous and enhanced secondary metabolite

production, through cell culture, by employing optimized culture conditions, serves as an important alternative to protect the plants from becoming extinct (Malik *et al.,* 2011).

Plant tissue culture involves the growth of plant cells and tissues *in vitro* in a microbe-free environment. Dedifferentiated plant cells are cultured in the form of callus or cell suspensions (Doran, 2009). Callus culture can be done from different vegetative organs such as the leaf, root, node, and stem. Young vegetative organs are more effective for callus induction. Successful callus culture also depends on the type of plant growth regulator, for eg, cytokinins and auxins are known to promote callus formation in tissue culture (Ribas *et al.,* 2011).

However, many factors such as genotype, composition of the nutrient medium, and physical growth factors such as light, temperature, humidity, and endogenous supply of growth regulators are important for callus induction. The general strategy for obtaining tissue culture lines for the production of a particular compound is establishing the culture strain from the explant obtained from the high production site (Wu *et al.,* 2003). To produce large quantities of products, high amounts of plant biomass are generally required. Once the amount biomass has been optimized for growth, then conditions are usually changed in order to stimulate the production of high amounts of products (Weathers *et al.,* 2010).

During *in vitro* studies of secondary metabolism, many methods for "upregulating" metabolism have been explored with promising results. Several major strategies have been explored for upregulation of metabolism including precursor feeding, hormonal signaling, elicitation, and nutrient stress. Elicitors *i.e.,* physical elicitors and chemical elicitors can be defined as signaling molecules triggering the formation of secondary metabolites in cell cultures by inducing plant defense, hypersensitive response and or pathogenesis related proteins (Baldi *et al.,* 2009).

Despite quercetin having widespread medicinal and culinary uses, the separation and purification of plant quercetin samples and standardization of cell suspension cultures as a new way to improve quercetin production from noncommercial plants have

not been attempted. Hence, in the present study, from six different families, six plants - *Abutilon indicum, Albizzia julibrissin, Caesalpinia pulcherrima, Clitoria ternatea, Euphorbia hirta,* and *Psidium guajava* were chosen.

Though several studies has been reported on various bioactivity assays of secondary metabolite content in these selected plants, studies on quercetin content and its production in cell suspension cultures have not been reported so far. Hence, the present study entitled "**Isolation, Purification and Structural Characterization of Quercetin and its Derivatives from *In Vitro* Suspension Cultures of Selected Plants and Its Comparison to *In Vivo* Plant Parts"** is aimed to meet the following objectives:

- To optimize the solvent system for extraction of flavonoids.
- To standardize chromatographic techniques for separation of quercetin and purification of quercetin glycosides.
- To optimize *in vitro* cultural conditions for callus induction and cell suspension cultures
- To augment quercetin biosynthesis in *in vitro* cultures
- To elucidate the structure of purified quercetin glycosides and
- To evaluate the bioactivity of quercetin glycosides isolated from suspension cultures.