1. INTRODUCTION

Subjective methods based on the morphological features such as shape, colour, texture and odour were used for the discrimination of herbal medicines in traditional systems. However, it is often difficult to accurately identify medicinal plants from wild populations (or) to differentiate species within the same genus based on this subjective evaluation. Furthermore, the use of chromatographic techniques and marker compounds to standardize herbal medicines is also limited because of variable chemical complexity, which is affected by growth, storage conditions, harvest times and variable sources (Joshi et al., 2004; Zhang et al., 2007).

The therapeutic effects of several plants and vegetables, which are used in traditional medicine, are usually attributed to their antioxidant compounds. Antioxidants are also used to preserve food quality mainly because they arrest oxidative deterioration of lipids. Plant based antioxidants are now preferred to the synthetic ones because of safety concerns (Sherwin, 1990). These factors have inspired the widespread screening of plants for possible medicinal and antioxidant properties, the isolation and characterization of diverse phytochemicals and the development and utilization of antioxidants of natural origin (Gulcin et al., 2002). A profile of the chemical composition of a plant together with knowledge of its antioxidant activity will give a fair estimate of its therapeutic potential.
Meanwhile, natural antioxidants, derived mostly from their plants have been reported for high potential in prophylaxis and treatment of many degenerative diseases caused by chain oxidative reactions such as atherosclerosis, coronary heart disease, aging and cancer (Finkel and Holbrook, 2000). An inverse relationship has been reported between consumption of natural antioxidants and mortality from such degenerative diseases (Govindarajan et al., 2005). Therefore, the search for nontoxic high potential natural antioxidants is of increasing interest. Many authors present their work about antioxidant activities from different plant sources (Okonogi et al., 2007).

The medicinal value of plants have assumed a more important dimension in the past few decades owing largely to the discovery that extracts from plants contain not only minerals and primary metabolites but also a diverse array of secondary metabolites with antioxidant potential. Antioxidant substances block the action of free radicals which have been implicated in the pathogenesis of many diseases including atherosclerosis, ischemic heart disease, cancer, Alzheimer’s disease, Parkinson’s disease and in the aging process (Aruoma, 2003; Dasgupta and De, 2004; Coruh et al., 2007).

In recent years, one of the areas, which have attracted a great deal of attention, is antioxidants in the control of degenerative diseases in which oxidative damage has been implicated. Several plant extracts
and different classes of phytochemicals have been shown to have antioxidant activity. The search for newer natural antioxidants, especially of plant origin, has ever since increased (Bergman et al., 2001). The existence of multiple molecular forms of antioxidant enzymes and any changes they may undergo in response to various environmental signals imply potential roles for these isozymes in the detoxification of ROS (Pinhero et al., 1997).

Manian et al., 2008 studied the methanol extracts of green tea, Ficus bengalensis and 70% acetone extract of F. racemosa contained relatively higher levels of total phenolics than the other extracts. The antioxidant potential of the extracts were assessed by employing different invitro assays such as reducing power assay, DPPH, ABTS+ and OH radical scavenging capacities, peroxidation inhibiting activity through linoleic acid emulsion system, antihemolytic assay by hydrogen peroxide induced method and metal ion chelating ability. Though all the extracts exhibited dose dependent reducing power activity, methanol extracts of all the samples were found to have more hydrogen donating ability. Similar line of dose dependent activity has been maintained in all the samples in DPPH and OH scavenging systems.

Genetic tools are using the hybridization, polymerase chain reaction (PCR) and sequencing techniques that provide more objective
and reliable methods for identification of herbal medicines (Shcher and Carles, 2008; Collard and Mackill, 2009). Randomly Amplified Polymorphic DNA (RAPD) analysis has become one of the most effective methods for estimating genetic diversity in plant populations or cultivars because it can reveal high levels of polymorphism. RAPD also has many advantages, such as its high speed, low cost and requirement of minute part of plant material (Williams et al., 1990; Penner et al., 1993). RAPD analysis has been applied in herbal medicine to discriminate between species in various genera (Shcher and Carles, 2008). However, it is less reproducible than other methods (Hosokawa et al., 2000). It has other disadvantages as well such as homoplasy, non homology, nested priming, hetero duplex formation, collision, nonindependence, artefactual segregation which lower its reliability (Bussel et al., 2005).

Paran and Michelmore (1993) used RAPD analysis to develop Sequence Characterized Amplified Region (SCAR) markers, a more accurate and reliable technique to avoid the above problems of RAPD. This technique can be used to develop markers that authenticate herbal medicines by using specific PCR primers derived from RAPD or AFLP fragments. These specific primers result in amplification of products from given samples and can be used to generate unique amplification products from closely related samples (Wang et al., 2001; Lee et al., 2006).
Proteolysis is an irreversible process of polypeptide cleavage with important physiological roles in number of cellular process where it is essential to confine the cleavage of peptides in space and time (Majerle and Jerala, 2003). Historically, several names have been assigned to enzymes that cleave peptide bonds, including proteinases, proteases, peptidases and proteolytic enzymes (Barrett et al., 1998).

Bremer (1988) studied the biological macromolecules gained an increasingly important role in evolutionary and systematic studies and reveal protein variation within and among species, protein electrophoresis and histochemical staining were used as early applications. Isozyme and allozyme electrophoresis are the most widely used approaches in molecular systematics. The detection of amino acid composition and sequence is also used for comparing different species.

The electrophoresis of protein is an effective technique for generating systematic data from macromolecules. This method has become increasingly popular among systematists (Crawford and Ornduff, 1989). Generally, electrophoretic methods have focused on two general forms of protein data, isoenzymes (also called isozymes) and allozymes. Isozymes are multiple forms of an enzyme that catalyze the same reaction, but differ from each other in amino acid sequence, substrate affinity, Vmax, and/or regulatory properties. They involve different molecular forms encoded by different genes (Matus and Hucl, 1999).
Some analytical methods have been used for the quality evaluation, such as qualitative and quantitative analyses of saponins by HPLC (Li et al., 2005; Fujioka et al., 2006) and analysis of volatile oil by GC-MS (Bertoli et al., 2004). Investigations of the phytochemical components of *Ulmus davidiana* stem and bark have resulted in the isolation of (+)-catechin, catechin, rhamnoside and catechin apiofuranoside (Kim et al., 2003), triterpene esters (Lee and Kim, 2001), sesquiterpene O-naphthaquinones (Kim et al., 1996), and lignan and neolignan glycosides (Lee et al., 2001). The bioactive ingredients from *U. davidiana* have been reported to have medicinal activities, such as neuroprotective effects (Lee and Kim, 2001), antitumor activity (Lee et al., 2004) and nitric oxide inhibition (Jun et al., 1998).

Quantitative determination of four marker compounds, i.e., sinomenine, paoniflorin, paeono and curcumin on qualitative fingerprinting analysis of QFGJS using HPLC-DAD method. In fingerprinting analysis, the chemical characteristics of four herbs present in QFGJS (excluding Radix *Aconiti Lateralis Preparata*) were found in the HPLC chromatographic file. The results showed the contents of these five marker compounds and HPLC fingerprint profiles of three batches of QFGJS products collected at 3 months after production in the stability testing were relatively consistent (Xie et al., 2007).
Pharmacological research on *B. monnieri*, little is known about the analysis of its bioactive compounds in plant material or products thereof. Except from a rather unspecific spectrophotometric determination of the hydrolyzed aglycones, one HPLC method enables the quantification of bacoside $A_3$ (Pal and Sarin, 1992; Pal *et al*., 1998). In a third report the use of LC-NMR in search for novel compounds in the plant is described (Renukappa *et al*., 1999).

*Bacopa monnieri* (L.) Wettst, ‘Brahmi’ belonging to the family Scrophulariaceae, is a small prostrate herb that grows wild in marshy and damp places near water logs. It is used in traditional medicine to treat various nervous disorders (Mathew *et al*., 2009). This plant also possesses antiepileptic, antipyretic, analgesic, anti-inflammatory, epilepsy, insanity and anticancer activities (Tripathi *et al*., 1996; Vohora *et al*., 1997). It is used for cognitive impairment, thus used as cure of Alzheimer’s disease (Dhansekaran *et al*., 2007; Stough *et al*., 2008).

*B. monnieri* was placed second in the priority list of most important medicinal plant in a sector study of (Export and Import bank of India, 1997), evaluated on the basis of their medicinal importance, commercial value and potentials for further research and development. The medicinal properties of *Bacopa* have been mainly attributed by the presence of different types of saponins such as bacosides A, B, C and D. These are the ‘memory chemicals’ responsible for cognitive effect and are basically triterpinoids (Rastogi *et al*., 1994; Singh and Dhawan, 1997).
Since the quality and therapeutic effect of herbal medicines are closely related to the authentic identification of species, it is necessary to find a way to distinguish genuine herbal medicine.

Objectives of the work

1. To evaluate the Phenological variation, Biochemical contents, enzymatic and non-enzymatic antioxidants from different ecotypes in Tamil Nadu.
2. To study the Invitro free radical scavenging capacity from the Butanolic fraction.
3. The assessment of genetic diversity among the *B. monnieri* (L.) Pennell ecotype variants.
4. To study the protein expression pattern by SDS - PAGE.
5. To quantify the active principle (Bacoside - A) by HPLC method from different ecotypes.