5. DISCUSSION

The present study was conducted to evaluate the Phenological variation, Biochemical contents and antioxidant potential, Free radical scavenging capacity, Genetic diversity, Protein profile and Bacoside-A content of the Bacopa monnieri ecotypes from Tamil Nadu were discussed.

5.1. Phenological variations

The phenological variations like growth, shape of the leaves and Colour of Stem and flowers among the ecotypes were noticed. Nature of growth varied the accessions except Vattakottai and Aliyar dam sample. Colour of flower similar in all accessions. Shape of the leaves was similar in all accessions except Kovai kuttrallam sample of Bacopa monnieri in Tamil Nadu. The similar studies were observed in olive (Hagidimitriou et al., 2005) and Almond cultivars (Ali and Nabulsi, 2003). Morphological traits were not reliable in estimating genetic relationships among diverse groups of cultivars (Hagidimitriou et al., 2005).

Erect type growth habit, open ear type, light green ear (glumes) color, enclosed grains by glumes, lower spikelet density and purple black seed color was predominant phenotypic classes for the traits and the variations of qualitative traits with respect to geographic location and agroclimate in Elucine coracana (Lule et al., 2012).
The difference observed across location and among genotypes collected from different regions could be due to the genetic factors, edaphic factors or other environmental condition of the area that influence the adaptive role of the traits (Bezaweletaw et al., 2006).

5.2. Chlorophyll

In maximum chlorophyll contents were observed in Vattakottai accession. Similar results were noticed by Ivanov et al., 2013. The chlorophyll and carotenoid content in steppe plants of South Ural growing along a latitudinal gradient from southern steppe to forest steppe. The chlorophyll (a + b) was 5 - 6mg per 1g of the leaf dry weight and did not depend on the latitude, whereas the carotenoid content is increased northward from 1.0 to 1.5mg/g dry wt. At the same time, the greatest changes occurred in the ratios between the forms of pigments, the chlorophyll a/b ratio increased 1.8 to 2.8mg and the chlorophyll/carotenoid ratio decreased 5.6 to 3.5mg/ml. The obtained results indicate that adaptation of the pigment apparatus of steppe plants growing along the latitudinal gradient occurs due to the transformation of the light harvesting complex (Ivanov et al., 2013).

Lisiewska et al. (2001) observed in Anethum graveolens content of chlorophylls ranged from 77 to 163mg in 100g fresh leaves, depending on the cultivar, growing time and the kind of usable parts such as leaves or leaves with petioles. Chlorophyll ‘a’ has been suggested as one of the most decisive factors which directly influence the photosynthetic
activity, with increase in the level of Chlorophyll increased the photosynthetic rate (Mao et al., 2007). S-adenosylmethionine (SAM) is the primary biological methyl-group donor methionine and regulates essential cellular processes such as cell division, synthesis of cell wall, synthesis of chlorophyll and membrane synthesis (Roje, 2006).

5.3. Carotenoid

The present study deals with the carotenoid content and the maximum content was noticed in Vattakottai accession. Due to the provitamin-A activity of some carotenoids, they also have other functions, such as antioxidants and enhancers of the immune response. Furthermore, some of them are involved in the cell communication and xanthophylls have shown to be effective as free radical scavengers. The great interest in studying these carotenoids is due to their physiological and biological functions which have been extensively and in detail revised by Berg et al. (2000).

Disruptions in leaf morphology and photosynthetic machinery affect chlorophyll concentration, which can result in alterations on the concentration of carotenoids (Camara et al., 2010). Carotenoids have several functions associated with photosynthesis, including accessory light harvesting and energy transfer (Ritz et al., 2000) and photoprotection (Adams, 1998). The quantity of several types of carotenoids is known to correlate with plant stress and photosynthetic capacity. The content of carotenoids increases during winter months in
overwintering leaves (Adams et al., 2002), in high irradiance and high temperature environments (Kirchgebner et al., 2003), low nitrogen availability (Adams and Adams, 1996) and onset of leaf senescence (Bosch and Penuelas, 2003).

Carotenoids are protection against oxidative damage and some are used as warning colours in plant defense system. The commercial interest for carotenoids used as colorant, nutraceutical or antioxidant in food and cosmetics. Carotenoids could possibly play an important role as anticarcinogenic drug and in the prevention of chronic diseases (Giovannucci et al., 2002; Perlman et al., 2002).

5.4. Biochemical analysis

5.4.1. Total soluble protein

The report on protein content in *B. monnieri* significantly varied among the accessions and the highest protein content were found in the leaves of Aliyar dam accession. This result is positively correlated with the following study. The primary products such as carbohydrates, lipids and proteins are common to all plants and are involved in primary metabolic processes and secondary metabolites content of the plant may vary with respect to their growing conditions (Kaufman et al., 1999).

*Solanum tuberosum* contains relatively good quality of protein and patatin and 11S globulin is a major storage protein with high level of lysine. However, Potato tuber protein contains relatively low amounts of sulphur containing amino acids, which may result in low nutritional value (Goo et al., 2013).
In *Triticum aestivum*, water soluble proteins are about 10% of total grain proteins and have specific functions in plant growth and development. The optimized RP-UPLC could be used as an effective and alternative method for rapid separation and characterization of water soluble proteins in wheat cultivar and germplasm evaluation, genetic and biochemical studies on grain proteins and environmental influence analysis of water soluble proteins (Yu *et al.*, 2013).

5.5. **Non-enzymatic antioxidants**

5.5.1. **Ascorbic Acid**

The ascorbic acid content was significantly increased in all accessions. The ascorbic acid content is maximum in the leaves of Nagercoil accession. The levels of vitamin C and flavonoids depending on species and variety, growing location, harvesting time, storage, processing and other conditions, with respect to methodological differences in *Malus sylvestris, Phaseolus vulgaris, Vigna unguiculata, Brassica oleracea, B. chinensis var. parachinensis, B. rapa var. chinensis, B. oleracea var. capitata, B. rapa var. pekinensis, B. oleracea var. rubra, Vaccinium macrocarpon, Vaccinium ssp., Solanum melongena, Citrus paradise, Vitis vinifera, Psidium guajava, Lactuca sativa, Mangifera indica, Allium cepa, Citrus sinensis, Carica papaya, Pisum sativum, Ananas comosus, Prunus domestica, Citrus maxima, Ipomoea batatas, Rubus idaeus, Spinacia oleracea, Fragaria spp., Citrus reticulate, Colocasia esculenta var. esculenta, Lycopersicon esculentum and Nasturtium officinale* (Franke *et al.*, 2004).
Zhang and Hamauzu (2004) observed high susceptibility of ascorbic acid in temperature rise. Antioxidative properties of the buffered water infusions were weaker than in the ethanol infusions. The reason could be that ethanol infusions had better antioxidant properties due to the antioxidative effect of the lipophilic compounds present in ethanol solutions. In the plant material the antioxidative properties may depend on the presence of the water soluble ascorbic acid as well as the semi polar polyphenolic compounds or unpolar compounds. Vitamin C has antioxidant activity when it reduces oxidizing substances such as hydrogen peroxide (Duarte and Lunec, 2005). It reduces metal ions, which lead to the generation of free radicals through the fenton reaction (Carr and Frei, 1999).

Ascorbic acid is a reducing agent and thereby neutralize oxygen species such as hydrogen peroxide (Padayatty et al., 2003). The non enzymic antioxidants and Vitamin C in orange, reduced glutathione in grapes and vitamin E in tomato were predominant antioxidants (Rani et al., 2004).

5.6. α-Tocopherol

In the present study, α-Tocopherol content in aerial parts of *Bacopa* plants was negatively correlated and the maximum content of α-tocopherol found in Athani sample. The plant tissues have different tocopherol content. In *Brassica napus* seeds often dominate other plant
parts in terms of the higher content of total tocopherols (T-tocopherol), although α-tocopherol (vitamin E) is most biologically active, is often only a minor component (Eitenmiller, 1997; Goffman and Becker, 2002).

Chinese genotypes of *Brassica napus* for seed tocopherols content and their analysis using gas chromatography has not been comprehensively reported. Variations detected in tocopherol contents among the genotypes signify the need to quantify a wide range of rapeseed germplasm for seed tocopherol dynamics (Hussain *et al*., 2013).

**5.7. Reduced glutathione**

The present investigation, the amount of reduced glutathione content in different parts of *Bacopa monnieri* accessions was found the maximum amount in Aliyar dam accession. This type of result was noticed in *Nicotiana tobaccum* by Herschbach and Rennenberg, 1994.

GSH is the predominant form of reduced sulfur in plants, homologues of GSH can be found in different plant species, whereas some of the constitutive amino acids differ from those found in GSH. For instance, homoglutathione (hGSH, L-glutamyl-L-cysteine-alanine) can be found in several tissues and organs of legumes (Matamoros *et al*., 1999).
Reduced glutathione and ascorbic acid play an important role in maintaining the intracellular redox status in plant cells. Both metabolites act in the so called ascorbate-glutathione cycle, helping to prevent and/or minimize damages caused by reactive oxygen species (Davey et al., 2000).

5.8. Total phenol

In the present study, the higher amount of phenol content was in Nagercoil accession. The variations in the quantity of total phenol were noticed in different accessions. These results were correlated with *Myrtus communis* (Wannes et al., 2010). The total phenol contents varied between different parts of the myrtle plants. Leaf extract had higher total phenol content than flower and stem extracts. Significant differences were also found in total tannin contents in myrtle parts.

Phenolic compounds are biochemically synthesized via the shikimate pathway, which produces the group of phenolics called phenylpropanoids (Singer et al., 2003). They can act as antioxidants by donating hydrogen to highly reactive radicals, thereby preventing further radical formation (Lapornik et al., 2005).

The phenolic and nutrient content and antioxidant capacity of the samples vary with respect to the growing localities of the plants. Similar results were observed in *Terminalia chebula* (Singh and Sharma, 2010). This indicates the effect of growing localities on the secondary metabolite and nutrient content of plants. Primary products
such as carbohydrates, lipids and proteins are common to all plants and are involved in primary metabolic processes. While secondary metabolites content of the plant may vary with respect to their growing conditions (Kaufman et al., 1999; Wink, 1999). Influence of genotype and climatic conditions on total phenolics, total anthocyanins and antioxidant capacity of different cultivars of *Vaccinium* species (Connor *et al.*, 2002; Moyer *et al.*, 2002).

**5.9. Antioxidant enzymes**

**5.9.1. Superoxide dismutase**

Superoxide dismutase enzyme activity is varied in among the accessions and their aerial parts of *B. monnieri*. The highest SOD activity was recorded in leaves of Aliyar dam accession. The principal defense systems against oxygen free radicals are SOD, GSH, GSH peroxidases, glutathione reductase, catalase and antioxidant nutrients. Superoxide dismutase converts O$_2$ to H$_2$O$_2$. Hydrogen peroxide is also produced through two electron reduction of O$_2$ by cytochrome P-450, D-amino acid oxidase, acetyl coenzyme-A oxidase or uric acid oxidase (Freidovich, 1999; Fang, 2002).

Cytochrome C and SOD catalyze the formation of O$_2$ from O$_2$ radical. A coproduct of SOD is H$_2$O$_2$, which is converted to H$_2$O by catalase and the selenium dependent GSH peroxidase. Lipid hydroperoxides are detoxified to alcohols by GSH peroxidase. Another type of GSH peroxidase acts on phospholipid peroxides in membrane
structures (Sies, 1999). Superoxide dismutase (SOD), peroxidase (POD) and ascorbate peroxidase (APX) was found different in roots and leaves of the metal treated plants. Iron induced stress was observed as indicated by high level of lipid peroxidation in leaves than in roots of *Bacopa monnieri* (Sinha and Saxena, 2006).

5.10. Catalase

The Catalase activity is higher in leaves of Aliyar dam accession and the activity is significantly varied among the accessions. Catalase has one of the highest turnover rates for all enzymes, one molecule of catalase can convert ~6 million molecules of hydrogen peroxide to water and oxygen per minute. The significantly decreased variety of tumours for detoxifying hydrogen peroxide is linked to a decreased level of catalase (Fridovich, 1986).

Gondim *et al.*, 2012 were observed in catalase is responsive of these enzymes to H$_2$O$_2$, with higher activity early (48 h) in the treatment, while guaiacol peroxidase and ascorbate peroxidase were responsive only at later stages (240 h) of treatment. Increased CAT activity appears linked to gene expression regulation. Lower malondialdehyde levels were detected in plants with higher CAT activity, which may result from the protective function of this enzyme. The pretreatment with H$_2$O$_2$ leaf spraying was able to reduce the deleterious effects of salinity on seedling growth and lipid peroxidation. These responses could be attributed to the ability of H$_2$O$_2$ to induce antioxidant defenses, especially CAT activity.
Periods of carbohydrate deprivation are commonly encountered by plant cells. Plants respond to this nutrient stress by the mobilization of stored carbohydrates and the reallocation of other cellular macromolecules to degradative pathways. A number of metabolic genes that are upregulated in *Arabidopsis thaliana* cells during sucrose starvation. AcylCoA oxidase-4 (ACX4) activity increases during sucrose starvation, indicating a shift to a catabolic breakdown of fatty acids as a source of available carbon. The degradation of short chain fatty acids in the response to sucrose starvation, leading in turn to the production of toxic H$_2$O$_2$. Catalase activity is increases during starvation as a direct response to the increase in oxidative stress caused by the rapid activation of alternative catabolic pathways, including a specific increase in ACX4 activity. Any disruption in ACX4 expression or in β-oxidation of fatty acids in general prevents this increase in catalase activity and expression. CAT3 activity increases to remove the H$_2$O$_2$ produced by alternative catabolic processes induced during the carbohydrate shortages caused by extended periods of low light conditions (Contento and Bassham, 2010).

Catalase activity plays a vital role in plant defense against salt induced oxidative stress. Influence of salt stress on the repair of PS II and the synthesis of D1 in wild type tobacco (*Nicotiana tabaccum* ‘Xanthi’) and in transformed plants comprising the CAT gene katE in *Escherichia coli* (Taweel *et al.*, 2007). On the other hand, such a positive relationship
between the activity of CAT and degree of salt tolerance has also been
drawn in transgenic plants of Arabidopsis (Willekens et al., 1997).

5.11. Peroxidase

Peroxidase enzyme activity in leaves and stem parts of the plant
of B. monnieri significantly varied in the different parts of Tamil Nadu
accessions. The highest peroxidase activity was observed in Aliyar dam.
POD catalyses the dehydrogenation of structurally diverse phenolic and
endiolic substrates by H$_2$O$_2$ and are thus often regarded as antioxidant
enzymes, protecting cells from the destructive influence of H$_2$O$_2$ and
derived oxygen species (Prasad et al., 1994; Vianello et al., 1997).
Enhance production of oxygen free radical is responsible for
peroxidation of membrane lipids and the degree of peroxidative damage
of cells is controlled by the potency of antioxidant peroxidase enzyme
system in spinach leaves (Ozturk and Demir, 2003).

Glutathione peroxidase plays a pivotal role in H$_2$O$_2$ catabolism and
the detoxification of endogenous metabolic peroxides and hydroperoxides,
which catalyses reduced glutathione (GSH) level. The decreased level of
 glutathione peroxidase due to either free radical dependent inactivation
of enzymes or depletion of its cosubstrates that is GSH and NADPH in
Thespesia populnea (Bailey et al., 2001). In POD isoenzyme pattern of
treated plants was in accordance with the activity change in time.
Several POD isoforms (P3, P4 and P9) were specifically induced by
salinity and drought in Centaurea ragusina (Radic et al., 2006).
Amongst various enzymes involved in quenching of reactive oxygen species, guaiacol peroxidase (GPX) and catalase have their importance in elimination of $\text{H}_2\text{O}_2$. The stimulated activities of these enzymes (GPX and catalase) and reduced APX activity found that elimination of $\text{H}_2\text{O}_2$ in *Occimum tenuiflorum* was achieved by GPX and catalase. Furthermore, GPX participates in the lignin biosynthesis and might build up a physical barrier against poisoning of the heavy metals (Roy *et al.*, 1992).

### 5.12. Polyphenol oxidase

Polyphenol oxidase activity was observed in leaves and stem of Aliyar dam and Urachikottai accession. The polyphenol oxidase enzyme activities expressed due to nutritional modification, enzymatic browning, mishandling and storage of fresh tissue. These results positively correlated with Gulcin *et al.*, 2005. In plant tissues, the browning pigments lead to organoleptic and nutritional modifications, thus depreciating the quality of the food products.

Polyphenol oxidase activity were catalyzes the hydroxylation of monophenols to O-diphenols through a monophenolase activity and a subsequent oxidation of these O-diphenols to the corresponding O-quinones by a catecholase/diphenolase activity (Mayer, 2006). These differences could be due to in concentrations of phenolics and their irreversible binding to the PPO protein, resulting interference with protein purification in certain fruits and vegetables (Papadopoulou and Frazier, 2004).
The inhibition of PPO is important in the food industry, due to its role in browning. The newly studied compound, cysteine hydrochloride, was structurally quite similar to the earlier studied inhibitor, cysteine and showed significant inhibition of PPO activity at a concentration much below the permissible level. Cysteine hydrochloride is derived from the amino acid cysteine and is highly soluble in water (Kux, 2010).

5.13. **Glutathione S-transferase**

Glutathione S-transferase enzyme activity was higher in the leaves of Aliyar dam accession. GST expressed at the time of detoxification of the natural and exogenous toxic compounds in the environment. The similar type of results was noticed in the following study. The plants have versatile detoxification systems to encounter the phytotoxicity of wide range of natural and synthetic compounds present in the environment. Glutathione S-transferase is an enzyme that detoxifies natural and exogenous toxic compounds by conjugation with glutathione (GSH) (Kim *et al.*, 2012).

Mannervik and Danielson (1988) were observed Glutathione S-transferase is a family of multifunctional proteins, catalyzing the formation of conjugates between reduced glutathione (GSH) and a wide variety of electrophilic compounds biotransformed from xenobiotics including carcinogens. GSH is present in plant cells in millimolar concentrations and regarded as a major determinant of cellular redox
Glutathione can be reversibly endured by oxidation and reduction in cells. The reduced glutathione (GSH) could be restored from oxidized glutathione disulfide (GSSG) by the catalysis of glutathione reductase (GR), which is encoded by GR1 gene (Martya et al., 2009).

5.14. **In vitro free radical scavenging activity**

5.14.1. **Phenol content**

The highest phenol content in the crude extract of Chidambaram accessions when compared with other accessions. Plants are the source of the most potent free radical scavengers such as phenolic compounds and vitamins. Therefore, the investigations of biological activity and chemical composition of medicinal plants as a potential source of natural antioxidants are numerous in the recent studies (Albayrak and Aksoy, 2010; Salas et al., 2010).

The phenolic compounds are considered the major determinants for the antioxidant capacity in plants (Velioglu et al., 1998; Dorman et al., 2003). Phenolic and carotenoids content, the Brazilian cherry fruit is a promising source of natural antioxidant compounds (Lima et al., 2002).

However, the abundant phenolic derivatives with diverse structural patterns found in *Laguncularia racemosa* effects on ecological interactions. In fact, the antioxidation and insecticide of the
diverse phenolic compounds from *L. racemosa* led to the prediction that the additional ecological functions may be related to herbivores, detritivores and/or pathogens through oxidative activation (Lovelock *et al.*, 1992).

### 5.15. DPPH

Highest percentage of DPPH radical activity observed in Aliyar dam accession. The DPPH radical has been widely used to test the ability of compounds as free radical scavengers or hydrogen donors and to evaluate the antioxidant activity of plant extracts and foods (Porto *et al.*, 2000). Percentage of DPPH radical scavenging activities of all the extracts were dose dependent. The strong DPPH scavenging activity of tea could be attributed in part to the tea catechins and some low molecular polyphenols (Zhu *et al.*, 2002).

Antioxidant properties of *Cassia fistula* in methanol seeds extract were evaluated to find a new natural source of antioxidant. DPPH radical is a commonly used substrate for fast evaluation of antioxidant activity because of its stability in the radical form and simplicity of the assay (Bozin *et al.*, 2008). This assay is known to give reliable information concerning the antioxidant ability of the tested compounds (Huang *et al.*, 2005). The principle behind this assay in the color change of DPPH solution from purple to yellow as the radical is quenched by the antioxidant (Karagozler *et al.*, 2008).
The DPPH scavenging activity of the methanol extract of *Pistacia lentiscus*, *Fraxinus angustifolia* and *Clematis flammula*. *Fraxinus excelsior* showed higher than that of hexane and dichloromethane extracts, suggesting that the hydrogen donating compounds are more likely to be present in polar solvents (Middleton *et al.*, 2005).

### 5.16. ABTS

The highest ABTS radical scavenging activity noticed in Nagercoil accession. The activity variation is due to the presence of phytochemicals in the extract. Higher concentration of the prynalated flavonoids was more effective in quenching ABTS free radicals (Pannala *et al.*, 2001). Generation of the ABTS (2, 2'-azinobis-3-ethylbenzo thiazoline-6-sulfonic acid) radical cation forms the basis of the spectrophotometric methods that have been applied to the measurement of the total antioxidant activity of solutions of pure substances, aqueous mixtures and beverages.

The original ABTS assay was based on the activation of metmyoglobin with hydrogen peroxide in the presence of ABTS to produce the radical cation, in the presence or absence of antioxidants. This has been criticized on the basis of the faster reacting antioxidants might also contribute to the reduction of ferryl myoglobin radical. A more appropriate format for the assay is a decolorization technique in that the radical is generated directly in a stable form prior to reaction with putative antioxidants (Wolfenden *et al.*, 1982; Salah *et al.*, 1995).
5.17. Superoxide radical

Superoxide radical scavenging activity was higher in Aliyar dam accession extract. These results were positively correlated by the following study. The scavenging activities of the crude aqueous extracts of fresh flowers in southern China, the polyphenolic contents against superoxide and hydroxyl free radicals. The results showed that the extracts of red rose flowers had stronger antioxidant activity (Youwei et al., 2008). Superoxide anions are the most common free radicals \textit{in vivo} and are generated in a variety of biological systems and the concentration of superoxide anions increases under conditions of oxidative stress (Lee et al., 2002).

Superoxide radical is known to be very harmful to cellular components as a precursor of more reactive oxygen species (Halliwell and Gutteridge, 1985). Photochemical reduction of flavins generates $O_2^-$, which reduces NBT, resulting in the formation of blue formazan. The extract was found the scavenging of superoxide radical generated in riboflavin-NBT-light system \textit{in vitro}. The extract inhibited the formation of the blue formazan and the percentage of inhibition was proportional to the concentration with an EC$_{50}$ value of 73.9\textmu g/ml. These results indicated that the tested extract had a notable effect on scavenging of superoxide when compared with ascorbic acid, which was used as positive control (Beauchamp and Fridovich, 1971).
5.18. Hydroxyl radical

Hydroxyl radical scavenging activity was higher in Aliyar dam sample. The deoxyribose degradation assay is widely used to evaluate the hydroxyl (OH) radical scavenging ability of food. They compared the hydroxyl radical scavenging activity of 25 antioxidant samples prepared in ethanol solution with samples prepared after removing the ethanol (residue). The data suggested that there was an approximately 9-fold difference between assay results for the ethanol solution and residue samples. The scavenging activities of 18 organic solvents (including ethanol) were measured by the deoxyribose assay. Most pure organic solvents (especially alcohols) could effectively scavenge hydroxyl radicals. As hydroxyl radicals have extremely high reactivity, they will quickly react with surrounding solvent molecules. This shows that any organic solvent should be completely evaporated before measurement. The proposed method is regarded as a reliable hydroxyl radical scavenging assay, suitable for all types of antioxidants (Li, 2013).

Similar results were found in ethanol can act as a free radical scavenger (Novogrodsky et al., 1982; Paz and Anderson, 1992) and can protect DNA from X-rays by scavenging hydroxyl radicals (Ellahuene et al., 2012).

Samak et al., 2009 reported that in Wagatea spicata, a woody legume shrub, widespread medicinal plant found in Western Ghats of India has significant abilities to scavenge highly reactive free radicals.
Shade dried leaf, bark and flower powder of this plant has been extracted with water and fractionated with different solvents. Extracts and their solvent fractions were found to be good scavengers of superoxide and hydroxyl radicals. Free radical scavenging action of *W. spicata* is due to its rich phenolic and flavonoid contents. Bark and leaf extracts showed significant scavenging action against superoxide radicals, whereas flower extracts efficiently inhibited hydroxyl radicals.

### 5.19. Genetic diversity

The present study indicates the low level of genetic variations in the accessions of *B. monnieri* in Tamil Nadu. These results were positively correlated with Mathur *et al.*, 2003. 27 accessions of *Bacopa monnieri* collected from semi temperate, subtropical and tropical environments at geographically distinct locations in north India were examined for genetic variability. Both the gross agroclimatic environment of a region and microenvironment in the vicinity of water bodies where a *Bacopa monnieri* genotype occurred must have interacted for natural selection of the genotype.

Tripathi *et al.*, 2012 observed with marker systems of RAPD and ISSR, individually or combined can be effectively used in determination of genetic relationship among *B. Monnieri* accessions collected from different parts of Central India. It could be concluded that genetic similarities and diversity among *Bacopa monnieri* accessions is necessary for their conservation and breeding programme.
Darokar et al., 2001 reported the collection of 24 *B. monnieri* accessions from different agro climatic zones of India and an introduction from Malaysia accession maintained in field gene bank at CIMAP were analysed for RAPD variation. The number of polymorphic bands generated in primer dependent, ranging from 2 to 8. It was possible to differentiate individual accessions, showing differences in morphological and growth properties at DNA level. The observed low levels of genetic variation were attributed to interplay of sexual and vegetative modes of reproduction and similarity of local environments in habitats of *B. monnieri*.

**5.20. Protein profile**

The significant qualitative difference in the expression of proteins from *Bacopa monnieri* accessions in Tamil Nadu. The ranges of 205 - to 52kDa proteins are well resolved in the analysis. The similar type of results was noticed by Antao and Malcata, 2005. Most plant serine protease found to be monomeric with the molecular weight in the range of 30-110kDa. Extreme conditions of denaturation prior to SDS-PAGE before concluding the oligomeric nature of such proteases by SDS-PAGE especially that are poorly characterized biochemically and biophysical properties.

Legumain like cysteine proteases cleave during germination period indicating their role as proprotein cleaving enzymes (Schlereth et al., 2000). During germination and the early growth stage of plants,
storage proteins are degraded by proteolytic enzymes. In mature organs like leaves, roots and dry seeds, the level of legumain is probably reduced compared to that of papain like proteases. The legumain specificity towards asparagine indicates a regulatory function for this protease. It would be expected that other proteases with less substrate specificity and involved in general protein catabolism, would be more abundant.

Proteolysis is a fundamental process during the whole life of the plant and its importance increases during senescence, when the nitrogen reserves of proteins are mobilized and translocated into the reproductive parts like seeds and leaves. The mechanism of balancing the level of protease activity in plants includes differential rates of protease synthesis and degradation, location of proteases and their substrates in different cell compartments and modulation the rate of proteolysis by different concentrations of protease inhibitors. The decrease of protease activity in plants results in altered growth and development of the plants and indicates the involvement of the proteases in different physiological processes. The high activity in whole plant and aerial part of plant could be a result of additional effects of different enzymatic activities that might occur in the whole plant and aerial part as compared to single plant part (Novak et al., 2002).
5.21. Bacoside- A quantification

The highest amount of Bacoside-A content was present in the Aliyar dam sample. The significant variation was noticed among the ecotypes of *B. monnieri* from Tamil Nadu. This similar type of results was noticed by Deepak et al., 2005. Bacoside concentrations from the analytes in the samples of *Bacopa monnieri* collected from different regions of India were Bacodide A$_3$ (0.14-0.85%), Bacopaside-II (0.12-0.69%), Jujubogenin (0.05-0.72%) and Bacosaponin-C (0.05-0.44%).

Brahmi is a traditional Indian medicinal plant known for its natural nootropic action of saponins present in large amount in its shoots (Darokar et al., 2001). In two triterpenoid glycosides have been isolated along with 10 known saponins from *Bacopa monnieri*. Structures of the compounds have been elucidated as $3\text{-O-}[\beta\text{-D-glucopyranosyl-}(1\rightarrow3)\beta\text{-D-glucopyranosyl}]$ Jujubogenin and $3\text{-O-}[\beta\text{-D-glucopyranosyl-}(1\rightarrow3)\beta\text{-D-glucopyranosyl}]$ pseudojujubogenin by high resolution NMR spectral data and chemical correlations. Further, the chemical compositions of Bacosides A and B have been delineated (Sivaramakrishna et al., 2005).

*B. monnieri* samples (extract, plant material and commercial products) were successfully analysed, each of them containing at least four of the seven reference compounds (Ganzera et al., 2004). Main components were either Bacoside A$_3$ or Bacopaside II, least dominant showed to be Bacopasides IV and V. The total saponin content in the
samples varied from 1.1 to 13%. Extrapolization of the observed quantitative variations indicate that reference standard of Bacoside A, if obtained from different source, could also exhibit variation in the contents of 1-4 (Renukappa et al., 1999).

The first analytical procedure permitting the analysis of individual bioactive saponins (bacosides) in *Bacopa monnieri* is described. Furthermore, the method was validated for limit of detection, linearity, precision, accuracy inter day variation. Several *B. monnieri* samples (extract, plant material and commercial products) were successfully analysed, each of them containing at least four of the seven reference compounds (Ganzera et al., 2004).

Several papers have been published on the link between alkaloid variability and abiotic factors, e.g. humidity and nitrogen availability (Saenz et al., 1993; Hunt et al., 2005), temperature (Salminen et al., 2005), light intensity and light quality (Flota and Deluca, 1998). However, this relationship is still controversial. Although wide variation in total alkaloid content has been detected in *A. crassiflora* leaves, no significant correlation was observed between alkaloid content and mean temperature (°C) or relative humidity (%).

Besides ecological aspects, the alkaloid profile of *A. crassiflora* suggests that the species has potential medicinal applications. Anonaine, romucosine and annoretine showed to exert significant
antibacterial activity (Paulo et al., 1992). These compounds also inhibit platelet aggregation (Chen et al., 2001) and are cytotoxic (Chang et al., 1998). Soil fertilization is critical, since over rich soils affects secondary metabolite content. Plants growing in sand rich soils produce more terpenes. This result indicates the high terpene producing germplasm and recommendations for plant cultivation in Centella asiatica (Devkota et al., 2010). Upadhyaya, 2013 reported the leaves of Paederia foetida collected from three districts of Assam were analysed for phytochemical constituents, antioxidant and antimicrobial activity and nutrient content. Saponin, tannin, phenol, flavonoid, terpenoid, cardiac glycoside, alkaloid and reducing sugar were detected in the samples. The order of total phenolic content, antioxidant activity and nutrient content of the samples were sample 1 > sample 3 > sample 2. It is noted that both primary and secondary metabolite content of plants varies with variation of growing localities.