DISCUSSION

Medicinal plants are referred to as plants that are used for their therapeutic or medicinal values. The most of the plants or their different parts could be valued for their therapeutic, medicinal, aromatic or savoury qualities. With this view, the pharmaocochemical characterization, GC-MS analysis and the pharmacological potentials of the whole plants of *Asystasia travancorica* and *Sonerila tinneveliensis* were studied.

PHARMACOCHEMICAL CHARACTERIZATION

Physicochemical constituents

**Ash values:** The evaluation of physical constants of the drug is an important parameter in detecting adulteration or improper handling of drugs (African Pharmacopoeia, 1986). Parameters such as extractive value, total ash, water soluble ash, acid insoluble ash and sulphated ash of whole plants of *A. travancorica* and *S. tinneveliensis* were determined by analysing powered samples of these plants. Ash values are useful in determining the quality and purity of crude drug, especially in the powder form. The extractive values are useful for the evaluation of the powdered plant drugs, especially when the constituents of drugs cannot be readily estimated by any other means. Further, these values indicate the nature of the constituents present in the crude drug. The total ash is particularly important in the evaluation of purity of drugs, i.e., the presence or absence of foreign organic matter such as metallic salts and/or silica (Musa *et al.*, 2006). Acid insoluble ash reflects the calcium oxalate content of the drug. In the present investigation, a considerable amount of total ash was noticed in the whole plants of *A. travancorica* (8.44%) and *S. tinneveliensis* (9.32%) and this finding can be employed as a quality parameter to evaluate *A. travancorica* and *S. tinneveliensis* biomass for any adulteration. The ash values are an indicative of
the impurities present in the drug. Since the ash values are constant for a given
drug, the values are also one of the diagnostic parameters of the drug. Both the selected
plant samples had more water soluble ash than the acid insoluble ash. The ash value is
generally an index of the purity as well as identity of the drug.

**Fluorescence analysis:** Many phytocompounds fluoresce when suitably illuminated.
The fluorescent colour is specific for each compound. A non-fluorescent compound
may fluoresce if mixed with impurities that are fluorescent. The fluorescence
analysis method is sensitive adequately and enables the precise and accurate
determination over a satisfactory concentration range without several time consuming
dilution steps prior to the analysis of pharmaceutical samples (Pimenta *et al.*, 2006).
The powdered samples of whole plants of *A. travancorica* and *S. tinnevelliensis* as such
fluoresced green under day light and yellowish green under short UV light (254 nm).
The powdered sample of *A. travancorica* fluoresced dark green under long UV light
(365 nm), whereas that of *S. tinnevelliensis* fluoresced dark blue under long UV light.

To understand the nature of the fluorescence emission from the crude
preparation, under different conditions, the preliminary phytochemical analysis of the
crude preparation was compared. The comparative analysis clearly showed a
correlation between the compounds present in it and their fluorescent behaviours
under different conditions. The major bioactive compounds present in the crude
preparations are the coumarins, steroids, flavonones, tannins, alkaloids, phenols and
saponins. Coumarin, especially hydroxyl amino acid derivatives like o-coumaric acid,
appears yellowish green in alkaline condition under short UV radiation. Flavonones
which are light yellow in aqueous condition, under UV light, turns to bright yellow
under alkaline conditions. Similarly, the phytosterols, when treated with 50% H₂SO₄
show green fluorescence under UV light. Terpenoids, especially sapogenins, exhibit
yellow green fluorescence under short UV light (Horborne, 1976). Quinine, aconitin, berberin and emetin show specific colours of fluorescence (Aconitin - light blue; berberin - light yellow; emetin - orange). Fixed oils and fats fluoresce least, waxes more strongly and mineral salts most of all (Evans, 1996).

Haydon (1975) studied the photophysical characters of coumarins. Hydroxy methyl coumarin fluoresced at 420 - 440 nm when observed in different solvents with increasing polarity (Chal-topudhyay et al., 2006). The fluorescence analysis of the crude drug prepared from the whole plants of *A. travancorica* and *S. tinneveliensis* exhibited clear fluorescence behaviours at different radiations which can be taken as standard fluorescence pattern.

**Phytochemical studies:** Presence or absence of certain important compounds in an extract is determined by colour reactions of the compounds with specific chemicals which act as dyes. This procedure is a simple preliminary prerequisite before going for the detailed phytochemical investigations. Various tests have been conducted qualitatively to find out the presence or absence of bioactive compounds. Phytochemical evaluation is one of the tools for the quality assessment, which includes preliminary phytochemical screening; chemo-profiling and marker compound analysis using modern analytical techniques. In the last two decades, HPTLC has emerged as an important tool for the qualitative, semi-quantitative and quantitative phytochemical analyses of herbal drugs and formulations. HPTLC method is fast, precise, sensitive and reproducible with good recoveries for standardization of herbal drugs.

The preliminary phytochemical screening of the whole plant methanol and ethanol extracts of both the experimental plants revealed the presence of alkaloids, anthraquinones, catechins, coumarins, flavonoids, phenols, quinones, saponins, steroids, tannins, terpenoids, sugars, glycosides and xanthoproteins in them. HPTLC
investigation also confirmed the presence of alkaloids, flavonoids, glycosides, saponins and steroids in whole plants of *A. travancorica* and *S. tinnevelliensis*. The presence of these useful secondary metabolites could make these plants useful for treating different human ailments and thus providing potential drugs for human use. This is because; the pharmacological activity of any plant is usually traced to a particular phytocompound.

Therapeutically terpenoids exert a wide spectrum of activities such as antiseptic, stimulant, diuretic, anthelmintic, analgesic and counter-irritant (Gokhale *et al.*, 2003). Many tannin containing drugs are used, in medicine, as astringent. They are used in the treatment of burns as they precipitate the proteins of exposed tissues to form a protective covering (Handa and Kapoor, 1992). They are also medically used as healing agents in inflammation, leucorrhoea, gonorrhoea and piles and also as antidote (Ali, 1994).

Saponins, a group of natural products, occur in both the plants selected for the present study. In plants, the presence of steroidal saponins like cardiac glycosides appear to be confined to many families and these saponins have great pharmaceutical importance because of their relationship to compounds such as the sex hormones, cortisones, diuretic steroids, vitamin D etc., (Evans and Saunders, 2001). Sapogenin, a synthetic steroid is prepared from plants and is used to treat a wide variety of diseases such as rheumatoid arthritis, collagen disorders, allergic and asthmatic conditions (Claus, 1956). Saponin reduces the uptake of certain nutrients including glucose and cholesterol, at the gut, through intra-luminal physicochemical interactions. Hence, it has been reported to have hypocholesterolemic effects and thus may aid in lessening metabolic burden that would have been placed in the liver (Price *et al.*, 1987).

Several authors have reported that flavonoids, sterols/terpenoids and phenolic
acids are known to be bioactive antidiabetic principles (Oliver-Bever, 1986; Rhemann and Zaman, 1989). Flavonoids are known to regenerate the damaged beta cells in the alloxan induced diabetic rats (Chakravarthy et al., 1980). Flavonoids act as insulin secretagogues (Geetha et al., 1994). Most of the plants have been found to contain substances like glycosides, alkaloids, terpenoids, flavonoids etc., which are frequently implicated as having antidiabetic effects (Loew and Kaszkin, 2002).

**GC–MS ANALYSIS**

GC-MS is a valuable tool for reliable identification of phytocompounds. Ten compounds were detected in the whole plant ethanol extract of *A. travancorica* and they include Levo-à-Elemene (41.97%), Tetra hydrospirilloxanthin (17.07%), Stigmasterol (12.25%), Phytol (8.23%), 2,6,10- Dodecartrien-1-ol, 3,7,11-trimethyl-[trans-farnesol] (7.03%), Ethyl iso-allocholate (4.62%), Heptadecane 9-hexyl (3.21%), Palmitic acid, ethyl ester (2.21%), 8,11,14, Eicosatrienoic acid, methyl ester, (z,z,z) – [methyl dihomo-c-linolenate] (2.01%) and Tetra tetracontane (1.41%). The nature and biological activities of some major phytocompounds obtained through the GC-MS study of whole plant of *A. travancorica* are tabulated below.

**Name, Nature and Biological activities of some detected compounds in the whole plant ethanol extract of *A. travancorica***

<table>
<thead>
<tr>
<th>S. No.</th>
<th>RT</th>
<th>Name of the compound</th>
<th>Nature of the compound</th>
<th>Biological activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11.45</td>
<td>Ethyl iso-allocholate</td>
<td>Steroids</td>
<td>Antimicrobial, Anticancer, Antiarthritic, Antiasthma, Hepatoprotective, Antiinflammatory</td>
</tr>
<tr>
<td>2</td>
<td>13.24</td>
<td>Palmitic acid, ethyl ester</td>
<td>Palmitic acid ester</td>
<td>Antioxidant, Nematicide, Flavor, Pesticide, Hypcholesterolemic, 5-Alpha reductase inhibitor</td>
</tr>
<tr>
<td>S. No.</td>
<td>RT</td>
<td>Name of the compound</td>
<td>Nature of the compound</td>
<td>Biological activity</td>
</tr>
<tr>
<td>-------</td>
<td>-----</td>
<td>------------------------------------------</td>
<td>------------------------</td>
<td>--------------------------------------</td>
</tr>
<tr>
<td>3</td>
<td>14.72</td>
<td>Phytol</td>
<td>Diterpene</td>
<td>Antiinflammatory, Antimicrobial, Anticancer, Diuretic</td>
</tr>
<tr>
<td>4</td>
<td>15.46</td>
<td>8,11,14 Eicosatrienoic acid, methyl ester, (z,z,z) -</td>
<td>Unsaturated fatty acid</td>
<td>Anti cholesterol, Cardio protective</td>
</tr>
<tr>
<td>5</td>
<td>24.31</td>
<td>Trans Farnesol</td>
<td>Sesquiterpene Alcohol</td>
<td>Antitumour, Analgesic, Antibacterial, Antiinflammatory, Sedative, Fungicide</td>
</tr>
<tr>
<td>6</td>
<td>30.52</td>
<td>Stigmasterol</td>
<td>Steroid</td>
<td>Antiinflammatory, Sedative, Antiviral, Antioxidant, Cancer preventive, Antihepatotoxic</td>
</tr>
<tr>
<td>7</td>
<td>31.74</td>
<td>Tetra hydrospirilloxanthin</td>
<td>Carotenoid compound</td>
<td>Antioxidant, Free radical scavenger, Improve immune system, Vitamin A precursor</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>Levo-á- Elemene</td>
<td>Sesquiterpene</td>
<td>Antitumor, Analgesic, Antibacterial, Antiinflammatory, Sedative, Fungicide</td>
</tr>
</tbody>
</table>

**Activity Source: Dr Duke’s Phytochemical and Ethnobotanical databases**

Among the identified phytochemicals, Phytol is one among the ten compounds in the present study. Phytol is observed to have antibacterial activities against *Staphylococcus aureus* by causing damage to cell membranes as a result there is a leakage of potassium ions from bacterial cells (Inoue et al., 2005). Phytol is a key acyclic diterpene alcohol that is a precursor for Vitamins E and K₁. It is used along with simple or corn syrup as a hardener in candies. Phytol, detected in *A. travancorica*, is also found to be effective at different stages of the arthritis. Ogunlesi et al. (2009) reported that phytol could give good as well as preventive and therapeutic results against arthritis.
Eight compounds were detected in whole plant ethanol extract of *S. tinneveliensis* and they include Tetra hydrospirilloxanthin (18.50%), Ethyl iso-allochoate (18.27%), Linolelaic acid, methyl ester (6.09%), Diisooctyl phthalate (6.09%), Stigmasterol (5.39%), Heptadecane, 9-hexyl (5.39%), Palmitic acid ethyl ester (5.15%) and Tetra tetra-acontane (2.34%). The nature and biological activities of some major phytocompounds obtained through the GC-MS study of whole plant of *S. tinneveliensis* are tabulated below.

**Name, Nature and Biological activities of some detected compounds in the whole plant ethanol extract of *S. tinneveliensis***

<table>
<thead>
<tr>
<th>S. No.</th>
<th>RT</th>
<th>Name of the compound</th>
<th>Nature of the compound</th>
<th>Biological activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11.45</td>
<td>Ethyl iso-allocholate</td>
<td>Steroids</td>
<td>Antimicrobial, Anticancer, Antiarthritic, Antiasthma, Hepatoprotective, Antiinflammatory</td>
</tr>
<tr>
<td>2</td>
<td>13.24</td>
<td>Palmitic acid, ethyl ester</td>
<td>Palmitic acid ester</td>
<td>Antioxidant, Flavour, Nematicide, Pesticide, Hypocholesterolemic, 5-Alpha reductase inhibitor</td>
</tr>
<tr>
<td>3</td>
<td>15.37</td>
<td>Linoleic acid methyl ester</td>
<td>Fatty acid ester</td>
<td>Hypocholesterolemic, Nematicide, Antiarthritic, Antieczemic, Hepatoprotective, Anti androgenic, Hypocholesterolemic, 5-Alpha reductase inhibitor, Antithistaminic, Anticoronary, Insectifuge</td>
</tr>
<tr>
<td>4</td>
<td>20.53</td>
<td>Diisooctylester</td>
<td>Plasticizer compound</td>
<td>Antimicrobial, Antifouling</td>
</tr>
<tr>
<td>5</td>
<td>27.37</td>
<td>Tetra hydrospirilloxanthin</td>
<td>Carotenoid compound</td>
<td>Antioxidant, Free radical scavenger, Improve immune system, Vitamin A precursor</td>
</tr>
<tr>
<td>6</td>
<td>30.56</td>
<td>Stigma sterol</td>
<td>Steroid</td>
<td>Antinflammatory, Sedative, Antiviral, Antioxidant, Cancer preventive, Antiepileptogenic</td>
</tr>
</tbody>
</table>

**Source: Dr.Duke’s Phytochemical and Ethnobotanical Databases**
Among the identified phytochemicals Ethyl iso-allocholate is the ester of a bile acid and can act as an emulsifying agent so that fat and oils can be digested by water-soluble digestive enzymes in the small intestine (Bruice, 1998). Stigma sterol is used as the precursor of semi-synthetic progesterone (Sunderaraman and Djerassi, 1977), a valuable human hormone that plays an important physiological role in the regulatory and tissue rebuilding mechanisms related to estrogens effect, as well as acting as an intermediate in the biosynthesis of androgens, estrogens and corticoids. It is also used as the precursor of Vitamin D₃ (Kametani and Furuyama, 1987).

Thus, GC-MS analysis is the first step towards understanding the nature of active principles in the medicinal plants and this type of study will be helpful for further detailed study. An exhaustive investigation about the pharmacological importance of whole plants of *A. travancorica* and *S. tinnevelliensis*, their diversity and detailed phytochemistry may add new knowledge to the information in the traditional medicinal systems.

**PHARMACOLOGICAL STUDIES**

*In vitro* antioxidant activity

Antioxidants are substances that combat free radicals, which are extremely reactive species that cause the oxidation of various biomolecules (lipids, proteins and nucleic acids), present in our organs. These free radicals may be the cause or the aggravating factor of their general picture (Halliwell, 1994). Thus, research seeks alternatives to reduce the harmful effects of free radicals and improve the body's antioxidant capacity, as a form of treatment and prevention of diseases and their complications.

Free radicals and other reactive oxygen species are considered to be important causative factors in the development of diseases of aging such as neurodegenerative
diseases, cancer and cardiovascular diseases. These free radicals may be the cause or the aggravating factor of their general picture (Halliwell, 1994). Phytochemicals have long been recognized to possess many properties including antioxidant, antiallergic, antiinflammatory, antiviral, antiproliferative and anticarcinogenic (Eastwood, 1999). This relationship has led to considerable interest in assessing the antioxidant capacity of foods, botanicals and other nutritional antioxidant supplements. As plants produce significant amount of antioxidants to prevent the oxidative stress caused by photons and oxygen, they represent a potential source of new compounds with antioxidant activity. Thus, research seeks alternatives to reduce the harmful effects of free radicals and improve the body's antioxidant capacity, as a form of treatment and prevention of diseases and their complications. Continued research is being undertaken all over the world on different plant species and their therapeutic principles.

Several studies are focused on the relationship between the antioxidant activity of the phenolic compounds, their chemical structure and as hydrogen donating free radical scavengers. It has been shown that the presence of the –CH=CH-COOH group in the hydroxylated cinnamates ensures greater H-donating ability and subsequent radical stabilization than the carboxylate group in the hydroxyl benzoates (Rice-Evans et al., 1996). Previous studies (Castellucio et al., 1995; Chen and Ho, 1997; Heinonen et al., 1998; Larson, 1988) showed that antioxidant activity correlated with hydroxycinnamic derivatives in nutritional plants and this corroborate with our results, although the antioxidant activity of phenolics mainly depends on the number and the position of hydrogen donating hydroxyl groups on the aromatic cycles of the phenolic molecules (Dziedzic and Hudson, 1983; Liens et al., 1999; Rice-Evans et al., 1996). Phenolic compounds are very important constituents of plants and their radical
scavenging ability is due to their hydroxyl groups (Hatano et al., 1989). The phenolic compounds may contribute directly to antioxidative action (Duh et al., 1999).

Antioxidants are substances that delay the oxidation process, inhibiting the polymerization chain initiated by free radicals and other subsequent oxidizing reactions (Halliwell and Aruoma, 1991). Phenolic constituents, such as flavonoids, phenolic acids and tannins are known as powerful chain breaking biomolecules and are well known for their high antioxidant activity (Shahidi and Wanasundara, 1992; Rice-Evans et al., 1996). Epidemiological studies suggest that the consumption of flavonoids rich foods protects against human diseases associated with oxidative stress, like coronary heart disease and cancer (Duthie et al., 2000; Lambert and Yang, 2003; Renaud and de Lorgeril, 1992). The protective effect provided by fruits and vegetables against cancer, cardiovascular and cerebrovascular diseases, has been attributed to their antioxidant compounds (Ames, 1983; Gey, 1990). The majority of the antioxidant capacity of plants is not only represented by vitamin C, vitamin E or β-carotene, but it is also due to other compounds such as polyphenols which have a strong antioxidant potential (Bors and Saran, 1987). Many studies indicate a linear relationship between total phenolics and antioxidant activity (Djeridane et al., 2006; Kim et al., 2003).

Ascorbic acid, the standard antioxidant used in the present study, acts as a chain breaking scavenging agent that impairs the formation of free radicals in the process of intracellular substances formation throughout the body, including collagen bone matrix and tooth (Beyer, 1994; Aqil et al., 2006). Several methods have been developed to estimate the antioxidant capacity of different plant materials (Guo et al., 2003). A single assay is not sufficient to evaluate the total antioxidant activity (Frankel and Meyer, 2000; Silva et al., 2006). Hence, in the present study, the whole plant extracts of A. travancorica and S. tinnevelliensis were investigated for their antioxidant activity.
using DPPH radical scavenging activity, hydroxyl radical scavenging activity, superoxide scavenging activity, ABTS radical cation scavenging activity and reducing power assay.

**DPPH radical scavenging activity**

Free radicals such as oxygen, superoxide and hydroxyl are biologically important substances which are naturally released from human tissues. The highly reactive radicals can cause oxidative damage to DNA, lipids and proteins (Fritz et al., 2003; Boveris et al., 2007). Therefore, free radicals result in many disorders like cancer, cardiovascular diseases and diabetes mellitus (Vaya and Aviram, 2001). Many compounds that carry out free radical scavenging are substances having antioxidant activity such as flavonoid and phenolic compounds or phenolic rich plant extracts. The radical scavenging activities of whole plant extracts of *A. travancorica* and *S. tinnevelliensis* were tested using the ‘stable’ free radical, DPPH. Unlike laboratory generated free radicals such as the hydroxyl radical and superoxide anion, DPPH has the advantage of being unaffected by certain side reactions, such as metal ion chelating and enzyme inhibition (Yen and Hung, 2000; Mau et al., 2002; Cheung et al., 2003; Amarowicz et al., 2004). DPPH radical scavenging is considered to be a good *in vitro* model widely used to assess antioxidant efficacy within a very short time. In its radical form, DPPH disappears, on reduction by an antioxidant compound or a radical species, to become a stable diamagnetic molecule resulting in the colour change from purple to yellow, due to the formation of diphenyl picryl hydrazine (DPPH), which could be taken as an indication of the hydrogen donating ability of the tested samples (Oktay et al., 2003; Shon et al., 2003; Lee et al., 2007). Substances which are able to perform this reaction can be considered as antioxidants and therefore they are radical scavengers (Brand-Williams et al., 1995). The relatively stable organic radical, DPPH,
has been widely used in the determination of antioxidant activity of single compound, as well as of different plant extracts (Katalinic et al., 2006).

In the present study, among the solvents tested, the whole plant ethanol extract of *A. travancorica* and the whole plant methanol extract of *S. tinnevelliensis* exhibited the highest DPPH radical scavenging activities. Similar results were obtained when the methanol extracts of *Cassia fistula* bark (Ilavarasan et al., 2005), *C. auriculata* bark (Mishra et al., 2011) and methanol extracts of *C. occidentalis* stem, leaf and seed (Arya and Yadav, 2011) were used. The results indicate that the plant extracts with their proton donating ability, could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants (Marxen et al., 2007).

**Hydroxyl radical scavenging activity**

Hydroxyl radicals are one of the quick indicators of the lipid peroxidation process by abstracting hydrogen atom from unsaturated fatty acids or simply auto-oxidation of polyunsaturated fatty acids found primarily in membranes (Kappus, 1991). Scavenging of OH\(^-\) is an important antioxidant activity, because of its very high reactivity, which can easily cross the cell membranes at specific sites and reacts with most of the biomolecules and furthermore cause tissue damage and cell death. Thus, removing OH\(^-\)is very important task for the protection of living systems (Yang et al., 2008).

In the present study, the whole plant petroleum ether extract of *A. travancorica* and the whole plant methanol extract of *S. tinnevelliensis* showed maximum hydroxyl radical scavenging activity when compared to standard ascorbic acid. Similar activities of hydroxyl radical were reported by Ilavarasan et al. (2005) and Dheeraj et al. (2010) in *Cassia fistula* and *C. sophera* respectively. The results of the present study showed a close agreement with hydroxyl radical scavenging activities of *Sauropus bacciformis*.
(Jenecius et al., 2012), *Begonia malabarica*, *B. floccifera* (Kalpanadevi and Mohan, 2012) and *Xanthosoma sagittifolium* (Nishanthini and Mohan, 2012 a).

**Superoxide radical scavenging activity**

Superoxide anion is a reduced form of molecular oxygen created by receiving one electron. Superoxide anion is an initial free radical from mitochondrial electron transport systems. Mitochondria generate energy using electron chain reaction, reducing oxygen to water. Some of the electrons escaping from the chain reaction of mitochondria directly react with oxygen and form superoxide anion. It plays an important role in the formation of other reactive oxygen species, such as hydrogen peroxide, hydroxyl radical or singlet oxygen in living systems (Lee et al., 2004).

Superoxide is oxygen centred radical with selective reactivity. Although a relatively weak oxidant, superoxide exhibits limited chemical reactivity, but can generate more dangerous species, including singlet oxygen and hydroxyl radicals (OH\(^{-}\)), which cause the peroxidation of lipids (Halliwell and Chirico, 1993). Superoxide anions are precursors to active free radicals that have potential for reacting with biological macromolecules, and thereby, inducing tissue damage (Halliwell and Gutteridge, 1984).

Superoxide is easily formed by radiolysis of water in the presence of oxygen, which allows accurate reaction rate constants that to be measured (Gulcin and Dastan, 2007). It has been implicated in several pathophysiological processes due to its transformation into more reactive species such as hydroxyl radical. Also superoxide has been observed to directly initiate lipid peroxidation (Wickens, 2001). It has also been reported that antioxidant properties of some flavonoids are effective mainly via scavenging of superoxide anion radical. \(\text{O}_2^{\cdot -}\) is the precursor of \(\text{H}_2\text{O}_2\), \(\text{OH}^{-}\)and singlet oxygen, which induce oxidative damage in lipids, proteins and DNA.
Superoxide radicals are normally formed first, and their effects can be magnified because they produce other kinds of free radicals and oxidizing agents (Pietta, 2000). Superoxide anions derived from dissolved oxygen by the riboflavin/methionine/illuminate system will reduce nitroblue tetrazolium (NBT) in this system. In this method, $O_2^{-}$ radical reduces the yellow dye (NBT$^{2+}$) to produce the blue formazan, which is measured spectrometrically at 560nm. Antioxidants inhibit the blue NBT formation (Parejo et al., 2002; Khanamn et al., 2004).

Superoxide anions are the most common free radicals formed 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). It was therefore proposed to measure the comparative interceptive ability of the methanol extracts to scavenge the superoxide radical. In the present study, superoxide scavenging activities of extracts were measured by auto-oxidation of hydroxylamine in the presence of NBT. The reduction of NBT at 560nm indicates the consumption (Khanamn et al., 2004). Overproduction of superoxide anion radical contributes to redox imbalance and associated with harmful physiological consequences (Pervaiz and Clement, 2007).

In the present study, the whole plant methanol extracts of A. travancorica and S. tinnevellensis possessed superoxide quenching ability. This result is in accordance with that of Baccharis grisebachii (Tapia et al., 2004) and Calendula officinalis (Preethi et al., 2006). Based on the results, it could be attributed that A. travancorica and S. tinnevellensis scavenge superoxide radicals by combining with superoxide radical ions to form stable radicals, thus terminating the radical chain reaction (Wang et al., 2009).
ABTS radical scavenging activity

The ABTS assay is based on the inhibition of the absorbance of radical cation ABTS, which has a characteristic longwave length absorption spectrum (Sreejayan and Rao, 1996). The ABTS chemistry involves the direct generation of ABTS\(^+\) radical mono cation with no involvement of any intermediary radical. It is a decolourization assay, thus the radical cation is performed prior to addition of antioxidant test system, rather than the generation of the radical taking place continually in the presence of antioxidant. The results obtained imply the antioxidant activity of the extracts either by inhibiting or scavenging the ABTS\(^+\) radicals since both inhibition and scavenging properties of antioxidants towards ABTS\(^+\) radicals have been reported earlier (Re et al., 1999). In the present study, the percentage scavenging activity and IC\(_{50}\) value of the investigated extracts, at 1min of the reaction time, were calculated. The highest percentage scavenging activity, at 1min of the reaction time, for the whole plant methanol extract of *A. travancorica* was 89.84% (800\(\mu\)g/ml) and for the whole plant ethanol extract of *S. tinnevelliensis* was 88.27% (800\(\mu\)g/ml. The IC\(_{50}\) values were found to be 24.97\(\mu\)g/mL and 20.51\(\mu\)g/mL for the whole plant methanol extract of *A. travancorica* and for the whole plant ethanol extract of *S. tinnevelliensis* respectively. Similar ABTS\(^+\) radical cation scavenging activities were reported by Tresina *et al.* (2012a, b) in *Eugenia singampattiana*, Mary Jelastin Kala *et al.* in *Eugenia floccoa*, Nishanthini *et al.* (2012) in *Suaeda monoica*, Kalpanadevi and Mohan (2012) in *Begonia malabarica* and *B. floccifera* and Murugan and Mohan (2012) in *Dioscorea esculenta*.

Reducing power Assay

Reducing power assay measures the electron-donating capacity of an antioxidant (Yen and Chen, 1995; Hinneburg *et al*., 2006). In this assay, the yellow
colour of the test solution changes to various shades of green and blue, depending on the reducing power of each compound. Presence of reducers causes the conversion of the Fe$^{3+}$/ferricyanide complex to the ferrous form which may serve as a significant indicator of its antioxidant capacity (Yildirim et al., 2000; Amarowicz et al., 2004). The existence of reductones is the key of the reducing power and the reductones exhibit their antioxidant activities through the action of breaking the free radical chain by donating a hydrogen atom (Singh and Ranjini, 2004). The reduction of the Fe$^{3+}$/ferricyanide complex to the ferrous form occurs due to the presence of reductants in the solution (Yen et al., 1993; Siddhuraju et al., 2002). Antioxidant components and their activity are highly dependent on extracting solvent and concentration of solvent, but they also vary within the samples. Many researchers have reported the relationship between phenolic content and antioxidant activity. In some studies, they found a correlation between the phenolic content and antioxidant activity (Velioglu et al., 1998; Yen et al., 1993; Kahkonen et al., 1999; Siddhuraju et al., 2002).

In the present study, the higher absorption at higher concentration indicates the strong reducing power potential of the extracts. It is suggested that the extracts have high redox potentials and can act as reducing agents. Several reports have conclusively shown close relationship between total phenolic content and antioxidative activity of the plant extracts (Deighton et al., 2000; Vinson et al., 1998; Velioglu et al., 1998). Since the chemical composition and structures of active extract components are important factors governing the efficacy of natural antioxidants, the antioxidant activity of an extract could not be explained on the basis of their phenolic content, which also needs their characterization (Heinonen et al., 1998). For instance, it has been reported that phenolic compounds with ortho and para dihydroxylation or a hydroxyl and a methoxy group are more effective than simple phenols (Frankel et al.,
Polyphenolic compounds such as flavonoids, phenolic acids and tannins are considered to be the major contributors to the antioxidant activity of fruits and vegetables of medicinal plants. Phenol and phenolic compounds such as flavonoids have been shown to possess significant antioxidant activities and their effects on human nutrition and health are considerable (Kessler et al., 2003). However, synergistic or additive actions of the phenolics present in the extracts cannot be ruled out. This is the first report that envisages the antioxidant activities of different solvent extracts of whole plants of *A. travancorica* and *S. tinnevelliensis*. Hence, the whole plants of *A. travancorica* and *S. tinnevelliensis* could be a good source of antioxidant.

**Anticancer activity**

Cancer is a disease characterized by uncontrolled cellular growth, local tissue invasion and distant metastases and the free radicals have been implicated in carcinogenesis (Gustavo and John, 2003). Supportive to this, many plant extracts containing antioxidant principles have been reported to possess antitumour activity. A large number of plants possessing anticancer properties have been documented (Jasmine et al., 2008; Abeeu and Abeeu, 1979; Radha et al., 2008; Dauod et al., 2004; Rajkapoor et al., 2004; Durairaj et al., 2009). The present study was carried out to evaluate the antitumour effect of the whole plant ethanol extracts of *A. travancorica* and *S. tinnevelliensis* in DAL-bearing mice. The animals treated with the whole plant ethanol extracts of *A. travancorica* and *S. tinnevelliensis*, at 200 mg/kg and 400 mg/kg body weight doses, significantly inhibited the tumour volume, packed cell volume, tumour cell count and brought back the haematological parameters to more or less normal levels.
In DAL-bearing mice, a regular and rapid increase in ascites tumour volume was noted. Ascitic fluid is the direct nutritional source for tumour cells and a rapid increase in ascitic fluid with tumour growth would be a mean to meet the nutritional requirement of tumour cells (Clarkson and Burchenal, 1965). Treatment with the whole plant ethanol extracts of *A. travancorica* and *S. tinnevelliensis* increased the percentage of trypan blue positive stained dead cells in tumour bearing mice. The reliable criterion for judging the value of any anticancer drug is the prolongation of the life span of animals (Hogland, 1982). The whole plant ethanol extracts of *A. travancorica* and *S. tinnevelliensis* decreased the ascitic fluid volume, viable cell count and increased the percentage of life span. It may be concluded that the whole plant ethanol extracts of *A. travancorica* and *S. tinnevelliensis*, by decreasing the nutritional fluid volume and arresting the tumour growth, can increase life span of DAL-bearing mice. Usually, the major problems that are being encountered in cancer chemotherapy are of myelosuppression and anaemia (Price and Greenfield, 1958; Hogland 1982; Rajeshwari *et al.*, 2005). The anaemia encountered in tumour bearing mice is mainly due to reduction in RBC or haemoglobin percentage and this may occur either due to iron deficiency or due to haemolytic or myelopathic conditions (Rajeshwari *et al.*, 2005; Sarada *et al.*, 2012 b). Repeated treatment with the whole plant ethanol extracts of *A. travancorica* and *S. tinnevelliensis* can reverse the changes in haematological parameters such as haemoglobin content, RBC and WBC counts near to normal levels. This indicates the protective action of the whole plant ethanol extracts of *A. travancorica* and *S. tinnevelliensis* on the haematopoietic system.

Plant derived compounds have played an important role in the development of several clinically useful anticancer agents (Cragg and Newman, 2003). GC-MS analysis of the whole plant ethanol extract *A. travancorica* confirmed the presence of
Ethyl iso-allochate, Phytol, Trans Farnesol, Stigmasterol and Levo-á-Elemene and the whole plant ethanol extract of *S. tinnevelliensis* showed the presence of Ethyl iso-allochate and Stigmasterol.

**Immunomodulatory and antitumour activity of *A. travancorica***

The relation between immune state and the occurrence, growth and decline of tumour is one of the essential problems in tumour immunology. Various biological response modifiers such as natural products having biological activity to enhance host defense system have been considered as a useful tool to inhibit tumour growth in cancer immunotherapy (Suto *et al*., 1994; Yoo *et al*., 1994). Immunity has been shown to be suppressed in cancer. Chemotherapy and radiation therapy, useful in cancer treatment, were found to deteriorate the immunity. In many cases, biological response modifiers activate immune-related cells, including NK cells, lymphokine cells and macrophages, to control cancer growth (Yoon *et al*., 1998; Sakamaki *et al*., 1992). Their clinical applications are to boost the body’s general vitality or to treat a debilitating condition. Thus, the traditionally used natural resources to potentiate immune system and to prevent tumour growth without direct cytotoxicity for tumour cells may be important for cancer therapy.

In the present study, the whole plant ethanol extract of *A. travancorica* (ATW) exerts *in vivo* immunomodulatory activities on S180-bearing mice, in a dose-dependent but nonlinear manner. Generally, the assessment of immunomodulatory activity against tumour was carried out by testing the humoral, cellular and nonspecific immune response to the antigenic challenge by sheep RBCs, lymphocyte proliferation and NK cell cytotoxicity and by macrophages function tests (Fulzele *et al*., 2003; Han *et al*., 1998).
The humoral defence via antibody response is mediated by B cells, while other immune cells are involved in antigen processing and immunization. The antigen-antibody complex can counteract toxin and defend the infection induced by pathogen. Cell-mediated immune defence is mediated specifically by T cells including cytotoxic T cells and by the activation of natural killer cells. They can kill tumours and produce many lymphocyte factors consisting of macrophage mobile factor, lymphotoxin, transfer factor and interferon, which can enhance macrophage and play an important role in host defence mechanism against tumours by killing them (Kang et al., 2001) and producing effector molecules such as nitric oxide and TNF-α (Hirazumi and Furusawa, 1999) which have been recognized for their cytostasis and/or cytotoxic properties against tumour cells (Keller et al., 1990). In the present study, ATW extract showed stimulatory effect on overall immune functioning in S180-bearing mice. The effect was significant in the enhancement of lymphocyte proliferations, NK cells cytotoxicity, macrophages function and the secretion of antibody, which were responsible for the inhibition of the cancer and metastasis in many cases (Yoon et al., 1998; Saiki, 2000). These observations suggest that ATW induced antitumour effects were at least partially indirect and were associated with modulation of immune functions.

The reliable criteria for judging the value of any anticancer drug are prolongation of lifespan and decrease of WBC from blood (Kala et al., 2011; Sangameswaran et al., 2012). The ATW treated animals, at 300 mg/kg body weight dose, showed a significant (p<0.05) enhancement in the bone marrow cellularity when compared to S180-tumour bearing mice indicating the simulative action of the ATW extract on the haematopoietic system. Moreover, there was an increase in the number of β-esterase positive bone marrow cells, indicating that treatment with ATW extract could also enhance the differentiation of stem cells. The plant extract was found to
increase the circulating antibody titre and antibody forming cells indicating its stimulatory effect on the humoral arm of immune system. Moreover, the plant extract was found to reduce the weight of spleen and thymus indicating its positive role in the production of immune cells in drug treated S180-tumour bearing mice.

The above discussions clearly showed that the whole plant ethanol extract of *A. travancorica* possessed immunomodulatory and antitumour activities on S180-tumour bearing mice. It can be concluded that *A. travancorica* may contain biologically active compounds with immunomodulatory and antitumour properties. However, a detailed analysis of the plant extract is needed to determine the nature and phytochemistry of biologically active compounds in the extract.

**Antidiabetic activity**

Diabetes mellitus is a very common and prevalent disease affecting the citizens of both developed and developing countries. It is estimated that 25% of the world population is affected by this disease. Diabetes mellitus is caused by the abnormality of carbohydrate metabolism which is linked to low blood insulin level or insensitivity of target organs to insulin (Maiti *et al*., 2004). Despite considerable progress has been achieved in the treatment of diabetes, by oral hypoglycaemic agents, search for newer drugs continues because the existing synthetic drugs have several limitations. The herbal drugs with antidiabetic activity are yet to be commercially formulated as modern medicines, even though they have been acclaimed for their therapeutic properties in the traditional systems of medicine (Wadkar *et al*., 2008). Type 2 diabetes usually occurs in obese individuals and is associated with hypertension and dyslipidemia. Thus, the treatment aims to reduce insulin resistance and to stimulate insulin secretion.

Diabetes is a metabolic disorder where in human body does not produce or
properly use insulin, a hormone that is required to convert sugar, starch, and other food into energy. Diabetes mellitus is characterized by the constant high levels of blood glucose (sugar). Human body has to maintain the blood glucose levels at a very narrow range which is done with insulin and glucagon. The function of glucagon is causing the liver to release glucose from its cells into the blood for the production of energy. Type 1 Diabetes leads to inability to release insulin resulting in low rates of glucose uptake into muscles and adipose tissue (Lehninger and Nelson, 2010). Traditional medicine (herbal) is used for treatment of diabetes in developing countries where the cost of conventional medicines is a burden to the population (Saravanan and Pari, 2008). Despite the introduction of hypoglycaemic agents from natural and synthetic sources, diabetes and its secondary complications continue to be a major medical problem. Many indigenous Indian medicinal plants have been found to be useful to manage diabetes successfully. One of the great advantages of medicinal plants is that these are readily available and have very low side effects. Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. The ethnomeddicinal information reports about 800 plants that may possess antidiabetic potential (Alarcon-Aguilera et al., 1998). Several herbs have shown antidiabetic activity when assessed using presently available experimental techniques (Jafri et al., 2000).

Diabetes mellitus causes disturbances in the uptake of glucose as well as glucose metabolism. Alloxan induced hyperglycaemia has been described as a useful experimental model to study the activity of hypoglycaemic agents (Junod et al., 1964). The whole plant ethanol extracts of A. travancorica and S. tinneveliensis, at 200 mg/kg and 400 mg/kg body weight dose, produced no significant hypoglycaemic effect in normal rats. But, the whole plant extracts of A. travancorica and
S. tinneveliensis (200 mg/kg and 400 mg/kg body weight) significantly decreased the blood glucose in alloxan induced diabetic rats. Thus, the extracts of the experimental plants enhanced glucose utilization.

The antihyperglycemic effect of whole plants of A. travancorica and S. tinneveliensis might be due to the potentiating insulin from existing β-cells of the islets of langerhans. The blood glucose lowering effect can be compared with that of glibenclamide, the standard antidiabetic drug, which has been used for many years to treat diabetes and to stimulate insulin secretion from pancreatic β-cells (Tian et al., 1998).

Diabetes mellitus is one of the common metabolic disorders with micro-and macrovascular complications that results in significant morbidity and mortality. It is considered as one of the five leading causes of death in the world (Vats et al., 2004; Kumar et al., 2006). In modern medicine, no satisfactory effective therapy is still available to cure diabetes mellitus (Sumana and Suryawashi, 2001). There is increasing demands by patients to use natural products with antidiabetic activity due to side effects associated with the use of insulin and oral hypoglycaemic agents (Holman and Turner, 1991; Kameswrrarao et al., 1997; Kameswara Rao et al., 2001). There are numerous traditional medicinal plants reported to have hypoglycaemic properties such as Allium sativum (Garlic), Azadirachta indica (Neem), Vinca rosea (Nayantara), Trigonella foenum-graceum (Fenugreek), Momordica charantia (Bitter ground) and Ocimum santum (Tulsi).

Diabetes mellitus is also associated with long term complications, including retinopathy, nephropathy, neuropathy and angiopathy and several others (Krishova et al., 2008). Alloxan, a beta cytotoxic, induces chemical diabetes (Alloxan diabetes) in a wide variety of animal species by damaging the insulin secreting pancreatic
β-cells, resulting in a decrease in endogenous insulin release, which paves the way for the decreased utilization of glucose by the tissues (Bierman et al., 1975; Omamoto et al., 1981; Baynes, 1991; Saravanan and Pari, 2005; Gurusamy et al., 2008). The prevention of diabetes is an urgent worldwide health concern. The period preceding the onset of Type 2 diabetes is typically characterized by obesity and insulin resistance induced by over reacting and physical inactivity.

The whole plant ethanol extracts of *A. travancorica* and *S. tinneveliensis* were administered to alloxan induced diabetic rats (Groups III, IV, V and VI). The results, based on biochemical parameters, were compared with normal control rats (Group I), diabetic control (Group II) and the positive control rats treated with glibenclamide (Group VII) after fourteen days of treatment. After the induction of diabetes with alloxan, the levels of glucose, insulin, lipid profiles, protein and antioxidant were restored to control levels with the administration of the known drug glibenclamide and plant extracts of *A. travancorica* and *S. tinneveliensis*. The results of the present study showed significant changes in biochemical parameters of the experimentally induced diabetes. Blood glucose, a serum insulin, urea and creatinine level of rats treated with the plant extracts and glibenclamide were compared with the control. When compared to the control rats, the drug treated rats showed a decreased level of blood glucose and an increased level of serum insulin level. The hypoglycaemic activity of the ethanol extract of *Butea monosperma* leaves was found to induce insulin release from pancreatic cells of diabetic rats (Sharma and Garg, 2009). Ahmed et al. (1991) fed the ethyl acetate soluble fraction of an absolute ethanol extract of *Pterocarpus marsupium*, which significantly lowered blood sugar level with corresponding increase in insulin level in alloxan induced diabetic rats. It is evident from this study that there is an increase in insulin level in diabetic rats treated with the plant extracts.
Many plants have been studied for their hypoglycaemic and insulin release stimulatory effects (Morrison et al., 1985; Hikino et al., 1989; Ivorra et al., 1989; Al-Hader et al., 1994).

Extensive research has been conducted in the last few decades on plants mentioned in ancient literature and used traditionally for antidiabetic activity. Grover et al. (2002) have reported 45 medicinal plants and their products that have been used in the Indian traditional system of medicine and shown experimental or clinical antidiabetic activity. The most effective and commonly used antidiabetic plants include Allium cepa, A. sativum, Aloe vera, Gymnema sylvestre, Syzygium cuminii, Ficus benghalensis, Rubia cordifolia and Tinospora cordifolia (Ziyyat et al., 1997; Grover et al., 2002; Mohana Rao et al., 2005).

A significant elevation in serum constituents, urea and creatinine were observed in alloxan induced diabetic control rats (Group II), when compared to normal control rats. The administration of alloxan induced diabetic rats with the whole plant ethanol extracts of A. travancorica and S. tinneveliensis orally, for fourteen days, reversed the increased levels of urea and creatinine to near normal. The administration of the standard antidiabetic drug, the glibenclamide also decreased the levels of urea and creatinine to more or less to the same extent like that of the plant extracts.

Alloxan is taken as an indication of an abnormal glomerular function where a single injection of cisplatin, at a dose of 5 mg/kg body weight in rabbits, caused a marked reduction in the glomerular filtration rates, which was accompanied by an increase in the serum creatinine level, indicating the induction of acute renal failure. It is confirmed that there is a significant increase in serum creatinine in albino rats, thirty days after alloxan administration. The present result showed that the treatment with the whole plant ethanol extracts of A. travancorica and S. tinneveliensis were
effective in preventing alloxan induced increase in serum creatinine level when compared to the control.

Glycosylated haemoglobin has been found to be increased over a long period of time in diabetes. During diabetes, the excess of glucose present in blood reacts with haemoglobin to form glycosylated haemoglobin (Alyassin et al., 1981). The rate of glycation is proportional to the concentration of blood glucose. In the present study, the diabetic rats had showed higher levels of HbA1C compared to those in normal rats. Treatment with the whole plant ethanol extracts of *A. travancorica* and *S. tinnevelliensis* showed a significance decrease in HbA1C levels in diabetic rats that could be due to an improvement in glycemic status.

It is very clear from the results presented in Table 27, a significant reduction in serum protein, albumin and globulin were observed in alloxan induced diabetic control rats (Group II), when compared to normal control (Group I) and glibenclamide treated rats (Group VII). With the administration of the whole plant ethanol extracts of *A. travancorica* and *S. tinnevelliensis* to the diabetic rats, the levels of protein, albumin and globulin were restored to normal. These results were in accordance with the effect of *Eugenia singampatiana* and *Polygala rosmarinifolia* in diabetic rats (Kala et al., 2012; Alagammal et al., 2012 d). The increased levels of serum protein, albumin and globulin, in alloxan induced diabetic rats, are presumed to be due to increased protein catabolism and gluconeogenesis during diabetes (Palanivel et al., 2001).

Alloxan had a profound effect on the activity of hepatic marker enzymes. The animals treated with alloxan developed hepatic damage which is evident from the increase in the enzyme activities. Pre-treatment with the whole plants of *A. travancorica* and *S. tinnevelliensis* and glibenclamide resulted in a decreased
transaminase activity, in alloxan treated rats. Chalasani et al. (2004) observed that the levels of serum AST and ALP increased as a result of metabolic changes in the liver due to administration of toxin, cirrhosis of the liver, hepatitis and liver cancer including diabetes. Similar elevated levels of serum SGPT and SGOT in alloxan induced diabetic rats were observed in the present study. It may be due to the leaking out of enzymes from the tissues and migrating into the circulation by the adverse effect of alloxan (Stanely et al., 1999). AST and ALP levels in serum were used as markers to assess the extent of liver damage in streptozotocin induced diabetic rats (Hwang et al., 2005).

This study revealed that the whole plant ethanol extracts of *A. travancorica* and *S. tinnevelliensis* regulated the activity of SGPT, SGOT and ALP in the liver of alloxan intoxicated rats. The effect of glibenclamide, on the recovery of hepatic enzyme activity in serum, was very similar to that of the earlier studies (Preethi and Kuttan, 2009; Maruthupandian et al., 2010).

The levels of serum lipid profiles, total cholesterol (TC), triglycerides (TG), HDL-C, LDL-C, VLDL-C and PL in control and experimental animals were investigated in the present study. When compared to normal rats, the alloxan induced diabetic rats showed a significantly increased serum lipid profiles except HDL-C. The glibenclamide and whole plant ethanol extracts of *A. travancorica* and *S. tinnevelliensis* treated rats showed a significant decrease in the content of lipid profiles comparing to diabetic control rats. Similarly, HDL-C level decreased in alloxan induced diabetic rats when compared to normal control. With the administration of the whole plant ethanol extracts of *A. travancorica* and *S. tinnevelliensis* and glibenclamide, to the diabetic rats, HDL-C level was found to be restored to normal. The present study reveals that the levels of serum lipid profiles are
usually raised in diabetic rats and such an elevation represents a risk factor for coronary heart diseases (Mironova et al., 2000). Lowering the serum lipid level through dietary or drug therapy seems to be associated with a decrease in the risk of cardio-vascular disease (Scott and Grundy, 1999).

During diabetes, there is an enhanced activity of the enzyme resulting in an increased lipolysis releasing more fatty acids into the circulation (Agarth et al., 1999). The increased fatty acid concentration also increases the \( \beta \)-oxidation of fatty acids, producing more acetyl Co-A and cholesterol during diabetes. In normal condition, insulin increases receptor-mediator removal of LDL-cholesterol and decreased activity of insulin, during diabetes, causes hypercholesterolemia. Hypercholesterolemia and hypertriglyceremia have been reported to occur in diabetic rats (Mironova et al., 2000). The increased concentration of free fatty acid may be due to lipid break-down and this may cause increased generation of NADPH-dependent microsomal lipid peroxidation. Phospholipids are increased in alloxan induced diabetic rats. Phospholipids are present in cell membrane and make up vast majority of the surface lipoprotein forming a lipid bilayer that acts as an interface with both polar plasma environment and non-polar lipoprotein of lipoprotein core (Cohn and Roth, 1996). Increased phospholipid level in tissues was reported by Venkateswaran et al. (2002) and Pari and Satheesh (2004) in streptozotocin induced diabetic rats. Administration with the whole plant ethanol extracts of \textit{A. travancorica} and \textit{S. tinneveliensis} and glibenclamide decreased the level of phospholipids.

The results of the present study showed increased lipid peroxidation (LPO) on serum, liver and kidney of alloxan induced diabetic rats. Earlier studies have confirmed that there is an increased lipid peroxidation in liver, kidney and brain of diabetic rats (Latha and Pari, 2003; Ananthan et al., 2004). This may be due to relatively high
concentration of early peroxidizable fatty acids contained by the tissues. In the present study, an increase in the level of LPO was found and this level was significantly reduced after the supplementation with the whole plant ethanol extracts of *A. travancorica, S. tinnevelliensis* and glibenclamide. This indicates that the whole plant extracts of *A. travancorica* and *S. tinnevelliensis* inhibit oxidative damage due to the antiperoxidative effect of ingredients present in them. This could be correlated with the previous studies of Pari and Latha (2002) on *Cassia auriculanta* flower, Prince and Menon (1998) and Prince *et al.* (2004) on *Syzigium cuminii*, Prince *et al.* (1999) on *Tinospora cordifolia* and Latha and Pari (2003b) on *Scoparia dulcis* indicating antiperoxidative and antihyperlipidaemic effects in diabetic animals. Apart from the regulation of carbohydrate metabolism, insulin also plays an important role in the lipid metabolism. Insulin is a potent inhibitor of lipolysis, since it inhibits the activity of hormone sensitive lipase in adipose tissue and suppresses the release of free fatty acids (Loci *et al.*, 1994).

The levels of superoxide dismutase (SOD), catalase (CAT), glutathione peroxide (GPx) and reduced glutathione (GSH) in the serum, liver and kidney of the control and experimental rats were studied. A highly significant reduction in the activity of scavenging mitochondrial enzymes is observed in alloxan induced rats. These adverse changes could be reversed to near normal with the treatment of whole plants of ethanol extracts of *A. travancorica, S. tinnevelliensis* and glibenclamide. The results were in accordance with the effect of *Polygala rosmarinifolia* (Nishanthini and Mohan, 2012 b). Mitochondria are the energy reservoirs of the cell. The damage inflicted in mitochondria would ultimately result in the reduction of energy production and thereby leading to cell death (Sohal and Dubey, 1994). Sub-cellular membrane, associated with thiol bearing enzymes, represents sensitive sites for detoxification.
causing perpetuation of cellular function (Kyu and Byung, 1997). Reactive oxygen species can themselves reduce the activities of antioxidant defence mechanism. It was observed from the present study that the whole plant ethanol extracts of *A. travancorica* and *S. tinnevelliensis* enhanced mitochondrial enzymatic antioxidant activity and suppressed lipid peroxidation.

Free radical reacts with lipids causing peroxidation, resulting in the release of products such as malondialdehyde, hydroperoxide and hydroxyl radicals. The plant extracts have the capacity to scavenge free radicals directly or interfering with generation of free radicals (Reddy and Lokesh, 1992; Dhuley *et al*., 1993). Thus, the inhibitory effects of these extracts on oxidative damage may be attributed to the suppression induced peroxidation (Selvendiran *et al*., 2004). It is well known that CAT, SOD and GPx play an important role, as protective enzymes, against free radical formation in tissues (Oberly and Buettner, 1974). Several investigators have reported that the reduced activities of CAT and SOD genes are induced by free radicals and also by certain humoral factors (Anderson *et al*., 1994; Slaga, 1995). The present study indicates the reduction in the activity of SOD, CAT, GPx, GR and GSH in alloxan induced diabetic rats (Group II). These results reveal the protective role of these plant extracts in decreasing lipid peroxidation and by normalizing antioxidant system.

It can be concluded that medicinal plants have been reported to possess antihyperglycemic activity. The preliminary investigation on the antidiabetic efficacy of whole plant ethanol extracts of *A. travancorica* and *S. tinnevelliensis* will be significant to proceed further, in this path, for the isolation of active principles responsible for antidiabetic activity.
Hepatoprotective activity

Liver is a vital organ of the body, weighing 1-1.5 kg and representing 1.5-2.5% of the lean body mass (Fauci et al., 1916). The major functions of liver are to produce and metabolize proteins, carbohydrates and fats, detoxification of metabolic wastes (eg. urea), ingested drugs and chemicals (Goldman and Ausiello, 2004). Now a days, liver diseases such as hepatitis, hepatic fibrosis, adenoma, cirrhosis and hepatocellular carcinoma have become the major causes of morbidity and mortality in human being (Hunter, 2002). Hepatotoxicity due to various drugs such as paracetamol, rifampicin, oral contraceptives, penicillamine, danazol (Hunter, 2002), lipid lowering drugs (Harbans and Sharma, 2011), methyldopa, estrogen, diclofenac, tamoxifen, sulfa drugs (Goldman and Ausiello, 2004), acetaminophen, alcohol, infections and autoimmune disorders (Adewusi and Afolayan, 2010) appears to be the most common causative factor for such diseases. About 20,000 deaths occur every year due to liver diseases worldwide. Hepatocellular carcinoma is one of the ten most common tumours in the world with over 2,50,000 new cases each year (Kshirsagar et al., 2011). The conventional medicine is quite limited in preventing or treating hepatic diseases except allopathic drugs which have side effects like insomnia, vomiting, fatigue, dry mouth, diarrhoea, constipation, dizziness, depression, anaemia, hair loss, impotency, confusion, fainting etc. This limitation of therapeutic options gives considerable interest to the search for active compounds from plants traditionally used for hepatobiliary diseases.

Drug induced liver disorders, occurred frequently, can be life threatening and mimic all forms of liver diseases (Watkins and Seef, 2006). CCl₄ produces an experimental damage that histologically resembles viral hepatitis. Toxicity begins with the change in endoplasmic reticulum, which results in the loss of metabolic
enzymes located in the intracellular structures (Recknagel, 1983). The toxic metabolite, CCl₄ radical is produced and further reacts with oxygen to give trichloromethyl peroxo radical. Cytochrome P₄₅₀ is the enzyme responsible for this conversion. This radical binds covalently to the macromolecule and causes peroxidative degradation of lipid membrane of the adipose tissue, which leads to leakage of serum marker enzymes. It is possible that hepatocellular damage occurs when the free radicals generation exceeds the cellular radicals scavenging capacity (Jadhav et al., 2010). Assessment of liver toxicity was done by measuring the marker enzymes such as SGOT, SGPT and ALP, which are originally present in high concentration in the cytoplasm. When there is hepatic injury, these enzymes leak into blood stream in conformity with extent of hepatotoxicity. The whole plant ethanol extracts of A. travancorica and S. tinnevelliensis, at 200 mg/kg and 400 mg/kg body weight doses, significantly restored the elevated levels of serum marker enzymes. The normalization of serum markers, by the whole plants of A. travancorica and S. tinnevelliensis, suggests that they are able to condition the hepatocytes so as to protect the membrane integrity against CCl₄ induced leakages of marker enzymes into the circulation. The above changes can be considered as the expression of the functional improvement of hepatocytes.

Protein metabolism is a major project of liver and a healthy functioning liver is required for the synthesis of the serum protein. Hypoproteinemia is a feature of liver damage due to significant fall in protein synthesis. The reduction in the serum albumin and globulin levels in CCl₄ intoxicated group might be due to liver damage. Hepatotoxicity impairs the synthetic function of the liver (David, 1999). Treatment with the whole plant ethanol extracts of A. travancorica and S. tinnevelliensis ameliorated the imbalance.
Serum bilirubin is one of the most sensitive tests employed in the diagnosis of hepatic diseases. Hyperbilirubinemia was observed due to excessive heme destruction and blockage of biliary tract. As a result of blockage of the biliary tract there is a mass inhibition of the conjugation reaction and release of unconjugated bilirubin from damaged and dead hepatocytes (Wolf et al., 1997). Administration with the whole plant extracts of *A. travancorica* and *S. tinnevelliensis* decrease the level of bilirubin and increased the level of protein suggesting that it offered protection.

γ-glutamyl transferase (GGT) is a microsomal enzyme, which is widely distributed in tissue including liver. The activity of serum γ-glutamyl transferase is generally elevated as a result of liver disease, since γ-glutamyl transferase is a hepatic microsomal enzyme. Serum γ-glutamyl transferase is most useful in the diagnosis of liver diseases. Change in γ-glutamyl transferase is parallel to those of amino transferases. The acute damage caused by CCl₄ increased the γ-glutamyl transferase level but, the same attains the normal after treatment with the ethanol extracts of *A. travancorica* and *S. tinnevelliensis* and it may be due to the antioxidant activity of the extracts.

The body has an effective mechanism to prevent and neutralize the free radicals’ induced damage. This is accomplished by a set of antioxidant enzymes such as glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase. When the balance between ROS production and antioxidant defence is lost, oxidative stress results, which through a series of events deregulate the cellular functions leading to various pathological conditions (Castro et al., 1974). Any compound, natural or synthetic, with antioxidant properties might contribute towards the partial or total alleviation of this type of damage.

Lipid peroxidation (LPO) has been postulated to the destructive process of
liver injury due to acetaminophen administration. In the present study, the elevation in the levels of end products of lipid peroxidation in the liver of the rat treated with CCl\textsubscript{4} was observed. The increase in melondialdehyde (MDA) levels in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defence mechanisms to prevent the formation of excessive free radicals. Treatment with the whole plant extracts of \textit{A. travancorica} and \textit{S. tinnevelliensis} significantly reversed these changes. Hence, it may be concluded that the mechanism of hepatoprotection, by the whole plant ethanol extracts of \textit{A. travancorica} and \textit{S. tinnevelliensis}, is due to the antioxidant effects of the plant drugs.

Glutathione (GSH), extensively found in cells, protects cells against electrophilic attacks provided by xenobiotics such as free radicals and peroxides. GSH deficiency leads to cellular damage in kidney, muscle, lung, jejunum, colon, liver, lymphocytes and brain (Orhan \textit{et al.}, 2007). The elevation of MDA level, which is one of the end products of lipid peroxidation in the liver tissue, and the reduction in hepatic GSH levels are important indicators of CCl\textsubscript{4} intoxicated rats. In this study, it is ascertained that MAD levels might have been suppressed, in the entire drug treated liver damaged rats when compared to CCl\textsubscript{4} intoxicated control group, and this might have prevented CCl\textsubscript{4} induced depletion of GSH.

Superoxide dismutase (SOD), a metallo-protein, is the most sensitive enzyme index in liver injury and one of the most important enzymes in the enzymatic antioxidant defence system. It scavenges the superoxide anion to from hydrogen peroxide and oxygen, hence diminishing the toxic effect caused by this radical (Cartis \textit{et al.}, 1972). In the present study, it was observed that the whole plant ethanol extracts of \textit{A. travancorica} and \textit{S. tinnevelliensis} significantly increased the SOD activity in CCl\textsubscript{4} intoxicated rats thereby diminished CCl\textsubscript{4} induced oxidative damage.
Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues and the highest activity is found in the red cells and in the liver. CAT decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals (Chance et al., 1952). Therefore, the reduction in the activity of these enzymes may result in the number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide.

Administration with the whole plant ethanol extracts of *A. travancorica* and *S. tinnevelliensis* increased the activities of CAT, in CCl$_4$ induced liver damage in rats, to prevent the accumulation of excessive free radical and protect the liver from CCl$_4$ intoxication. Glutathione peroxidase (GPx) is a seleno enzyme and it protects the cells from damage due to free radicals like hydrogen and lipid peroxides (Zaltzber et al., 1999). It catalyzes the reduction of hydroperoxides with reduced glutathione to form glutathione disulphide.

The results of this study demonstrate that the whole plant ethanol extracts of *A. travancorica* and *S. tinnevelliensis* have potent hepatoprotective action against CCl$_4$ induced hepatic damage in rats. Their mode in affording the hepatoprotective activity against CCl$_4$ induced liver damage may be due to cell membrane stabilization, hepatic cells regeneration and enhancement of antioxidant enzymes such as catalase, superoxide dismutase and glutathione peroxidase production. The heaptoprotective and antioxidant potentials of the extracts might be due to the presence of various phytochemical principles like flavonoids, alkaloids, phenolics and tannins present in the whole plants of *A. travancorica* and *S. tinnevelliensis*. The results of this study clearly prove that *A. travancorica* and *S. tinnevelliensis* have significant protection on CCl$_4$ induced hepatotoxicity.
Antifertility activity

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources (Cragg GM, Newman, 2001). The World Health Organization suggested that effective, locally available plants be used as substitutes for drugs. Since the population explosion is a leading cause of poverty and pollution in developing countries, they created a population control programme, which includes studies of traditional medical practices. Fertility control is an issue of global and national public health concern. Current methods of contraceptive result in an unacceptable rate of unintended pregnancies. Approximately 50% of all pregnancies are unintended at conception; 50% of those occur in the 94% of sexually active couples who report that it is because of using some method of contraception (Henshaw, 1998). The only male-specific contraceptive methods currently available are withdrawal, condoms, and vasectomy. As concerns regarding side effects and inconvenience of these existing methods prevent their universal acceptance (Beckman and Harvey, 1996; Moore et al., 1996), the development of additional methods of fertility control for males can provide tremendous social and public health benefits.

The results of the present study revealed a little change in the body weight of rats treated with the whole plant extracts of *A. travancorica* and *S. tinneveliensis*, at doses of 200 mg/kg and 400 mg/kg body weight, for fourteen days. The weight of testis and other accessory sex organs were decreased significantly during the experiment. Among the accessory sex organs, a significant weight reduction was noticed in the testis, caput (*p* < 0.001) and caudal epididymis (*p* < 0.01) segments and the weight reduction was dose dependent. Reduction in the weight of testis and other accessory sex organs might be due to low level of androgen, which was not.
enough to maintain the weight of gonads and accessories (Sharma and Jacob, 2001).

It is known that the accessory sex organs viz., epididymis and vas deferens are androgen dependent target organs and manifest differential sensibility to androgens for maintenance of their structure and function. It is also known that any change in circulating androgens would affect the internal micro-environment of epididymis and thereby lead to the alternation in sperm motility and metabolism (Khan and Awasthy, 2003).

In the present study, the rats treated with whole plant ethanol extracts of *A. travancorica* and *S. tinneveliensis* showed decreased sperm motility and sperm density in caudal and caput epididymal segments. Drastic effect on the nature of the sperms was observed, in the caput and caudal region, of rats’ treated with the ethanol extract of *A. travancorica* and *S. tinneveliensis*. Further, tail region of the sperm was much affected in all the treated groups (Groups II, III and IV) than the head regions. The development of normal and mature sperm is the key to optimum male fertility. The production of the sperm cells (spermatozoa) and testosterone in the testis are mainly regulated by the follicle stimulating hormone (FSH) and luteinizing hormone (LH), which are released from the anterior pituitary (Steinberger, 1971). FSH stimulates spermatogenesis in the sertoli cells, while LH stimulates the production of testosterone in the leydig cells of the testis (Kerr and Klester, 1975). Many studies on the testis of rat treated, with plant extracts, have also revealed the inhibitory activity on the proliferation of spermatogonia in mammals (Steinberger *et al.*, 1964; Mancini *et al.*, 1967; Krueger *et al.*, 1974). Spermatogenesis is therefore, a complicated process, covering proliferation of the spermatogonia, long-lasting process of the tissue meiosis and numerous changes in the spermatids during their preformation (Steinberger, 1971 and Kerr and Klester, 1975). The results of the
present study suggest that ethanol extracts of *A. travancorica* and *S. tinnevelliensis*, on continuous oral administration for fourteen days, may affect the normal function of the sertoli and leydig cells.

Sex cells can occur during the reproductive phase, mitotic division of the spermatogonia or during the maturation of the spermatozoa, thereby affecting the number and quality of the sperm cells produced in the testis. Among the ethanol extract treated groups, Group III and V rats (400 mg/kg body weight) produced a significant reduction in total sperm count and viable sperms. This may be as a result of the ability of the extract at the given dose, to either interfere with spermatogenetic process in the seminiferous tubules, epididymal functions or activities of testosterone on hypothalamic release factor and anterior pituitary secretion of gonadotropins which may result in alteration of spermatogenesis (Bowman and Rand, 1985; William, 2000). The presence of immature sperms was also observed in the experimental rats treated with ethanol extracts of *A. travancorica* and *S. tinnevelliensis*, at the dose of 400 mg/kg body weight. This observation suggested that the 400 mg/kg body weight dose level could affect the maturation of the spermatozoa in the male rats, which might also be a contributory factor to the decrease in the mean total sperm count. The data generated in the present study, by and large, confirm to those already reported and studied with various plant extracts (Njar et al., 1995; Raji and Bolarinwa, 1997; Parveen et al., 2002). The decrease in the caudal epididymal sperm count is a clear indication that ethanol extracts of *A. travancorica* and *S. tinnevelliensis* can affect one or more aspects of spermatogenesis as well as spermiogenesis. Though a direct effect of ethanol extracts of *A. travancorica* and *S. tinnevelliensis* on the cellular mechanisms of spermatogenesis cannot be concluded, it is likely that the impairment of the hormonal mechanisms concerned with the
regulation of spermatogenesis may be the underlying cause.

The various other sperm abnormalities, like sluggish motility, coiled tail and sperm maturation are also due to ethanol extract of *A. travancorica* and *S. tinneveliensis* toxicity. The hitherto unreported abnormal sperm morphology, coiled tail and malformed head could be attributed to both testicular and epididymis effects of *A. travancorica* and *S. tinneveliensis* extracts. Coiling of the sperm tail is usually the product of abnormal axoneme and/or the outer dense fibril. The outcome of the present study affirms the toxic effects of *A. travancorica* and *S. tinneveliensis* extracts, on male reproductive system, when applied as a therapeutic agent. Since male reproductive toxicology and male contraception are two sides of the same coin, the negative consequence of *A. travancorica* and *S. tinneveliensis* extracts on the sperm may be taken as an advantage for further study. By the treatment employed in the study, no toxic effect was produced in the liver and kidney. It is because the liver and kidney are neither directly involved on the development nor functioning of the male reproductive system / reproductive organs.

A significant decrease in the sperm density and motility was observed, in the present study, in the caudal epididymis of all the treated groups, which leads to proven impairment of fertility in all the treated groups. The results observed in this study also indicated that the treatment of male rats with the whole plant ethanol extracts of *A. travancorica* and *S. tinneveliensis* reduces the number of female’s impregnation. In addition, the number of implantations and the number of viable foetuses were also decreased. This decrease in viable foetuses may be due to the decrease in sperm motility and sperm density observed in this study. This may be due to the effect of the given plant extracts on the enzymes involved in the oxidative phosphorylation process.
The present study revealed a decrease in the level of testosterone. This observation was similar to the earlier findings of Udoh and Kehinde, (1999); Udoh and Ekipeyong (2001) and Udoh et al. (2005a). The reduction in the level of testosterone, observed in this investigation, could be probably due to the decrease in the levels of LH/FSH. Leydig cells secrete testosterone by the stimulatory effect of LH (Udoh and Udoh, 2005; Udoh et al., 2005b). In males, the reduction of testosterone level may impair spermatogenesis and cause male infertility. The study also revealed a dose dependent increase in the serum estrogen level. This increase might probably be due to the conversion of testosterone to estrogen (Carr and Blackwell, 1993; Chinoy and Padman, 1996).

Treatment with the ethanol extracts of *A. travancorica* and *S. tinnevelliensis* (200 mg/kg and 400 mg/kg body weight) was highly effective in producing reversible functional sterility. The drug treated male rats clearly indicated structural and functional alteration in testis, epididymis and seminal vesicle. Depletion of sperm count and sperm motility in the drug treated rats suggested the alteration in sperm production in the testis and maturation in the epididymis. Changes in both sperm count and motility resulted in a partial infertility. This resulted in abnormal sperm function which ultimately gave rise to complete male sterility. Among the plant based contraceptives, inhibition of male fertility after administration of natural substances has been related to decreased spermatozoa density (Watcho et al., 2001). For male contraception, it is not necessary to stop spermatogenesis, but it is enough to eliminate the fertilizing ability of the spermatozoa by causing changes in the morphology or in the function of the sperm (Dwivedi et al., 1990).

The antifertility activity of *A. travancorica* and *S. tinnevelliensis* has been attributed to the action of various steroidal saponins. Saponins are important mainly
because of their steroid structure. They are precursors for the hemi-synthesis of birth control pills (with progesterone and estrogens) as well as similar hormones and corticosteroids (Crabbe, 1979). Recently, many laboratories are engaged in developing male contraceptives from plants (US National Academy of Sciences, 1992). Plant products, as contraceptives, will be more acceptable for economic reasons in terms of self reliance and the possible practicability for a male pill approach in countries where population pressure is high. Recently, extensive efforts have been made to study the antifertility drugs from plants (Handelsman, 1994; Khan and Awasthy, 2003; Upadhyay et al., 1993). The present study showed that treatment with the extracts of A. travancorica and S. tinneveliensis produced marked alterations in the male reproductive organs. Further studies are needed to prove whether the alterations are reversible or permanent after cessation of treatment and for understanding the exact mechanism.

**Antiinflammatory activity**

Inflammation is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. The classical signs of acute inflammation are pain, heat, redness, swelling and loss of function. Inflammation is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process. Inflammation is not a synonym for infection, even in cases where inflammation is caused by infection. Although infection is caused by a microorganism, inflammation is one of the responses of the organism to the pathogen. However, inflammation is a stereotyped response, and therefore it is considered as a mechanism of innate immunity, as compared to adaptive immunity, which is specific for each pathogen (Ferrero-Miliani et al., 2007; Abbas and Lichtman, 2009).

Inflammation can be classified as either acute or chronic. Acute inflammation
is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system and various cells within the injured tissue. Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process (Cotran Kumar, 1998).

Many synthetic drugs were now available in market to treat inflammation and pain, leading to side effects. So, search for the herbal drugs of the utmost important is essential and there is a need for the production of novel herbal drugs.

The whole plant ethanol extract of *A. travancorica*, at 250 mg/kg and 500 mg/kg body weight doses, decreased the edema significantly ($p<0.001$) by 80.83% and 82.56% respectively, at 3rd h after administration, whereas the whole plant ethanol extract of *S. tinnevelliensis*, at 250 mg/kg and 500 mg/kg body weight doses, decreased the edema significantly ($p<0.001$) by 66.50% and 78.11% respectively (Table 41). This antiinflammatory effect of the selected plant extracts was comparable to the antiinflammatory effect of the standard allopathic drug indomethacin (82.71%). The present study revealed a dose dependent inhibition of paw edema that was induced by the treatment with ethanol extracts of whole plants of *A. travancorica* and *S. tinnevelliensis*.

Carrageenan-induced edema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The early phase (1 to 2 h) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged surrounding tissues. The late
phase (3 h) is sustained by prostaglandin release and is mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages (Chavan et al., 2011; Olivera de Melo et al., 2006). Prostaglandin-E2, a powerful vasodilator, synergizes with other inflammatory vasodilators such as histamine and bradykinin and contributes to redness and increased blood flow in the areas of acute inflammation. The significant ($p<0.001$) suppressive activity of the whole plant ethanol extracts of *A. travancorica* and *S. tinnevelliensis*, in late phase, shows their potent antiinflammatory effect. This result is quite similar to the one observed for indomethacin (82.71%), at 10 mg/kg body weight concentration. Therefore, it is suggested that the mechanism of action of the extract may be related to histamine and prostaglandin synthesis inhibition. Further studies can be carried out to isolate and characterize antiinflammatory chemical constituents present in the ethanol extract.

**Immunomodulatory activity**

Immunomodulatory agents of plant and animal origin enhance the immune responsiveness of an organism against a pathogen by activating the immune system. However these agents and the polyhedral formulations should be subjected to systematic studies to substantiate the therapeutic claims made with regard to their clinical utility.

The presence study showed an overall stimulatory effect of the whole plant ethanol extracts of *A. travancorica* and *S. tinnevelliensis* on the immune function in mice. Stimulatory effects were observed on both humoral and cellular immunity. In the HT test, the whole plant of extracts of *A. travancorica* and *S. tinnevelliensis* showed an increased response with all the tested doses, but this increase was only significant at 400 mg/kg body weight dose. This activity could be due to the presence of flavonoids
or coumarins, which can augment the humoral response by stimulating the macrophages and B-lymphocytes involved in antibody synthesis (Makare et al., 2001). The treatment with whole plant of ethanol extracts of *A. travancorica* and *S. tinnevelliensis* improved the haemagglutinin antibody titre reflecting an overall elevation of humor immune response. The DTH response which directly correlates with cell mediated immunity (CMI) was found to be the highest at the maximum dose (400 mg/kg) tested. The mechanism behind this elevated DTH during cell mediated immunity response could be due to sensitised T-lymphocytes. When challenged by the antigen, they are converted to lymphoblast and secrete a variety of molecules including proinflammatory lympokines, attracting more scavenger cells to the site of reaction (Mitra et al., 1999). The infiltrating cells are probably immobilized to promote defensive (inflammatory) reaction (Dash et al., 2006). Treatment with *A. travancorica* and *S. tinnevelliensis* extracts enhanced DTH reaction, which is reflected from the increased foot pad thickness, as compared to control group, suggesting heightened infiltration of macrophages to the inflammatory site.

Recent reports indicate that several types of flavonoids stimulate human peripheral blood leukocyte proliferation. They significantly increase the activity of helper T-cells, cytokines, interlukin-2, gama interferon and macrophages and are there by useful in the treatment of several diseases caused by immune dysfunction (Kawakita et al., 2005). Some reports have suggested that these compounds affect the health as immunostimulating agents i.e. directly enhancing the lymphocyte activation and / or secretion / recreation of multipoint cytokine IFN-r. Some of these constituents also possess antioxidant properties and they may induce the immunostimulant effect, as several antioxidants have been reported to possess immunomodulatory properties. (de la Fuente and Victor, 2000; Ruby et al. 1995; Devasagayam and Sainis, 2002). This study
may be supporting the possible role of *A. travancorica* and *S. tinnevelliensis* in assisting cell mediated immune response.

The ethanol extracts of *A. travancorica* and *S. tinnevelliensis* enhanced the production of WBC and SGPT, SGOT and ALP. Results of the present study also revealed that no significant difference was observed in other blood parameters. Findings of the present study establish that *A. travancorica* and *S. tinnevelliensis* also have appreciable immunostimulatory activity. Their reported immunomodulatory effects warrant further investigation for their use in the cases of clinical immunosuppression.

These investigations validate the use of whole plants of *A. travancorica* and *S. tinnevelliensis* as herbal drug and confirming their antioxidant, anticancer, antidiabetic, hepatoprotective, antifertility, antiinflammatory and immunomodulatory activities.