DISCUSSION
The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as phenols, saponins, steroids, tannins etc. The preliminary phytochemical screening of plant extracts will direct to characterize the plants qualitatively and lead to the discovery of bioactive constituents. The present study has revealed and supplemented the phytochemical properties of medicinal plants of Tamil Nadu. Recently, a number of plants have been reported to have antimicrobial properties across the world (Selvamaleeswaran et al., 2010; Haripriya et al., 2010; Johnson et al., 2010; 2010a; 2011). In the present investigation, A. lanata collected from Coimbatore, Tamil Nadu, India has been screened for phytochemical properties. The various phytochemical compounds detected are known to have beneficial importance in medicinal sciences. Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds etc. (Gordon and David, 2001). The medicinal properties of the plants depend on the combination of secondary products. Many naturally-occurring compounds found in plants have been shown to possess antimicrobial functions and could thus serve as a source of both traditional and orthodox medicine (Akinyemi et al., 2005). Plant derived natural products such as phenolics, tannins, steroids and saponins etc., have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and antitumor activity. Phenolics are anti-oxidative, anti-diabetic, anti-carcinogenic, anti-microbial, anti-allergic, anti-mutagenic and anti-inflammatory (Jang et al., 1997; Rauha et al., 2000; Fang et al., 2003; Perez, 2003; Mei et al., 2005; Padilla et al., 2005; Dos Santos et al., 2006). In the present study, the methanolic extracts of A. lanata showed the presence of phenolics in high concentrations. It suggests that A. lanata can be used as antioxidative, antidiabetic, anti-carcinogenic, anti-microbial, anti-allergic, anti-mutagenic and anti-inflammatory agent. The results of the present investigation confirm the pharmacological proper-
ties of *A. lanata*. Tannins are known to possess general antimicrobial and antioxidant activities (Riviere *et al.*, 2009). Recent reports show that the tannins may have potential value as cytotoxic and antineoplastic agents (URL, http://en.wikipedia.org/wiki/tannin). Tannin is present in the methanolic extracts of *A. lanata* and it has antimicrobial properties. Saponins are used in hypercholestrolaemia, hyperglycemia, antioxidant, anticancer, anti-inflammatory and weight loss, etc. It is also known to have anti-fungal properties (De-Lucca *et al.*, 2005) Saponins have been implicated as a bioactive antibacterial agent of plants (Mandal *et al.*, 2005). The results of antibacterial and anti-fungal activity against *Escherichia coli* and *Staphylococcus aureus* and *Aspergillus niger* confirms the saponins presence in the *A. lanata* plant. Steroids are known to be important for their cardiotonic activities and also possess insecticidal and antimicrobial properties. In the present investigation, presence of steroid is confirmed in *A. lanata* by the phytochemical screening. The results of phytochemical screening analysis confirmed the ethnobotanical applications of *A. lanata* leaves used as expectorant, antipyretic, anti-diabetic agents and for skin disease. The whole plant of *A. lanata* possesses antibacterial, hypoglycaemic antispasmodic and antiasthmatic properties.

6.1 Aminoacids

The basic components of living cells are proteins, with building block material, amino acids. The requirement of amino acids in essential quantities is well known as a means to increase the yield and overall quality of plants. About 20 important amino acids are involved in the process of each plant function. Studies have proved that amino acids can directly or indirectly influence the physiological activities of the plant. L - Histidine helps in proper ripening of fruits (http://www.servpro.com.my/Folio_intro.pdf). L - Alanine, L - Valine and L – Leucine are essential to improve the quality of the fruits (http://www.agrowchem.com/lang_english/_research_and_data/pdf/organics_Agriculture_Production_research.pdf).
L-Proline and Hydroxy Proline acts mainly on the hydric balance of the plant strengthening the cellular walls in such a way that they increase resistance to unfavourable climatic conditions (http://www.aopww.com/plant_science.html). L-Glutamic acid and L-Aspartic acid, by transamination give rise to the rest of the amino acids. L-methionine is precursor growth factors that stabilize the cell walls of the microbial flora (http://www.priyachem.com/effect.html). L-Proline helps in fertility of pollen. L-Lysine, L-Methionine, L-Glutamic acid is essential amino acids for pollination (http://www.servpro.com.my/Folio_intro.pdf).

L-Glycine and L-Glutamic acid are known to be very effective chelating agents (http://www.priyachem.com/effect.html). L-Methionine is precursor of ethylene and of growth factors such as Espermine and Espermidine, which are synthesize from 5-Adenosyl Methionine. L-Arginine induces synthesis of flower and fruit related hormones. Amino acids have a chelating effect on micronutrients. L-Tryptophan is the precursor for auxin synthesis (http://www.priyachem.com/effect.html).

In addition, Lysine ensures adequate calcium absorption and maintains a proper nitrogen balance in adults; helps to form collagen (which makes up cartilage and connective tissue); aids in the production of antibodies which have the ability to fight cold sores and herpes outbreaks; lowers high serum triglyceride levels.

Arginine is considered "The Natural Viagra" by increasing the blood flow to the penis; retards the growth of tumors and cancer by enhancing the immune system; increases the size and activity of the thymus gland, reduces the effects of chronic alcohol toxicity; used in treating sterility in men by increasing sperm count; aids in weight loss because it facilitates an increase in muscle mass and a reduction of body fat; aids in stimulating the pancreas to release insulin (http://www.realtime.net/anr/aminoacid.html).
Aspartic acid increases stamina and is good for chronic fatigue and depression; rejuvenates cellular activity, cell formation and metabolism, which gives you a younger looking appearance; protects the liver by aiding the expulsion of ammonia; combines with other amino acids to form molecules that absorb toxins and remove them from the bloodstream; helps to facilitate the movement of certain minerals across the intestinal lining and into the blood and cells; aids the function of RNA and DNA, which are carriers of genetic information (http://www.realtime.net/anr/aminoacid.html).

Glutamic acid is an excitatory neurotransmitter for the central nervous system, the brain and spinal cord; important in the metabolism of sugars and fats; aids in the transportation of potassium into the spinal fluid; acts as fuel for the brain; helps correct personality disorders, and is used in the treatment of epilepsy, mental retardation, muscular dystrophy and ulcers. Glycine retards muscle degeneration; improves glycogen storage, thus freeing up glucose for energy needs; promotes a healthy prostate, central nervous system, and immune system; useful for repairing damaged tissue and promotes healing (http://www.realtime.net/anr/aminoacid.html).

Alanine plays a major role in the transfer of nitrogen from peripheral tissue to the liver; aids in the metabolism of glucose, a simple carbohydrate that the body uses for energy; guards against the buildup of toxic substances that are released into muscle cells when muscle protein is broken down quickly to meet the energy needs, such as what happens with aerobic exercise; strengthens the immune system by producing antibodies. Methionine is a powerful anti-oxidant and a good source of sulfur, which prevents disorders of the hair, skin, and nails; assists the breakdown of fats, thus helping to prevent a buildup of fat in the liver and arteries, which might obstruct blood flow to the brain, heart, and kidneys (http://www.realtime.net/anr/aminoacid.html).

Phenylalanine is used by the brain to produce norepinephrine, a chemical that transmits signals between nerve cells in the brain; promotes alertness and vitality; elevates mood; decreases
pain; aids memory and learning; used to treat arthritis, depression, menstrual cramps, migraines, obesity, Parkinson's disease, and schizophrenia. Tyrosine is important to overall metabolism; is a precursor of adrenaline, nor epinephrine, and dopamine, which regulate mood and stimulates metabolism and the nervous system; acts as a mood elevator, suppresses the appetite, and helps reduce body fat; aids in the production of melanin and in the functions of the adrenal, thyroid, and pituitary glands; has been used to help chronic fatigue, narcolepsy, anxiety, depression, low sex drive, allergies and headaches (http://www.realtime.net/anr/aminoacid.html).

Serine is needed for the proper metabolism of fats and fatty acids, the growth of muscle, and the maintenance of a healthy immune system; is a component of the protective myelin sheaths that cover nerve fibers; is important in RNA and DNA function and cell formation; aids in the production of immunoglobulins and antibodies. Valine is needed for muscle metabolism and coordination, tissue repair, and for the maintenance of proper nitrogen balance in the body; used as an energy source by muscle tissue; helpful in treating liver and gallbladder disease; promotes mental vigor and calm emotions (http://www.realtime.net/anr/aminoacid.html).

Threonine helps to maintain proper protein balance in the body; is important for the formation of collagen, elastin and tooth enamel; aids liver and Lipotropic function when combined with aspartic acid and methionine; prevents the buildup of fat in the liver; assists metabolism and assimilation. Isoleucine is needed for hemoglobin formation; stabilizes and regulates blood sugar and energy levels; is valuable to athletes because it aids in the healing and repair of muscle tissue, skin and bones; has been found to be deficient in people suffering from certain mental and physical disorders (http://www.realtime.net/anr/aminoacid.html).

All essential amino acids such as valine, threonine, methionine, isoleucine, leucine, lysine, phenylalanine, histidine, and arginine, which are not synthesized in children, were observed in the amino-acid composition. The results of HPTLC profile revealed the presence of lysine, argi-
nine (anti-cancer), aspartic acid (hepatoprotective), asparagine, glycine, glutamic acid, alanine, cystine, threonine, valine (liver and gallbladder disease), methionine (anti-oxidant), isoleucine (increase haemoglobin synthesis), serine (immunomodulatory), tyrosine and phenyl alanine in the vegetative and reproductive parts of *A. lanata* confirmed and supplemented the pharmacological application of the *A. lanata*. The combined components suggest that the extracts of *A. lanata* can be used as medicinal preparations to treat inflammatory diseases, catarrh of upper respiratory tracts, bronchial asthma (http://www.realtime.net/anr/aminoacid.html).

6.2 Alkaloids

Alkaloids are produced by a large variety of organisms, including bacteria, fungi, plants and animals especially by higher plants about 10 to 25% of those contain alkaloids and are part of the group of natural products (also called secondary metabolites). They often have pharmacological effects and are used as medications, as recreational drugs, or in entheogenic rituals (Rhoades and David, 1979). Depending on the type of plants, the maximum concentration is observed in the leaves (black henbane), fruits or seeds (Strychnine tree), root (*Rauwolfia serpentina*) or bark (cinchona) (Rhoades and David, 1979). Furthermore, different tissues of the same plants may contain different alkaloids (http://en.wikipedia.org/wiki/Alkaloid). Similar to the previous observations; in the present investigation, we also observed 29 different alkaloids in the different parts of *A. lanata*. Medicinal use of alkaloid plants has a long history, and thus when the first alkaloids were synthesized in the 19th century, they immediately found application in clinical practice (Rhoades and David, 1979). Many alkaloids are still used in medicine, usually in the form of salts, including the following: anti-arrhythmic, anti-cholinergic, anti-tumor, vasodilating, antihypertensive, cough medicine, anesthetic and antiprotozoal agent (Rhoades and David, 1979). The results of the present study confirms the folkloric usage and pharmacological studies of the medicinally important plant *A. lanata* and suggest that some of the plant extracts
possess compounds with bioactivity properties that can be used as active principles or agents in new drugs for the therapy of infectious diseases. A recent review shows that the HPTLC techniques can be used to rectify many qualitative and quantitative analytical problems in a wide range of fields including medicines, pharmaceutical, chemistry, biochemistry and toxicology (Rhoades and David, 1979).

6.3 Flavonoids

Flavonoids are ubiquitous in photosynthesising cells and therefore occur widely in the plant kingdom (Deshmukh et al., 2008). They are found in fruits, vegetables, nuts, seeds, stems and flowers and represent a common constituent of the human diet. The results of the present study confirm the flavonoids presence in the methanolic extract of the root, stem, leaf, flower and seed of *A. lanata*. They have been reported to possess many useful properties, such as anti-allergic activity, antioxidant activity etc., (AppiaKrishnan et al., 2009; Deshmukh et al., 2008; Manokaran et al., 2008; Shirwaikar et al., 2004). In traditional medicines, medicinal plants have contributed hugely to the traditional and western medicines through providing ingredients for drugs or having played central roles in the drug discovery. The evaluation of a crude drug is an integral part of establishing its correct identity. Before any crude drug can be included in herbal pharmacopoeia, pharmacognostical parameters and standards must be established. Chromatographic finger printing of phyto constituents can be used for the assessment of quality consistency and stability of herbal extracts or products by visible observation and comparison of the standardized fingerprint pattern (Rajkumar and Sinha, 2010).

6.4 Glycosides

In the present study, different types of glycoside presence are confirmed in the methanolic extracts of root, stem, leaves and reproductive parts (Flower and seed) of *A. lanata*. Glycosides comprise a very wide range of compounds that are of common and ubiquitous occur-
rence in almost all plants. Glycosides play important roles in our lives. Many plants store medically important chemicals in the form of inactive glycosides. The non-sugar portion contains the biochemically active properties of medical interest. Once the glycoside is split into its two components (sugar and non-sugar parts), the non-sugar component is free to exert its chemical effects on the body. For example, digitalis is a glycoside that causes the heart to contract (pump) more forcefully when ingested. A considerable number of glycosides are of great medicinal value, all of which are of natural origin. These pharmaceutically valuable glycosides contribute to almost every therapeutic class, cardiac drugs, laxatives, counter irritants, analgesics, renal disinfectants, anti-rheumatics, anti-inflammatory, anti-tuberculosis, expectorant and antispasmodic action (http://www.pua.edu.eg.,2010).

6.5 Saponins

Saponin is an important class of natural products that can be found primarily in roots, petals and foliage of many plants, as well as in some marine animals (Hostettmann and Marston, 1995). In the present study, saponin’s presence in the root, stem, leaves, flower and seeds of *A. lanata* was confirmed. Their structures are characterized by the presence of a steroid or triterpene group, referred to as the glycone, linked to one or more sugar molecules. The presence of both polar (sugar) and non-polar (steroid or triterpene) groups provides saponins with strong surface-active properties which then are responsible for many of its adverse and beneficial biological effects (Lilian, 1993).

In cultivated crops, triterpenoid saponins are generally predominant, while steroid saponins are common in plants used for their health-promoting properties (Akiyama *et al.*, 1972). Triterpenoid saponins have been detected in many legumes such as soyabees, beans, peas, lucerne, etc. and also in alliums, tea, spinach, sugar beet, quinoa, descending liquorice, sunflower, horse chestnut, and ginseng. Steroid saponins are found in oats, capsicum peppers, aubergine,
tomato seed, alliums, asparagus, yam, fenugreek, yucca and ginseng. *Yucca schidigera* is the most common commercial source of steroid saponins (Fenwick *et al.*, 1992). Saponins are generally known as non-volatile, surface active compounds that are widely distributed in nature, occurring primarily in the plant kingdom (Kerem *et al.*, 2002; Oleszek, 2002; Lasztity *et al.*, 1998). Saponins have a diverse range of properties, which include sweetness and bitterness (Hostettmann and Marston, 2005; Kitagawa and Licoriceroot, 2002; Grenby, 1991), foaming and emulsifying properties (Heng *et al.*, 2006), pharmacological and medicinal properties (Price, 1987), strong haemolytic properties, as well as antimicrobial, insecticidal, and molluscicidal activities (Attele *et al.*, 1999). Saponins have found wide applications in beverages and confectionery, as well as in cosmetics (Sparg *et al.*, 2004) and pharmaceutical products (Uematsu *et al.*, 2000). Saponins have a potential as pharmaceutical synthons and have been used in hormone synthesis (Hardman, 1975). Most saponins possess a variety of bioactivities (e.g., cardiac, antifungal, hemolytic activities and abilities to affect metabolism and biosynthesis); they are among the major effective components in nutraceutical products (Yoshiki *et al.*, 1998). The results of the present study and previous studies confirm the presence of saponins in root, stem, leaves, flower and seeds of *A. lanata*. They have confirmed the pharmacological activities of *A. lanata* and suggest that the plant can be used to control insects, molluscs etc. The biological and biochemical properties of saponins suggest the *A. lanata* possesses cardiac, antifungal, hemolytic activities and abilities to affect metabolism and biosynthesis.

### 6.6 Steroids

A large number of plants produce secondary metabolites such as alkaloids, flavanoids, phenols, terpenes, steroids and quinines that are used in pharmaceuticals, cosmetics and pesticide industries. Steroids (naturally occurring or synthetic) such as methylprednisolone, hydrocortisone, gluco-cortisteroids, corticosteroids, squalamine, oestrogens, androgens, are also used for the
treatment of various diseases such as allergic reactions, arthritis, some malignancies, and diseases resulting from hormone deficiencies or abnormal production. In addition, synthetic steroids (e.g., mifepristone) that mimic the action of progesterone are widely used as oral contraceptive agents. Other synthetic steroids (e.g., oxandrolone) are designed to mimic the stimulation of protein synthesis and muscle-building action of naturally occurring androgens. The results of the present study revealed that the 30 different types of steroids in the different parts of A. lanata.

6.7 Tannins

The tannin compounds are widely distributed in many species of plants, where they play a role in protection from predation, perhaps also as pesticides and in plant growth regulation. Tannins are mainly physically located in the vacuoles or surface wax of plants. These storage sites keep tannins active against plant predators, but also keep some tannin from affecting plant metabolism while the plant tissue is alive; it is only after cell break down and death that the tannins are active in metabolic effects. Tannins are found in leaf, bud, seed, root, and stem tissues. In the present study also, the presence of tannin in the root, stem, leaves, flower and seeds of A. lanata was documented. Previous biological and pharmacological studies on tannins showed that tannins possess anti-inflammatory, anti-viral, anti-bacterial, anti-parasitic, anti-oxidant, antihelmintic, anti-cancer, anti-septic, anti-diuretic properties (Harborne, 1973; Adebajo et al., 1983; Kurosoki and Nishi, 1983; Bajaj, 1988; Akiyama et al., 2001; Lu et al., 2004; Kolodziej and Kiderlen, 2005; Souza et al., 2006; Banso and Adeyemo, 2007). These results of the present study confirmed the presence of tannins in the stems, leaves, flowers, seeds and roots of A. lanata. The presence of tannin confirmed the pharmacological applications of A. lanata.

6.8 Terpenoids
Terpenoids constitute one of the largest families of natural products accounting for more than 40,000 individual compounds of both primary and secondary metabolisms. Most of them are of plant origin, and hundreds of new structures are reported every year (Sacchettini and Poulter, 1997; Penuelas and Munne, 2005; Withers and Keasling, 2007).

Currently, there is an increased interest in natural products with valuable and significant medicinal properties, such as terpenoids (hydrocarbon composition) and multiple \( \text{C}_5\text{H}_8 \). Plant terpenoids are used extensively for their aromatic qualities. They play a role in traditional herbal remedies and are under investigation for antibacterial, antineoplastic, and other pharmaceutical functions.

Sometimes terpenoids are added to proteins, e.g., to enhance their attachment to the cell membrane; this is known as isoprenylation (Terpenoid [http://en.wikipedia.org/wiki](http://en.wikipedia.org/wiki), 2012). These compounds and their derivatives also belong to other drugs such as validol, bromkamfora, menovasin, turpentine, etc. Basic research about terpenoids by various methods, including chromatography, was carried out in the early 60’s and late 70’s of the last century (Yermakov et al., 2010). A comprehensive review about terpenoids, their sources, structures, uses can be found (Heras et al., 2010). Although comparatively a few of these substances have been investigated in depth, they are thought to serve primarily in ecological roles, providing defense against enemies and acting as attractants for animals that disperse pollen or seeds or as inhibitors of germination and growth of neighbouring plants (Wink, 2010). After the discovery of the mevalonate (MVA) pathway in yeast and animals, it was assumed that IPP was synthesized from acetyl-CoA via MVA and then isomerized to DMAPP in all eukaryotes and some gram-positive prokaryotes (Penuelas and Munne, 2005; Withers and Keasling, 2007). Some exceptions have been described showing that interactions between the two biosynthetic pathways may exist (Dudareva et al., 2005). Before 1993, the MVA pathway was the only known source of terpenoids. After isotope-
labeling studies by Rohmer et al., 1993, it has been shown that there is an alternate pathway to terpenoids that does not originate from acetyl-CoA. The complete pathway has been finally elucidated in 2002 (Rohdich et al.). This alternative MVA-independent pathway has been named the methyl erythritol phosphate (MEP) pathway, which has been identified in both bacteria and plants (Penuelas and Munne, 2005; Withers and Keasling, 2007).

Moreover, there is evidence that a certain degree of crosstalk between the MVA and MEP pathways can occur, implying that these pathways are not completely autonomous (Laule et al., 2003). In addition to universal physiological, metabolic, and structural functions, many specific terpenoids function in various situations, including communication and defense. Members of the isoprenoid group also include industrially useful polymers (e.g., rubber and chicle) and agrochemicals (e.g., pyrethrins and azadirachtin). Terpenoids are a large and diverse class of naturally occurring organic chemicals found in all classes of living organisms. Plant terpenoids are used extensively for their aromatic qualities and play a role in traditional herbal remedies. They are currently under investigation by numerous groups for antitumor, antibiotic, anticancer, antineoplastic, antibacterial, anti-inflammatory and other therapeutic properties (Heras et al., 2010).

In recent times, in addition to morphological markers, anatomical, cytological, biochemical and molecular markers are used to classify the organisms. Chromatographic fingerprint has been suggested to be practical and comprehensive approach for identifying authenticity and evaluating the quality, consistency and the stability of raw herbal materials and herbal extracts (Koll et al., 2003). HPTLC is a valuable tool for reliable identification of the medicinally important plants (Rakesh et al., 2009; Sampathkumar and Ramakrishnan, 2011). In the present study also the HPTLC profiles were developed for the medicinally important plant A. lanata. The HPTLC method developed for the identification of A. lanata is simple, precise, specific, accurate, rapid and cost effective. Developed HPTLC chromatogram of methanolic extracts of vege-
Vegetative and reproductive parts of *A. lanata* may be treated as chromatographic finger prints and could be used efficiently for identification, and quality assessment of the plant.

Each and every metabolite has a specific role and functions in harmony with other metabolites within the organizational framework of cells in the defense mechanism of the plants. Metabolites often exhibit tissue or cell specificity. The results of the present study have shown the presence of different types of metabolites with different Rf values in different parts of *A. lanata*. The profile of root, stem, leaves and reproductive parts (flowers and seeds) showed uniqueness and variation in the metabolites composition between the parts of the same plant.

Considering the wide therapeutic applications and importance of *A. lanata*, an HPTLC method was developed to ensure the identity and quality of commercial samples. This shall help to obtain monograph of the medicinally active plant. The method was validated by determining linearity, peak purity and limit of detection and repeatability of alkaloids, flavonoids, tannins, steroids, glycosides, saponins, terpenoids and aminoacids from vegetative and reproductive parts extract of *A. lanata*. For developing analytical method, pure active chemical constituents should be isolated in further study and identification on the basis of reference standard shall be made. These profiles helps in setting in house standards of the medicinal plants used extensively by herbal manufacturers and used to distinguish the medicinally important plant from its adulterant. By isolating and identifying these bioactive compounds new drugs can be formulated to treat various diseases.

The crude powder subjected to FT-IR analysis is used for the identification of functional constituents present in *A. lanata*. The FT-IR analysis revealed the similarity and variation between the various parts of *A. lanata* based on the functional group presence and absorption spectrum. The results of FT-IR studies clearly show that although they show substantial overlap of
each absorption spectrum of various components, each band represents an overall overlap of some characteristic absorption peaks of functional groups in the samples.

Spectral differences are the objective reflection of componential differences. By using the macroscope fingerprint characters of FT-IR spectrum, the origin of different extracts were identified effectively, trace the constituents in the extracts, identify the medicinal materials true or false and even evaluate the qualities of medicinal materials. So, FT-IR spectrum reflecting objectively the panorama of chemical constituents in complex system is a most credible method to validate and identify the mix-substance systems such as traditional medicine and herbal medicine. The results of the present study spectrum also revealed that the functional constituents present in the crude powder of *A. lanata*. Many workers applied the FT-IR spectrum as a tool for differentiating, classifying and discriminating closely related plants (Ellis *et al.*, 2002; Liu *et al.*, 2006; Janakiraman *et al.*, 2011; Rebuffo *et al.*, 2006; Lamprell *et al.*, 2006; Lu *et al.*, 2004; Kim *et al.*, 2004; Hori and Sugiyama 2003; Gidman *et al.*, 2003; Dorado *et al.*, 2001; Goodacre *et al.*, 2000; Timmins *et al.*, 1998; Naumann *et al.*, 1991 and Helm *et al.*, 1991). The results of the present study also supplemented the previous observations and provided the similarity and variation in functional groups at parts (leaves, stem, root and flower) level also. Therefore, the present work on *A. lanata* has displayed novel phytochemical markers as useful analytical tool to check not only the quality of the powder but also to identify the medicinally important plant.

GC-MS analysis on methanolic extract of root, stem, leaves, flowers and seeds showed the existence of various compounds with different chemical structures. The prediction of the biological activities was confirmed with the previous observations which supplemented the traditional usage of *A. lanata*. (Hema *et al.*, 2011; Praveen Kumar *et al.*, 2010; Maruthupandian and Mohan, 2011). Thus, the present study suggests that methanolic extract is a potent therapeutic
agent. It paves the way for the development of several treatment regimens based on different extracts. Further work is needed to isolate and identify these bioactive compounds.

The present study results revealed the anti-bacterial and anti-fungal potential of *A. lanata*. The beneficial medicinal effects of plant materials typically result from the secondary metabolites present in the plant although, it is usually not attributed to a single compound but a combination of the metabolites. The medicinal actions of plants are distinctive to a particular plant species or group, reliable with the concept that the combination of secondary metabolites in a particular plant is taxonomically distinct (Wink *et al.*, 1999). The possibilities for the higher antibacterial activity of ethanolic extract are because of the the nature of biologically active compounds and stronger extraction capacity that may yield a greater number of active constituents (Ghosh *et al.*, 2008). *S. aureus* can cause a range of illnesses from minor skin infections to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), chest pain, bacteremia, and sepsis (Alam Sher, 2009).

The antimicrobial activity found in the plant extracts has been attributed to some of the secondary metabolites (Giwa *et al.*, 2010). In general, Gram-negative bacteria are more resistant to antibiotics than Gram-positive bacteria (Chowdhury *et al.*, 2004). The resistance of Gram negative bacteria towards antibacterial substances is related to the hydrophilic surface of their outer membrane which is rich in lipopolysaccharide molecules, presenting a barrier to the penetration of numerous antibiotic molecules. The membrane is also associated with the enzymes in the periplasmic space which are capable of breaking down the molecules introduced from outside (Shan *et al.*, 2007). However, the Gram positive bacteria do not possess such outer membrane and cell wall structures (Kalamba and Kanicka, 2003).

Natural plant product based antibacterial drug discovery has attained paramount importance as newly discovered drugs are likely to be effective against multi drug resistant mi-
The compounds present in *A. lanata* are known to be biologically active and therefore aid the antibacterial activities. The antimicrobial potency of *A. lanata* is believed to be due to the presence of steroids, phenolic compounds and flavonoids. The methanolic and ethyl acetate extracts of *A. lanata* show interesting antimicrobial activity (Chowdhury *et al.*, 2002). In the present study also, the methanolic extracts of *A. lanata* has shown antimicrobial activity with reference to the concentration of the extracts. Higher concentration (25 µg) of *A. lanata* extracts showed the maximum zone of inhibition against the selected pathogens.

Muthukumaran *et al.*, (2011) observed that the antimicrobial susceptibility reveals methanolic and aqueous extracts of *A. lanata* having promising antibacterial activity against gram positive bacteria (*B. subtilis* and *S. aureus*) as compared to gram negative bacteria (*E. coli* and *P. aerugenosa*). Similar to Muthukumaran *et al.*, (2011) observation in the present study also the methanolic extracts of *A. lanata* showed high degree of activity against *S. aureus*. In addition the results of the present study supplemented Muthukumaran’s observation.

Gurumurthy *et al.*, (2009) observed the maximum degree of activity in methanolic extract of *A. lanata*. They observed good activity in the methanolic extracts against *P. aeruginosa* *A. tumefaciens*, *S. aureus* and *B. subtilis*. The present study revealed that the methanolic extracts showed maximum activity against *E. coli*, *S. aureus* and *A. niger*. Very similar observation was made by Gurumurthy *et al.*, (2009). Britto *et al.*, (2011) observed the antibacterial activity of *A. lanata* methanolic and aqueous extracts against *Xanthomonas campestris* and *A. hydrophila*. Contrary to Nishanta Rajakaruna *et al.*, 2002 observations, the present study results showed moderate antibacterial activities against *S. aureus*, *P. aeruginosa* and *E. coli*.

It is interesting to note that even crude extracts of plants showed good activity against multidrug resistant strains where modern antibiotic therapy has limited effect. The results of the present study have given as most valuable information and also support the continued sustainable
use of *A. lanata* in traditional systems of medicine. Moreover, a continuous and progressing research is to be conducted to prove the biological ingredients and test the safety, efficiency and to determine the types of compounds responsible for the antibacterial effects of *A. lanata*.

The high content of polyphenolics compounds present in the ethanolic extracts of the selected species should be able to chelate transition metal because of the high charge density of the phenoxide group generated on deprotonation. The finding of the study established that the extracts could chelate irons and the values are substantial. The transition metal ion is capable of generating free radicals from peroxides by Fenton reactions and may be implicated in human cardiovascular diseases (Chung *et al.*, 2002).

Since Fe\(^{2+}\) has been shown to cause the production of oxyradicals and lipid peroxidation, minimizing Fe\(^{2+}\) concentration in Fenton reactions affords protection against oxidative damage. Chelating agents are effective as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ion (Gulcin *et al.*, 2007). The high contents of polyphenolic compounds present in the extracts should be able to chelate transition metals because of the high charge density of the phenoxide group generated on deprotonation (Lai and Lim, 2011). The findings of the present study have established that the methanolic extracts of different parts of *A. lanata* could chelate ions and the values are substantial.

The hydrogen/electron transfer from antioxidants to DPPH radical and Mo (VI) complex occur in the DPPH radical and phosphomolybdenum assays, respectively (Hyder *et al.*, 2001). The results of the present study demonstrated that the methanolic extracts of different parts of *A. lanata* possess significant antioxidant and free radical scavenging activities. Previously Muthukumaran *et al.*, (2011) Ragavendran *et al.*, (2012) and Arthi *et al.*, (2012) studied the antioxidant properties of *A. lanata* using the aerial parts. They reported that the methanolic and aqueous extracts of *A. lanata* have promising remarkable antilipid peroxidation property.
In the present study, the antioxidant activities of different parts of *A. lanata* was studied using DPPH⁺, ABTS⁺, FRAP, SO, NO and phosphomolybdenum reduction which revealed more scavenging properties. Hence, it is very clear that different parts of *A. lanata* is having widespread use and also extraordinary potential for the pharmaceuticals. These findings justify the increasing demand for *A. lanata* extracts for use as a dietary supplement or as a functional ingredient in nutraceutical and pharmaceutical products.

The extracts studied in this work showed significant lethality against brine shrimp, which has been successfully used as a simple biological test to guide the fractionation process of plant extracts in order to detect antitumour compounds (SudhaKesavan et al., 2011). This bioassay has good correlation with the human solid tumour cell lines (Anderson et al., 1991). These extracts can be regarded as a promising candidate for a plant-derived antitumour compound. Cytotoxic property of plant material is due to the presence of antitumor compounds (Ara et al., 1999). Many of the secondary metabolites produced by the medicinal plants are well known for their cytotoxic property. As noted by Harada and Kamei (1997) the extract from a red alga, *Amphiroa zonata* exhibited strong cytotoxicity to human leukemic cell line. El-Baroty et al., (2007) demonstrated the cytotoxic activities of powdered *Asparagopsis taxiformis* and its water extract on *Daphna magna*. Halogenated monoterpenes, isolated from *Plocamium cartilagineum* exhibited cytotoxic activity (De Ine’s et al., 2004). Bromophenols from *Polysiphonia lanosa* possesses in-vitro cytotoxic activities against DLD-1 cells (Nagwa et al., 2004).

Brine shrimp bioassay is considered as a rapid preliminary screening for the presence of biochemical activity and was used to determine the crude extract’s toxicity. The present study supports that brine shrimp bioassay as a reliable method for the assessment of bioactivity of the medicinal plants and lends support for their use in pharmacology. According to Meyer et al., (1982) extracts derived from the natural products which have LC50 ≤ 1.0 mg/mL are known to
possess toxic effects. In this study, the plotted graphs show that the LD50 value of the crude ex-
tract is 8.33 and 20 μg/mL for 96 h and respectively. Thus, these results prove that the
methanolic extracts of A. lanata are not toxic and show that the A. lanata root, stem, leaf, flower
and seed extract may be further explored for the development of natural product-based pharma-
ceutical products.

Brine shrimp lethality is a general bioassay, which is indicative of cytotoxicity, antibacte-
rial activities, pesticidal effects and various pharmacologic actions. The results indicate the abil-
ity of the plant extract to kill cancer cells in cell cultures, kill pests, and exert a wide range of
pharmacologic effects (MacLaughin et al., 1991). The presence of saponins, alkaloids and car-
diac glycosides may be responsible for lethality activities of the extracts by brine shrimps. The
presence of saponins, alkaloids and glycosides is confirmed through the phytochemical studies.
The brine shrimps lethality studies further supports the antibacterial activities of A. lanata on
some pathogenic organisms observed in this study.

Free radicals have been reported to stimulate platelet aggregation by interfering with
several key steps of platelet functions (Ambrosio, et al., 1997; Bakdash and Williams,
2008). The beneficial effects of antioxidants on the inhibition of platelet activation and aggrega-
tion have also been reported (Krotz, et al., 2004; Sobotková, et al., 2009). Furthermore, re-
searchers (Mary, et al., 2003; Lin and Hsieh, 2010; Anjana, et al., 2010) have linked the anti-
platelet aggregation activity of some plants with their antioxidant activity.

Data from various studies indicate that medicinal plants contain a wide variety of natural
antioxidants such as phenolics, flavonoids and tannins which possess more potent antioxidant
activity than common dietary plants. The anticoagulant or anti-platelet aggregation activity of
tannins has been demonstrated by various researchers (Dong et al., 1998; Mekhfi et al., 2006;
Tognolini et al., 2006; Kee et al., 2008; Kim and Choi, 2008). The phytochemical compo-
dition of the plants, particularly the presence of polyphenolic compounds is therefore worth noting.

Muthukumaran et al., (2011), Ragavendran et al., (2012) and Arthi et al., (2012) reported the anti-oxidant activity of A. lanata. The results of anti-platelet aggregation activity confirms very similar previous observations (Mary et al., 2003; Lin and Hsieh 2010; Anjana et al., 2010 and Mosa et al., 2011). Platelets readily aggregate in response to a variety of endogenous substances and they can initiate thrombus formation, leading to ischaemic diseases. In addition, the interactions between platelets and blood vessel walls are important in the development of thrombosis and cardiovascular diseases (Dinerman and Mehta 1990; Hirsh 1987). Therefore, inhibition of platelet function represents a promising approach for the prevention of thrombosis.

In Type -II diabetes, hyperglycemia is a condition characterized by an abnormal increase of blood glucose level in postprandial state (Marles and Farnsworth, 1995). Many natural resources have been investigated for the suppression of glucose production from carbohydrates in the gut or glucose absorption from the intestine thereby reducing post prandial hyperglycemia (Fernando et al., 1991). Therefore, the retardation and delay of carbohydrate absorption with a plant-based α-glucosidase inhibitor offers a prospective therapeutic approach for the management of Type -II diabetes mellitus (Mc Cue et al., 2005). Present study revealed the enzyme inhibitory effect of the four extracts of A.lanata, studied to find out the possible mechanism of its antidiabetic action. Among the four parts studied, root and flower extract showed satisfactory inhibitory effect on α-amylase and α-glucosidase enzymes respectively though higher inhibitory effect was shown on amylase compared to α-glucosidase activity.

These different inhibitory activities may be attributed to significant differences in phenolic content among the fractions. As reported previously, polyphenols or classes of polyphenols may have other beneficial effects, independent of their antioxidant capacities, by directly influ-
encing the activities of key enzymes. There have been reports that polyphenolic fractions from plants can cause insulin-like effects in glucose utilization (Mc Dougall et al., 2005). Many researchers have investigated polyphenolic extracts from a number of plants with α-glucosidase inhibitory activity (Matsui et al., 2001). Recently, Zhang et al., (2007) determined that polyphenol-rich extracts from *Ascophyllum* inhibit glucosidase and show promising antidiabetic effects in mouse models.

Literature has shown that advance glycated end-product (AGEs) is now known to be the source of free radicals in diabetes, thus aggravating the state of an increased oxidative stress in diabetes mellitus (Adisa et al., 2004), hindering its formation implies lower level of free radicals in diabetes, and reduced diabetic complication. It is concluded that the administration of *A. lanata* methanolic leaf extract inhibits glycosylation of hemoglobin as such and the formation of AGES may be inhibited by the plant extract. This observed effect might be attributed to the presence of bioactive compounds in the plant extract such as flavonoids, alkaloids, phenols and sterols.

Sometimes some reduced compounds like phenols can cause oxidation of hemoglobin producing metahemoglobin thus finally causing hemolysis (Bukowska et al., 2004). Measuring hemolytic activity is important as it is an indicator for cytotoxicities. The *in vitro* hemolysis test has also been employed by many different groups for the toxicological evaluation of different plants (Gandhi and Cherian, 2000). Mechanical stability of erythrocyte membrane is a good indicator of various *in vitro* cytotoxicities (Sharma and Sharma, 2001). Performing hemolytic assay is important to determine whether a drug possessing antioxidant and other bioactivities can be used in pharmacological applications (Kalaivani et al., 2010). In the present study, all the extracts showed less percentage of hemolytic activity and so can be considered as safe for the human erythrocytes.
SUMMARY AND CONCLUSION
In pharmacognosy, the phytochemical assessment is one of the important and vital tools for quality assessment, which includes preliminary phytochemical screening, chemoprofiling and marker compound analysis using modern analytical techniques such as fluorescence, UV-VIS, FT-IR, HPLC, HPTLC and GC-MS. The spectrometric and chromatographic screening methods could provide the needed preliminary observations to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations. The present study was intended to produce the phytochemical marker for the medicinally important plant *A. lanata* (L.) Juss. ex. Schultes. collected from Kadathur. In addition to this, various biological activities are also performed to evaluate the active principles.

Preliminary phytochemical screening was done by following the standard method described by Harborne (1998). In addition to this, FTIR, HPTLC and GC-MS analysis were also performed. Anti-microbial activities of the methanolic extracts of *A. lanata* were carried out by disc diffusion method against the pathogens viz., *Escherichia coli*, *Staphylococcus aureus* and *Aspergillus niger*. The antioxidant activity was evaluated by DPPH radical, green phosphomolybdenum and superoxide radical scavenging activity. Cytotoxicity of the extracts was determined against the brine shrimp *Artemia salina*. Alpha amylase inhibition activities were assayed according to the procedure portrayed by Jayasri *et al.*, (2009). Hemolytic effect of different methanolic extracts on human and rat erythrocytes was evaluated by using washed erythrocytes (RBCs).

Preliminary phytochemical screening of *A. lanata* revealed the presence of aminoacids, alkaloids, flavonoids, glycosides, phenols, saponins, steroids, sugar, tannins, terpenoids etc. in different crude extracts. HPTLC aminoacids profile showed various aminoacids at different Rf values viz., lysine (0.02), arginine (0.05), aspartic acid (0.21), asparagin (0.25), glutamine / alanine / cystine (0.35), valine (0.54), methionine and isoleucine (0.6) were commonly present in all the parts.
of the *A. lanata*. In general, more degree of alkaloids, flavonoids and glycosides diversity has been observed in the vegetative parts when compared to the reproductive part. The maximum number (12) of alkaloids has been observed in the leaves followed by the root (11). Highest number of flavonoids has been observed in the leaves followed by the root and the leaves. Maximum number (12) of glycosides has been observed in the roots followed by the stem (9).

HPTLC profile of saponins, steroids, tannins and terpenoids of *A. lanata* confirmed the diversified presence in more number in the vegetative parts. The maximum number (9) of saponins has been observed in the roots followed by the stem (8). Highest number (11) of steroids has been observed in the leaves followed by the root (10). Maximum number of tannins (10) has been observed in the flowers and seeds followed by the leaves (9). More number (11) of terpenoid was observed in the leaves followed by the stem (9).

The proposed HPTLC profile can be used for the identification of the medicinally important plants and for distinguishing it from its adulterant. The results of the present study, HPTLC profile (chemical profile) of the methanolic extracts of *A. lanata* also confirms and supplements the previous observation of *A. lanata*. The FTIR analysis results of *A. lanata* root, stem, leaves, flower and seeds validated the presence of amide, alcohols, phenols, amines, alkanes, ketones, primary amines, nitro compounds, alcohols, carboxylic acids, esters, ethers, alkyl halides and aliphatic amines. GC-MS analysis of *A. lanata* showed the existence of various compounds with different chemical structures. Methanolic root, stem and leaf extracts of *A. lanata* revealed the presence of 23 different compounds. The methanolic flower and seed extract of *A. lanata* showed the presence of 25 different compounds and their biological potentials were predicted using PASS. The prediction of the biological activities was confirmed with the previous observations which supplemented the traditional usage of *A. lanata*.
These spectroscopic profiles will act as pharmacognostic marker to distinguish its adulterants. The results of the present study confirms the folkloric usage and pharmacological studies of the medicinally important plant *A. lanata* suggesting that some of the plant extracts possess compounds with bioactivity properties that can be used as active principles or agents in new drugs for the therapy of infectious diseases.

The methanolic extract of *A. lanata* showed the antibacterial activity against the selected two pathogens viz., *E. coli* and *S. aureus*. The methanolic extracts of *A. lanata* exhibited the antifungal activity against the selected fungi, *A. niger* with the zone of inhibition 15 mm. The results showed considerable activity against the tested pathogens with the maximum inhibition in the highest concentration.

The total phenolic and flavonoid content of methanolic extracts of leaves, stems, roots, flowers and seeds of *A. lanata* were also estimated. The best free radical scavenging activity was exerted by leaves of *A. lanata* (IC$_{50}$ 8.83 mg/mL) which was lower than the free radical scavenging activity of standard ascorbic acid (8.9 μg / ml). Even though, all the parts of *A. lanata* exhibited good ABTS radical scavenging activity, stem (5mg/ml of ethanolic extracts) showed the highest activity (95.07%) whereas the methanolic extracts of flower, root and stem extracts exhibited comparable levels of activity i.e., 94.19%, 91.50 and 91.36% respectively. The methanolic leaves extracts of *A. lanata* registered higher FRAP antioxidant activity (84.42%) followed by flower, stem and root i.e., 66.92%, 57.33% and 19.83% respectively. The methanolic extracts of *A. lanata* flower (22.56 %) exhibited higher ability in scavenging superoxide anion radical, when compared to those of other parts of *A. lanata*. Active fraction of *A. lanata* methanolic extracts of flower, stem, root and leaves showed prominent result in brine shrimp cytotoxicity assay. The LD$_{50}$ value of the methanolic extracts was 8.33 μg/ml flower, stem and root of *A. lanata*. The
IC$_{50}$ values of the crude extracts with anti-platelet activity are 0.84 mg/ml and 0.86 mg/ml for methanolic extracts leaves and stem respectively.

The methanolic extracts of A. lanata leaves and flower showed more anti-platelet aggregation activity than the heparin. Among the four methanolic extracts studied (root, stem, leaves, flower and seeds) stem extract showed maximum alpha amylase inhibitory effect of about 24.39%, whereas root extract showed 21.06% of inhibition at a concentration of 1mg/ml. Among the four extracts tested methanolic extracts of A. lanata leaves showed higher alpha glucosidase inhibitory (22.51% at 20mg) effect when compared with the other extracts. Among the four methanolic extracts studied (root, stem, leaves, flower and seeds) the stem extract showed the maximum haemoglobin glycosylation of about 44.63%, followed by leaf extracts of A. lanata showing 38.35% of inhibition at a concentration of 500µg/ml. The highest percentage (10.3%) of human erythrocyte hemolysis was observed in 1 mg methanolic extract of A. lanata stem followed by leaves (7.79%) and flower (7.77%).

Thus, the present study suggests that methanolic extract is a potent therapeutic agent. It paves the way for the development of several treatment regimens based on the different extracts. Further work is needed to isolate and identify these bioactive compounds.