7. DISCUSSION

Indian traditional system of medicine is based on pragmatic facts of the observations and the experience over millennia. Traditional medicine, being a significant element in the cultural patrimony, still remains the main choice for a large majority of people for treating various diseases. The WHO has estimated that 80% of the population of the developing countries depends on traditional medicine mostly derived from plants for their primary health care needs. The demand of medicinal plants is increasing throughout the world. 90% of the drugs used in Indian systems of medicine and Homeopathy are plant based and collected from wild sources. In recent years traditional drugs are receiving great attention all over the world; therefore, a great emphasis has been laid to revive the heritage knowledge on the medicinal plants. Medicinal plants have been man’s oldest friends in his efforts at health and healing. Hence, in the present study, herbal plants curing various ailments were surveyed in the Mookanur hill at Semmanalli area of Dharmapuri District. From the surveyed plants, a sporadic plant *Tarenna asiatica* (L.) kuntze ex K.Schum. Used for skin diseases (Chopra *et al.*,1956) was selected and the leaves were used for investigating the anatomy, physico-chemical, pharmacological activities and conservation studies and the results are discussed.

7.1. Survey of Medicinal Plants

The survey indicated that the hill and the surrounding area have moderate assemblage of medicinal plants of low density to treat wide spectrum of human ailments. Southern tropical mixed dry deciduous forest of the hill is commercially more valuable due to the occurrence of *Santalum album* sporadically. The hill is retrogressed and often degraded to dry deciduous and scrub formations due to indiscriminate exploitation. It is evident from the study conducted in Mookanur hill at Semmanalli area, knowledge of medicinal plants is limited to a few traditional healers and elderly persons who are living in the surrounding areas. The study also points out that certain species of medicinal plants such as *Scilla indica* bulb and *Gloriosa superba* rhizome are being exploited by the local residents who are unaware of the importance of medicinal plants in the ecosystem. Younger generation simply opt for modern medicine. Only aged people who are not
influenced by urbanization are still practicing green therapy. Thus it becomes essential to
gather the available information on indigenous medicinal plants as remarked by Schultes
(1963) “Our challenge is to salvage some of the medico-botanical lore before it becomes
over entombed with the culture that gave it birth”

**Temperature, Rainfall and Relative Humidity**

Each species of plants has its own maximum and minimum temperature beyond
which its life activities cease. It is known that the temperature exceeding 45.9°C has
inimical effect on plant growth. In the study area, the highest temperature was 35.11°C
and 35.10°C in 2009 and 2010 respectively which never exceeded the critical limit and is
therefore not a limiting factor. So the plant growth is safe from this damaging effect.
Rainfall variation is the most important ecological factor in determining the distribution
of plants. The intensity, quantity and periodic distribution of rainfall are all important for
vegetations as reported by Mehra and Khan (1976). The study area had received the
mean annual rainfall of 69.1 and 76.6 mm respectively in 2009 and 2010. The thick
vegetative cover during the winter months indicates the availability of soil moisture
content sufficiently.

A greater part of the hill area received normal rainfall which causes the new flush
of herbs and is covered with mixed dry deciduous forests. This is in consonance with the
report of Mehra and Khan (1976). During January and February, though rains were
received but in small quantity, it had a far reaching effect in improving the moisture
conditions for the growth of plants especially herbs. The relative humidity was higher in
the winter months in both 2009 and 2010. Relative humidity was lower in summer. The
winter months were generally marked by high rainfall which made the hill and the
surrounding area a humid one which resulted in lush growth of plants in winter season.

**Soil**

Joffe (1949) reported that soil is a natural body of vegetation, mineral and organic
constituents. The red loamy and sandy loam soil with neutral to slightly alkaline pH in
the study area reflect the degree of soil fertility which enrich the plant growth.
Survey

Survey of medicinal plants curing various diseases were carried out by Vajravelu and Krishnan (1967), Apparanantham and Chelladurai (1986) in Dharmapuri District and reported more than 200 plant species, but in the present survey approximately 165 plants of medicinal value have been explored. Almost all parts of the plant from subterranean roots to flowers at top including latex of plants and entire plant are used to treat various diseases.

The survey recorded 154 Dicotyledons, 8 Monocotyledons and 3 ferns. Within the Dicotyledons, 74 species belong to Polypetalae, 54 species are Gamopetalae and 26 species are Monochlamydeae. Despite the fact that the area is environmentally degraded, moderate number of medicinal plants categorized in diverse genera (140) and families (55) were recorded. This demonstrates the presence of high taxonomic diversity of medicinal plants grown in the hill at Semmanalli area as well as the immense knowledge associated with the plants. Family-wise analysis of the drug indicates the predominance of Euphorbiaceae followed by Papilionaceae, Acanthaceae, Amarantaceae, Caesalpiniaceae and Mimosaceae. Loranthaceae is recorded as the least in position.

The results on the medicinal plants survey indicate the maximum representation of Angiosperms (162), there are 3 Pteridophytes and Gymnosperms in natural condition are totally absent. This reveals a favourable climatic and edaphic condition for Angiosperms. The family Euphorbiaceae accounted for the highest number of medicinal plants could probably be due to their high species. Viewing the habit, herbs (66 species) represent the predominant vegetation and the density of herbs is high during rainy days. Trees often do not grow very tall and the dry period shows maximum trees and shrubs of medicinal value.

More than half of the plant remedies were reported to have been obtained from herbs. This might indicate that the people have come to rely on herbs because they are relatively common in the area as compared to shrubs and trees. The reliance on herbal medicine might cause the disappearance of some medicinal herbs growing in the area and...
will add more plants to the list of the endangered plant species. The high usage of herbs as medicinal plants could also indicate their better abundance as compared to trees and shrubs. Studies conducted in other parts of India (Rajaram, 2004; Shukla, et al., 2001; Gupta, et al., 1999 and Jha, 2001) also indicated the common usage of herbs as sources of medicine. Among the different plant parts, the leaves were the most frequently sought part for the treatment of diseases as they contain more active principles. Similar findings were reported by Giday, 2001; Jain et al., 2009 and Muthu et al., 2006.

Concerning the medicinal plants for human use, many plants are directly used from the wild. 165 plant species, distributed across 55 families, are reported to be used against skin diseases, respiratory disorders, urinary infection, cancer, diabetes, constipation, diarrhoea, dysentery, liver disorders, scurvy, spasmodism, inflammation, snake bite, bone fracture, kidney stone, rheumatism, arthritis, fever, indigestion, intestinal worms, fertility, hypertension, elephantiasis, stomachache, headache and allergy.

The use of 62 medicinal plants against skin related ailments may be attributed to the high preponderance of these disorders. The plants cited below with their medicinal uses are reported for the first time and were not reported by earlier works. The leaf paste of Adiantum caudatum, Actiniopteris radiata and Cheilanthes mysurensis is applied externally for healing wounds. The information available on the utility of medicinal plants was compared with other literatures. Many facts relating to the use of plants coincide with the findings of the earlier works (Chopra et al., 1956 & Rajendran et al., 2006). Rajendran et al. (2006) reported that Adhatoda zeylanica, Tridax procumbens, Phyllanthus amarus, Cardiospermum halicacabum, Solanum nigrum and Vitex negundo are used to treat respectively asthma, wounds, jaundice, rheumatism, ulcer and headache.

These remedial measures are being practiced by the traditional healers of Semmanallli area. Geyid et al. (2005) have confirmed the usage of Ageratam conizoides against wounds. Giday (2001) report confirms the efficacy of the Achyranthes aspera leaf against boils and abscess. Singh et al. (2008) have confirmed the usage of Acacia nilotica, and Santalum album against skin diseases. Auti et al. (2004) report confirms the utilization of Acalypha indica, Tridax procumbens and Jatropha gossipifolia against skin
diseases. The investigation of Suresh and Kujur (2009) in Jharkand on medicinal plants confirmed the usage of Clitoria ternatea, Rauwolfia serpentina and Commelina benghalensis against constipation, blood pressure and leprosy respectively.

Many findings from Mookanur hill are identical and surprisingly similar with the above mentioned earlier researchers. Minor deviations of usages of the medicinal plants surveyed in the hill to treat various diseases are also reported by other researchers. Wondimu et al. (2007) reported that Carica papaya, Cissus quadrangularis, Dichrostachys cinerea, Melia azedarach and Xanthium strumarium are used to treat respectively wounds, toothache, snakebite, diarrhoea and fungal disease on skin. Ram and Saha (1998) reported that Achyranthes aspera, Aloe barbadensis and Aristolochia bracteata are used against dysentery. In the present study the above mentioned plants are used to cure boils, rheumatism and ulcer respectively. Das and Devi (2004) reported that Bodo tribe of Assam using Cissampelos pareira against Jaundice, but in the present study traditional healers of Semmanalli area using the Cissampelos pareira root for controlling diarrhoea. It is noteworthy that many wild edibles are widely used throughout the world by various tribes though they are less known or not known to urban or civilized population. This may be due to the non-availability or the bitter taste as in the leaves of Gynandropsis pentaphylla or sourness in Oxalis corniculata.

Among the plants surveyed, Amaranthus spinosus, Amaranthus viridis, Boerhaavia diffusa, Gynandropsis pentaphylla, Celosia argentea, Cassia tora, Commelina benghalensis and Solanum nigrum are widely eaten by rural population throughout India (AlQuran, 2009). In India culinary value of these herbs was reported by Jain and De (1966) from Purulia, West Bengal. Considering the fruits, Aegle marmelos, Lantana camara, Lantana indica, Phyllanthus emblica, Pithecolobium dulce, Zizyphus jujuba and Zizyphus oenoplia fruits are widely relished by the shepherds of the area.

A survey of active principles through literature for all these medicinal plants brings out the constituents of these plants which again support their pharmacological efficacy. Gathering pharmacological data on the known chemical constituents of a drug plant further proves the efficacy of particular drug.
Rauwolfia serpentina, Ricinus communis and Withania somnifera are the species that were reported by many researchers against the same ailment. The fact that medicinal plants are used for the same purpose by more than one community might indicate their pharmacological effectiveness. The majority of the remedies are applied in the form of juice, paste or decoction which is also a common practice in different parts of India and other countries (Das and Devi, 2004; Ram and Saha, 1998; Kadavul and Sujesh, 2005, AlQuran, 2009 and Giday, 2001). As the majority of remedies are prepared from fresh materials, it is much easier and quicker to prepare them in juice, paste or decoction form. Most remedies prepared from single plant species, are in agreement with the results of similar studies done by Giday, 2001; Jain et al., 2009 and Nandanakunjidam, 2004.

Wester and Yongvanit (1995) reported that literate people were found to be less knowledge on the type of medicinal plants as compared to illiterate people due to modernization. Similar condition was observed in the study area. The study revealed that men have better medicinal plant knowledge than women and this could probably be due to the reason that men are interested and preferred than women in the transfer of the knowledge. The study further indicated that younger people have less medicinal plant knowledge as compared to older ones. Results of many studies conducted in Ethiopia revealed similar findings (Gedif and Hahn, 2002 and Giday, 2001). Majority of the informants agreed that they kept their medicinal knowledge secret as reported by Gedif and Hahn (2002). The secrecy of medicinal plant knowledge is also a common practice in different parts of the country. Medicinal plants have been grouped from the view point of their conservation status as presently safe, sporadic, threatened and gregarious patches.

The status column shows that presently safe and sporadically occurring species form a good percentage of species followed by threatened and species in gregarious patches. Efforts should be taken to conserve the threatened species, as the hill and the surrounding area is under heavy biotic pressure due to grazing and browsing. Many species are constantly decreasing in this area. It therefore, seems appropriate to manage the grazing and browsing system. The fact that most of the remedies are only found in the wild poses a significant threat to their existence and the mass destruction of their habitats continues. The continued cutting of plants for different reasons has resulted in a scarcity
of some medicinal plants in the hill and the surrounding area (*Withania somnifera*, *Rauwolfia serpentina* and *Santalum album*).

Thus incidence of similar usage of plants from various parts of India and over the world invariably point out the therapeutic effectiveness of the above mentioned plants. This also proves, beyond doubt their efficacy and the wider acceptability of phytomedicines in Semmanalli which have no tribal linkages. So the next move on the right direction will be a thorough phytochemical screening and clinical trials of these plants for getting novel drugs.

### 7.2. Macro and Microscopical Studies on Leaf

The leaf macro and micro-characters of *Tarenna asiatica* can be used for distinguishing the species even when they are fragmented. The presence of crystals of calcium oxalate within the leaf tissues and nonglandular trichomes are pointers to medicinal lodgments of bioactive principles of the plants. Some useful diagnostic features for the species are their hypostomatic leaves, paracytic stomata and deposition of cell inclusions. Ogundipe and Wujek (2004) reported that these features have been used for taxonomic distinction and recognition in the angiosperm family. This is in line with the present findings. The presence or absence of crystals is one of the important characters for understanding the evolutionary relationships of plant species as reported by Franceschi and Horner (1980).

The results of these investigations could serve as a basis for proper identification, collection and investigation of the plant. The micro-morphological features of the leaf described, distinguishes it from other members of the genera. Any crude drug which is claimed to be *Tarenna asiatica* but whose characters significantly deviate from the accepted standard above would then be rejected as contaminated, adulterated or downright fake.

### 7.3. Physico-Chemical Analysis

#### 7.3.1. Organoleptic Characters of Leaf Powder

The organoleptic or sensory characters describe the colour, odour, taste and texture of the raw materials which are used for drug preparation, identification and adulteration.
Present organoleptic investigation of leaf powder exhibited a characteristic smell and bland taste. The fresh leaves are dark green in colour and upon drying and powdering, the leaves turned greenish brown. The organoleptic profile is one of the many diagnostic parameters in the proper identification of raw materials (Deshmukh and Beal, 1984).

7.3.2. Moisture Content

In the present study the leaf sample contains 54.12% moisture. Moisture content (loss on drying) determines the water drying off from the drug. Drug containing excess moisture will lead to the activation of enzymes and gives suitable condition for the proliferation of living microorganisms. Higher water content indicates the presence of larger amount of mucilage or starch and paves way for more chances of microbial degradation and if the value is not too high, it indicates less chances of microbial degradation (Trease and Evans, 1983).

7.3.3. Ash Content

The presence of ash in raw material is determined as total ash, acid insoluble and water soluble ash and sulphated ash. The determination of ash value is useful for detecting exhausted drugs and excess of sandy and earthy matter. The total ash usually consists of carbonates, phosphates and silicates of silica. Ash value determination is a good index of quality and is also helpful to some extent in the detection of adulteration. An increase in the ash value when compared to the standardized value is indicative of contamination or adulteration (Trease and Evans, 1983).

In the present study, the total ash content of the sample was 9.12%. Acid insoluble ash value can be used to determine the silica impurities admixed with the drug while collection or harvesting during rain. Water soluble ash value helps in determining the added mineral matter and helps in the interpolation of analysis of powdered drug for their quality. It is a good indicator of either previous extraction of the water soluble salts in the drug or incorrect preparation (Pruthi, 1980).
7.3.4. Extractive values and Successive solvent extraction

The determination of extractable matter refers to the amount of constituents in a given amount of raw material extracted with suitable solvents. Such extractive values provide an indication of the extraction of the polar and non-polar components present in the material. These values have significance in the evaluation of drugs (Miller, 1973). In the present study, extractive value analysis of the leaf sample of *Tarenna asiatica* revealed that the percentage of extractability was maximum in ethanol (19%) followed by water (14%) and acetone (12.12%). Extractive value profiles help in the detection of adulterants during the process of authentication of crude and raw drug materials. Earlier work had revealed the extractive value profiles in several other medicinal plants (Khatoon *et al.*, 2006).

7.3.5. Mineral Studies

Human body contains 29 different elements. Minerals constitute about 8% of body weight. These minerals are essential for a number of metabolic processes like blood coagulation, muscle contraction and enzyme action (Murugesh, 1998). Nitrogen is an essential constituent of protein and nucleic acids and many other organic molecules which play an important role in plant life. Being essential for the formation of protoplasm, which is predominantly proteinaceous, the deficiency of nitrogen inhibits cell division and cell enlargement. In the present study, the nitrogen content was maximum in the leaf sample. Similar reports were made by Sanchez (2005) in *Faramea occidentalis* and Prasad and Bisht (2011) in *Pavetta indica*. Phosphorus is an essential constituent of lipoprotein membranes of the cell nucleoproteins, many coenzymes and organic molecules. The presence of higher concentration of phosphorus may also helpful in the healing process of skin. The high concentration of calcium (77mg/100g) in the leaf sample is a positive sign to skeletal muscular problem. Calcium is necessary for neuromuscular mechanism (Chaudhari and Gokhale, 1991), formation and growth of bones and teeth (Murugesh, 1998). Deficiency of calcium leads to hypocalcaemia, rickets (Dandiya and Sharma, 1996), osteoporosis and parathyroid deficiency (Deb, 2004).
Magnesium was present in appreciable amount (110.5 mg/100g) in *Tarenna asiatica* leaf sample. Many enzymes would function efficiently in the presence of inorganic activators like Ca++, Mn, Mg and Cl. They actively take part in the enzyme substrate complex (Deb, 2004). Dietary supplements of Mg have been reported to be effective in treating depression. Magnesium which exerts neuromuscular irritability, is a cofactor for a number of enzymes and it is a constituent of bones, dental enamel and dentin (Murugesh, 1998).

Among the macro elements, less amount (40.1mg/100g) of sodium was recorded in the sample. Sodium maintains the osmotic pressure, irritability of muscle, permeability of the cell, heart beat and glucose absorption (Dandiya and Sharma, 1996).

The leaf sample contained 114.0 mg/100g potassium. Potassium plays a crucial role in physiological function like conduction of nerve impulses, cardiac function, maintenance of osmotic pressure, protein biosynthesis and muscular activity (Murugesh, 1998 and Deb, 2004). The minerals present at levels less than 0.05% are defined as micro minerals. The micro minerals are also known as the trace elements. Iron is physiologically active in the ferrous state. It occurs universally in plants in small amount, but is more abundant in physiologically active regions such as leaves and flowers. It is believed that iron influences the chloroplast structure by controlling the synthesis of chloroplast protein.

Manganese is present in low concentration (0.3mg/100g) in the test sample analysed. It is essential for normal bone structure, reproduction and the normal functioning of central nervous system. Manganese deficiency, results in bone deformities and liver arginase activity in animals (Deb, 2004). Zinc required for insulin secretion, stimulates the release of vitamin A from liver and also necessary for wound healing, normal growth and reproduction. In the present study, 0.35mg/100g zinc was recorded. Presence of zinc may be attributed to the wound healing activity of *Tarenna asiatica*. The sample contains 0.12mg/100g selenium and cobalt and boron respectively 0.01mg/100gm and 0.004mg/100g. Boron is thought to be helpful in carbohydrate
transport and fat metabolism of plants. Similar analysis of minerals was carried out by Thirumurugan et al. (2008).

7.3.6. Heavy metal Analysis

Moreno et al. (1993) have reported that heavy metals are usually higher in areas of industrial activity. Since there is no industry in the Semmanalli area, the forest is free from heavy metal pollution. The impact of heavy metals on plants and forest decline due to heavy metals have been well documented by Larison et al. (2000), Ravindran et al. (2005) and Jordan (1975). In the present study, cadmium, mercury, chromium were found to be absent. Absence of these elements and traceable amount of lead in the sample analysed indicating the water pollution free edaphic condition of Mookanur hill.

7.3.7. Vitamin Studies

Vitamins are a group of unrelated organic substances occurring in many foods in small amounts and are necessary in trace amounts for the normal metabolic functioning of the body. In the present study vitamins such as B1, B2, B3, B6, B12, E and C in trace were recorded. Vitamin C is a reducing agent that function in the body as antioxidant. The activities of plants may be attributed to the presence of Thiamin (B1), Riboflavin (B2) and Niacin (B3) in the test sample.

Decarboxylation, Deamination, Transamination, Nicotinic acid formation and haemoglobin synthesis are carried out by Vitamin B6. Vitamin B6 was found to be maximum in present study. 0.008 mg B12 vitamin is present in the leaf sample. This maybe helpful in nucleic acid synthesis and therefore for the growth and division of cells.

Vitamin E is a fat soluble vitamin, it is able to mix with and protect lipid molecules from oxidation. It is considered to be the body’s first line of defense against a specific form of lipid peroxidation. In this role, vitamin E protects cell membrane against oxidizing free radicals. 0.12 mg vitamin E was present in the leaf sample analysed. The antioxidant activity of sample may be due to the presence of vitamin E and C in trace amount. The antioxidant properties of vitamin E are believed to explain its role in
inhibiting the formation of lipofuscin, a pigment that accumulates within the tissues during ageing (Srilakshmi, 2008).

The presence of vitamins B, C and E and certain microelements as selenium, zinc and manganese confirms the wound healing and antioxidant activity of *Tarenna asiatica* leaf as reported by Cadenas and Packer (2001) who stated that manganese, copper, selenium, zinc, vitamin B and C are non enzymatic antioxidants.

**7.3.8. Qualitative Phytochemical Screening**

The medicinal plants have some chemical substance called phytochemicals that produce a physiological action on the human body. Phytochemical screening is an essential step towards the discovery of new drugs as it provides the information regarding the presence of a particular primary and secondary metabolites in the plant extract of clinical significance. In the present study, the ethanolic leaf extract screening revealed the presence of carbohydrates, proteins and amino acids, alkaloids, flavonoids, glycosides, phenols and tannins, saponins, steroids and sterols, anthraquinones, triterpenoids and volatile oil. The aqueous extract showed negative response to saponins, steroids and sterols, anthraquinones and volatile oil.

The positive response of the above mentioned compounds to the ethanolic extract may be due to the dissolution capacity of phytochemicals in the organic solvents. The preliminary data collected, helpful in the preparation of an authentic preliminary phytochemical profile. The bioactive properties of *Tarenna asiatica* may be attributed to the presence of various primary and secondary metabolites. Similar studies were carried out by Hsu *et al.* (1981) in *Strumpfia maritima*, Heitzman *et al.* (2005) in *Uncaria species* and Abere *et al.* (2007) in *Mitracarpus scaber*.

**7.3.9. Quantitative Estimation**

**7.3.9.1. Total Alkaloids**

Alkaloids comprise the largest class of secondary plant substances at present, numbering more than 7000. In higher plants some families like Papaveraceae, Liliacee,
Solanaceae, Rubiaceae, Rutaceae, Boraginaceae and Asclepiadaceae are plants contain rich alkaloids (Mothes et al., 1985). In the present study, the Rubiaceae member *Tarenna asiatica* leaf sample contains 3.68mg alkaloid. They are stored predominantly in tissues which are important for survival and reproduction which include actively growing young tissues, root and stem bark, flowers, seedlings and photo synthetically active tissues. Daniel (2008) reported that alkaloids are confined to Rubiaceae, Apocynaceae and Asclepiadaceae. This is in consonance with the present study. These alkaloids are defensive agents as reported by Mothes et al. (1985). The presence of alkaloids is conferring the wound healing activity of *Tarenna asiatica* leaves. Presence of alkaloids in Rubiaceae plants was confirmed by Nagakura et al. (1993) in *Cephaelis ipecacuanha*, Daniel (2008) in *Cinchona* Spp. and Niranjan et al. (2000) in *Anthocephalus cadamba*.

### 7.3.9.2. Total Phenolic content

Phenolics are of great importance as cellular support materials. They form an integral part of cell wall structures. With their formulation, plants became adapted to terrestrial life by building rigid organs such as woody stem and conducting cell elements for water transport (Dey and Harborne, 1997). The leaf sample has recorded 94.3mg total phenolics in the present investigation. The occurrence of phenolics in Rubiaceae plants was well documented by Hamerski et al. (2003), Kannan et al. (2009) and Hsu et al. (1981). It is also mentioned here that an increase of the phenolic metabolism in *Tarenna asiatica* leaves may be related to the slightly hard climatic conditions (hot temperature), high solar exposure and dryness as reported by Djeridane et al. (2006) in some Saharan plants. Mongrand et al. (2005) reported that Rubiaceae species are known to be used as bio producers of alkaloids, phenols, tannins, saponins, terpenoids and flavonoids. This is in agreement with the present findings. The food, ornamental and pharmaceutical importance of Rubiaceae species have been also confirmed by Karou et al. (2011).

### 7.3.9.3. Tannin Content

Tannins are water soluble plant polyphenols which cause protein precipitation from aqueous solutions, located in vacuoles (Dey and Harborne, 1997). This group of
compound has received a great deal of attention in recent years, since it was suggested that the consumption of tannin containing beverages especially green teas and red wines can cure or prevent a variety of illness. In the present study 65.2mg TAE/g extract tannin content was recorded. This is in line with the report of Kannan et al. (2009) in Rubia cordifolia.

7.3.9.4. Total Flavonoid Content

Flavonoids are well known phytochemicals having the biological effects such as free radical scavenging, modulation of enzymatic activity, potential utility as antibiotic and anti-inflammatory agents. In the present study 19.3 mg/g rutin equivalent total flavonoids was registered. The occurrence of flavonoids in Rubiaceae plants has been confirmed by Hamerski et al. (2003) in Randia dumetorum, Kannan et al. (2009) in Rubia cordifolia, Hsu et al. (1981) in Strumpfia maritima, Lopes et al. (2004) in Chiococca braquiata and Heitzman et al.(2005) in Uncaria sp. From the analysis it is deduced that the leaves of Tarenna asiatica are moderate in flavonoid content. The presence of total phenolics and total flavonoids indicate that the polyphenolic compounds are important contributors of wound healing and antioxidant potential of Tarenna asiatica leaves.

Natural products have played an important role in the development of drug for various diseases. Until 1990’s scientists thought that most of the compounds produced by plants were useless waste products. These waste compounds are called as secondary metabolites. But later found that these perform a huge array of functions. The secondary metabolites have complex stereo structure with many chiral centers which may be essential for biological activity. Many of these cannot be synthesized economically on a commercial basis (Farnsworth and Morris, 1976). The secondary metabolites from natural sources are good candidates for drug development because being elaborated within the living systems, they are perceived to exhibit more similarities to drugs and show more biological friendliness than totally synthetic drugs (Shoeb, 2006).
7.3.10. HPTLC Analysis

HPTLC finger printing profile of ethanolic extract of *Tarenna asiatica* leaf was recorded with different peaks, Rf values, height and area. The assigned substance is unknown in peaks 1, 2, 3 and 6. Among them, peak 7 was found as polyphenol. Brown colour zone detected in UV after derivatization in the chromatogram confirms the presence of polyphenols. This is in consonance with the study of Sasikumar *et al.* (2009) who reported that blue, brown colour zone in UV after derivatization in the chromatogram is mainly due to the presence of polyphenols. Similar studies were made by Saxena *et al.* (2000) in *Andrographis paniculata*, Nayak and Mengi (2010) in *Morinda citrifolia* and Singh (2010) in *Citrullus colocynthis*.

7.3.11. GC-MS Analysis

GC-MS is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosive investigation and identification of unknown samples. GC-MS can also be used in airport security to detect substances in luggage (Manjamalai *et al*., 2011).

GC-MS chromatogram of the ethanolic extract of leaf of *Tarenna asiatica* showed 19 peaks indicating the presence of 19 phytochemical constituents. On comparison of the mass spectra of the constituents with the NIST library, 19 phytoconstituents were characterized and identified. Five major phytochemical constituents were identified as 4-methylmannitol, (30.66 peak area %), Sorbitol (24.26%), D-mannoheptulose (13.63%) and 1, 2, 3, 5-cyclohexanetetrol (5.51%) and n-Hexadecanoicacid (5.44%). The major constituent was found to be 4-methylmannitol at retention time of 10.70. The sugar sorbitol was found in next higher concentration at the retention of time of 14.66. D-mannoheptulose, 1, 2, 3, 5- Cyclohexanetetrol and n-Hexadecanoicacid were identified respectively at 8.20, 9.71 and 13.25 retention time. The components may be grouped into aliphatic, aldehyde, sugars, alcohols, ketone, fatty acid, terpenoids, phenols, esters, toco pherol and aromatic compound. Terpenoids are an important part of volatiles from plants.
The occurrence of various components in GC-MS analysis and their biological activities were studied by Hanbali et al. (2005) in *Pulicaria odora*, Lacikova et al. (2007) in *Staphylea* species, Aboutab et al. (2010) in *Macfadyena unguis-cati*, Mothana et al. (2011) in *Boswellia* species, Maruthupandian and Mohan (2011) in *Pterocarpus marsupium* and Ramalakshmi and Muthuchelian (2011) in *Tabebuia rosea*. The various active compounds present in the leaf and their biological activities indicate the phytopharmaceutical importance of *Tarenna asiatica*. The presence of various bioactive compounds justifies the use of plant for skin disease by traditional practitioners. However isolation of individual phytochemical constituents and subjecting it to biological activity will definitely give good results. So it is recommended as a plant of phytopharmaceutical importance.

### 7.4. Pharmacological Investigation

#### 7.4.1. Antimicrobial Activity

The antimicrobial activity is the capacity of the substance to kill or inhibit microorganisms. The results indicate a strong antimicrobial activity of ethanol and aqueous leaf extracts of *Tarenna asiatica* against the tested organisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Aspergillus niger*, *Aspergillus flavus* and *Candida albicans*. The ethanol extract showed greater level of inhibition as evident from the size of inhibition zone than the aqueous extract against all the organisms.

#### 7.4.1.1. Antibacterial activity

The results indicate a broad spectrum antibacterial activity of *Tarenna asiatica* against human and plant pathogenic bacteria. The demonstration of antibacterial activity against both gram positive and gram negative bacteria is an indication that the plant is a potential source for production of drugs with a broad spectrum activity.

The test organisms used in this study are associated with various forms of human infections. From a clinical point of view, *Klebsiella pneumoniae* is the most important member of the *Klesiella* genus of Entero-bacteriaceae and it is emerging as an important...
cause of neonatal nosocomial infection. *Escherichia coli* causes septicemias and can infect the gall bladders, meninges, surgical wounds, skin lesions and the lungs, especially in debilitate and immuno deficient patients (Black, 1996). *Proteus mirabilis* causes wound infections and urinary tract infections in the elderly and young males often following catheterization and cystoscopy and it is a secondary invader of ulcers and pressure sores (Cheesebrough, 2000).

Hence the usage of leaf extract of *Tarenna asiatica* against the human pathogenic bacteria may claim the therapeutic value of the plant. The results of the experiment thus justify the use of *Tarenna asiatica* in the traditional system of medicine for the treatment of various infectious skin diseases caused by human pathogenic bacteria. The results reveal that both ethanol and aqueous extracts were more active against *Bacillus subtilis* and *Staphylococcus aureus* than *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis*. Among the extracts, ethanol leaf extract had exhibited higher inhibitory activity than the aqueous extract. The results of this study showed that the ethanol extract was more effective than aqueous extract. This may be due to the better solubility of the active components in organic solvents as reported by de Boer *et al.* (2005).

Successful prediction of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The traditional healers make use of water primarily as solvent, but the present study showed that ethanol extract was certainly much better and powerful.

This observation can be rationalized in terms of the polarity of the compounds being extracted by the solvent in addition to their intrinsic bioactivity, by their ability to dissolve or diffuse in the different media used in the assay. The growth media also seem to play an important role in the determination of the antibacterial activity. Lin *et al.* (1999) reported that Muller- Hinton agar appears to be the best medium to explicate the antibacterial activity and the same was used in the present study.

Soneja *et al.* (2009) reported that the ethanol fruit extract of *Mitragyna* did not exhibit any antibacterial potential against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*. But in the present investigation ethanol leaf extract was more potent in
inhibiting the *Staphylococcus aureus* and *Bacillus subtilis*. The inhibitory activity of the extract may be due to the presence of wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, phenols and saponins. This is in agreement with the report of Srinivasan *et al.* (2001) who stated that the presence of bioactive substances have been reported to confer resistance to plants against bacteria, fungi and pests and therefore explains the demonstration of antibacterial activity by the plant extracts used in this study.

Karou *et al.* (2011) stated that more than 60 species of Rubiaceae exhibited antimicrobial activities as they contain indole alkaloid, terpenoids and anthraquinones. In the present investigation the wide variety of components of leaf of *Tarenna asiatica* would have conferred the antibacterial activity. Several reports (Nino *et al.*, 2007; Parthasarathy *et al*., 2009; Thapliyal *et al.*, 2000; Houghton *et al.* 2000; Mandal *et al*., 2007; Scortichini and Piarossi, 1991 and Bohra and Bohra, 2009) reveal that the secondary metabolites of plants are antimicrobial in nature. This is in consonance with the present findings.

Basavaraja *et al.* (2011) reported that *Mussaenda frondosa* bark extract exhibited almost equipotent antibacterial activity as compared with that of standard drug. This is in line with the present study that the positive control Gentamycin showed more or less equipotent zone of inhibition with the ethanol extract only. The inhibitory activity may be due to the inhibitory effect of compounds on cell wall synthesis and nucleic acid production as reported by Hammer (1999). The above said antibiotic is reported to have inhibitory effect on cell wall synthesis and nucleic acid production.

Generally glycosides, terpenoids and flavonoids are implicated in general resistance as anti pathogen principles. Such principles are present in the *Tarenna asiatica*. In classifying the antibacterial activity as gram positive and gram negative, it would generally be expected that a much greater number would be active against gram positive than the gram negative *(Rabe et al.*, 2002).*
In the present findings, the leaf extract showed more inhibitory activity against gram positive bacteria as supporting the above view. Usually most of the gram negative bacteria are more resistant than gram positive bacteria (Barberan, 1988).

7.4.1.2. Antifungal activity

The results of the antifungal activity presented in Table-19 show that all extracts exhibited antifungal properties by inhibiting the growth of all fungal organisms. Human pathogenic fungi like *Aspergillus niger*, *Aspergillus flavus* and *Candida albicans* were used to find out the antimycotic activity of *Tarenna asiatica*. *Candida albicans* was the most sensitive, this was closely followed by *Aspergillus flavus*. *Aspergillus niger* was the least sensitive in this assay. Highest growth inhibition against *Aspergillus niger* (40.2%), *Aspergillus flavus* (43.2%) and *Candida albicans* (58.2%) was demonstrated by 75% ethanolic extract of *Tarenna asiatica*. 50% concentration in both the extracts showed mild inhibitory activity.

Aqueous extracts did not inhibit much the growth of *Aspergillus flavus*, *Aspergillus niger*. This is interesting as water is one of the media through which traditional healers utilize medicinal plants to their patients. The extract in ethanol was found promising against all the fungal organisms.

A wide variety of plant extracts are known to possess antifungal properties and in many cases this activity is due to the presence of chemical constituents which exert membrane damaging of microbial strains and also stimulates leakage of cellular potassium ions which provide evidence of a lethal action related to cytoplasmic damage as reported by Mandal *et al.* (2007). The observed antifungal activity may be due to the presence of potent phyto constituents in the extracts. Even though the leaf extract is chemically active, it is moderately successful in inhibiting the fungal growth.

Subramani *et al.* (2004) reported that the phenolic acids and flavonoids are active against fungi like *Aspergillus niger*. Thapliyal *et al.* (2000) reported that crude protein extract of *Rauvolfia* inhibited *Aspergillus flavus*. These reports are in consonance with the present findings. Sanjay *et al.* (2007) reported that the alcoholic and aqueous extract
of *Anthocephalus cadamba* showed a significant antimicrobial activity against almost all the organisms especially against dermatophyte fungi.

In the present study, the aqueous extract effect was less than the ethanol extract which means that ethanol extracted all the active components of the leaf. The wound causing dermatophyte fungi *Candida albicans*, *Aspergillus flavus* and *Aspergillus niger* were moderately controlled by the ethanol extract. The results also confirm the observation of Bhardwaj (2011) who reported the antimicrobial potency and synergistic activity of Indian medicinal plants. Cowan (1999) reported that many natural products including pigments, enzymes and bioactive components are soluble in water, which explains the highest yield of extract. But in the present study aqueous extract is not highly successful in controlling the fungal growth. *Candida albicans* was more susceptible than *Aspergillus niger* and *Aspergillus flavus*.

Conclusively both ethanol and aqueous extracts have displayed antimicrobial activities from the present study. These findings support the traditional knowledge of local users and it is a preliminary scientific validation for the use of *Tarenna asiatica* leaf for skin diseases.

In the present work, it was confirmed that the phytochemicals of *Tarenna asiatica* leaf possess better antibacterial property than antifungal property, subsequently, pure chemical compounds responsible for this activity have to be identified; isolated and finally using these compounds as prototypes, synthetic chemical entities will be developed that will possess even greater bacterial and fungal activity.

Further possible isolation of pure compounds of the therapeutic antimicrobial from the leaf of *Tarenna asiatica* and pharmacological evaluations are the future challenges.

7.4.2. Wound Healing Activity

7.4.2.1. Excision wound-wound contraction

Wounds are common clinical entities in day to day life which may be either major or minor. The process of wound healing can be classified in to five phases such as
cellular (granulation), narrowing of wound area (wound contraction), collagen deposition (collagenation), epithelial covering (epithelialization) and scar remodelling (cicatrisation). These phases are concurrent but independent of each other. Any agent which accelerates the process is a promoter of wound healing as reported by Smith (1985). The process of wound healing consists of an integrated cellular and biochemical events leading to reestablishment of structural and functional integrity with region of strength of injured tissue. It is an established fact that plants and plant based medicaments have immense potential for the management and treatment of wounds and induce healing and regeneration of tissue by multiple mechanism and needs to be exploited to minimise the undesired consequences of injuries.

Many studies (Kerr, 2002; Mohideen et al., 2003; Manjunatha et al., 2005; Sanjay et al., 2007; Ayyanar and Ignacimuthu, 2009; Karodi et al., 2009; Sheeba et al., 2009; Joshi et al., 2010; Paul et al., 2010; Patil and Joshi, 2011; Senthil et al., 2011 and Nirmala and Karthiyayini, 2011) indicate that plants and plant products are potential agents for wound healing and largely preferred because of the absence of unwanted side effects. 70% of the wound healing Ayurvedic drugs are of plant origin, stated to be effective in different conditions such as wounds, ulcers, abscess and erysipelas (Biswas and Mukherjee, 2003). In the present study, it is shown that the topical application of 5% and 10% w/w test ointment formulated from ethanol leaf extract of Tarenna asiatica promoted the wound healing activity in excision wound model and leaves are mostly used in wound healing as they are active in photosynthesis and production of metabolites as reported by Ghorbani (2005).

Chopda and Mahajan (2009) reported that leaves of many Rubiaceae members including Tarenna asiatica are used for healing wounds in human and domestic animals. This is in consonance with the present investigation. The healing property of Tarenna asiatica leaf is also confirmed by Sudarsanam et al. (1995) who observed that the leaf infusion of Tarenna asiatica heals plough injuries. The results showed that wound contraction was accelerated by applying ointment of Tarenna asiatica which was highlighted by the full thickness coverage of the wound area by an organized epidermis. Early dermal and epidermal regeneration in healed animals also confirmed that the test
ointment had a positive effect towards cellular proliferation, granular tissue formation and epithelialization as reported by Reddy et al. (2002).

The enhanced capacity of wound healing with the test ointment prepared from Tarenna asiatica could be explained on the basis of anti-oxidant effects of the plant that are well documented by Sang et al. (2001) in Morinda citrifolia and Senthil et al. (2011) in Guazuma ulmifolia. Thiem and Grosslinka (2003) also reported that topical application of plant based compounds with free-radical scavenging properties in patients have shown to improve significantly wound healing and protect tissues from oxidative damage. In the present investigation, presence of free radical scavenging enzymes has been proved, which are a cyto protective enzymal group that has an essential role in the reduction, deactivation and removal of reactive oxygen species as well as in the regulation of the wound healing process as reported by Baboir (1978).

Excision wound study on animal model showed enhanced rate of wound contraction and drastic reduction in healing time (16 day in 10%w/w test ointment) than simple ointment (control) which might be due to enhanced epithelialization. Wound contraction is the process of mobilizing healthy skin surrounding the wound to cover the denuded area. This centripetal movement of wound margin is believed to be due to the activity of myofibroblast. Since Tarenna asiatica test ointment enhanced the wound contraction, it would have either enhanced contractile property of myofibroblasts or increased the number of myofibroblasts recruited in to the wound area as reported by Sidhu et al. (1999).

Significant wound contraction on 16thday at 10% test ointment of Tarenna asiatica may also be due to the participation of various inflammatory cells such as macrophages and neutrophils in the repair process and may also promote the migration and proliferation of endothelial cells, leading to neovascularization of connective tissue cells, which synthesize the extracellular matrices including collagen and of keratinocytes leading to reepithelialization of the wounded tissue as reported by Rasik et al. (1999). It is well accepted that secondary metabolites play an important role in wound healing
process (Sang et al., 2001; Kerr, 2002; Somava et al., 2003, Sumitra et al., 2005 and Ghorbani, 2005).

In the present study the qualitative screening of leaf extract revealed the presence of alkaloids, triterpenoids, phenols, flavonoids, glycosides, anthraquinones, saponins and tannins. Wound contraction in animal model may be due to the presence of the above mentioned compounds. Similar reports were made by Joshi et al.(2003), Manjunatha et al. (2005) and Senthil et al. (2011). Flavonoids are known to reduce lipid peroxidation not only by preventing or slowing the onset of cell necrosis but also by improving vascularity. Hence any drug that inhibits lipid peroxidation is believed to increase the viability of collagen fibres, increasing the circulation, preventing the cell damage and promoting the DNA synthesis (Getie et al., 2002).

Somava et al. (2003) and Scortichini and Piarossi (1991) also reported that tannins, flavonoids, triterpenoids and sesquiterpenes are known to promote the wound healing process mainly due to their antioxidant, astringent and antimicrobial properties which seem to be responsible for wound contraction and increased rate of epithelialization. The sesquiterpene lactones are known to possess antioxidant property which may also contribute to the wound healing process (Manjunatha et al., 2005). Thus the wound healing potency of Tarenna asiatica may be attributed to the phytoconstituents present in it, which may be either due to their individual or additive effect that fastens the process of wound healing.

7.4.2.2. Incision wound-Tensile strength

The tensile strength of a wound represents the degree of wound healing. Usually wound healing agents promote a gain in tensile strength. The results on tensile strength revealed that upon application of 5% and 10% w/w test ointment, there was an increase in the tensile strength or breaking strength of skin as compared to control (simple ointment). The findings of present study showed a significant increase in tensile strength of animals treated with 10% test ointment. This is more or less comparable with standard drug nitrofurazone. Similar studies were reported by Sheeba et al. (2009) in Cassia

Patil and Joshi (2011) showed 293g tensile strength by the application of Mussaenda frondosa leaves, but in the present study 298.3g and 370g tensile strength was recorded respectively in 5% and 10% test ointment. Beloz et al. (2003) reported that Hamelia patens increased the breaking strength of wounds by double incision wound healing assay. This is in agreement with the present findings.

Researchers (Soneja et al., 2005; Sang et al., 2001 and Sussman, 2007) who have explored the complex dynamics of tissue repair have identified several nutritional cofactors involved in tissue regeneration, including vitamins C, E and micro minerals like zinc and iron. In the present investigation the presence of vitamins like B1, B2, B6, B12, E and trace amount of vitamin C and micro minerals such as zinc, manganese and iron might have played a significant role in enhancing the wound contraction. Vitamin C and Iron are required for the synthesis of strong collagen. Zinc reduces the size of the wound and shortens the healing time (Sussman, 2007). In the present study, the reduction in the healing time may be attributed to the presence of zinc in both the models.

It is well established that sufficient protein (Hanna and Giacopelli, 1997) is necessary for wound healing. Diallo et al. (2002) stated that polysaccharides are partially responsible for the process of wound healing. The presence of protein and carbohydrates in the leaf sample confirms the healing property of Tarenna asiatica.

In the present study, the animals treated with 5% and 10% test ointment showed significant results when compared with simple ointment treatment (control). 10% test ointment treated animals showed faster epithelialization of wound than the 5% test ointment treated animals. From the above result of excision and incision wound model, it is evident that 10% test ointment was found to be superior to 5% test ointment in wound contraction and increasing the tensile strength.

The observation of the current study confirms the traditional use of Tarenna asiatica leaves for wound healing properties. The formulation of ethanol extract applied
augments the healing process by strengthening the tensile strength and promoting the wound contraction.

7.4.3. Antioxidant Activity

Since ancient time, the medicinal properties of plants have been investigated in the recent scientific development throughout the world due to their potent antioxidant activities. Antioxidants have been reported to prevent oxidative damage caused by free radicals (Robak and Marcinkiewicz, 1995 and Basile et al., 2005). Free radicals can be traced back to 3.5 billion years ago when the basic components of life were being produced by the free radicals with the help of solar reaction. Now the same free radicals responsible for the initiation of life have become a threat to our very existence of life. Many experimental investigations as well as clinical findings have provided evidence (Husain et al., 1987; Farber, 1994; Lugasi and Hovari, 2003 and Rajneesh et al., 2008) supporting the role of reactive oxygen metabolites or free radicals such as singlet O₂, superoxide O₂, hydrogenperoxide and hydroxyl radical in the etiology of diseases.

Human body is equipped with certain antioxidants such as superoxide dismutase and catalase which can counteract the deleterious actions of the reactive oxygen species and protect tissues against cellular and molecular damage as reported by Singh et al. (2003). Recently there has been an upsurge of interest in the therapeutic potentials of plants as antioxidants in reducing free radical induced tissue injury. Hence in the present investigation considerable attention has been directed towards the identification of antioxidant ability in the leaf extract of Tarenna asiatica.

7.4.3.1 In vitro Antioxidant Activity

DPPH radical scavenging activity

DPPH is a molecule containing a stable free radical. The DPPH free radical scavenging assay measures free radical scavenging capacity of the leaf extract of Tarenna asiatica. Parthasarathy et al. (2009) reported that DPPH assay is often used to evaluate the ability of antioxidants to scavenge free radicals which are known to be a major factor in biological damages caused by oxidative stress. Since DPPH assay has been largely
used as a quick, reliable and reproducible parameter to search the *in vitro* general antioxidant activity of pure compounds as well as plant extracts, the potential antioxidant activities of leaf extract of *Tarenna asiatica* were evaluated. The principle of the assay is based on the colour change of the DPPH solution from purple to yellow as the radical is quenched by the antioxidant system and this assay measures the ability of antioxidants to scavenge free radical (Roginsky and Lissi, 2005).

The ethanol extract of *Tarenna asiatica* showed a high effective free radical scavenging in the DPPH assay at 200µg concentration. Hence this extract exhibited a great antioxidant effect. Mothana *et al.* (2009) reported that 1000µg *Acacia pennivenia* methanol extract exhibited 94% free radical scavenging effect. In the present study 200µg concentration exhibited 86.41%. This result supports the idea that *Tarenna asiatica* can be a promising source of potential antioxidant. The antioxidant activity may be due to the presence of phenol compounds. Dutra *et al.* (2008) reported that the essential oil of *Pterodon emarginatus* seeds containing phenol showed DPPH scavenging activity with IC$_{50}$ value 163.22. This is in line with the present investigation. The phenolic compound may reduce the oxidative stress which causes damage to lipids, proteins, carbohydrates and nucleic acids as reported by Pryor *et al.* (2006). DPPH inhibition (Parthasarathy *et al.*, 2009) of *Mitragyna*, a Rubiaceae member confirms the present investigation.

**Superoxide radical scavenging activity**

Superoxide radical scavenging activity was also high at 160µg and 200µg concentration when compared to other concentrations. This result reveals that 160µg and 200µg concentrations had inhibitory activity against superoxide radicals and this good scavenging activity may be due to phenolic contents present in the extract. The superoxide radicals generated from dissolved oxygen by PMS-NADH (Phenazine methosulphate-nicotinamide adenine dinucleotide) coupling can be measured by their ability to reduce NBT. The decrease in absorbance at 560nm with the plant extract and the reference compound quercetin indicates their abilities to quench superoxide radicals in the reaction mixture.
As shown in the Table-24 the IC$_{50}$ value of the leaf extract and quercetin on superoxide scavenging activity was 364.2 and 52.1 respectively. The IC$_{50}$ value of the extract was more than that of the standard. At 40µg/ml, the percentage inhibition of the leaf extract was 10.08 whereas that of quercetin was 18.2 at 20µg/ml. Superoxide anion is very harmful to cellular components. Robak and Glyglewski (1988) reported that flavonoids are effective antioxidants mainly because they scavenge superoxide anions. In the present study, the superoxide radical scavenging activities of the plant extract are increased gradually with increasing concentrations.

The results suggest that the plant extract is a potent scavenger of superoxide radical. Halliwell, (2001) reported that the antioxidant system is composed of superoxide dismutase which converts the superoxide radical into hydrogen peroxide and catalase and glutathione peroxidase, both of which detoxify the hydrogen peroxide. This is in agreement with the present findings. The antitumour effects of plant flavonoids have been reported to induce cell growth inhibition and apoptosis in a variety of cancer cells (Dicarlo et al., 1999). Anti collagenolytic and antielastase activity were thought to be due to the flavonoids present in the polyphenol extract (An et al., 2005). Cook and Samman (1996) reported that phenolic compounds are very important plant constituents because their hydroxyl groups confer scavenging ability. This confirms the present investigation.

**Nitric Oxide radical scavenging activity**

The results on nitric acid radical scavenging activity revealed that the leaf extract of *Tarenna asiatica* is a better scavenger of nitric oxide free radicals rather than the superoxide radicals. The activity is attributed to the presence of bioactive compounds. It is well known that nitric oxide has important role in various diseases. Sustained levels of production of this radical are directly toxic to tissues and contribute to the vascular collapse associated with septic shock, whereas chronic expression of nitric oxide radical is associated with various inflammatory conditions including diabetes, arthritis and sclerosis. The toxicity of nitric oxide increases greatly when it reacts with superoxide radical, forming the highly reactive peroxynitrite anion. The nitric oxide generated from sodium nitroprusside reacts with oxygen to form nitrite (Huie and Padmaja, 1993). The
leaf extract inhibits the nitrite formation by directly competing with oxygen in the reaction with nitric oxide. The present study proved that the extract studied has more potent nitric oxide scavenging activity.

**Hydroxyl radical scavenging activity**

Hydroxyl radical is a highly reactive radical formed in biological systems and capable of damaging almost every molecule found in living cells (Halliwell, 2001). Hydroxyl radicals are the major active oxygen species causing lipid peroxidation and enormous biological damage. They were produced in this study by incubating ferric-EDTA with ascorbic acid. When *Tarenna asiatica* leaf extract was added to the reaction mixture, it removed the hydroxyl radicals from the sugar and prevented the reaction. The IC$_{50}$ value indicates that the leaf extract of *Tarenna asiatica* is a better hydroxyl radical scavenger.

Hydroxyl radical has the capacity to induce carcinogenesis, mutagenesis and rapidly initiates lipid peroxidation (Rajneesh *et al*., 2008). If any plant extracts or drug scavenges the hydroxyl radical, they may either scavenge the radical or may chelate the Fe$_{2+}$ ion making them unavailable for the fenton’s reaction. Plant extracts containing particularly polyphenols are reported to quench oxygen derived free radicals by donating a hydrogen atom or an electron to the free radical (Kahkonen *et al*., 1999) or neutralize free radicals by their chelating ability due to their high nucleophilic character of the aromatic ring (Robak and Marcinkiewicz, 1995).

Joharapurkar *et al*. (2003) reported that ethanol extract of *Rubia cordifolia* has antioxidant activity. In present study, since *Tarenna asiatica* leaf extract scavenge the hydroxyl radical it confirms the antioxidant activity. Similar study carried out on *Rubia cordifolia* by Tripathi *et al*. (1998) confirms the present investigation. In this study the ethanol leaf extracts of *Tarenna asiatica* were found to exhibit a dose dependent hydroxyl radical scavenging activity. The radical scavenging property of *Tarenna asiatica* as evidenced from the studies provides a platform for its future evaluation as a drug in skin diseases.
Metal chelating capacity

Metal chelating capacity is significant since it reduces the concentration of the transition metal that catalyses lipid peroxidation (Duh et al., 1999). Although metal chelating agents are not antioxidants, they play a vital role in the stabilization of fatty acids against rancidity (Yen and Duh, 1994). The ethanol extract of leaf of Tarenna asiatica demonstrated the ability to chelate ions. The extract may be able to play a protective role against oxidative damage by sequestering iron (II) ions that may otherwise catalyse fenton-type reactions or participate in metal catalysed hydroperoxide decomposition reactions.

Phosphomolybdenum Assay

It is based on the reduction of Mo (VI) to Mo (V) in the presence of antioxidant compounds and the subsequent formation of a green phosphate Mo (V) complex at acid pH (Prieto et al., 1999). The good antioxidant activity might be attributed to the presence of phytochemicals present in the ethanol leaf extract of Tarenna asiatica.

Ferric Reducing/Antioxidant Power Assay

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the activities of antioxidants have been attributed to various mechanisms such as prevention of chain initiations, decomposition of peroxides, reducing capacity and radical scavenging (Cook and Samman, 1996). Antioxidants can be referred to as reductants which inactivate oxidants. They are involved in redox reactions in which one reactions species (oxidant) is reduced at the expense of the oxidation of the antioxidant (reductant). Reductants also react with certain precursors of peroxide, thus preventing peroxides formation. Siddhuraju et al. (2002) have reported that the reducing power of bioactive compounds was associated with antioxidant activity. In present study, the polyphenolic compounds of the ethanol extracts appear to function as good electron and hydrogen atoms donors. The results indicate that Tarenna asiatica leaf extract contains good amounts of flavonoids and phenolic compounds. Both these classes of compounds have good antioxidant potential and their
effects on human health are considerable. The mechanism of action of flavonoids is mainly through the radical scavenging or chelating process. Phenolic compounds are also very important plant constituents because their hydroxyl groups confer scavenging ability.

7.4.3.2 *In vivo* Antioxidant Activity

**Superoxide dismutase (SOD) activity**

Superoxide dismutase is considered as primary antioxidant enzyme, since it is involved in direct elimination of reactive oxygen metabolites (Rajneesh *et al.*, 2008). Fridovich (1978) has demonstrated in his study that the reduction in superoxide dismutase activity increases the toxic effects of O$_2$ and this might lead to severe cellular damage. Superoxide dismutase, which causes superoxide breakdown and the subsequent production of hydrogen peroxide, has a central role in regulating reactive oxygen species levels (Pryor *et al.*, 2006). One unit of SOD is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical. In the present study, superoxide dismutase activity (high dose) was recorded as 2.48Units/min/mg protein as this may counteract the deleterious actions of reactive oxygen species and may protect the body against cellular and molecular damage as reported by Singh *et al.* (2003). The chemical screening showed the presence of Terpenoids, Tannins, Phenols, Flavonoids in the leaves of *Tarenna asiatica* which might exhibited the antioxidant effect as reported by Frankel (1995); Kahkonen *et al.* (1999) and Aderogba *et al.* (2005).

As mentioned by several researchers (Mano *et al.*, 1997; Halliwell, 2001; Halliwell and Gutteridge, 1997; Singh *et al.*, 2003 and Sanjay *et al.*,2007) superoxide dismutase is a naturally occurring enzyme which protects the cell from the reactive and damaging O$_2$ by dismuting it in to O$_2$ and H$_2$O$_2$. The results suggest that the presence of SOD activity has the potential to prevent radical related injuries and diseases. The SOD activity may be related to phenolic content as reported by Cook and Samman (1996), Kahkonen *et al.* (1999) and Sanjay *et al.* (2007)
**Catalase (CAT) activity**

Catalase is a homotetrametric heme-containing enzyme that catalyzes the conversion of hydrogen peroxide into water and oxygen with one of the highest turnover rate known in enzymology. Heme catalase is a H$_2$O$_2$ scavenging enzyme with optimal activity at high H$_2$O$_2$ concentration (Kinnula *et al.*, 1995).

In the present study, 16.37µ moles of H$_2$O$_2$ consumed/min/mg protein activity suggested that the leaf extract has potential to prevent radical related injuries and diseases. Iwata *et al.* (1984) have reported that catalase prevents chromosomal aberration caused by hypoxanthine/xanthine oxidase in Chinese hamster cells. Singh *et al.* (2003) reported that catalase can counteract the deleterious actions of reactive oxygen species and protect tissues against cellular and molecular damage. This is in consonance with the present study. The catalase activity was brought to near normal in *Tarenna asiatica* leaf extract treated rats. This evidently shows the antioxidant property of the ethanol extract of *Tarenna asiatica* against oxygen free radicals.

**Glutathione peroxidase (GPx) activity**

The cytosolic enzyme, Glutathione peroxidase is a tetramer and 85,000 dalton protein containing selenium that eliminates H$_2$O$_2$. The primary function of glutathione peroxidase is that of detoxifying low levels of H$_2$O$_2$ in cells (Quinlan *et al.*, 1994). Ethanol leaf extract treated rats recorded 31.58 and 32.81µ moles of GSH oxidized/min/mg protein respectively in low dose and high dose. The increased activity in high dose might be due to the higher concentration of secondary metabolites in the ethanol extract. The main biological role of this enzyme is to protect the organism from oxidative damages as reported by Meister (1988). The biochemical function of glutathione peroxidase is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxidase to water. It is suggested that this antioxidant enzyme might have destroyed reactive oxygen species so that oxidative stress is not severe enough to create cell damage.
Glutathione-S-Transferase (GST) activity

Glutathione-s-transferase, a soluble protein located in cytosol plays an important role in detoxification and excretion of xenobiotics by conjugating them with glutathione. As compared to control values, GST activity in the low dose is insignificant and it is increased in the high dose. The increased activity of GST can be related to the scavenging effect of the reactive oxygen species. Glutathione, together with its related enzymes, comprises a system that maintain the intracellular-reducing environment and acts as primary defense against excessive generation of harmful ROS. The oxygen radiant scavenging activity of glutathione directly facilitates ROS neutralization and the repair of ROS induced damage (Pastore et al., 2003). In this study, the ethanol leaf extract of *Tarenna asiatica* as antioxidant supplement is vital to combat oxidative damage.

Lipid peroxidation (LPO) activity

Lipid peroxidation is a free radical mediated phenomenon in biological tissues where poly unsaturated fatty acids are generally abundant and is one of the most frequently used parameters for assessing the involvement of free radicals in cell damage. The elevated level of serum lipid peroxide may be due to defective antioxidant system which lead to the accumulation of lipid peroxides in tissue which are released into the blood serum (Kumaraguruparan et al. 2002). In the present study, the decreased lipid peroxidation activity in the serum might be due to the antioxidant compounds present in the leaf extract as reported by Lugasi and Hovari (2003). These compounds might have reduced the reactive oxygen species, which have harmful effects such as the peroxidation of the membrane lipids, aggression to tissue proteins and membranes and damage to DNA and enzymes (Husain et al., 1987).

Stambullian et al. (2007) reported that Vitamin E, a potent chain breaking lipid soluble antioxidant reacts with lipid peroxyl radicals eventually terminating the peroxidation chain reaction and thereby reducing oxidative damage. Hence, the lipid peroxidation activity might be attributed to the presence of Vitamin E in the ethanol leaf extract of *Tarenna asiatica*. The result of the *in vivo* antioxidant study provides a strong
evidence regarding the definitive role of ethanol leaf extract in reducing the reactive oxygen species.

7.4.4. Skeletal Muscle Relaxant Activity

The skeletal muscles are composed of a large number of muscle fibres which are attached to the skeleton give shape, form and appearance to the body. They protect the vital organs of the body, keep the joints in proper position and produce movements of the body. The loss of muscle grip is an indication of muscle relaxation. A muscle relaxant is a drug which affects the skeletal muscle fraction and decreases the muscle tone. It may be used to alleviate symptoms such as muscle spasms and pain. Drugs acting as effective muscle relaxants are desirable in a wide variety of conditions. Thus skeletal muscle relaxants are able to reduce unwanted spasm or spasticity without interfering with consciousness. Such drugs would also be valuable to surgeons during operative procedures for achieving satisfactory muscle relaxation (Satoskar et al., 2001). The rotarod is used to evaluate the activity of drugs interfering with motor coordination. Dunham and Miya (1957) suggested that the skeletal muscle relaxation induced by a test compound could be evaluated by testing the ability of mice or rats to remain on a revolving rod.

The rota rod test has been used by many researchers (Rakotonirina et al., 2001); Ganatra et al. (2011), Rathor and Ram (2010) and Deoliveira et al. (2012) to screen centrally acting muscle relaxants. In the present study the test drug exhibited a marked muscle relaxant activity as compared to control. A moderate myorelaxant effect was observed with the higher dose (200mg/kg) of ethanol leaf extract of Tarenna asiatica as compared to standard drug.

Ethanol leaf extract treated mice did not show any significant motor performance alterations with the dose of 100mg/kg i.p (45.9% decrease in time) as compared to 200mg/kg dose treated animals (63.1% decrease in time) and standard drug in the rotarod test. Abid et al. (2006) reported that the low dose of drug (75mg/kg) did not show any significant effect on the motor coordination. This is in line with the present
findings. As expected, Diazepam (positive control and standard drug), the most widely used benzodiazepine derivative causes sedation and reduced the motor performance of wistar mice significantly after 30min of treatment as reported by Deoliveira et al. (2012).

The decrease in motor activity gives an indication of the level of excitability of the CNS and this decrease maybe related to sedation resulting from depression of CNS. So the observed CNS depressant activity may be attributed to the presence of secondary metabolites in the test samples as reported by Masur et al. (1971).

The phytochemical analysis revealed the presence of anthraquinones, saponins, terpenoids, flavonoids, phenols, alkaloids and tannins in the leaf extract of Tarenna. Therefore, the observed skeletal muscle relaxant activity may be attributed to these compounds. Jha et al. (2011) reported that the presence of different chemical compounds in the plants reduces the fall off time. This is in consonance with the present study.

A number of scientific reports indicated that triterpenoids and flavonoids produced CNS depressant action (Masur et al., 1971; Chattopadhyay et al., 2003; Yasuda et al., 2005 and Jha et al., 2011). Therefore, the presence of terpenoids and flavonoids in the ethanolic leaf extract of Tarenna asiatica may be responsible for the skeletal muscle relaxant activity. It is demonstrated that the leaf extract of Tarenna asiatica showed muscle relaxant activity in albino wistar mice model by rota rod test, that are predictive of muscle relaxant activity in humans.

Pranit et al. (2010) reported that 400mg/kg Leucas longifolia showed more skeletal muscle relaxation in animal model, but in the present study 200mg/kg ethanolic leaf extract showed reduction in fall off time after 30 min of drug administration. Similar findings were reported by Kumar et al. (2008) and Ganatra et al. (2011).

The exact mechanism by which normal skeletal muscle tone is regulated is not known, but it is probably dependent on the stretch reflex. The reticular information on the brain stem is believed to exert augmentory as well as inhibitory effects on the muscle tone through the internuncial neurons of the spinal cord (Satoskar et al., 2001).
To the best of my knowledge, this is the first report on the skeletal muscle relaxant activity of leaf extract of *Tarenna asiatica* in wistar mice. Previously the leaf extract was reported to cure skin diseases, however, no antispasmodic and skeletal muscle relaxant effects have been reported in wistar rat and mice by *Tarenna asiatica* leaf.

Dhawan *et al.* (2003) reported that many standard psychoneural drugs adversely affect the respiratory, digestive and immune system of body and the chronic treatment with Diazepam often proved more harmful in the longer run. In this context a resurgence of interest in medicine from natural sources, mainly plant products, is seen and there is tremendous hope that drugs of plant origin will have significantly less side effects than that observed with synthetic drugs. To conclude, the ethanolic leaf extract of *Tarenna asiatica* possesses muscle relaxant property. Further studies are necessary to elucidate the exact mechanism of skeletal muscle relaxant activities and to isolate the active constituents responsible for this pharmacological activity.

7.4.5. Antispasmodic Activity

Spasms are continuous involuntary smooth muscle contractions, may be induced due to endogenous acetylcholine and histamine. They can lead to discomfort, uneasiness and could result in irritation and inflammation of tracheal tract posing a major health problem in the human being (Satoskar *et al.*, 2001). For centuries herbs have been used in the treatment of bronchospasm disorders. The use of plant extracts as bronchodilators for the relaxation of bronchial muscle contraction induced by histamine has been proved beyond doubt by several workers (Parmar *et al.*, 2010; Kumar *et al.*, 2010; Ninave *et al.*, 2011 and Chauhan *et al.*, 2012). In this context, the aim of the present investigation becomes relevant and significant, since it attempts to analyse the ethanol leaf extracts of *Tarenna asiatica* as bronchodilator.

Histamine induced broncho constriction is the traditional immunological model of antigen induced airway obstruction. Histamine when inhaled causes hypoxia and leads to convulsion in wistar rats and causes very strong smooth muscle contraction, profound
hypotension and capillary dilation in cardiovascular system. A prominent effect caused by histamine leads to severe bronchoconstriction in the wistar rats that causes asphyxia sometimes death (Armitage et al., 1961). Hence broncho dilators are used to delay the occurrence of these symptoms. The results obtained from the preliminary study of antispasmodic activity confirmed the bronchodilator properties of the leaf extract of Tarenna asiatica.

The present findings reveal that the antihistaminic activity of leaf extract of Tarenna asiatica may be due to the inhibition of histamine induced bronchoconstriction. This is in line with the report of Ninave et al. (2011) who stated that Randia dumetorum plant extract offered protection against anaphylactic shock induced bronchospasm in rats. In the present study exposure of wistar rats to histamine aerosol caused convulsion due to bronchoconstriction. This is in agreement with the report of Bosquet et al. (2000) who stated that histamine, acetylcholine, leukotrienes and prostaglandins mediators directly cause acute bronchoconstriction. In the present study the standard drug promethazine gave good protection against histamine aerosol as reported by Armitage et al. (1961). It has gained widespread clinical use with many different indications. The marked antihistaminic effect of the drug is responsible for its use in treating bronchospasm disorders.

The test drug in crude form offered only 43.08% protection against histamine aerosol and prolonged the convulsion time. Chauhan et al. (2012) observed 47.45% protection of root extract of Clitoria ternatea against histamine induced bronchoconstriction. This is in line with the present findings. Similar observation was made by Parmar et al. (2010). One of the possible mechanisms for the spasmolytic activity of the extract could be mediated through the inhibition of histaminic receptors. The less percent protection offered by leaf extract of Tarenna asiatica might be due to the crude nature of the extract. This observation corroborate the views of several authors (Parmar et al., 2010; Bigovic et al., 2010 and Chauhan et al., 2012).

There is growing evidence that the spasmolytic effect of the extract is associated with the presence of phenolic compounds. Flavonoids are one of the most numerous and
widespread groups of phenolics present in the *Tarenna asiatica*. Based on this report, the spasmolytic activity of *Tarenna asiatica* could be attributed to flavonoids and other phenolic compounds. The key role of phenolic compounds and other secondary metabolites as spasmolytic agents has been emphasized in several reports (Gharzouli and Holzer, 2004; Ghayur *et al*., 2005; Goze *et al*., 2009 and Cimanga *et al*., 2010).

The results obtained in this study clearly demonstrate that the leaf extract of *Tarenna asiatica* possesses antispasmodic activity against histamine induced broncho constriction.

### 7.5. *In vitro* Regeneration Technique

Conservation and propagation of medicinal plants are important from the standpoint of bioprospecting as well as bio-resource management. *Tarenna asiatica* reported to be rich in phenolic compounds and flavonoids, is used in skin diseases, especially the leaves are known to possess, antimicrobial, wound healing properties, antioxidant activity, skeletal muscle relaxant activity and antispasmodic activity. To date, studies on the *in vitro* regeneration of *Tarenna asiatica* are not thoroughly documented. Owing to the immense medicinal value, the demand for the supply of the plant is on an increase. As part of the conservation and management strategy of medicinal plants, an *in vitro* protocol for micro propagation and regeneration of *Tarenna asiatica* is presented.

**Effect of 2, 4 - D on Callus Induction**

In the present study, the leaf explants gave differential response to different concentrations of 2, 4-D and produced yellowish to greenish yellow compact calli on medium fortified with 1.5-2.5mg/l 2, 4-D alone. 0.5mg/l and 1.0mg/l concentrations of 2, 4-D did not induce callus and found inferior in inducing the callus whereas good results were obtained at 1.5 and 2.0mg/l concentrations. The maximum amount of callus tissue per explant was produced on MS-medium with2.0mg/l 2, 4-D with 60% response by showing greenish white, hard, compact calli. Similar kinds of proliferation of callus tissue in 2, 4-D supplemented media under *in vitro* conditions were also noted in other
medicinal plants including *Ixora chinensis* (Noreen *et al*., 2001); *Morinda citrifolia* (Selvaraj *et al*., 2006) and *Tragia involucrata* (Dharmendra and Sudarshana, 2010).

Among the three concentrations, 2.0mg/l was found satisfactory for enhancing hard, compact pigmented calli on leaf explants after 20 days of inoculation. Noreen *et al*. (2001) reported that callus initiation was higher in MS medium having 2, 4-D at 3mg/l concentration, but in the present study, 2.5mg/l concentration produced low amount of calli and 2.0mg/l 2, 4-D induced high amount of calli. Thus 2.5mg/l 2,4-D maybe slightly inhibitory in the production of calli. This is in consonance with the report of Dharmendra and Sudharshana (2010) who stated that 2.0mg/l 2, 4-D induced light yellow or greenish yellow callus with 83.3 % response and higher concentration produced friable callus with 41.6% response. Beneficial effect by the addition of activated charcoal (0.1%) towards inhibition of exudation of poly phenols was achieved as reported by Poornima and Shivamurthi (2008). Young tissues are less prone to browning on excision than older ones as reported by George and Sherrington (1984). In the present study, the young leaf explants showed very good response towards inhibition of polyphenols on the addition of activated charcoal.

**Effect of BAP+KIN on Shoot Induction**

In order to induce differentiation in the leaf derived tissues, they were further cultured on different concentrations of BAP alone and on different concentrations of BAP+KIN combinations. The callus tissue underwent differentiation and produced shoot buds on MS medium supplemented with 1.5-2.5mg/l BAP either alone or in combination with Kinetin. The highest number of shoot buds (5.6±0.11)/mass of culture was observed in the medium containing 2.0+2.0mg/l BAP+ KIN combination.

The highest proliferation rate (50%) was also found in this combination of medium. Development of shoot buds via induction of callus tissue indicated the occurrence of indirect organogenesis and such differentiation of tissue was also noted in other medicinal plants including *Rudgea jasminoides* (Stella and Braga, 2002); *Gardenia jasminoides* (AlJubooriy et al.,1998) and *Morinda citrifolia* (Selvaraj *et al*., 2006).
Efficiency of the media was assessed in terms of enhancement of shoot bud production. Such enhancement of shoot bud has been noted in many other medicinal plants including *Mitragyna parvifolia* (Roy *et al*., 1988), *Gardenia jasminoides* (AlJubooriy *et al*., 1998) and *Ixora coccinea* (Lakshmanan *et al*., 1997). Subramani *et al*., (2007) clearly indicated that it is the hormonal combinations which are very vital for *in vitro* response, BAP alone for shoot initiation, Kinetin along with BAP for multiple shoot formation. This is in consonance with the present findings. The present views are also corroborate the findings of Venkateshwarlu (2007) who observed callus and shoot bud initiation from the leaf explants of *Zizyphus mauritiana* on MS medium supplemented with BAP+ Kinetin. In the present study 2.5+2.5 mg/l BAP and Kinein slightly inhibited the shoot bud formation and showed reduced percent response. This is in line with the report of Dasilva *et al*., (2008) who recorded a similar pattern of shoot bud differentiation and suggested that the higher concentration of BAP had an inhibitory effect on shoot bud formation. In the present investigation a maximum of 5.6 shoots were formed per culture. This may be due to the shubby nature of plants.

**Effect of NAA and IBA on Root Initiation**

Spontaneous rooting was observed in secondary medium containing NAA alone and in NAA and IBA combination. Root induction of cultured plantlets was observed. Within 15 days of culturing, there was no initiation of root at 0.2mg/l concentration of NAA and at 0.2+0.1 mg/l concentration of NAA+IBA. Response of shoots to rooting was very much dependent on the concentration of NAA and combinations of NAA and IBA provided. The highest mean number (4.9) of roots per culture was noted in half strength MS medium supplemented with 0.8mg/l NAA+0.4mg/l IBA. IBA was the most effective auxin. At all the tested concentrations, except 0.2 + 0.1, it showed some rooting, however, the best rooting was observed when shoots were transferred to 0.8+0.4mg/l NAA+IBA concentrations.

Success of IBA for efficient root induction was also reported by Alam *et al*., (2010) in *Paederia foetida*, a Rubiaceae member at 0.3mg/l concentration of IBA. This is in line with present findings. Turker *et al*., (2001) suggested the efficacy of NAA at lower
concentrations on *in vitro* rooting. In present study, rooting was induced at 0.6 mg/l concentration of NAA. Rooting of shoots through basal callusing is not recommended because in most cases proper acclimatization of the plantlet could not be achieved. Thus direct root induction in micro shoots is described as the most advantageous for successful establishment of the plant in natural conditions. Higher concentrations of NAA alone or NAA +IBA concentrations produced less number of roots. Similar observations were recorded by Alam *et al.* (2010) in *Paederia foetida* and Chaudhuri *et al.* (2004) in *Tylophora indica*. The influence of IBA for induction and proliferation of root growth has been reported by Roy *et al.* (1988)

**Acclimatization**

Acclimitisation is the most crucial stage for success of any *in vitro* regeneration protocol. Successful establishment of *in vitro* regenerated plantlets in field conditions requires great care.

Plantlets with fully expanded leaves and well developed roots after removing the nutrient medium through gentle washing were transferred to thermocol cups for the purpose of hardening. The *in vitro* grown complete plantlets were then successfully transferred from the culture room to earthen pots outside through a process of successive phases of acclimatization. Most of the regenerated plants survived. Although the survival rate was 70%, the surviving plants have grown to a mature stage. Phenotypic variations in regenerated and acclimatized plants were not observed and the plants behaved normally.

The overall result of the preliminary study revealed that the propagation of *Tarenna asiatica* is possible through induction of organogenesis in leaf originated tissue. It was further proved that induction of tissue growth and its differentiation was dependent on growth regulator supplements in the medium. Though the percentage survival of transferred plantlets to the outside environment was not very high, this technique can be adopted to conserve *Tarenna asiatica*, a valuable skin disease plant. Further experiments are in progress to pinpoint the factors related to the improvement of this culture technique and establishment procedure.