5. DISCUSSION

GENERAL DISCUSSION

Nature has been a source of medicinal agents for thousands of years and continues to be an abundant source for novel chemo types and provides novel chemical scaffolds for elaboration by combinatorial approaches (Cragg et al., 2005). Indian and Chinese systems of traditional medicine date back to 3000 years and majority of its medicinal plants have been investigated for pharmacological targets such as HIV, cancer, malaria, cardio vascular diseases, neurological disorders, etc (Ramawat et al. 2007). WHO has estimated that at least 80% of the population globally relies on traditional medicine to meet their primary health care needs (Bannerman et al., 1982). Materia Medica of India provides lots of information on the folklore practices and traditional aspects of therapeutically important natural products. Traditional folk medicine uses the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to its cultures, for maintenance of health. It holds a heritage of community acceptance, and is solely based on the expertise gained by local herbalists over a period of time (Ayensu et al., 1986).

Current research in drug discovery involves a multifaceted approach like botanical, phytochemical, biological and molecular techniques to provide new and important leads for drug candidates (Balunas et al., 2005). Plant extracts used in ethno medical treatments enjoy great popularity, however, lacks scientific validation. Therefore, ethno botanists, all over the world, have been actively working to collect, document and conserve the indigenous medicinal plants. Studies on the use of and hunt for plant based drugs have accelerated in recent times as they are safe and have fewer side effects. Ethno-pharmacologists, botanists, microbiologists, and pharma-chemists are to comb in the hunt for novel bioactive compounds "leads" which could be developed as an effective drug for
treatment of various infectious diseases (Cowman et al. 1999, Pushpangadan et al., 1984). India is sitting on a gold mine of well-recorded and traditionally well-practiced knowledge of herbal medicine. This country is perhaps the largest producer of medicinal herbs and is rightly called the botanical garden of the world (Dubey, 2004).

India has over 84.3 million tribals, belonging to diverse ethnic groups. In India, there are 550 communities of 227 ethnic groups as per the classification made by the anthropologists on linguistic basis (Pushpangadan 2008). In Kerala, the major tribal communities are nearly forty in number. The major tribes of Kerala are Kani, Kurichiar, Kurumar, Eravallan, Kattunaikkan, Muthuvan etc. (Joseph & Antony, 2012). Forest have been the home for many of these tribes and they have deep rooted association with the forest and nature around. Their relationship with the forest has always been harmonious and their whole life revolves around forest and forest resources. They have acquired unique knowledge about the use of many wild flora and fauna through generations most of them are lesser known or hitherto unknown to the outside world. This treasure of traditional knowledge if subjected to scientific scrutiny could be highly beneficial to tribals, the country and the humankind in many ways. There are several medicinal plants, it enables traditional healers to develop effective therapies against various ailments. These plants are used only at the tribal level are found to have very good antibacterial and wound healing properties. But scientific validation and authentication of these species is needed to establish with evidence based therapeutic activity. It was in this background the present exercise of survey, documentation and scientific validation of medicinal plant, commonly used for the skin diseases by the tribes and local health practitioners of Malabar region of Kerala.

Skin disorders affect 20-30% of general population in the world. Socio demographic factors play a pivotal role in determining the pattern of skin diseases. More over school going children are more frequently exposed to various risk factors. Since prevalence of skin diseases is more among children from low
socioeconomic classes and developing countries like India. Studies on skin
diseases are inevitable.

Herbal therapy for skin disorders has been used for thousands of years.
Specific herbs and their uses developed regionally, based on locally available
plants and through trade in ethno botanical remedies. Systems of herbal use
developed regionally in Europe, the Middle East, Africa, India, China, Japan,
Australia, and the Americas (Ghazanfar 1994; Behl and Srivastava 2002). Two
well-known systems still in use are the Ayurvedic herbs in India and herb
combinations developed as part of traditional Chinese medicine (TCM) in China
(Kapoor 1990; Xu 2004). In Europe and the United States, use of herbs declined
as purified extracts and synthetic chemical drugs became available. In recent
years, there has been a resurgence of the use of herbs due to the following
reasons: the side effects of chemical drugs became apparent, there was a call to
return to nature, natural remedies became a part of the green revolution, and there
was a return to organic produce. Herbal remedies, including those for skin
disorders, are currently gaining popularity among patients and to a lesser degree
among physicians. In Asia, especially in China and India, herbal treatments that
have been used for centuries are now being studied scientifically.

In addition to documenting the traditional knowledge related to medicinal
plants, scientific validation of traditional medicinal plants has been an important
path of recent research (Uprety et al, 2010). Validation is performed by in-vitro
or in-vivo experiments or by isolation of important secondary metabolites that are
useful for treating particular types of diseases or disorders (Taylor et al, 1996;
Adzu et al, 2004; Rokaya et al, 2012). In addition, previously published studies
can also aid in establishing links between traditional uses and modern scientific
knowledge (Gaire et al, 2011). The practice of seeking evidence helps in
identifying important medicinal plants and may also lead to the development of
new or important pharmaceutical drugs with future bio prospecting potential

Skin is the largest organ of the body, accounting for about 15% of the total
adult body weight and a major part of the body contact with the outside world.
Plants and plant extracts have been used for the treatment of skin disorders for centuries (Augustin and Hoch, 2004; Avalos and Maibach, 2000; Schempp et al., 1999). Because of increasing resistance to antibiotics of many bacteria, plant extracts and plant compounds are of new interest as antiseptics and antimicrobial agents in dermatology (Augustin and Hoch, 2004; Blaschek et al., 2004; Norton, 2000). Therefore, many folk remedies from plant origin are to be tested for their potential antibacterial properties causing skin disease in experimental models. It was in this background the present exercise of survey, documentation and scientific validation of plant, commonly used for the skin ailments by the traditional healers of Malabar region of Kerala

5.1. SURVEY AND TAXONOMIC STUDIES

The objective of the present study was to find out a scientifically unexplored, effective, reliable and non-toxic medicinal plant used for the skin disease of Malabar area of Kerala. As per the survey conducted in this area revealed that, the tribes are using several plants for skin ailments but many of these plants are common to many skin disease.

The Malabar region of Kerala was explored during the ethno botanical survey. Survey was conducted in the study area from March 2008 – June 2011. Malabar is endowed with more than one-half of the geographical area starting from Thrissur to Kasaragod in the northern part of Kerala and also blessed with more than one-half of the total inhabitants of the state. Geographically, the Malabar Coast, especially on its westward-facing mountain slopes, comprises the wettest region of southern India as the Western Ghats intercept the moisture-laden monsoon rains.

The results of the present study demonstrate the persistence of folk medicine for different kinds of skin diseases in Malabar region of Kerala. The people are still depended on indigenous knowledge for primary health care. The interviewees mentioned 16 different skin diseases which are treated with different plant-based medication (Figure 3). Impetigo (32 interviewees) are by far the most recorded skin problem treated (Fig.4) followed by Eczema (26 interviewees),
Boils (20 interviewees), Scabies (14 interviewees), wounds (13 interviewees) and Ringworm infection (10 interviewees). Several ethanobotanical studies had been conducted in the Malabar region of Kerala, but the survey of medicinal plants used for skin diseases in particular was the first time and it was published by the present authors. (Deepthy and Remashree, 2014). The survey is conducted among the traditional physicians (Nattu vaidyas). The traditional physicians may belong to a forward cast or other backward cast or belongs to a tribal community. The nattuvaidyas practicing in rural areas are neither qualified nor registered with any state counsel. They acquire the knowledge of treatment only from experience. Vaidyas use bhasmas (ash) and gutikas (tablets), asavas (fermented medicine) and kashayas (decoctions) to cure the diseases.

Treatments are done with either single plant formulation, combinations of several plants or even with many parts of the same plant. From this survey, it is revealed that out of 113, 17 formulations involved multiple drugs obtained from two or more different plant species. In the treatment of skin diseases the traditional physicians will give advices according to the intensity of the disease. For internal use most of the traditional physicians of the study area follows Ayurvedic treatises for drug preparations. For external application they keep their uniqueness. In most of the case leaves are frequently used plant parts for the drug preparations (48%) (Fig. 2). Of all the drug preparations, paste is the most preferred method followed by fresh juice extraction (Fig. 3).

In India Nagariya(2010) made a detailed review on plants having medicinal properties to cure skin disease. Pradeep Bhatt et. al. (2013), have reported medicinal plants used for curing skin diseases in coastal parts of central Western ghats, Karnataka district. Bhatt et al. (2012) conducted a survey on ethno medicinal practices in different communities of Uttara Kannada district of Karnataka for treatment of wounds. Sivaranjini et. al. (2012) documented the traditional use of medicinal plants in treating skin diseases in Nagapattanam district of Tamilnadu. They have reported the use of 50 species belongs to 26 families. Manisha yadev et. al. (2012) reported 23 plant species belongs to 17 families having medicinal property for curing skin diseases. The studies of Sanjeet

In Kerala, general survey was conducted and no specific studies related to skin diseases. Silja et al. (2008) have studied the ethno medicinal plant knowledge of the mullukuruma tribe of Wayanad district, Kerala. They reported 18 plant species to cure Skin diseases. Deviprasad (2012) and Shyma, (2013) conducted field general survey among the tribes of Vythiri thaluk and Mananthavady thaluk of Wayanad district. Lincy (2013) surveyed the ethno medicinal practices of Tribal inhabitants of Attappady and Vakkodan Hill regions of Kerala and reported 13 plants for treating skin diseases. Udayan et al. (2008) have reported some common plants used by Kurichiar tribes of Tirunelly forest, Wayanad district, Kerala in medicine and other traditional uses.

Several ethanobotanical studies had been conducted in the Malabar region of Kerala, but the survey of medicinal plants used for skin diseases in particular was the first time reported by Deepthy and Remashree (2014). The present study revealed the use of 113 species of plants distributed in 103 genera belonging to 45 families which were commonly used by most of the traditional healers for the treatment of 16 types of ailments. The prominent family was Fabaceae with 14 species, followed by Lamiaceae with 9 species and Euphorbiaceae with 7 species (Figure.5). Each reported species are provided with botanical name, family, local
A large number of crude drugs used in Ayurvedic system and for skin disease employ plants belonging to the family Fabaceae. The family Fabaceae popularly known as legumes, is the third largest order of seed-plants containing about 600 genera with 12,000 species. The family includes the greatest number of Legumes, comprising 400 genera with about 7000 species. It is an extremely important family and its members yield nutritious food, fiber, shelter, valuable medicines and also virulent poisons (Datta and Mukherji, 1952). From the study, 14 Fabaceae members are used for the skin disease in the selected study area.

The Lamiaceae (Labiatae) is one of the most diverse and widespread plant families in terms of ethno medicine and its medicinal value is based on the volatile oils concentration. The Lamiaceae plant family is one of the largest families among the dicotyledons, many species belonging to the family being highly aromatic, due to the presence of external glandular structures that produce volatile oil. This oil is important in pesticide, pharmaceutical, flavouring, perfumery, fragrance and cosmetic industries (Giuliani et. al., 2008). The present survey reveals that 9 members from this family used against skin diseases in Malabar area.

Species of Euphorbiaceae have been used by local population of many countries in folk medicines as remedies against several diseases and complaints such as cancer, diabetes, diarrhoea, heart diseases, hemorrhages, hepatitis, jaundice, malaria, ophthalmic diseases, rheumatism and scabies etc., (Ayensu, 1978; Baslas, 1982). The survey shows that seven plants from Euphorbiaceae are used against skin diseases in the selected study area.

The medicinal uses of plants gathered in this study were compared with the previously published information from other parts of world and other parts of India. From the investigation it was found that there were 48 new claims about the plant species from the study area such as Beloperone plumbaginifolia L., Amaranthus viridis L., Aristolochia indica L., Bischofia javanica Blume., Cissus **
quadrangularis L., Justicia beddomei (Clarke) Bennet, Holigarna arnottiana Hook. f., Anethum graveolens L., Cocos nucifera L., Areca catechu L., Ayapana triplinervius Vahl., Elephantopus scaber L., Salacia fruticosa Heyne ex Lawson, Commelina erecta L., Shorea roxburghii G.Don., Briedelia stipularis (L.) Blume., Flueggea virosa (Roxb. ex Willd.) Voigt., Tragia involucrata L., Phyllanthus emblica L., Cullen corylifolium (L.)Medik., Derris scandens (Roxb.)Benth., Desmodium motorium(Houtt.), Merr., Desmodium triflorum Anisomeles indica (L.) O. Ktze., Plectranthus hadiensis (Forssk.) Schweinf.var.tomentosus (Benth.ex.Emey.) Codd., Premna serratifolia L., Vitex negundo L., Allium cepa L., Grewia tiliifolia Vahl., Pavonia odorata Willd., Tinospora cordifolia (Willd.) Miers ex Hook. F. & Thoms., Naragamia alata Wight & Arn., Myristica fragrans Houttt., Ficus racemosa L., Piper betle L., Oldenlandia umbellata L., Spermacoce lattifolia Aubl., Glycosmis pentaphylla (Retz.)DC., Citrus limon (L.)Burn.f., Ventilago madraspatana Gaerh..Fruct., Smilax china L., Bacopa monnieri (L)Pennell., Picrorrhiza kurroa Royle ex Benth., Symplocos cochinchinensis (Lour)Moore., Dendrocnide sinuata (Blume) Chew., Zingiber zerumbet (L) J.E Smith., Marsilea minuta., were reported for the first time from the study area. (Table 1). However, no plants were reported as a new medicinal plant as all the plants were reported with different uses.

Habit-wise analysis of the plants indicates that herbs are highly used followed by trees, shrubs, and climbers (Fig. 1). In most of the case leaves are frequently used plant parts for the drug preparations (48%) followed by whole plant (17%), bark (9%), root (7%), fruit, rhizome (5%), seed (4%), and flower, endocarp, stem (2%), resinous exudation (1%) (Fig. 2). The reason why leaves were used mostly is that they are collected very easily than underground parts, flowers and fruits etc. (Giday et al., 2009) and in scientific point of view leaves are active in photosynthesis and production of metabolites (Ghorbani, 2005). In the present survey 48% leaves are used for treating the disease. The leaves are recommend as sustainable part for harvesting.

Of all the drug preparations, paste is the most preferred method (31%) followed by fresh juice extraction (17%), oil extract (15%), Infusion (12%), root
paste (9%), decoction (4%), fruit juice, rhizome (3%), seed oil, whole plant juice (2%), ash (1%) (Fig. 3).

Like many other forms of indigenous knowledge, the nattu vaidya tradition suffers from lack of support, and the rapidly diminishing number of local folk medical practitioners or the vaidyas and their existence is also threatening (Dubey et al., 2004). Hence all these traditional knowledge acquired from the present study required further scientific evaluation and documentation.

5.2 QUANTITATIVE ANALYSIS OF DATA

The information such as botanical name, family, part used, and Use value are provided for each species (Table 2). Informants consensus factor (Table 3), fidelity level (Table 4), Correlation between fidelity level and citation of the plants are tabulated in (Table 5) separately. The ICF was used to analyse the agreements degree of the informants knowledge about each category of ailments (Heinrich, 1998). Fidelity level was employed to determine most important species of plants used to cure skin diseases by traditional physicians and elderly resident people (Alexiades 1996).

Use value’ is the positive correlation between the importance value of a plant (measured by the number of informants for a particular plant) and the number of uses cited by the informants. The use value (UV), a quantitative method that demonstrates the relative importance of a species known locally.

As indicated in Table-1, UV is calculated for all the species and the single use by the single informant is not considered for analysis. The plant species, *Derris scandens, Aristolochia indica* scored a high UV (1.50 each), followed by *Myristica fragrance* (1.33 each), then *Vitex negundo* and *Bischofia javanica* (1 each) respectively. Plant with high UV is the indication of its importance in the herbal medicine practice.

The informant consensus factor (ICF) was used to identify the ethno pharmacological importance of the collected plant species. ICF values will be low (near 0), if plants are chosen randomly or if informants do not exchange
information about their use. A low value indicates that the informants disagree on the taxa to be used in the treatment within a category of illness. Values will be high (near or more than 1), if there is a well defined selection criterion in the community and/or if information is exchanged between informants or high value (close to 1.0) indicates that relatively few taxa are used by a large proportion of the informants.

The treatment for different types of skin diseases by the herbal healers are classified in to 16 categories and the ICF values for each category are given (Table 2). ICF values obtained for the reported categories indicate the degree of shared knowledge for the treatment of each category of ailment. The highest ICF is scored for Impetigo (ICF=0.58). 14 plants are used to treat this ailment and were cited by the 32 practitioners. Most of the practitioners quoted leaves of *Aristolochia indica*, *Derris scandens*, and *Myristica fragrans* to treat the disease. Boils scored the second highest value of ICF (0.36). It is a very common type of skin infection in the study area. 13 plants with 20 citations are recorded to treat the ailment. Leaves of *Vitex negundo*, *Bischofia javanica* and *Centella asiatica* are extensively used to treat the infection. Psoriasis recorded the third highest value (ICF=0.25). 7 plants with 9 citations are recorded to treat the ailment. Eczema recorded the third highest value (ICF=0.2) Comparatively low scores are for insect bite, Foot crack, Athlete's foot disease, Tinea infection, Leucoderma, Ringworm infection, Wound healing, anti-inflammatory activity, Chicken pox, Scabies, Burns. They scored ‘zero’ or less than zero as the informants did not share their knowledge to treat these ailments. ICF value is a proportion between the uses of different plants by various herbal healers for a particular disease category suggesting the level of sharing the knowledge about the use of medicinal plants amongst the healers. High ICF value indicates the use of same plants by many healers. Whereas negative value means use of different plants by many healers and ICF value would be ‘zero’ when different plants are employed by few herbal healers.

The fidelity level (FL) is useful for identifying the inhabitants’ most preferred species in use for treating certain ailments. FL values vary from 1.0% to
100%. Generally, a FL of 100% for a specific plant indicates that all of the use-reports mentioned the same method for using the plant for treatment.

Fidelity level is calculated for the plants which are used to treat most frequently reported ailment categories for both higher ICF values (ICF=0.2 and above) and lower ICF values (ICF=0.00), as both have scored a highest fidelity value (50% or above). The plants cited only once by a single informant are not considered for the Fidelity level analysis. In the higher ICF category 6 plants for boils, 5 plants for Impetigo, 6 plants for Psoriasis, and 2 plants for Eczema scored 100% fidelity level. On the other hand only 5 plants for Scabies, 3 plants for Ringworm infection scored 100% fidelity level in case of lower ICF category (Tables 4 and 5).

Correlation between fidelity level and Use mention (Np) of the plants: Comparative analysis of the data is an important criterion for evaluating the medicinal value of a particular plant. A correlation between FL and Np revealed that, plants with highest FL may score low Np and such plants are of less significance. Whereas, plants which score high FL and high Np for a particular disease are worth taking up for further phytochemical analysis. (Table5).

As a result of survey report, review of literature and quantitative analysis of data, the plants with high Use value, high fidelity level, five traditionally important plants such as Aristolochia indica, Derris scandens, Myristica fragrans, Bischofia javanica, Vitex negundo are used for the skin diseases and these species were selected for further scientific validation.

5.3. VALIDATION

PHARMACOGNOSTIC STUDIES

A) Aristolochia indica L.,

Pattar and Jayaraj (2012) reported the pharmacognostic and phytochemical details of whole plant of Aristolochia indica and character of powder of Aristolochia indica L. with different chemical reagents. T.S of leaf support his findings. But the present investigation shows some differences.
Similar to the previous study anisocytic stomata present in the lower epidermis but paracytic stomata are not found. The following characters like, the presence of 1-2 layers of collenchymatous cells are seen in between palisade cells, crescent shaped vascular bundle, Medullary rays are uniseriate, sclerenchymatous bundle sheath, large prismatic crystals of calcium oxalate in the midrib are not mentioned in the previous study.

No previous powder microscopic studies was reported in the selected plant. The major features of powder microscopy are anisocytic stomata in the lower epidermis; Groups of crystals fibres associated with xylem elements; entire and fragments of trichomes, fragments of scleried; scattered prismatic calcium oxalate crystals. The histochemical studies shows the presence of tannin and lignins

**B) *Derris scandens* Benth.**

Metcalfe and Chalk (1972) studied the anatomical details of family Fabaceae. The characteristic feature of the Genus *Derris* shows that the epidermis of the leaf is characterized by the occurrence of angular folds in the anticlinal walls. The presence of secretary canals, lower epidermis is pappillus or sub pappillus. The central layer of mesophyll occupied by cells containing little chlorophyll and often but not invariable filled with tanniferous content which are coloured brown in dried materials in petiole and mesophyll. Small crystalline grains were present in the mesophyll is the characteristic of the genus.

The diagnostic features of T.S of leaf are single layer of upper and lower epidermis with a thin layer of cuticle. Cells of the upper epidermis are large oval or round in C.S and devoid of stomata. Cells of the lower epidermis are comparatively small in size with a few unicellular finger like pappillose trichomes and a number of anisocytic stomata. In the midrib portion just below the upper epidermis 1-2 layers of collenchymatous cells are seen in between palisade cells which extends in to the midrib. In the lower region collenchymatous cells are seen in single layer just inner to the lower epidermis crescent shaped vascular bundle
is embedded in the parenchymatous tissue. Vascular bundle consists of single row of xylem vessels arranged in radial rows towards the lower side. Medullary rays are uniseriate. The vascular bundle covered by 3-4 layers of sclerenchymatous bundle sheath. Many of the parenchyma cells of the midrib contain large and small prismatic crystals of calcium oxalate.

In the lamina portion palisade cells are in two layers and are followed by 4-5 layers of spongy mesophyll. At intervals vascular strands are present with sclerenchymatous covering which extends upto the upper epidermis towards the upper side. Small prismatic crystals of calcium oxalate are present in the mesophyll cells but Metcalfe and Chalk (1950) mentioned it as crystalline grains. Histochemical studies show the presence of lignin in the xylem vessels and phloem fibers on upper and lower side of the bundle. In petiole a few starch grains are present in the ground parenchyma cells of midrib and petiole.

Powder microscopy shows fragments of upper and lower epidermis in surface view. Fragments of upper epidermis seen associated with underlying palisade cells; Lower epidermis possess anisocytic stomata; Groups of crystals fibres associated with xylem elements; cross sectional view of fragments of epidermis with palisade cells; entire and fragments of trichomes, fragments of scleried, prismatic crystals of calcium oxalate seen scattered throughout the section.

C) *Myristica fragrans* Houtt.

Mary Helen et al (2012) reported the histochemistry of *Myristica fragrans* leaf and pointed out the presence of oil and phenolics. General anatomical feature and powder microscopy of leaf were not previously reported. The characteristic features of the leaf are very specific. T.S shows slightly oval in shape with a small wide, concave depression or flat at the adaxial side with two lateral upward projections. Lower side of the petiole is convex or with two small projections forming a shallow notch in the centre. There are three large and two small bundles arranged in the form of a crescent. Each bundle shows radially arranged xylem vessels towards the adaxial side and phloem towards the abaxial side. A single layer of chlorenchymatous cells partially
encircles or borders the bundles towards the lower side. Epidermis single layered and is followed by 4-5 layers of collenchymatous hypodermis. Lateral projections are composed of collenchymatous hypodermis. The ground tissue is parenchymatous arranged loosely with intercellular spaces.

T.S of leaf shows a dorsiventral nature with distinct lower and upper surface. Upper and lower side of the midrib is convex with single layered epidermis and collenchymatous hypodermis. In the upperside collenchymatous hypodermis forms a patch just below the epidermis. The extension of palisade cells enters in to the midrib up to collenchymatous patch. Centre of the midrib is encircled by a large bundle with many xylem vessels arranged in scattered manner. A few thin walled pericycle fibers are seen outside the phloem. A small patch of wide sclerenchymatous cells present above the vascular bundle. Ground tissue is parenchymatous with intercellular spaces.

T.S of lamina shows a single layer of comparatively large epidermal cells with thick cuticle. Upper epidermis is followed by a single layer of compactly arranged palisade cells. Spongy parenchyma is 4-5 layered and loosely arranged. Lower epidermis is single layered and possess uniseriate multicellular trichome with hook shaped tips. Lower epidermis possess anomocytic stomata.

Histochemical study for lignin showed the presence of xylem vessels as lignified cells in petiole. In leaf lignin is present in the xylem vessels and phloem fibers on upper and lower side of the bundle. A few starch grains are seen in the parenchyma cells of petiole and midrib. Oil cavities are present in the lamina.

Characteristic features of powder study shows fragments of upper epidermis with cells having straight walls, fragments of lower epidermis with anomocytic stomata and wavy walled epidermal cells, surface view of lower epidermal cells with trichome base fragments, reticulate vessels, Cluster crystals of calcium oxalate.

D) Bischofia javanica

Metcalfe and Chalk (1972) studied the anatomical details of family Euphorbiaceae. In leaf, marginal pit to the epidermal cells, presence of
mucilage cavity are reported characteristic feature of the genus *Bischofia* by Metcalfe and Chalk (1972). No previous pharmacognostic study was reported in this plant.

The characteristic features of leaf are shield shaped with wavy ring of vascular bundle in the centre having infolding towards the adaxial side. The adaxial side of the petiole is flat and abaxial side is deeply convex. Epidermis is double layered with thick cuticle. Ground tissue is parenchymatous with intercellular spaces. Some of the cells contain tannin deposition and prismatic crystals. Vascular bundles are conjoint, collateral and closed. Each vascular bundle consists of radially arranged xylem vessels towards the centre and phloem towards periphery. Tannin depositions are more in phloem region and xylem vessels when compared to ground parenchyma. A narrow strip of pericyclic fibers are present external to the phloem in each vascular bundle.

T.S of lamina shows dorsiventral nature. Pallisade cells are extended in to the midrib up to the collenchymas patch. One layered palisade tissue, where reset of the lamina portion is occupied by oval shaped spongy parenchyma. Large tangentially elongated schizogenous cavity lined by 2-3 rows of epithelial cells. Large rosette crystals and tannin cells are embedded in the mesophyll region. Lower epidermis possess a large number of Anisocytic stomata.

Histochemical study for lignin showed the presence of xylem vessels as lignified cells in rachis and petiole. In leaf lignin cells are represented as a continuous band of pericyclic fibers. Oil globules are present in the trichome. Starch grains are present outer to the vascular bundle.

Powder shows fragments of tannin deposition, longitudinal view of pericyclic fibers, fragments of lower epidermis with stomata, rosette crystals xylem with spiral thickening, longitudinal view of pitted trachieds, fragments of upper epidermis.

**E) Vitex negundo**

The histochemical study was reported by Dhale (2011). Quality standards
of leaf was reported by Gupta (2005). All the pharmacognostic characters are
complies with the earlier report by Gupta (2005). The major parts are rachis,
petiole, leaf and lamina. The diagnostic feature of the rachis is circular in
outline. The epidermis is covered with plenty of trichomes. The hypodermis is
collenchymatous. The hypodermis at its upperside slightly protrudes inwardly.
Xylem and phloem forms a crescent shape in the centre and it is embedded in the
parenchymatous ground tissue. The two ends of xylem and phloem is connected
to the two vascular bundles situated underneath the chlorenchymatous band; The
pericycle is discontinuous due to the presence of lignified fibers. cells of the pith
are pitted and lignified: cluster crystals of calcium oxalate are present throughout
the parenchymatous cells of the section.

The cross section of petiole is oval in outline. Plenty of trichomes are
present in the epidermis like that of rachis. The adaxial side of the petiole slightly
concave with two wings on either side. The hypodermis is collenchymatous. The
xylem and phloem forms a crescent shape similar to rachis. The pericycle fibers
are present in the centre of the ground tissue. Pith shows the presence of Lignified
and pitted cells. Calcium oxalate crystals are present in the parenchyma cells.

The section of leaf is strongly convex on its abaxial side and somewhat
flattened on its adaxial side. The xylem and phloem forms a U-shape in the centre.
3-5 row of palisade cells are present in the lamina portion and it extends up to the
centrally located vascular bundle. Below the epidermis lies the collenchymatous
tissue: the remaining ground tissue of the midrib being parenchymatous. The
upper epidermis of the lamina composed of polygonal, cells devoid of stomata:
thin cuticle and bears few trichomes; the cells of lower epidermis are much
smaller in size, stomata and trichomes are plenty. Both simple, unicellular, and a
few multicellular uniseriate 2-6 celledtrichomes are present. The parenchymatous
cells contain rosette crystals and oil globules.

Histochemical study for lignin showed the presence of xylem vessels as
lignified cells in rachis and petiole. In leaf lignin cells are represented as a
continuous band of pericyclic fibers. Oil globules are present in the trichome.
Starch grains are present outer to the vascular bundle.
Powder shows various types of plenty of trichomes consisting of simple, unicellular, short, conical, with pointed or blunt apex, collapsed or warty, multicellular trichomes are 2-7 celled ,often abruptly bent at its apex, collapsed or warty, occasionally branched; glandular trichomes are sessile with globular 4 celled or one celled head ,or stalked one, with unicellular head and unicellular stalk and unicellular stalk with bicellular head; occasionally transversely cut fragments of an arc of epidermis covered with trichomes, fragments of transversely cut lamina, upper epidermal thick cells of lamina devoid of stomata, lower epidermal cells in surface view with plenty of anomocytic stomata and trichomes are present. Rectangular parenchymatous cells of the rachis containing calcium oxalate crystals, lignified pitted pith cells of the rachis ,vascular strands of the rachis with pitted squarish cells of the parenchyma are seen.

5.4. PHYTOCHEMICAL STUDIES

The plants selected for scientific validation are subjected to detailed phytochemical screening. The crude extracts of leaves of *Aristolochia indica*, *Derris scandens*, *Myristica fragrans*, *Bischofia javanica*, and *Vitex negundo* revealed the presence of alkaloids, flavonoids, terpenoids, steroids, phenolics, carbohydrates, amino acids and quinines. In *D. scandens* and *M. fragrans*, the test for the alkaloids given negative results. Carbohydrates, Phenolics and steroids were present in all the 5 plant extracts. The preliminary phytochemical screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. Further, these tests facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds (Varadarajan.,2008).

Structurally, phenolic compounds comprise an aromatic ring, bearing one or more hydroxyl groups and range from simple phenolic molecules to highly polymerized compounds. Polyphenols may be associated with various carbohydrates and organic acids. These compounds exhibit a wide range of physiological properties, such as anti-inflammatory, antimicrobial and antioxidant effects (Manach 2004). In the present study presence of phenolics may be the reason for the antibacterial activity of the selected five plants.
The phenolic compounds caused severe damage to the bacteria. In addition, the modes of action of bacterial agents depend on the type of microorganisms and are mainly related to their cell wall structure and to the outer membrane arrangement. This study and many previous studies (Smith et. al 1998; Ceylan et. al 2004; Lopez et. al 2005; Shan et. al 2005) indicated that the most bioactive compounds of plant extracts were more active against Gram-positive bacteria than Gram-negative bacteria. This is likely due to the significant differences in the outer layers of Gram-negative and Gram-positive bacteria. Gram-negative bacteria possess an outer membrane and a unique periplasmic space which is not found in Gram positive bacteria (Duffy and Power 2001). Phenols and Tannins otherwise called carbolic acids are aromatic alcohols consisting of a benzene ring bonded directly to a hydroxyl group (OH) (De Ruiter, 2005). They are weakly acidic and have long history of roles in antisepsis and disinfection (Okigbo et al., 2009). They are used as the starting ingredients in the industrial production of drugs, herbicides, synthetic resins and additives to inhibit microbial growth in various ranges of pesticides (Greener Industry, 2009). Phenolics cause slow growth, block microbial cell division and enzyme activity. According to Okwu et al. (2007), they caused swelling of fungal hyphae tips, plasma seeping and leaking around hyphae tips; cell wall distortions, abnormal branching or fusion of hyphae surface. The presence of phenolics and tannins were observed in the five selected plants.

The alkaloids are known to have antimicrobial and antiparasitic properties. In the present study presence of alkaloid observed in A. indica, Bischfia javanica and Vitex negundo. Verpoorte (1998) have reported about 300 alkaloids showing such activity. Similar results on antibacterial activity were reported on related species of the genus Mahonia by Duraiswamy et al. (2006), Livia et al. (2004) and Li et al. (2007). Generally, the plant extracts inhibited the Gram-positive bacteria better than the Gram-negative ones. This is in agreement with reports on plant extracts by Tomas-Barberan et al. (1988), Vlietinck et al. (1995), Rabe and Van Staden (1997). The reason could be attributed to the presence of extra outer membrane in their cell wall acting as barrier for the compound(s) to diffuse into the bacterial cells.
Phytochemicals of the group alkaloids have complex structure. The presence of alkaloids is interesting, as significant quantities are used as antimalarials, anti bacterial analgesics and stimulants (Duke and Ayensu, 1985). They are the most physiologically active compounds of medical importance found in plants. Alkaloids and their derivatives are used as basic starting points for drugs. They possess antifungal and bactericidal properties (Okwu and Uchendu, 2009). Karlovsky (2008) reported that alkaloids can inactivate enzymes, block ion channels, interfere with neurotransmission and cause loss of electrical coordination (ataxia) in affected organisms.

Flavonoids are polyphenolic compounds possessing 15 carbon atoms made up of two benzene rings joined by linear carbon chain. They represent the most common and widely distributed class of plant phenolics. Flavonoids are a class of secondary metabolites known most commonly for their antioxidant and free radicals scavenging activities. A side of preventing oxidative cell damage; flavonoids also play roles in combating allergies and microbes (Okigbo et al., 2009).

The antibacterial activity of flavonoids is being increasingly documented. Crude extracts from plants with a history of use in folk medicine have been screened in vitro for antibacterial activity by many research groups. Flavonoid rich plant extracts from species of Hypericum, Capsella and Chromolaena have been reported to possess antibacterial activity. Many other phytochemical preparations with high flavonoid content have also been reported to exhibit antibacterial activity. Presence of flavonoids was observed in 4 plants except A. indica.

Saponins are glycosides of both triterpenes and steroids known for the soap-like foaming they produce in aqueous solutions. Saponins can ward off microbes and this makes them good candidates for treating yeast, viral and fungal infections (Enyiukwu et al. 2014). They are known to play a role in cytolysis by complexing with cell membrane bilayers (Okwu and Njoku, 2009) sometimes creating pores on them (Rongai et al., 2012) and pure saponin fraction exhibited
remarkable antibacterial activity when compared to crude extracts (Krishnan et al. 2008). Steroids and saponins were also present in the selected 5 plants.

Terpenoids have been found to be useful in the prevention and therapy of several diseases, including cancer, and also to have antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, anti hyperglycemic, anti inflammatory, and immunomodulatory properties (Rabi and Bishayee 2009).

Preliminary phytochemical analysis of Aristolochia indica L. has revealed the presence of alkaloids, tannins, cardiac glycosides, steroids, flavonoids and saponins (Vaghasiya and Chanda, 2007; Pattar and Jayaraj 2012). Most of the compounds are reported to be antibacterial by the earlier workers.

Komal kumar et al (2012) conducted studies on preliminary phytochemical analysis of Derris scandens. The ethanolic extracts of these plants indicated the presence of tannins, saponins, steroids, terpenoids, anthraquinones and phlobatonins. In the present study, the presence of anthraquinone, flavanoids and glycosides were observed in D. scandans.

The phytochemical screening of the Bischofia javanica was done by Khan et al (2001). They reported the presence of flavonoids, sterols, triterpenoids from the petroleum ether extract, alkaloids, flavonoids, saponins, sterols, tannins, triterpenoids from chlorophorm extract, and alkaloids, flavonoids, saponins, and tannins from ethanol extract. All the compounds are showing the antibacterial property.

Preliminary phytochemical analysis of leaves of Vitex negundo by Dhole (2011). In the present analysis positive result were obtained to alksoids, Glycosides, steroids, Phenolics, tannins, flavonoids triterpenes, and Coumarins and all the class of compounds posses antibacterial activity.

In the present investigation all the crude extracts contain Carbohydrates, glycosides, tannin, phenolics, steroids. The leaves of Aristolochia indica, Bischofia javanica, Vitex negundo contain alkaloids. The presence of alkaloids indicates their antifungal and bactericidal efficacy for treating bacterial and fungal
infections (Okwu and Uchendu, 2009). Except *Aristolochia* all the plants show the presence of flavonoids. Flavonoids also play roles in combating allergies and microbes (Okigbo et al., 2009). All the plant extract contain the tannin. Tannin-rich plants extracts are used by Asian natives for the treatment of ulcers (Peru, 2001; Dharmananda, 2007). So the above said plants are recommended for treating ulcers and wounds. Except *Aristolochia indica*, all the plants contain terpenoids. So the plants may have antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, anti hyperglycemic, anti inflammatory, and immunomodulatory properties (Rabi and Bishayee 2009). Quinones are not present in the plant extracts. Saponin compounds are reported in *Aristolochia indica* and *Bischofia javanica*. Plants with saponins have good anti-microbial potential. The presence of Coumarin is reported in the leaves of *D. scandans*.

**TLC Comparison Studies**

Khatoon *et al.*, (1993) used TLC finger printing technique and identify that the market samples 'Ratanjot' is derived from *Arnebia nohilis*. Asif & Shafiullah, (1993) analyzed 175 herbal drugs with infrared spectrum and evolved a method for checking the purity of herbal drugs. Quality standardisation of Medicinal Plants, Pharmacopoeia of different countries are being used TLC as a major criteria for quality control parameter (Gupta *et al.*, 2008).

The HPTLC comparison revealed of 5 plants revealed that the finger printing profiles of leaf consists of prominent bands corresponding to chemical constituents at 254 and 366 nm. Each plant showed specific bands at specific Rf values. In two plants, *M. fragrance* and *V. negundo* the leaf contains lot of volatile oils hence the GC was performed.

**A). Aristolochia indica**

The plant mainly contains aristolochic acid, methyl esters of 12-non acosenoic acid, besides n-heptadecane, n-triacontane, palmitic acid, hexa cosannic acid, stigma st-4-en-3-one, friedelin, cyclo eucalenes and rutin (Wealth of India 2000, Chotra et al, 2006). In HPTLC profile 5 prominent bands were observed at 254 nm and 9 prominent bands at 366nm. The TLC plates after derivatisation showed 4 prominent bands.
B) *Derris scandens*

Several anti dermatophyte active isoflavones and anti-inflammatory and cancer chemo-preventive rotenoids have been also reported from *Derris scandens* (Sekine *et al.*, 1999; Laupattarakasem *et al.*, 2004). A variety of biological active compounds have been identified from the extract of this plant. The major active constituents of *D. scandens* are benzyls and isoflavones, including genistein, coumarins, scandinone, scadenin, prenylated isoflavones, and isoflavone glycosides (Rukachaisirikul *et al.*, 2002; Laupattarakasem *et al.*, 2004; Mahabusarakam *et al.*, 2004; Rao *et al.*, 2007). The main compounds showing intestinal α-glucosidase inhibitory and free radical scavenging activity are scandinone, scadenone, scadenin A, scadenin B, and 4’, 5’, 7-trihydroxybiprenyl isoflavone (Rao *et al.*, 2007). Ganapathy et al (2006) isolated flavonoids, ovaliflavanone and lupinifolin from the leaf extracts.

In the present study HPTLC profiling showed 8 prominent bands at 254 and 366 nm with little variation in the Rf values. In addition to all the compounds percentage of umbelliferone was quantified and its percentage ranges from 0.041 to 0.045 in the samples analyzed. It is the first report in his plant. The best solvent system for the profiling was Toluene: Ethyl acetate: Formic acid in the ration of 7:3:0.3. The best solvent system for the profiling was Ethyl acetate: Toluene: Formic acid in the ration of 7:3:0.2.

C). *Myristica fragrans*

Maya *et al* studied HPLC of leaf amino acids. Dry leaf samples were refluxed with alcohol. Using the HPLC technique 11 amino acids were separated from the leaves of *Myristica fragrans*. The HPTLC profiling shows that 6 prominent bands at 254 nm and 9 bands at 366nm and 5 bands in the derivatised plants. The best solvent system for the profiling was Ethyl acetate: Toluene: Formic acid in the ration of 7:3:0.3.

Volatile oil from the leaves of *Myristica fragrans* was isolated and characterized by Gas Chromatography- Mass Spectroscopy (Mary Helen *et al*, 2012). Twenty constituents from 20 peaks were identified beta- pinene (22.69%),
alpha-pinene (14.06%), alpha-thujen (13.93%) and p-menth 1-en-4-ol (10.53%) are as the major constituents and this leaf oil contains the minor constituents like camphene (3.92%), α-terpinene (3.42%), Limonene (9.56%) p-cymene (6.86%) Linalool (7.41%), beta myrcen (4.81%), alpha fellandrene (3.14%), 3-carene (3.54%), allyl catechol methylene (8.32%), myristicin (7.20%), elermicin (9.85%), beta ocimene (4.74%), gamma terpinene (4.75%), alpha terpinolene (4.38%), p-menth 2-en-1-ol (3.30%) and α-terpineol (6.00%).

From the investigation 41 compounds were identified in leaf oil, of which α-pinene predominates in the oil with 13.78%. 4-terpeneon (6.81%), Terpenolen (5.22), Elemicin (4.54%) were the other major compounds present in the oil analysed. All the remaining compounds were considered as minor.

**D. Bischofia javanica**

Gupta *et al* (1988) investigated the chemical constituents of *B. javanica*. It has been shown to have antiulcer, anthelmintic and antidysenteric activities. Extracts of leaves yielded n-triacontane, β-amyrin, friedelene, β-sitosterol, ursolic acid, chrysoeriol, fisetin, quercetin, luteolin-7-O-glucoside and ellagic acid. Tartaric acid (8-10%), tannin and vitamin C (136/100g) also present in this plant.

The HPTLC profiling using Ethyl acetate: Toluene: Formic acid solvents in the ration of 7:3:0.3 showed 6 bands in 254 nm and 9 bands at 366 and 8 bands in derivatised TLC plates.

**E. Vitex negundo**

*Gautam et al* (2008) conducted Bioactivity guided phytochemical investigation of methanolic extract of leaves of *Vitex negundo* resulted in the isolation of eight compounds under silicagel VLC, CC and preparative TLC. They were identified as negundoside, agnuside, vitegnoside, 7,8 dimethyl herbacetin 3-rhamnoside, 5,3’-dihydroxy-7,8,4’-trimethoxy flavanone, 5-hydroxy-3,6,7,3’,4’-pentamethoxy flavone, 5,7 dihydroxy-6,4’ dimethoxy flavonone, and 5 hydroxy-7,4’ dimethoxy flavone. The structures of pure compounds were elucidated by MP, Rf values, Co- TLC, Colour reactions (Cerrie sulphate spray), mild acid
hydrolysis and spectroscopic methods (Mass, UV, IR, 1H, 13C and 2D NMR). HPTLC was performed using Ethyl acetate: Toluene: Formic acid solvents in the ratio of 7:3:0.2 showed 8 bands in 254 nm and 12 bands at 366 and 7 bands in derivatised TLC plates.

Essential oils from fresh leaves, flowers and dried fruits of *Vitex negundo* were obtained by hydrodistillation. The chemical constituents of essential oil of leaves, flowers and dried fruits were analyzed by GC-FID and GC/MS techniques. Main constituents identified in leaves oil were delta-guaiene, carryophyllene epoxide and ethyl-hexadecenoate (Khokra et al, 2008).

In the present study about 39 compounds were identified in leaf oil using GCMS, of which Ledol predominates in the oil with 17.14%. Caryophellene (13.77%), β-Phyllandrene (7.39%), 4-terpeneon (6.81%), Terpenolen (5.22), Elemicin (4.54%) are the other major compounds present in the oil analyzed.

### 5.5. Pharmacological Properties of Documented Plants

All the selected plants having anti-microbial, anti-inflammatory. All the plants having anti-cancerous properties except *Derris scandens* Benth. Among 55 plants having high Use value, all the documented plants possess anti-microbial properties, 44 having anti-oxidant activity, 37 plants having anti-inflammatory activity, 28 having anti-cancerous activity, 2 plants having anti-allergic activity.

The antimicrobial properties of certain Indian medicinal plants were reported based on folklore information (Ahmad *et al.*, 1998; Mehmood *et al.*, 1999; Perumal Samy *et al.*, 1998, 1999). The substances that can inhibit pathogens and have little toxicity to host cells could be considered candidates for developing new antimicrobial drugs (Bajpai *et al.*, 2005). These compounds find in various medicinal plant organs such as stems, roots, leaves, barks, flowers, fruits and seeds (Cutter, 2000). The most important of these medicinally compounds are alkaloids, tannins, flavonoids and phenolic compounds (Amal *et al.*, 2009).
There is an urgent need to explore and discover new antimicrobial compounds with diverse chemical structures and novel mechanism of action for new and reemerging infectious diseases (Rojas, Brotz, 2003). Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads for developing better drugs against microbial infections (Benkeblia, 1996). The increasing failure of chemotherapeutics coupled with antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Colombo, Bosisio. (1996). Several factors are known to influence the active principle present in the plant. Polarity of the extracting solvent greatly influences the antimicrobial property. The activity of plant extracts against both gram positive and gram negative bacteria may be an indicative of the presence of broad spectrum antibiotic compounds or simply general metabolic toxins in the plant. Generally gram negative bacteria are resistant than gram positive bacteria (Rabe, Van Staden, (1997), Parekh, Chanda, (2005).

We screened five plant extracts for their antimicrobial effects on Bacteria utilizing commercially available alcoholic, water for extracts. Traditional practitioners make use of water preliminary as solvent, but our studies showed that the ethanolic extracts of this plant parts were certainly much better and powerful. Burapedjo S, Bunchoo A (1995). Antimicrobial activity of tannins from Terminalia citrine. Planta Med. 61:365-366. These Observations can be rationalized in terms of polarity of compounds being extracted by each solvent and, in addition to their ability to dissolve or diffuse in different media used in assay. The growth media also seem to play an important role in the determination of antibacterial activity (Lin, et al, Opoku, (1999).

Lin reported that Muller- Hinton agar appears to be the best medium to explicate the antibacterial activity and same was used in present study.

In general, the plant antibiotic substances appear to be more inhibiting to gram positive organisms than gram negative type. Unlike Gram positive bacteria, the lipopolysaccharide layer along with proteins and phospholipids are the main
reason for their inhibitory action. Most of them being inhibited to Gram positive bacteria unlike gram positive bacteria, lipopolysaccharide layer along with proteins and phospholipids are the major component in the outer surface in gram negative bacteria (Burn, 1988). Access of most components to the peptidoglycon layer of cell wall is hindered by the outer lipopolysaccharide layer. This explains the resistance of the Gram negative bacteria to the lytic action of most extracts exhibiting the activity. Presence of the phytochemical constituents such as alkaloids, flavanoides, tannin, and phenolic compounds has been reported to be important compounds in many other medicinal plants (Barnabas and Nagarajan, 1988), Burapedjo, Bunchoo, (1995).

In the present investigations, ethanol extract of leaves posses alkaloids, flavanoides, tannin, and phenolic compounds which might shows antibacterial activity. Previously some reports are there concerning the antibacterial activity of all the selected plants but our findings support the efficacy.

Demands of the scientific community have necessitated experimental evidence to further underline the medicinal importance of these five plants. Taking clue from these traditional and folk systems of medicine, scientific studies have been designed and conducted in order to pharmacologically validate these claims.

A) Aristolochia indica

In the present study, the aquous extract of leaf showed maximum inhibitory activity against test organisms in different degrees except against P. aeruginosa K.phemoniea and these two are gram negative bacteria. The presence of aristolochic acid may be the active principle responsible for the activity.

B) Derris scandens

In the present study the ethanolic extract of leaf showed inhibitory activity against test organisms in different degrees. The result clearly indicate that gram positive strains Staphylococcus aureus and Bacillus subtilis are highly susceptible
to ethanolic extract of leaf of *D. scandens*. The gram negative strains, *Klebsiella pneumoniae* shows moderate activity. The strain *Pseudomonas aeruginosa* and *Escherichia coli* is resistant to ethanolic extract of leaf of *D. scandens*.

**C) Myristica fragrans**

In the present study, the aqueous extract of leaf showed inhibitory activity against test organisms in different degrees. The result clearly indicate that gram positive strains *staphylococcus aureus* is highly susceptible. *Bacillus subtilis* shows less activity compared to *staphylococcus aureus*. The gram negative strains *Escherichia coli*, gram positive strain *Pseudomonas aeruginosa* shows moderate activity. *Klebsiella pneumoniae* is resistant to aqueous extract of leaf of *Myristica fragrans*. In the chemical investigation the percentage of myristicin is 3.9 and is reported to be a good antibacterial agent.

**D) Bischofia javanica**

In the present investigation, the ethanolic extract of leaf showed inhibitory activity against test organisms. The result clearly indicate that gram positive strains *staphylococcus aureus* and gram negative strains *Escherichia coli*, *Klebsiella aerogenes*, is highly susceptible to ethanolic extract of leaf of *Bischofia javanica*. It is the best among other 4 plants tested against gram positive and negative bacteria. The chemical compounds like quercetin and ellagic acid are good antibacterial agents.

**E). Vitex negundo**

The fresh juice of leaf showed inhibitory activity against test organisms in different degrees in the present screening. The result clearly indicate that gram positive strains *staphylococcus aureus*, *Bacillus subtilis* and gram negative strains *Escherichia coli*, *Klebsiella aerogenes* susceptible to aqueous extract of leaf of *Vitex negundo*. The gram negative strain *Pseudomonas aeruginosa* is resistant to aqueous extract of *Vitex negundo*.

There is no doubt that the 113 recorded plant species are awaiting search for new uses. So, further scientific assessment of these medicines for
phytochemical, biological and clinical studies is however greatly needed. The present research work indicates that research projects should be designed in priority on this area for the pharmacological evaluation and conservation of medicinal plants in the study area. These studies even provide a significant frame work for utilization of such traditional knowledge in deriving conservation strategies.